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Protistology

Senoma gen. n., a new genus of microsporidia, with the type species *Senoma globulifera* comb. n. (syn. *Issia globulifera* Issi et Pankova 1983) from the malaria mosquito *Anopheles messeae* Fall.

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Summary

In 1983, a new species of microsporidia from mosquitoes was attributed to the genus *Issia* and described as *I. globulifera* sp. n. basing upon the taxonomic criterion of this genus: formation of two binucleate spores within a sporophorous vesicle at the end of development. However, the presence of octospores in sporophorous vesicles (besides forms with two spores) on the smears of the type species of the *Issia* genus testifies to its dimorphic development. Thus, the monomorphic species *I. globulifera* does not satisfy the criteria of the genus. A new genus *Senoma* should be established, with the type species possessing the following unique set of characters: 1) the *Nosema* type of development; 2) the presence of small vesicles, tubular structures and granules over the cell plasma membrane and its numerous extensions; 3) a stable coupling of spores by a large spherical homogenous mass (globule); 4) the absence of a sporophorous vesicle.

Key words: microsporidia, mosquitoes, ultrastructure, life cycle, *Anopheles, Senoma* gen. n.

Introduction

In 1983, a new species of microsporidia from a malaria mosquito *Anopheles messeae* Fall. (erroneously referred to as *A. maculipennis*) was described on the basis of light microscopy data (Issi and Pankova, 1983) and the diagnosis was specified, basing on the microsporidian ultrastructure (Issi et al., 2001). The development of the new microsporidium was shown to occur in the

midgut epithelium of the mosquito larvae (Issi and Pankova, 1983). The infection, not accompanied by clear symptoms, was revealed by chance in the course of genetic research. Examination of the infected tissue smears showed the new microsporidium's development to be similar to that of the *Nosema* species, with the only exception: sporoblasts and spores were coupled into pairs with the globule-forming material. After Giemsa staining, spores were surrounded with a dark blue vesicular border, mistaken for a sporophorous vesicle envelope during that investigation.

According to these features, the microsporidium matched perfectly the criteria of the *Issia* genus with the type species *I. trichopterae* (Weiser, 1946) (junior synonyms *Perezia trichopterae* and *Glugea trichopterae*) from the fat body of the caddisfly *Plectrocnemia geniculata* (Trichoptera), collected in the Dubravka River near Goetheborg, Czechoslovakia (Weiser, 1961, 1977). In the species description (Weiser, 1961), frequent mixed infection of the host with this parasite and another microsporidium (from the *Thelohania* genus) is mentioned.

Later, after revealing di- and polymorphic microsporidian life cycles, with formation of two and even three types of spores in the same host, the *Issia* genus was attributed to forms with dimorphic development (Canning and Vavra, 2000). As a result, the microsporidium species with monomorphic type of development, described by us, no longer complies with the diagnosis of this genus. Basing upon a unique fine structure of its developmental stages and spores, we describe a new genus of these parasites.

Material and Methods

To reveal microsporidia, IV instar larvae, pupae and imago of malaria mosquito *Anopheles messeae* Fall. (Diptera, Culicidae) were examined. For light microscopy (LM) smears of infected insect tissues were fixed with methanol for 2 min and stained according to Giemsa. For transmission electron microscopy (TEM), tissue pieces were fixed with 2.5 % glutharaldehyde solution in phosphate or cacodylate buffer for 1-2 h and postfixed with 1 % buffered tetroxide osmium solution for 1 hr. The tissues were dehydrated in a graded ascending ethanol series and absolute acetone and embedded into epon or araldite resin.

Ultrathin sections were cut using Ultratome-III (LKB) and stained with 2 % uranylacetate in 50 % ethanol and lead citrate for 10-20 min. The material was examined using electron microscope JEM-100 CX II at an accelerating voltage of 80 kV.

For scanning electron microscopy (SEM), abdominal larval somits fixed with glutharaldehyde in cacodylate buffer were smeared on a glass slide, dried and coated with a thin silver layer, using thermic evaporation in a vacuum unit. The material was examined using scanning device ASID-4D at an accelerating voltage of 10-20 kV.

Results and Discussion

Until now, no diplokaryotic microsporidia with disporoblastic sporogony have been known to have mature spores coupled, which warrants a description of a new genus.

Description of *Senoma* gen. n.

Type species: *Senoma globulifera* comb. n. (syn. *Issia globulifera* Issi et Pankova, 1983).

Type host: malaria mosquito *Anopheles messeae* Fall. (Diptera, Culicidae). IV instar larvae and pupae. Dyplokaryotic meronts have also been found in the ovary tissues of adult mosquitoes, but their species was not determined.

Localization: gut epithelium of larvae and pupae.

Recovery site: flood plain pond of the Chulym River (a tributary of the Ob' River), Tomskoye Priobye, Western Siberia.

Ways of transmission: Transmission is probably per os, since the earliest developmental stages are found in pupae, indicating the possibility of elder instar larvae infection.

Time of recovery: July, 1978- 2003, almost every year.

Infection symptoms: decrease in locomotory activity of larvae not accompanied by any changes in colour (characteristic of the fat body infection).

Life cycle. Merogony

Light microscopy. On the tissue smears of the infected pupae, the earliest life cycle stages are found: rounded cells of meronts 2-5 μm in size, with dark blue cytoplasm and 2-4 small compact brightly stained nuclei of diplokaryotic disposition. During the second merogony, whose stages are abundant in the cells of the infected larvae, ribbon plasmodia up to 10 μm long are being formed. Each ribbon plasmodium is a chain of binucleate cells. At the end of merogony, a ribbon-like meront divides to give rise to cells with a highly vacuolated cytoplasm and a large diplokaryon, occupying almost the entire cell volume (Fig. 5 A).

Electron microscopy. The earliest stage found on ulthrathin sections is that of diplokaryotic cells. They are linked to each other like beads on a string due to uncompleted division of the ribbon-like merogonial plasmodium about 10 μm long (Fig. 1 A). In most contact areas, two cytoplasmic membranes can be seen (Fig. 1 D). As a rule, the end cells (2.0 × 1.2 μm in size) are the

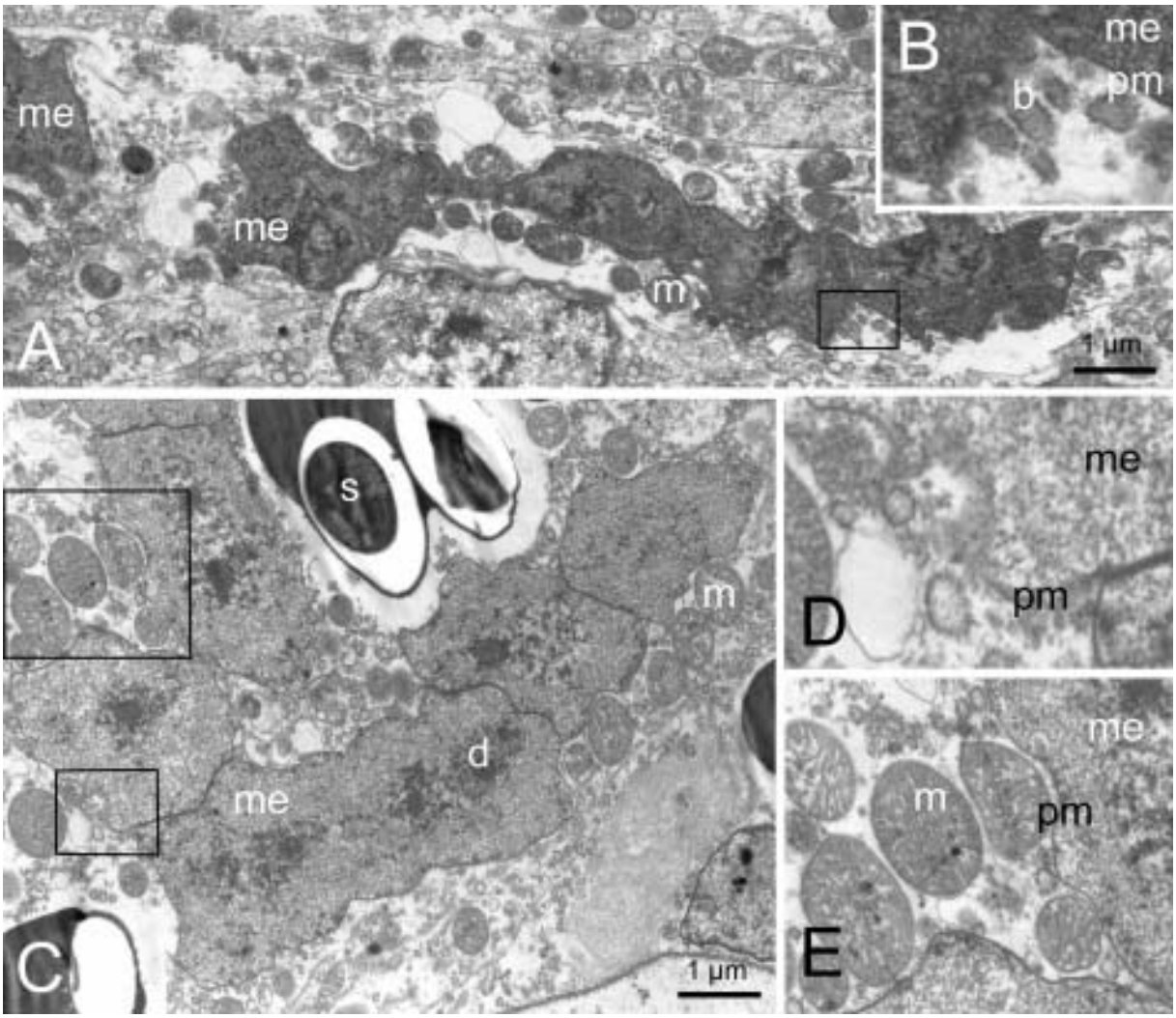


Fig. 1. Fine structure of merogony stages of the microsporidium *Senoma globulifera*. A - a young ribbon-like meront surrounded by host cell mitochondria; the meront's cytoplasm is filled with numerous ribosomes; B - tubular protrusions of the meront cell; incrustation of the cell and its protrusions with granules and thin tubular structures can be seen; C - mature ribbon-like meront with sporont cells detaching from its ends; D - an area of sporont's cytoplasmic membrane; E - contacts of parasite cell and host cell mitochondria. *Abbreviations*: b - bundle of tubular structures, d - diplokaryon, m - mitochondria, me - meront, pm - plasma membrane of meront; s - spores.

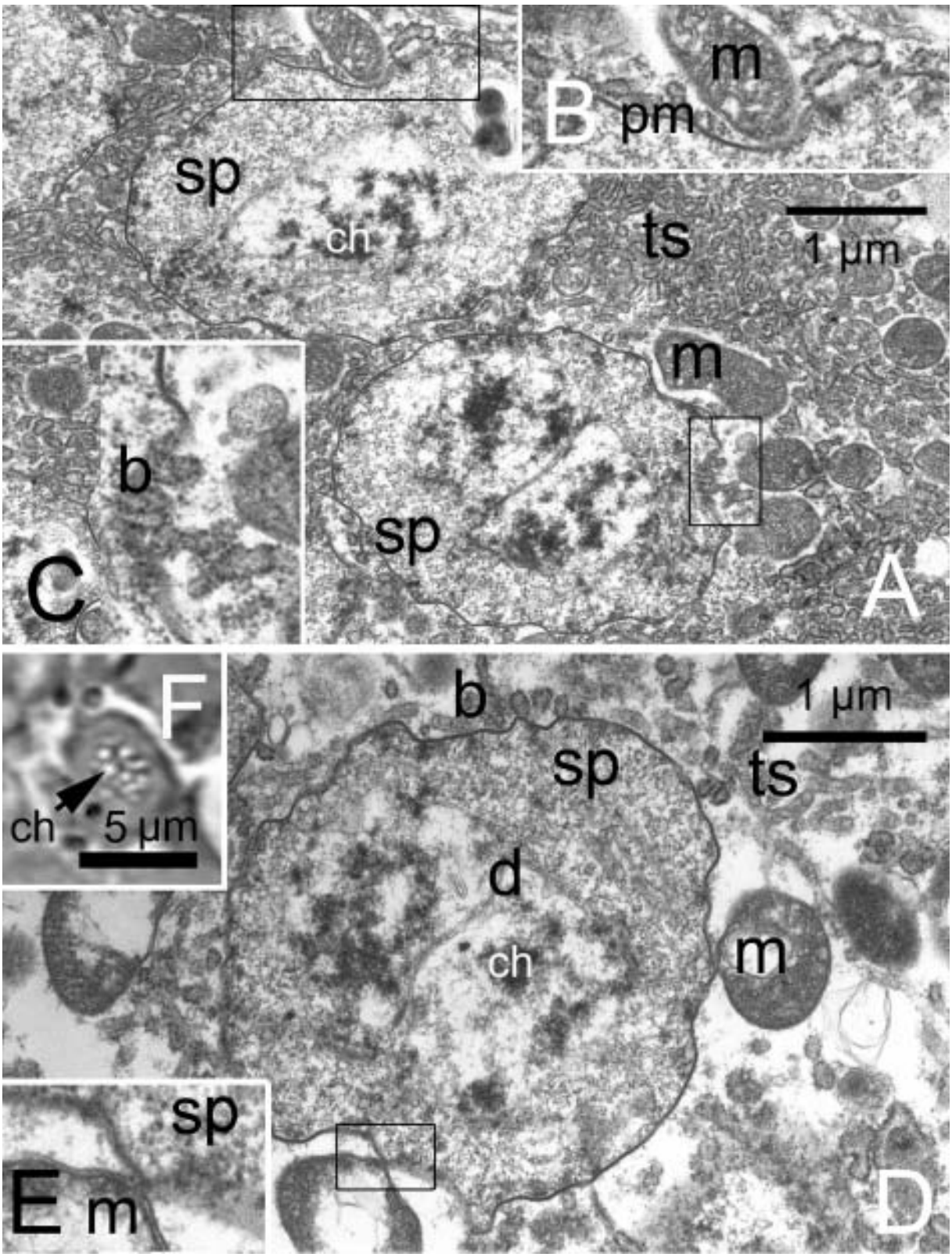
first to detach, while in the central part of the plasmodium there is a row of several diplokarya (Fig. 1 A, C). At this stage, the cell cytoplasm has a high electron density and contains poorly developed endoplasmatic reticulum, multiple ribosomes and several Golgi complex structures.

Small (about 1 μm) compact nuclei possess clearly expressed nucleoli. The cytoplasmic membrane of the parasite cell is incrusted with vesicles and granules and forms bundles of tubular extensions into the host cell (Fig. 1 B). The merogonal plasmodium is surrounded with numerous mitochondria of the host cell (Fig. 1 A, E).

Endoplasmatic reticulum and unchanged mitochondria are retained in the cytoplasm of the host cell, infected with meronts.

The next phase of merogony is also represented by ribbon-like forms, but their cytoplasm is less electron-dense and their nuclei, with clearly expressed nucleoli, are larger. Detaching cells are noticeably larger, 2.5-3.0 × 1.6-2.2 μm in size (Fig. 1 C). Host cell mitochondria are often in contact with the cytoplasmic membrane of the parasite cell (Fig. 1 E).

Merogony finishes with formation of cells with large diplokarya, occupying a considerable cell part, and with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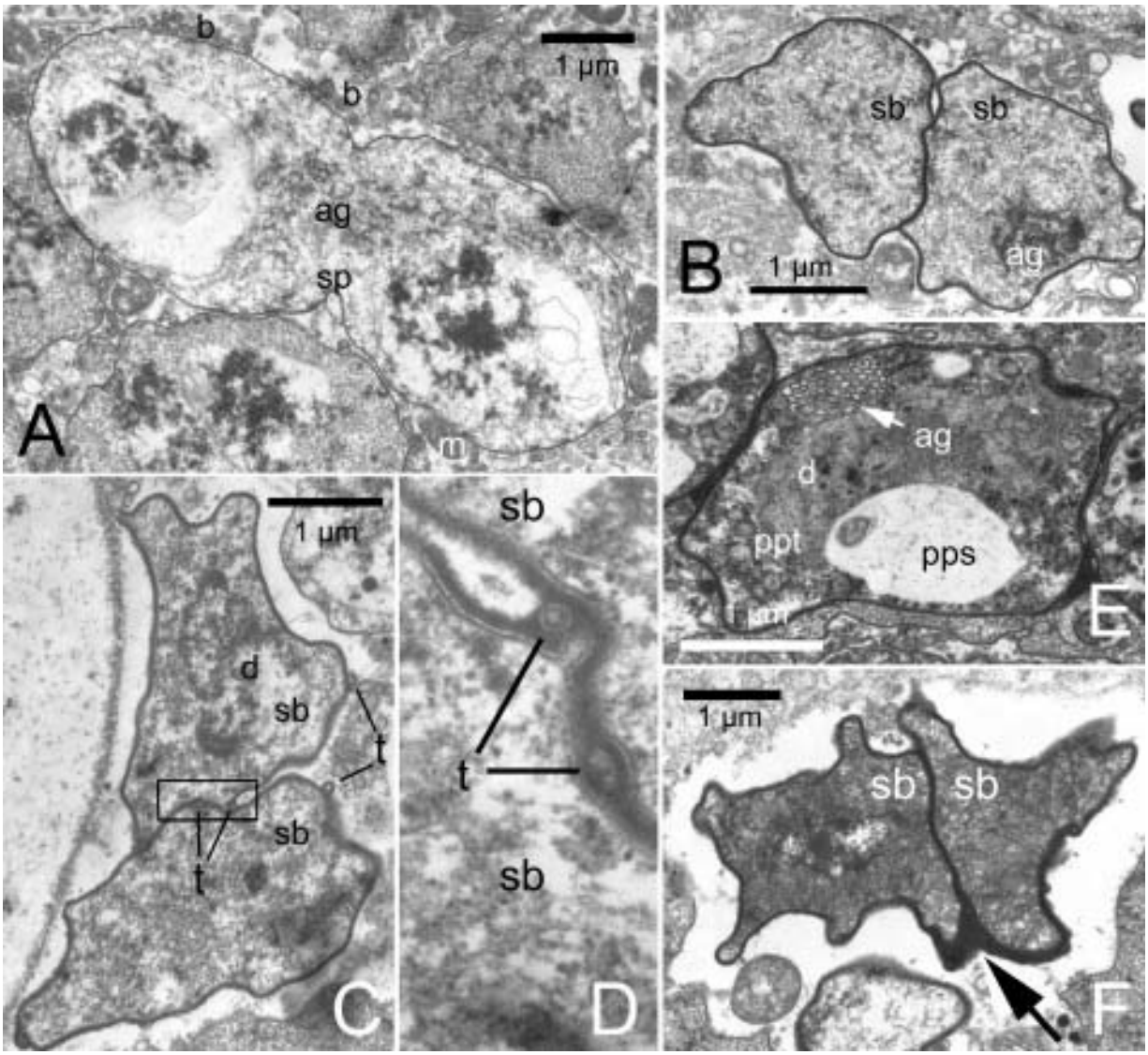


Fig. 3. Fine structure of sporoblasts of the microsporidium *Senoma globulifera*. A - sporont division into two sporoblasts; B - separation of sporoblasts and thickening of their membrane; C - appearance of tubular structures, responsible for secretion of the globule material; D - tubules, bounded by double membrane, around which globule material is deposited; E - sporoblast with an inchoate extrusion apparatus; F - beginning of globule formation (indicated by an arrow). *Abbreviations*: ag - Golgi apparatus, pps - primordium of polar sac, ppt - primordia of polar tubes, t - globule secreting tubules; for explanation of other symbols see figs 1 and 2.

➤ **Fig. 2**. Sporonts of the microsporidium *Senoma globulifera* (A-E - transmission electron microscopy, F - light microscopy). A - young sporonts, surrounded with mitochondria and tubular structures; B - contact of a mitochondrion and a parasite cell; C - bundle of tubular structures; granules and thin tubules are found both on the parasite cell membrane and on the tubular structures; D - late sporont, contacting vacuolized mitochondria; E - parasite's contact with mitochondria in detail; F - sporont with chromatin in the nuclei of its diplokaryon. *Abbreviations*: ch - chromosomes, sp - sporont, ts - tubular structures; for explanation of other symbols see fig. 1.

the vacuolated cytoplasm filled with ribosomes. We consider these cells as sporonts, the early sporogony stage (Fig. 2 A).

Sporogony

Light microscopy. Numerous cells are usually present on the smears. They are 3.5-4.5 μm in size and possess large nuclei, with chromatin often arranged in patterns resembling chromosome division (Fig. 2 F). Less commonly, these cells are found on the smears in the process of division into two sporoblasts with small compact nuclei.

Electron microscopy. On the TEM microphotos, sporonts are seen as large cells, from 3.2 ×2.7 μm (Fig. 2A) to 4.2 μm (Fig. 2 D) in size. Their cytoplasmic membrane bears granules and thin tubular structures on its surface and forms bundles of tubular extensions into the host cell cytoplasm (Fig. 2 C). The external layer of the membrane, bounding the bundles of tubular extensions, is also covered with rows of granules and small vesicles. At a distance from the cell, the extensions appear as stellar structures on transverse sections and as tubular-vesicular structures on longitudinal sections (Fig. 2 D). Unfortunately, it was impossible to determine whether these structures retained contact with the parasite cell.

The host cytoplasm is filled with ribosomes and the cisterns of the Golgi apparatus. It is often vacuolated. The nuclei are large, 1.5 to 2.0 μm in diameter, with chromatin organised in lumps. Host cell mitochondria, whose external membrane contacts the membrane of these cells, are strongly vacuolated (Fig. 2 B, D, E).

Sporogenesis

Light microscopy. During division of sporont into two sporoblasts, a rapidly growing sphere, which colours bright blue with Giemsa stain, appears at the posterior pole of cells (Fig. 4 B). The size of the sphere (globule) ranges between 6 and 10 μm, reaching sometimes 13-15 μm. In rare cases, it incorporates not two spores but one or four. In the latter case, each pair of spores lies in a distinct plane. On the smears examined under the light microscope, the spores are surrounded with a dark lilac sheath that often has a vesicular structure.

Electron microscopy. As the sporont starts to divide into two sporoblasts (Fig. 3 A), changes occur in form and fine structure of the membrane, the cytoplasm and the nucleus of the parasite cell. The cell becomes oval, 3.4 × 2.3 μm in size. The number of tubular extensions of the cytoplasmic membrane decreases drastically and it becomes covered with a layer of amorphous material, up to 200 nm thick in some regions (Fig. 3 B, E). The contents of the nuclei become homogenous and moderately electron-dense. In the sporoblast cytoplasm, primordia of the polar sack and the anchoring disk, as well as vesicular and tubular structures of the Golgi apparatus filled with secretory material can be seen (Fig. 3 E).

Between cytoplasmic membranes of the two separated sporoblast cells, rare tubular structures are found, with a diameter of 100 nm. They are surrounded by a membrane, identical to the plasmatic one (Fig. 4 C, D). Intensive deposition of amorphous material of the globule starts around these tubules. Secretory material is deposited more intensively on the one side of the sporoblasts and immature spores, so that the spores are pressed to one of the hemispheres of the globule and the angle between longer axes of the spores becomes more acute (Fig. 3 F). Later the sporoblasts are usually deformed, which prevented examination of extrusion apparatus morphogenesis.

Spore

Light microscopy. The spores have a regular oval shape, with an average size of 3.6 × 2.4 μm (3.3-4.4 × 2.2-2.8 μm). The anterior vacuole (the polaroplast) occupies more than a third of the spore. Rounded nuclei are located in the posterior third part of the spore or along its longer axis, forming a rhombus with acute angles. Posterosome of the young spores is brightly stained. Spores are submerged into the globule for about a fourth of their length (Fig. 4 A, B). Extruded polar tubes never exceed 40 μm in length. In the most cases, the pair-wise coupling of the spores remained undamaged on the smears prepared from both alive and dead insects.

Electron microscopy. The spores retain their wide oval shape, their size is 3.0 × 2.3 μm (Fig. 4 B). The exospore is bilaminate, the external layer is thinner and more electron-dense. The endospore thickness reaches 300-400 nm (Fig. 5 C, D). The anchoring disk is mushroom-shaped, the polaroplast is well developed and occupies almost a half of the spore volume. The polaroplast has two parts, the anterior one consisting of tightly packed long plates, while the posterior one, of tightly packed short plates (Fig. 4 D). Two nuclei are located in the posterior part of the spore, close to its centre. The polar tube is slightly anisophilar with 10-12 coils (8-10 coils have a diameter of about 120 nm, and 2-4 coils, 80 nm), arranged in 2-3 rows (Fig. 4 C). At the posterior pole, a large posterosome can be seen (Fig. 5 E). Electron microscopic analysis also shows an extant tubular system in the body of the globule, presumably involved in its morphogenesis (Fig. 4 C, E, F, G).

On the smears prepared from alive insects, the globules are intensively Giemsa-stained in bright turquoise colours typical of protein granules observed

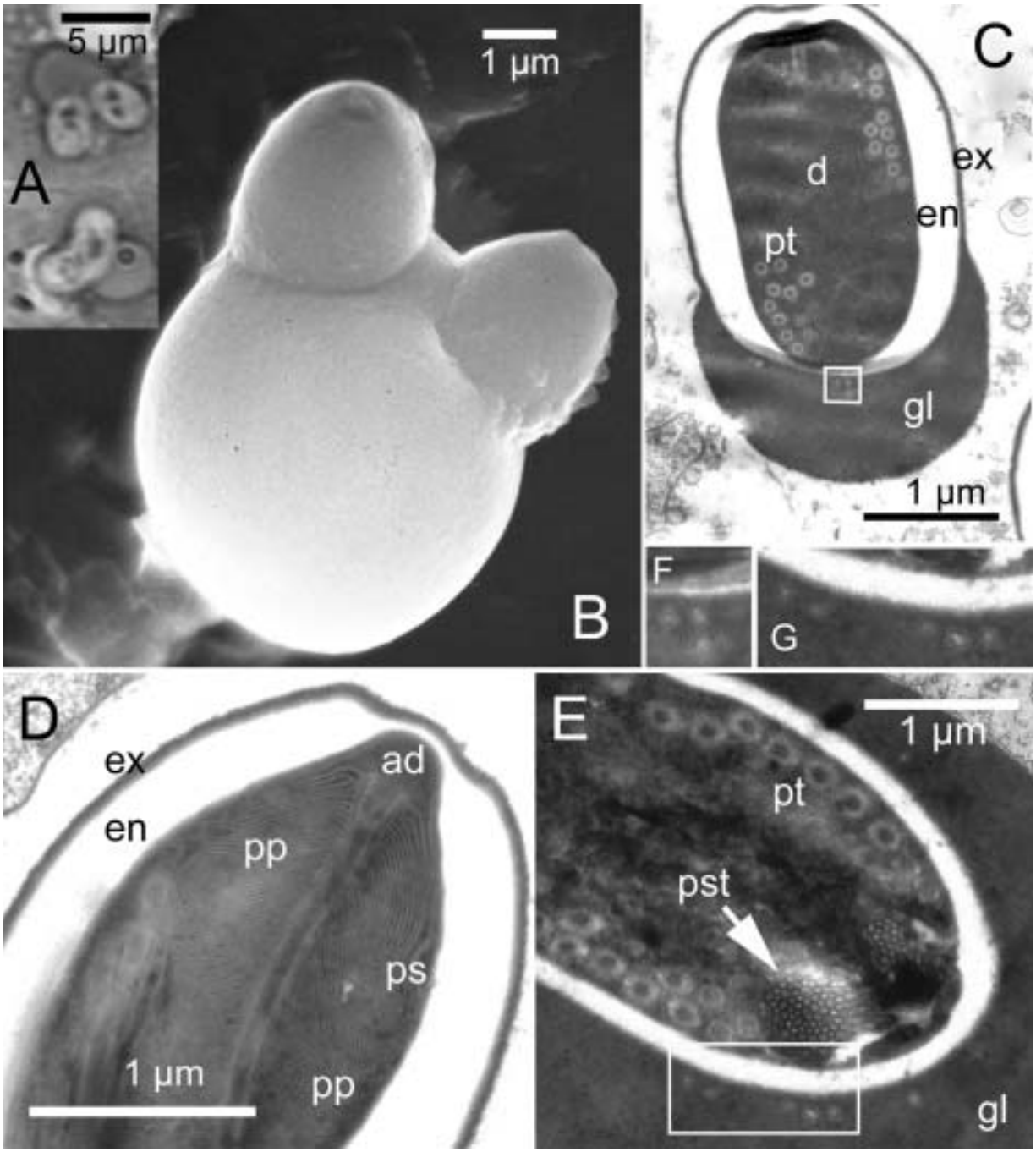
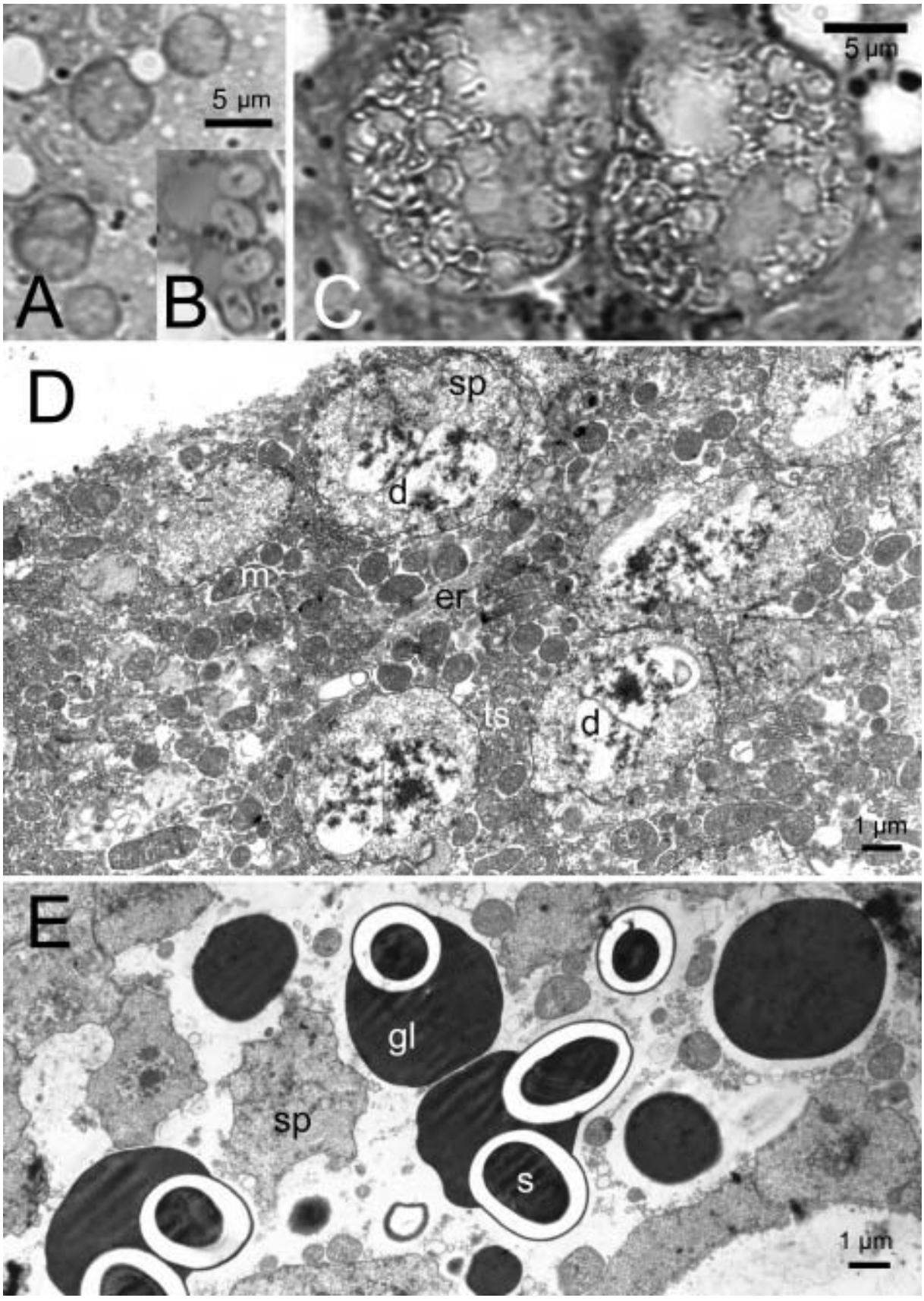


Fig. 4. Spore structure of the microsporidium *Senoma globulifera* (A - light microscopy, B - scanning electron mycroscopy, C-G - transmission electron microscopy). A - normal and undivided spores on a smear; B - spores under SEM, anchoring disc on the top of the spore can be seen; C - longitudinal section of a spore; a thick exospore can be seen; note a different structure of the anterior and the posterior coils of polar tubes; D - anterior pole of a spore with a mushroom-like anchoring disc and a lamellar polaroplast; E - posterior pole of a spore with a well developed posterosome (= reduced Golgi apparatus); F and G - tubular structures, retained in the body of the globule at the posterior poles of the spores shown on C and E. *Abbreviations*: ad - anchoring disc, en - endospore, ex - exospore, gl - globule, pp - polaroplast, ps - polar sac, pst - posterosome, pt - polar tube; for other abbreviations see fig. 1.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in the host fat body cells. On the smears taken from dead insects, both protein granules and microsporidian spores are stained dark-blue. This allows us to presume the protein nature of the globule material. Similarity in structure and electron density of the external proteinaceous exospore layer and the globule matter supports this idea.

Many sections show that sporonts and spores are "tiled" with host cell mitochondria (Fig. 5D, E). In the first description of this microsporidium, based on the light microscopy data, the dark blue vesicular border was interpreted as an envelope of the sporophorous vesicle. At present it can be assumed that the border was made up by mitochondria surrounding the parasites.

Basing on the structure of the life cycle stages of this species, the diagnosis of a new genus *Senoma* is proposed.

Diagnosis of *Senoma* gen. n.

Monoxenic and monomorphic microsporidia. Peroral transmission probable. Life cycle similar to that of *Nosema*. All stages with diplokaryotic nuclear apparatus. Ribbon-like merogonial plasmodia producing 4-5 sporonts. Meronts and sporonts forming bundles of tubular protrusions into host cell cytoplasm. Cytoplasmic membrane of parasites and its tubular protrusions covered with small vesicles, thin tubular structures and granules. Sporogony disporoblastic. During sporogenesis, homogenous material deposited between posterior poles of spores, forming a globule coupling the spores tightly. Spores with lamellar polaroplast and slightly anisophilar polar tube. Parasites of gut epithelium of *Anopheles* mosquitoes.

Type species: *S. globulifera* (Issi et Pankova, 1983) from gut of malaria mosquito *Anopheles messeae*. Ovoid spores 3.6 × 2.4 μm in size (light microscopy). Polar tube with 10-12 coils arranged in 2-3 layers. Size of the globule coupling the spores within the range of 6-15 μm. Type place and time of recovery: Tomsk region, Western Siberia, July.

The genus name is an anagram of *Nosema*.

Pathogenicity. One can judge upon the pathogenicity of the microsporidium from selective death of infected individuals during 1-1.5 h transportation of natural samples containing larvae and pupae. On the cellular level, pathology of microsporidiosis starts at the end of merogony. It is expressed in disappearance of all the organelles from the host cell cytoplasm, except the nucleus and mitochondria, whose number increases drastically (Fig. 5 D). If the host cell is infected slightly, mitochondria "tile" the parasite cells, from meronts to mature spores. Heavy infection causes decrease in the number of mitochondria from the beginning of sporogony (Fig. 5 E). When the membranes of parasite cells and host mitochondria come into contact, the latter undergo vacuolization (Fig. 2 D). The host cell is filled with microsporidian spores (Fig. 5 C), the nuclei lose chromatin and become electron-transparent.

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References

Canning E.U. and Vavra J. 2000. Phylum Microsporida Balbiani, 1882. In: An illustrated guide for the Protozoa. 1, 39-126.

Issi I.V. and Pankova T.F. 1983. New species of microsporidian *Issia globulifera* from malaria mosquito *Anopheles maculipennis*. Parasitologiya. 17, 3, 189-194 (in Russian with English summary).

Issi I.V., Pankova T.F. and Sokolova Y.Y. 2001. Diagnosis specification for *Issia globulifera* Issi, Pankova 1983 (Microsporidia, Nosematidae) basing on microsporidian ultrastructure. Parasitologiya. 35, 2, 165-168. (in Russian with English summary).

Fig. 5. Gut cells of the larvae of the malaria mosquito *Anopheles messeae* infected with the microsporidium *Senoma globulifera* (A-C - light microscopy, D, E - transmission electron microscopy). A - sporonts and B - spores on the gut smears; C - two infected cells of the insect, filled with prespore stages of the parasite and retaining their integrity; D - part of an enterocyte with multiple mitochondria and endoplasmatic reticulum of the host and sporont cells with tubular structures; E - enterocyte with spores and sporogonial stages of the microsporidium, the number of mitochondria is decreased; cell matrix and endoplasmatic reticulum are absent. *Abbreviations:* er - endoplasmatic reticulum, for other abbreviations see figs 1, 2, 4.

Weiser J. 1961. Die Mikrosporidien als Parasiten der Insekten. Monographien zur Angew. Entomologie. 17, 5-149.

Weiser J. 1977. Contribution of the classification of Microsporidia. Vestn. Cs spolec. zool. 41, 4, 308-320.

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