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Light and Electron Microscopic Observations on Life Cycle Stages of *Adelina grylli* Butaeva 1996 (Sporozoa, Adeleidae) from the Fat Body of the Cricket *Gryllus bimaculatus*.

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Summary

Adelina grylli Butaeva, 1996 (Sporozoa, Adeleina) was described from crickets *Grillus bimaculatus* from Middle Asia (Ashhabad). The parasite infected the laboratory population of *Gryllus bimaculatus*. Zoites, dividing schizonts, macrogamonts, microgamonts and oocysts are studied in light and electron microscopes (EM). The infective stage is a sporulated oocyst with 4 - 20 sporocysts, each containing 2 sporozoites. Sporozoites, like other generations of zoites, display ultrastructure typical for the coccidian motile stages with apical complex, pellicle, micronemes and subpellicular microtubules. Sporozoites penetrate through the intestinal epithelium inside the fat body cells, the primary infection site. There they are enveloped in a parasitophorous vacuole (PV) that often contains inclusions derived from both the host cell and the parasite. The growing parasite loses its apical organelles to convert into meront. Meronts undertake schizogony with the formation of bundles of merozoites. Merozoites of the second generation give rise to meronts, macrogamonts or microgamonts. A tubular-like secretory material resembling the tubular network of *Toxoplasma* PV is discovered inside the PV containing microgamonts of *A.grylli*. Micro- and macrogamonts are associated in a syzygy. The zygote contains abundant amylopectin and numerous protein granules and vacuoles. The host haemocytes are involved in the oocyst wall formation. Cellular response to the infection with *Adelina grylli*: proliferation of haemocytes, melanization, infiltration of the infected tissue with haemocytes, is very prominent, especially on sporogony stages of parasite development. Only 3 adeleid coccidian species have been so far studied in EM. The present study provides new data on the ultrastructure of life cycle stages of *Adelina grylli*, aimed to additional diagnostic criteria for *Adelina* species.

Key Words: Coccidia, Adeleidae, *Adelina grylli*, *Gryllus bimaculatus*, ultrastructure

Introduction

Almost all known homoxenus adeleid coccidians belong to the genus *Adelina* Hesse, 1911 originally found in Oligochaetes *Slavinia appendiculata.* All *Adelina* species are parasites of invertebrates , predominantly of the Arthropods. Fourteen from 20 known species of this genus are parasitizing in Insects (Purrini, 1984; Malone, Dhana, 1988), others are harboured by mites, myriopodes and oligochaetes. Representatives of the genus *Adelina* have been studied much less than other Apicomplexa, due to their comparatively low practical importance. Despite their vast distribution among insects *Adelina* species are of great interest for plant protection, as they obviously play an essential role in the natural control of pests (Malone, Dhana 1988). For example, infection with *Adelina* blocks pupa-

tion and reduces fecundity in scarabeids (Weiser & Beard,1959; King, Mercer, 1979). Coccidians of the genus *Adelina* often cause severe infestations in laboratory cultures of insects (Yarwood, 1937). In our laboratory *A.grylli* commonly infects *Grillus bimaculatus, Achaeta domesticus* and *Locusta migratoria migratorioides.* The systematic of adelins is based on life cycle peculiarities, host and tissue specificity, the number and size of the sporogonic stages. So far only three *Adelina* species have been studied ultrastructurally and no ultrastructural diagnostic criteria of *Adelina* species are available. The present paper is aimed to contribute to the development of such criteria.

Adelina grylli Butaeva, 1996 (Sporozoa, Adeleina) was described from crickets *Grillus bimaculatus* on the basis of light microscopic studies of alive and fixed material (Butaeva ,1996a). The life cycle of *A.grylli* involves the following events : mature oocysts penetrate inside the intestine of a new host with food due to cannibalism, necrophagia or experimental infection. Sporozoites are liberated from the oocysts and pass through the gut epithelium to enter the fat body cells, where they transform into meronts. The meronts give rise to merozoites (zoites) invading new fat body cells. The next generation of zoites may be transformed into either new meronts, microgamonts (in this case meront divides to form 2-4 gamonts) or macrogamonts. Microgamonts move actively towards macrogamonts to be eventually associated in a syzygy, that later transforms into a zygote. The latter divides to give rise to sporocysts. Each sporocyst forms 2 sporozoites. The duration of the whole life cycle is 45-55 days. The fine morphology of young schizonts, female gamonts and free sporozoites of *A.grylli* was reported elsewhere (Butaeva, 1996b). This paper fills the gap in the present knowledge of the *A.grylli* life cycle, providing both light an electron microscopic evidence.

Material and methods

Adelina grylli Butaeva, 1996 was initially discovered in the crickets *Gryllus bimaculatus* in the environments of Ashhabad (Turkmenia) in the 80-s. Several crickets were brought to St.Petersburg to establish the laboratory cultures of *G. bimaculatus* and *Adelina grylli* at the Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences (St.Petersburg). The cricket culture was maintained as described elsewhere (Kniasev, 1985). The infection is commonly transmitted with tissues of dead or enfeebled crickets containing ripe sporocysts with infective sporozoites. Non infected (control) crickets, used for the experimental infections, were reared from thoroughly washed eggs (Butaeva, 1996a), as the infection is not transmitted transovarially. Peroral experimental infection was performed by spreading mature (sporulated) oocysts in food. The third instar larvae were most susceptible to the infection. Besides «natural» infection that permanently occurred in the laboratory culture, was also examined. For the present study, infected female crickets were taken 1-5 days after the last molt . Light microscope studies of alive coccidians were performed on smears from infected fat bodies. Slides were examined in Opton-46 microscope equipped with an interference contrast objective. Measurements were made using an ocular micrometer. For electron microscopy small pieces of infected fat bodies were fixed in 1% glutaraldehyde in cacodilate buffer, washed up in the same buffer, postfixed in 1% osmium tetroxide, dehydrated in ascending ethanol series and pure acetone, embedded in Epon-Araldite. Sections were stained in saturated solution of 2% uranyl acetate in 50% etanol followed by lead citrate and viewed in a Hitachi 300 electron microscope.

Results

Light microscopy. The most abundant developmental stages observed were the sporogonic stages: unsporulated oocysts, sporulated oocysts, sporoblasts, sporocysts and sporozoites (Fig.1-5). Unsporulated oocysts measured 36.3 ± 2.39 [8] [1)] µm in diameter. Their nuclei were not visible, the cytoplasm was granulated and refractive (Fig. 1-4). Eventually a thick multilayered wall was built around the oocyst which divided to form 4-10 sporoblasts (Fig. 3). The cytoplasm of sporoblasts was homogeneous, and their walls were comparatively thin. The sporoblast transformed into a sporocyst (13.3 ± 0.32 µm [7]) with thick walls. Each sporocyst contained laterally located two sporozoites with granulated residual bodies inbetween (Fig.4). Often the remnants of microgamonts were observed still attached to the oocyst wall on very late stages of sporogony (Fig. 4). Macrogamonts (20.5 ± 2.89) [42] X 17.1 ±2.78 µm [49] and 1-2 round microgamonts 9.6 ± 0.78 [23] X 7.45 ± 0.75 µm [28]) were often seen associated in a syzygy (Fig. 5,6). After fertilization microgamonts changed their shape to become more „flatten“ (Fig. 4). The consequent stages of schizont maturation were observed: an early „morula-shaped» schizonts (Fig. 8), an „intermediate stage“ (Fig. 9) and a late „barrel shaped» schizont (fig.10) with elongated merozoites inside. All schizont stages measured nearly the same (25.6 ± 0.98 [5] x 16.4 ± 0.82 [5] µm). Two or three types of zoites were observed on smears, though not well different from each other (Fig. 3,7) . Broader (25 ± 0.77 [9] x 5.5 ± 0.2 [11] µm) zoites were presumably microgamonts, whereas thinner and elongated parasites (22.6 ± 1.34 [18] x 2.4 ± 0.23 µm [39]) were sporo- or merozoites. Zoites commonly move by either typical screw-like motion or periodical bending of two ends. The latter type of move-

[1)] Figures in square brackets indicate the number of measurements

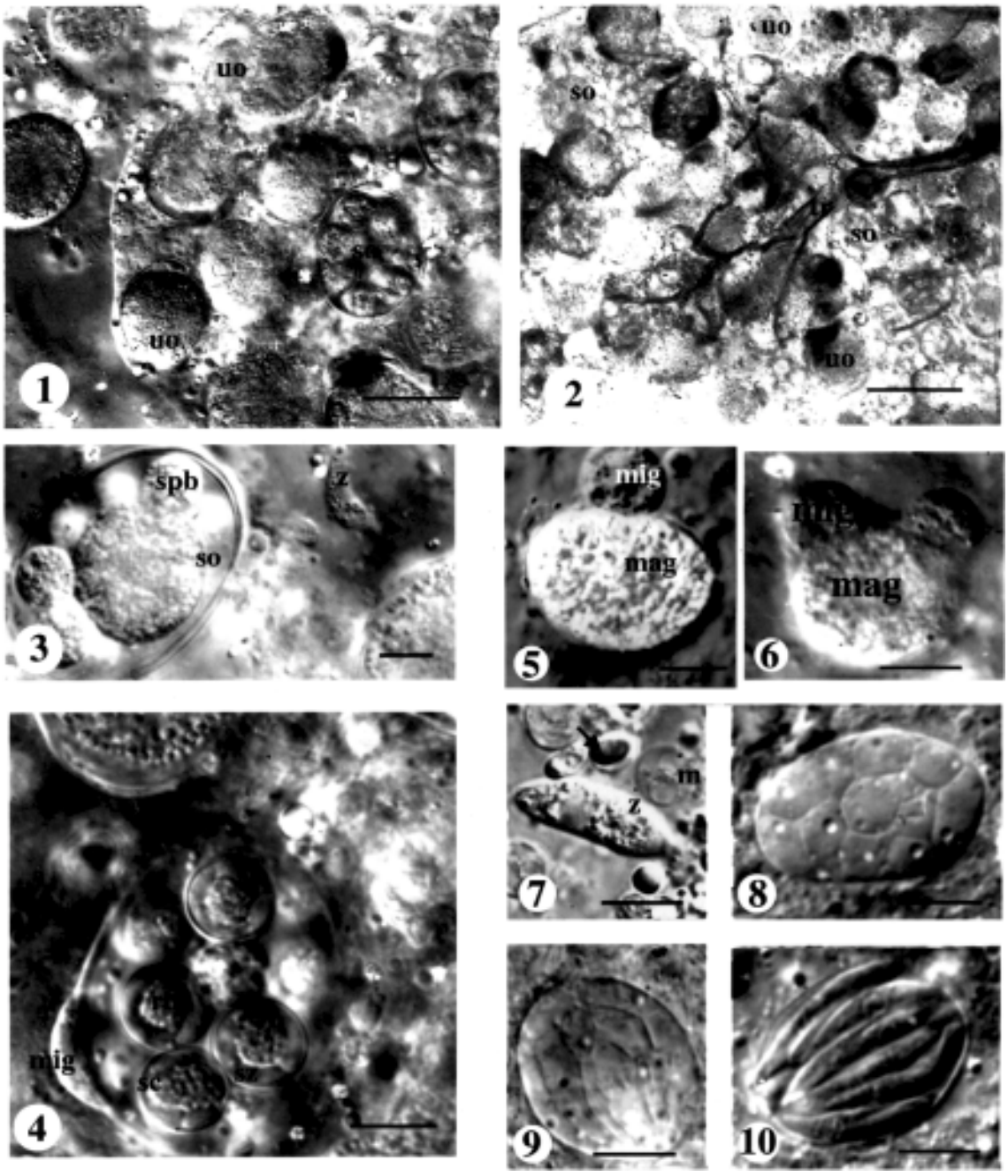


Fig. 1-10. Light microscopy of *Adelyna grylli* stages

Fig. 1 - Region of heavily infected fat body (FB) filled with sporogony stages of *A.grylli*. SO - sporulated oocyst; UO - unsporulated sporocyst. Scale bar: 20µm

Fig. 2 - Parasites accociated with intracellular tracheoles (TR). SO - sporulated oocyst; UO - unsporulated oocyst. Scale bar: 45µm

Fig. 3 - „Partially“ sporulated oocyst (SO). Spb - sporoblast. Scale bar: 10 µm

Fig. 4 - Sporulated oocyst (SO) with the remnants of microgamont (MIG) attached to the wall. Sporocysts (SC) contain two sporozoites (SZ) and residual body (RB). Scale bar: 10 µm

Fig. 5 - Macrogamont (MAG) and micragamont (MIG) in the syzygy. Scale bar: 10 µm

Fig. 6 - Two microgamonts (MIG) associated in a syzygy with one macrogamont. Scale bar: 10µm

Fig. 7 - Zoites (Z) of different types on smears of the infected cricket fat body. M – a microsporidian meront. Arrows indicate microsporidian spores. Scale bar: 10 µm

Fig. 8-10 - Stages of the schizont maturation:

Fig. 8 – a „morula-shaped“ schizont; Fig. 9 – an „intermediate stage“; Fig. 10 – a mature schizont. Scale bar: 10 µm

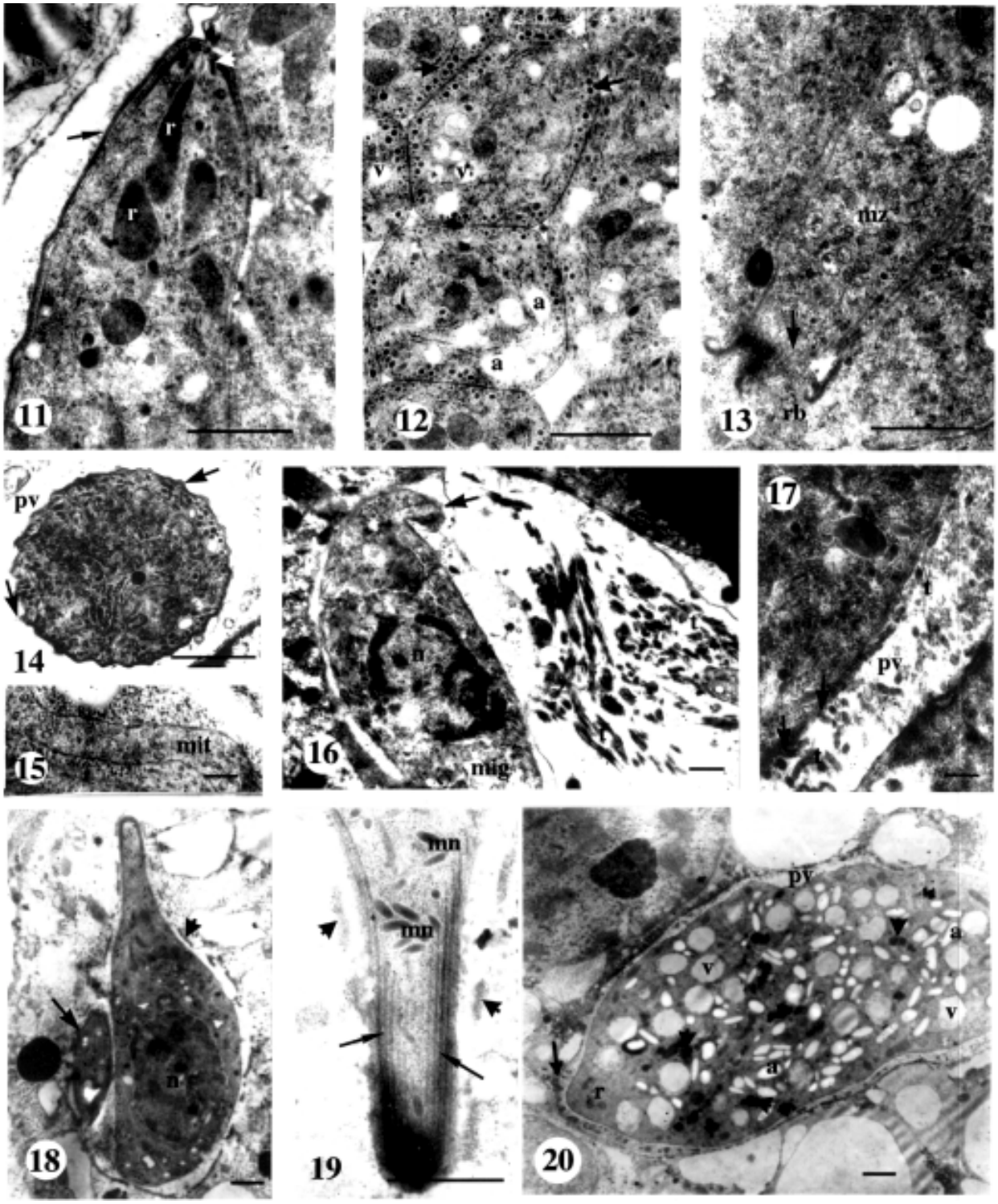
ment is especially characteristic of microgamonts. Besides *Adelina grilli* the fat body of *Gryllus bimaculatus* is often infected with microsporidian *Nosema grylli* (Sokolova et al., 1994). Spores and developmental stages of *N.grylli* are permanently observed simultaneously with the coccidian stages (Fig.5).

Ultrastructural observations. No differences were revealed between intracellular „free" sporozoites of *A.grylli* (Butaeva, 1996b) and its merozoites undergoing schizogony and packed in bundles. Unlike those in bundles the cross-sected free zoites whithin the parasitophorous vacuoles, often exhibit «undulating" superficial contours (Fig.14) with electron dense ridges on the periphery, suggesting that the latter are more motile than the former within the cell. *Adelina grylli* zoites contain the same set of organelles as motile vermiform stages of other coccidian species (Fig. 1: 3-layered pellicle, that consists of an outer membrane, an inner membranous complex and a layer of subpellicular microtubules (Fig. 11-13). The apical complex includes a short conoid and a polar ring. The layer of microtubules derives from the polar ring (not shown here, see Butaeva,1996b). Microtubules reach the distal part of the cell (Fig. 13) as it is well seen in merozoites not yet separated from the schizont residual body. Electron dense rhoptries numbering 8-10 extend posteriorly from the apical pole (Fig11). Abundant micronemes are located mostly underneath the microtubular layer (Fig.12,14). Zoites of *Adelina grylli* exhibit a usual set of intrinsic organelles of eukaryotic cells: a large nucleus with chromatin patches, occupying nearly the whole width of the zoite; a mitochondrion with tubular crystae; ribosomes and endoplasmic reticulum (Fig.11-16). In sporo- and merozoits amylopectin granules and electron lucid vacuoles are distributed randomly, though in a lesser number, than in macrogamonts and oocysts (Fig.11-13). Like other intracellular stages, each zoite within the host cell is surrounded by an envelope of a parasitophorous vacuole (PV), which is composed of 3 layers (two inner membranous and an outer - electron dense one) (Fig. 14, 17). Within the space of the PV tubules of c.30-40 nm in diameter are noticed, being especially abundant in the PV, harboring developing **microgamonts** (Fig.16-17). These tubules seem to derive from the parasite surface. Microgamonts bear some common features of both zoites and meronts: an elongated apical edge with numerous microtubules, a layer of electron dense material underlying its front region and micronemes (Fig.18-19). The posterior edge of the microgamont is coiled backwards and this „bending" figure is permanently observed on sections (Fig.16,18). The nucleus with chromatin granules being distributed throughout the nucleoplasm, is large and occupies nearly the whole width of the cell. Two microgamonts, probably deriving from one merozoite are often seen to share the same PV (Fig.16). However each zoite is enclosed in its own vacuole encircled by the common PV membrane. Patches of electron dense material, underlie the outer membrane of the PV (Fig.18,19). Microgamonts are often observed near **macrogamonts.**

After penetrating the fatbody cell, a previously elongated „female" zoite looses its apical complex and subpellicular microtubules, though a small anterior process and rhoptries often remain visible (Fig. 20). Eventually the parasite cell becomes spherical (Fig.21). Each macrogamonts possesses a large nucleus with prominent nucleolus , mitochondria end endoplasmic reticulum. Amylopectin granules, electron lucid vacuoles and dense protein granules are abundant (Fig.20,21). PV The PV surrounding a macrogamont, displays the multilayered structure. Some electron dense material is accumulated outside and inside the PV. This material seems to enter the PV, when its membrane makes deep invaginations. (Fig.20) . No tubules similar to those derived from microgamonts, are visible within the PV.

Micro (MIG)- and macrogamonts (MAG) in syzygy. The internal structure of both cells inside a syzygy is very much alike. Although they differ in size (MIG is smaller), in electron density (MIG cytoplasm is more electron dense); in distribution of granules (MIG has obviously less protein and amylopectin granules (Fig.22,24). The process of microgamete formation and fertilization has not been observed in the present study. After fertilization the appearance of both gamonts undergoes striking changes. MIG elongates, looses most of its amylopectin granules and actually becomes part of a newly formed **zygote** (**oocyst)** wall, being located between the layers of the wall (Fig.23). The oocyst wall is impermeable to fixative solutions and embedding materials, which accounts for the poor preservation of inner structures of the oocyst, particularly cytoplasm organelles, except a big amount of electron lucid granules.

Localization in the host and host response to the invasion. The parasites are localized mostly in the fat body, and in heavily infected crickets may occupy nearly the whole volume of this organ, primarily around intracellular tracheoles (Fig.2). The infected fat body is getting less in volume, and contains fewer lipid droplets and protein granules. Its specific gray color is due to the presence of numerous melanized capsules around oocystes formed by closely packed insect haemocytes and the products of their secretion. The early intracellular stages (zoites, meronts and gamonts) are hardly visible within the host cells (because of the same electron density), and no alterations in the host cell cytoplasm can be noticed at that period. The cell response seems to be activated by sporogonic stages. At this period the fat body is being infiltrated with haemocytes of different types: granulocytes, oenocytes, plasmotocytes (according to the classification of Beard,1953) They actively participate in the formation of capsules around zygotes and oocysts (Fig.25) and cause melanization in infected tissues.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Discussion

Light microscopy; characteristic features of t *Adelina* species. The morphology of various *Adelina* species from different hosts as revealed by light microscopy, is very similar (Leger, 1904 (from Embioptera); Bhatia, 1937 (from Coleoptera) ; Yarwood, 1937 (from Blattoptera); Weiser & Beard,1959 (from Coleoptera); Tuzet et. al., 1965 (from Oligochaeta); Purrini, 1984 (from Apterigota) ; Butaeva, 1996a (from Orthoptera)). At the same time the reported differences in body sizes, in the number of sporozoites per sporocysts or sporocysts per oocysts; in the life cycle patterns, and host and host-tissue specificity have provided enough evidence to consider *Adelina grylli* as a separate species (Butaeva, 1996a). All the mentioned above criteria of distinction should be applied very carefully for diagnosis of new species. Size fluctuations of particular stages, as well as differences in the number of sporocyst per oocyst vary significantly in different *Adelina* species and may depend on the temperature and other environmental factors (Yarwood, 1937); besides, cytochemical studies and experimental infections, that are badly needed to correctly distinguish between different development stages , i.e. microgametes, are not always technically possible (Butaeva, 1996a). The predominant site of infection in *Adelina* species is the fat body, though *A.sericesthis* may also infect connective tissues (Weiser & Beard, 1959); *A.creptocerci* or *A.tribolii* were found in all examined tissues of their hosts (Yarwood, 1937; Bhatia, 1937). As it concerns the host specificity, these parasites are far from being host specific. *A. sericesthis* infects larvae of several scarabaeides in nature (Weiser & Beard, 1959). Malone & Dhana (1988) reported that lepidopterans served an alternative host for *A.tenebrionis* (from *Heteronichus arator* (Coleoptera)). *Achaeta domesticus* and *Locusta migratoria* were easily infected with *A.gryll*i in our experiments.

In terms of the host taxonomy and ecology, in addition to the mode of host cell infection, general morphology of endogenous stages, and the life cycle peculiarities, *A.grylli* has much in common with *A.cryptocerci* (Yarwood, 1937). These two species differ from each other in host species, host tissue specificity, and in sizes of homologous stages. But the most fundamental difference between them lies in the mode of gametogony: whereas merozoites of *A.grylli* give rise to two different types of schizonts, producing male and female gametes (Butaeva, 1996a), the male and female gametes of *A.cryptocerci* derive from the same type of schizonts to differentiate later (Yarwood, 1937). Although the ultrastructural analysis of *A.cryptocerci* was not available, excellent drawings in Yarwood's paper make it possible to compare the data on *A.cryptocerci* histology with those on *A.grylli* ultrastructure suggesting a very close similarity in the fine morphology between these two species.

Ultrastructure. So far only three *Adelina* species have been studied with electron microscope: two of these - *A.tribolii* (Zizka, 1969, 1982,) and *A.tenebrionis* (Malone & Dhana, 1988) from insects and the third one - *A.dimidiata* - from myriapods (Tuzet, 1970). The organelles of motile stages (nucleus, mitochondria, rhoptries, micronemes, apical complex) are very much alike in all the species studied . The „folded motile stage", presumably a microgametocyte, reported for *A.tenebrionis* was also found in the life cycle of *A.grylli*, where it was seen displayed nearly the same form. The arrangement of zoites in bundles is more tight in *A.grylli*, than in *A.tenebrionis* (Malone & Dhana,1988). No crystalline bodies were revealed in *A.grylli* zoites, which differ from the record for *A.tribolii* sporozoites (Zizka,1985). The zoites of *A.grylli*, similar to any intracellular stages of other *Adelina* species are surrounded by 3-layer envelope of parasitophorous vacuole (PV). The PVs containing the *A.grylli* zoites, especially around microgamonts, often contain numerous membranous tubules, extending from the parasite. This fact has never been reported before for other *Adelina* species. The diameter (c.30-40 nm) and location of the tubules fit well the pattern of a „tubular network" recorded for tachyzoites of *Toxoplasma gondi* (Sibley et.al., 1986; Halonen et al., 1996). In *T.gondii* these tubules are supposed to derive from membranous secretions originating from dense granules. In *Toxoplasma* this secretion starts during the invasion to continue throughout the whole period of tachyzoite development. This type of secretion is presumably shared by all coccidians, and no wonder that it may also occur in *Adelina* species. The ultrastructure of macro- and microgamonts (gametocytes) of *A.grylli* is much alike that of other studied species,

Fig. 11 - 17 Ultrastructure of *Adelina grylli* zoites

Fig.11 Longitudinal section of a merozoite inside the bundle. Arrow shows the pellicle of zoite. R - rhoptries. Arrowhead points to a conoid;.; arrow – to a pellicle; Scale bar: 1 µm

Fig.12 Cross sectioned merozoite bundle. A - amylopectin granules; V - vacuoles. Arrow indicates a row of micronemes underlaying the pellicle. Scale bar: 1 µm

Fig.13 Cross sectioned merozoite bundle. Merozoites (MZ) still connected with a residual body (RB) of the schizont. Subpellicular microtubules (arrow) reaching the posterior end of a zoite. Scale bar: 1 µm

Fig.14 Cross section of a merozoite. PV - parasitophorous vacuole. Arrows indicate undulations of the parasite pellicle. Scale bar: 1µm

Fig.15 Mitochondtion (MIT) with tubular cristae. Scale bar: 1 µm

Fig.16 Parasitophorous vacuole containing two microgamonts (MIG) and filled with tubular secretory material (T). Arrow indicates a coiled posterior edge of the microgamont. N – nucleus of the microgamont. Scale bar:1µm

Fig.17 Parasitophorous vacuole (PV) with tubules (T) at higher magnification. Arrows shows the tubules contacting the parasite envelope. Scale bar: 0.2 µm

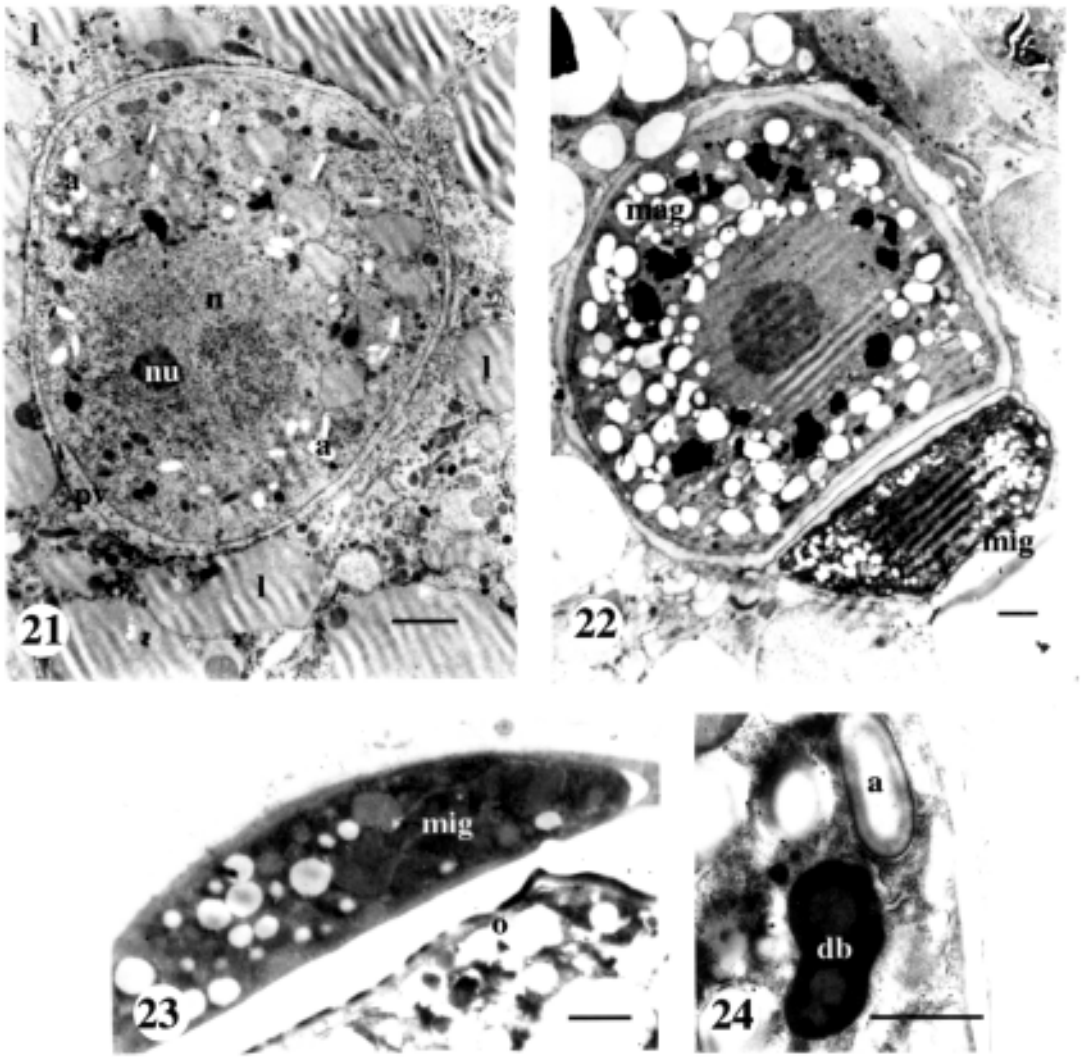

Fig.18-24 Ultrastracture of *Adelina grylli* gamonts

Fig. 18 – A microgamont inside the host cell. N - nucleus. Arrow shows a coiled posterior edge of the microgamont. Arrowhead points to the outer electron dense layer of the PV envelope. Scale bar: 1 μm

Fig.19- The anterior edge of a microgamont. MN - micronemes. Arrow indivates subpellicular microtubules. Arrowhead points to the outer electron dense layer of the PV envelope. Scale bar: 1 μm

Fig.20 - Macrogamont in the fat body cell with its apical edge with rhoptries (R) still noticeable. Cytoplasm with numerous vacuoles (V), amylopectin granules (A) and dense bodies (arrowheads). Arrow shows invaginations of the PV envelope indicating a possible way of transport of nutrients inside PV. Scale bar: 1 μm

Fig.21 - Round macrogamont inside the host cell: A - amylopectin granules; L - lipid droplets of the adipocyte; PV- parasitophorous vacuole; N - nucleus; N - nucleoulus; V -vacuole. Scale bar: 1 μm

Fig. 22 Macrogamont (MAG) and microgamont(MIG) associated in the syzygy . Scale bar: 1 μm

Fig. 23 Microgamont (MIG) attached to the oocyst (O) wall. Scale bar: 1 μm

Fig.24 Dense bodies (DB) and amylopectine granules (A) at higher magnification . Scale bar: 1 μm

though with a few differences. In *A.grylli* the macrogamont cytoplasm contains less amylopectin but more electron dense granules than in *A.tenebrionis.* The latter granules, obviously undergoing processing as macrogamonts mature, are transfer eventually into „multivesicular bodies" described for *A. triboli* (Zizka,1969). The arrangement and ultrastructure of macrogamonts and microgamonts of *A.grylli* associated in a syzygy displays much similarity in all examined *Adelina* species. Earlier investigations (Butaeva, 1996a) showed that nuclear division in microgamonts gives rise to 4 microgametes. Cytochemical studies revealed an intensive PAS reaction in macrogamonts and oocysts suggesting a high polysaccharide storage in these (Butaeva, 1996a). That is in accord with electron microscope data, demonstrating that amylopectin granules are more abundant in macrogamonts, than in microgamonts. More basophilic staining of microgamonts on histological preparations (Butaeva,1996a) corresponds to a higher electron density of microgamonts of all studied adelins.

Host-parasite interactions. In *A.grylli* as in *A.sericesthis* (Weiser & Beard, 1959), a striking increase in the number of haemocytes, and more intensive melanization of the haemolymph exposed to the air, was noticed in the infected crickets. With light and electron microscopes we observed the increased number of haemocytes just in the vicinity of the infected cells. The majority of haemocytes were identified as oenocytes or cystocytes as they contain crystalline inclusions inside large vacuoles. Oenocyte-like haemocytes are considered to be the main source of tyrosinase in insects. They seem to participate in the processes of melanization during infection. Cellular capsules around mature oocysts are formed by plasmotocyte-like haemocytes . By the end of the development mature oocysts are surrounded by thick capsules formed by the sporocyst multilayered wall, and several layers of flattened haemocytes, cemented by melanins. A question arises if this is a true or inverted defense reaction, and whether the cysts are still alive under such a cellular sheet? Our studies can not give an unequivocal answer to these questions. Weiser & Beards (1959) provided evidences that the cysts were not destroyed inside such sheets. This opinion is in accordance with observations of other authors (Moroff, 1906; Yarwood,1937). Our observations support an idea put forward by Weiser & Beard (1959), that some metabolites might be secreted by adelines at the end of sporogony or immediately after fertilization, which stimulate a very strong haemocyte response resulting in the appearance of numerous melanized capsules enveloping oocysts.

Simultaneous occurrence of microsporidia *Nosema grylli and Adelina grylli* in the fat bodies of crickets. These two intracellular parasites are permanently present in the fat bodies of laboratory cultured crickets. The simultaneous presence of microsporidia and coccidia in the same hosts was reported earlier (Purrini,1982,), and can be explained by structural and biochemical peculiarities of these intracellular parasites. The fact that coccidians possess mitochondria and stored nutrients while the microsporidians lack both, may indicate to the different host metabolic pathways involved in the interactions between the host and the parasite. Preliminary studies showed that microsporidia and coccidia influence in a different way on the activities of studied enzymes of carbohydrate and energy metabolism (Dolgikh et al, 1995; 1996). It is also possible that coccidian infection may occur on the

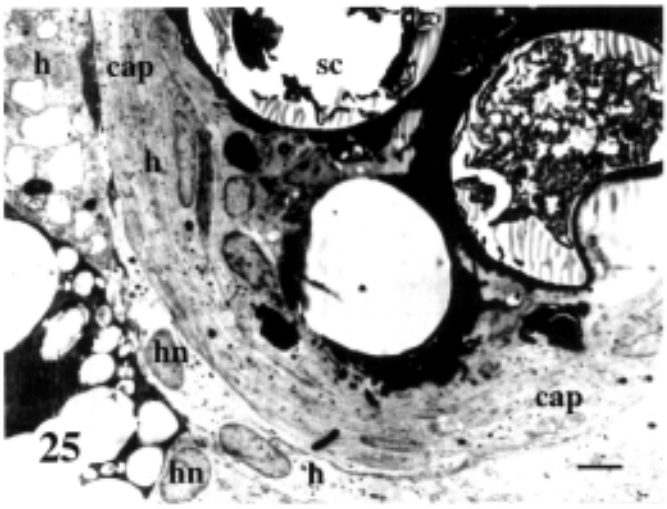

Fig.25 Capsule (Cap) around the oocyst composed of host haemocytes (H). Sc –sporocyst, HN –haemocyte nucleus. Scale bar: 1 μm

background of suppressed host immunity due to presence of microsporidia (or vice versa). The haemolymph response to the infection with either coccidians or microsporidians is significantly different. Further biochemical and immunological studies are to elucidate additional peculiarities of interactions of Coccidia and Microsporidia with host cells and a host organism.

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