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New Species of Microsporidia from Bloodsucking Mosquitoes Inhabiting Small Reservoirs†

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Seven species of microsporidia of the genus *Amblyospora* (*A. verna*, *A. firma*, *A. certa*, *A. rustica*, *A. aectiva*, *A. media*, and *A. ukrainica*) were found in four species of bloodsucking mosquito *Aedes* (*Ae. minus*, *Ae. punctor*, *Ae. cinereus*, and *Ae. c. caspius* and *Ae. c. dorsalis*) from Ukraine. Basing on the character of inclusions in sporonts and sporophore cysts, it was proposed to isolate two new subgenera (*Lanicysta* and *Amblyocysta*) in the genus *Amblyospora* and to consider the genus *Hyalinocysta* as its subgenus.

Introduction

Preimaginal forms of amphibian dipterans, bloodsucking mosquitoes, inhabit various water bodies: shallow littoral regions of lakes and bogs, small lakes and ponds, pools resulting from inundation or rains, etc. Most of these water bodies lack mosquito-eating animals, and the use of pesticides to prevent mosquito development is not commonly possible and is undesirable from the ecological standpoint. The use of mosquito parasites, including microsporidia, seems promising for long-term programs aimed at limiting the population numbers of bloodsucking mosquitoes. Microsporidia are studied in Ukraine since 1970–1972 [2].

Recently, the employment of electron microscopy allowed a significant progress in the studying of the species composition and ecology of these protozoan organisms. Some of the obtained results are discussed in this work.

Materials and Methods

Material (larvae, pupae, and imago of bloodsucking mosquitoes infested with microsporidia) was collected in Ukraine (Kievskaya, Odesskaya, and Chernigovskaya oblasts) from 1988 to 1990. Light microscopic preparations were obtained from insects with clearly detectable infestion according to the standard methods. To obtain electron microscopic preparations, insects were fixed with 2.5% glutaraldehyde in cacodylate buffer and then with 2% osmium tetroxide. Fixed preparations

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were treated with ethanol-acetone mixtures and embedded in Epone-812. Ultrathin sections were treated with uranyl acetate and lead citrate.

Some larvae and pupae were developed to imago in the laboratory. After exclusion, adults were reared in gauze cages and fed 5% honey.

Microsporidia from the collection of the Laboratory of Ecology and Toxicology, Kiev State University, were also studied.

Results

Microsporidia isolated from mosquitoes were classed with the genus *Amblyospora* Hazard et Oldacre, 1975, basing on the formation of octospore sporophore cysts during sporogony, morphology of developing and mature spores, spore ultrastructure (laminated polaroplast, aniso- or heterophyllary polar tube), and development of monospores in female ovaries.

Sporonts and sporophore cysts of this species of microsporidia commonly include metabolic granules varying in size and in number [4, 5, 9]; small masses of tubular secret occur more rarely. Hazard and Oldacre isolated microsporidia containing mucous electron-light mass in their sporophore cysts in a new genus, *Hyalinocysta* Hazard et Oldacre, 1975. Other morphological and ultrastructural traits of this genus are similar to those of the genus *Amblyospora*, which is the most complex of all known genera of microsporidia. Its representatives display three types of sporogony in their life cycle: they produce haploid octospores in insect larvae, diploid monospores in adult insects, and haploid mono- and tetraspores in Crustacea. These microsporidia also have an extremely broad host range. Basing on studies of octospores, they described more than 20 microsporidia species in bloodsucking mosquitoes (and more than 30 forms of microsporidia were identified within genera), 6 species in buffalo gnats (Simuliidae), 3 species in Chironomidae, and 1 species in freshwater shrimp.

This posed a problem of the diagnosis of spores of these microsporidia [1]. To solve it, new diagnosis criteria must be determined and used together with traditional ones. The character of inclusions in sporonts and sporophore cysts, the thickness of exo- and endospores, their ratio, the structure and diameter of various regions of the polar tube, and organization of the polaroplast were used as such criteria in this work.

Electron microscopic studies of the sporophore cysts showed that the space between spores is filled with fibrillar mass of a medium electron density in two out of seven tested species of microsporidia. Large metabolic granules were observed in sporonts before this structure is formed. Basing on the difference in the structure of inclusions, we consider it possible to isolate two subgenera in the genus *Amblyospora*: *Lanicysta*† sub. gen. n. with the typical species *A.* (*L.*) *rustica* Kilotchitskyj sp. n. (i.e., microsporidia containing fibrillar inclusions in sporophore cysts) and *Amblyospora* sub. gen. n. with the typical species *A.* (*A.*) *opacita* Hazard et Oldacre, 1975 (i.e., microsporidia containing metabolic granules). We also believe it justifiable to consider the genus *Hyalinocysta* as a subgenus belonging to the genus *Amblyospora* and displaying mucous mass in sporophore cysts.

† *Lana* is Latin for hair.

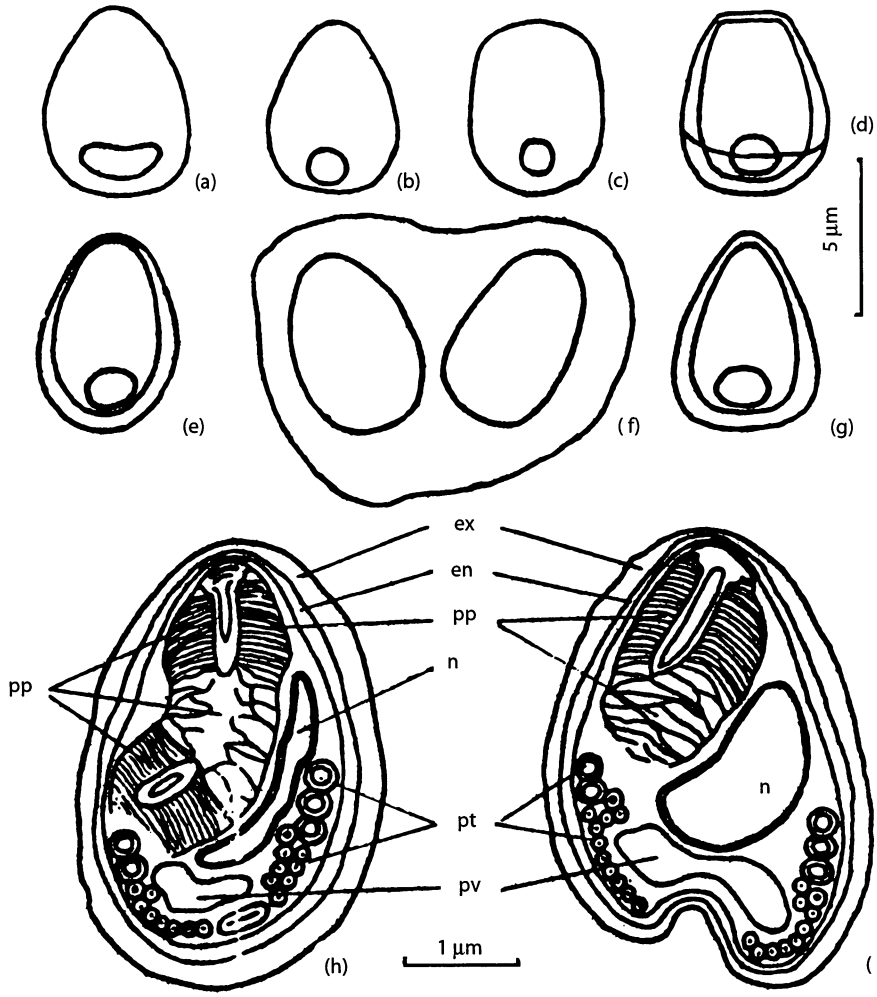

Fig. 1. Morphology and ultrastructure of *A. verna* spores: (a–g) native spores; (h, i) ultrathin sections; pv, posterior vacuole; pt, polar tube; pp, polaroplast; ex, exospore; en, endospore; n, nucleus.

Amblyospora (*Amblyocysta*) *verna*† Kilotchitskyj, sub. gen. et sp. n.

Synonyms: Thelohania opacita Kudo, 1922 [6]; Amblyospora sp. [7].

Type material: hapantotype (specimen No. 04-9) and paratypes are stored in the collection of the Laboratory of Ecology and Toxicology, Kiev State University.

Host: bloodsucking mosquito *Ae. communis* (typical host); forth-instar larvae (males and females) and adults (females).

† Verna is Latin for spring.

Histotropism: fat body in male and female larvae, ovaries in adults.

Type locality: Kievskaya oblast (Dnieper basin), Ukraine; shaded and semishaded, temporary and semipermanent small pools.

Species description. Of early developmental stages of microsporidia, electron microscopy revealed diplokaryons of 11×8 μm.

Dividing sporonts with one to eight nuclei were 12.5×15.0 μm (Romanovsky-Giemsa staining) or 11.2–11.4·10.0 μm (electron microscopy). Sporonts contained few to 10–15 small metabolic granules. Sporogony results in the formation of sporophore cysts, which contained eight spores each and were 12.5–14.0 μm (native) or 15–18 μm (Romanovsky-Giemsa staining). The cyst wall was unstable and degraded after spore maturation.

Spores varied in shape from ellipsoidal (typical) to pointed at one end or prism-shaped (Figs. 1a–1g, 9a, 9b). Diameter of the small posterior vacuole was about one-fifth of the spore length. Several thin-walled capsules including one or two spores were observed on aqueous preparations (Fig. 1f). Spore size (especially length) varied significantly (Table 1). Macrospores (8.1–11.0× 6.3–8.1 μm) and teratospores (12.5×8.8–9.8 μm) were also found. Spores isolated from female larvae were surrounded by liquid mucocalyx detectable in ink suspension (Fig. 9b).

Fixation of electron microscopic preparations resulted in reduction of spore size (Table 2). The spore wall was 340–530 nm thick and consisted of two layers; deformation of the posterior pole of spores was caused by fixation. Laminar polaroplast was dimorphic, i.e., involved regions of loosely

Table 1

Characterization of new species of microsporidia by light microscopy

Microsporidia species	Host species, developmental stage, sex	Site and date of collection	Size of native spores, μm
A. (A.) verna	*Aedes communis* Deg., L IV, ♀	Kievskaya oblast, Pushcha Voditsa, 3.03.1989	6.3–7.0×4.8–5.0, 6.56±0.29×4.93±0.1
A. (A.) firma	*Ae. punctor* Kirby, L IV, ♂	Kievskaya oblast, Vita Pochtovaya, 18.04.1990	6.3–7.5×4.8–5.0, 6.97±0.48×4.90±0.11
A. (L.) rustica	*Ae. c. dorsalis* Mg., L IV, ♂	Chernigovskaya oblast, Siberezh, 30.09.1990	6.3–6.9×3.8–4.4, 6.6±0.22×4.05±0.22
A. (L.) certa	*Ae. c. cinereus* Mg., L IV, ♂	Kievskaya oblast, Vita Pochtovaya, 16.05.1991	4.8–5.0×2.5–2.6, 4.97±0.14×2.54±0.06
A. (A.) aestiva	*Ae. c. dorsalis*, L IV, ♀	Chernigovskaya oblast, Siberezh, 27.07.1990	3.8–4.3×2.4–3.1, 4.02±0.38×2.91±0.28
A. (A.) media	*Ae. c. dorsalis*, L IV, ♂	Kievskaya oblast, Kruglik, 17.04.1990	6.3–6.9×4.4–4.8, 6.38±0.26×4.53±0.18
A. (A.) ukrainica	*Ae. c. caspius* Pall., L IV, ♂	Kievskaya oblast, Yurovka, 16.05.1991	6.0–6.5×3.5–4.0, 6.25±0.16×3.80±0.15

or closely packed laminae (Figs. 1h, 1I). The polar tube consisted of thick basal, thin distal, and intermediate central regions (Table 2). The diameter of the basal region was twice as large as that of the distal region. This was most clearly seen in immature spores (Fig. 9e). The length of the spontaneously extruded polar tube was 63–100 μm; the computed length of the tube within spores was 140–150 μm. Tube twists were inclined at an angle of 45 degrees to the longitudinal axis of a spore.

The large nucleus was in the center of a spore, above the posterior vacuole.

Ellipsoidal diplokaryons of 6.9×5.0 μm, spherical diplokaryons of 6.3 μm in diameter, and individual piriform spores of 7.5–9.8×2.5–3.1 μm with the large posterior vacuole were observed in ovaries of females developed from larvae and pupae in the laboratory.

Microsporidia were detected in 1–10% forth-instar larvae and 25% adults. The intensity of invasion significantly varied in larvae. Generalized infestation was observed in some larvae, while microsporidia were in only thoracic and several abdominal segments in some others. In adults, the intensity of invasion was 60–100 spores per female.

Differential diagnosis. This species is unique in morphology and ultrastructure of octo- and monospores.

Distribution. Microsporidia of this species were found in Ukrainian Poles'e, Ukrainian forest-steppe zone, and near Petrozavodsk in Karelia.

Amblyospora (Amblyocysta) firma† Kilotchitskyj, sub. gen. et sp. n.

Synonyms: *T. opacita* Kudo, 1992 [6]; Amblyospora sp. [7].

Type material: hapantotype (specimen No. 36-01) and paratypes are stored in the collection of the Laboratory of Ecology and Toxicology, Kiev State University.

Host: bloodsucking mosquito *Ae. punctor* (typical host); male and female larvae of the forth instar.

Histotropism: fat body.

Type locality: Kievskaya oblast (Dnieper basin), Ukraine; small semishaded temporary pools.

Species description. Electron microscopy revealed lines of ellipsoidal diplokaryons, each of 8.6×5.7 μm. Spherical (7.4 μm in diameter), ellipsoidal mononuclear (8.0×6.9 μm), and ellipsoidal multinuclear (11.4×7.4 μm) sporonts were more numerous. Sporonts with one or two nuclei contained one or two large metabolic granules; tetra- and octonuclear sporonts contained four or five smaller granules. Sequential divisions of nuclei resulted in the formation of octonuclear sporonts and octospore sporophore cysts, which were 8.6–8.9 μm in diameter.

† *Firma* is Latin for tolerant.

Table 2

Morphometric parameters of spores of microsporidia revealed by electron microscopy

Spore size, μm	Twist number in polar tube				Diameter of polar tube regions, nm			Thickness of spore wall	
	Total	in individual regions							
		basal	medial	distal	basal	medial	distal	exospore	endospore
				A. (*A.*) *verna*					
5.0–6.2× 3.1–4.0	14–17	2–3	1–2	10–12	200–280	140–170	60–140	190–350	140–180
				A. (*A.*) *firma*					
4.3–5.6× 3.2–3.9	10–13	2–3	1–2	7–9	250–290	160–220	130–170	250–410	130–180
				A. (*L.*) *rustica*					
5.3–5.8× 3.3–3.9	8–10	4–6	0.5–1	3.5–5	260–290	200–220	140–160	130–150	150–210
				A. (*L.*) *certa*					
4.3–4.6× 2.2–2.4	6	2–3	–	3–4	220–250	–	130–140	90	110
				A. (*A.*) *aestiva*					
2.5–3.2× 3.3–3.8	4–6	2	0.5–1	2.5–3	160–230	190	140–180	130–250	70–140
				A. (*A.*) *media*					
3.6–4.2× 2.8–3.2	13–14	3–4	1–2	8–9	220–260	120–130	100–120	230–250	80–100
				A. (*A.*) *ukrainica*					
4.3–4.7× 5.0–5.4	9–11	3–4	1–2	4–6	200–300	150–160	130–160	160–350	120–180

Native spores were ellipsoidal and more or less pointed at the apical end and had a large ellipsoidal posterior vacuole (Figs. 2a–2c, 9c, 9d). Native and fixed spores significantly differed in size (Tables 1, 2). Spores isolated from female larvae were surrounded with liquid mucocalyx. Macrospores of 10.0–10.6×7.5–8.1 μm were few in number.

The wall of mature spores was of 400–600 nm thick and involved several layers. Exospore was 2.0–2.5 times thicker than endospore. The laminar polaroplast occupied a great deal of spore volume and consisted of loosely and closely packed laminae (Figs. 2e, 2f, 9f). The polar tube included the basal, medial, and distal regions (Table 2). Tube twists were inclined at an angle of 60 degrees. Spontaneous extrusion of the polar tube was rare.

The large nucleus formed a semicircle round the polaroplast in the central region of a spore, above the posterior vacuole.

Up to 10% larvae were infested. The intensity of invasion was extremely high: almost all regions of the fat body were infected.

Differential diagnosis. This species is most similar morphologically to microsporidia *Amblyospora punctor* Weisre et Zizka, 1991, found in mosquito *Ae. punctor* in the former Czechoslovakia [11]. *A. punctor* ellipsoidal spores are 7×6 μm and have the anisophyllary polar tube, whose major region is twisted three times and the thin flagellar region, nine times. The two species differ in shape and size of native spores, structure and diameter of the polar tube, and in structure of the polaroplast. We believe that the similarity of these species is explained by their relatively recent divergence. A high variation observed in *A. firma* suggests that these processes are still in progress.

Distribution. The species was found in Ukrainian Poles'e and the forest-steppe zone.

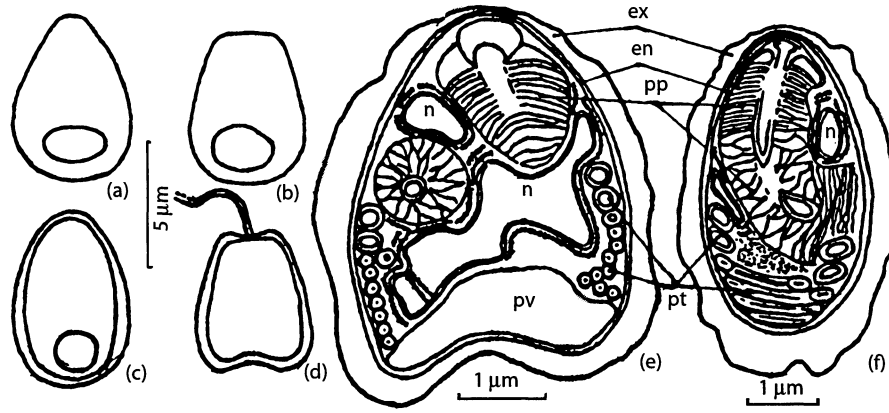

Fig. 2. Morphology and ultrastructure of *A. firma* spores: (a–d) native spores; (e, f) ultrathin sections; pv, posterior vacuole; pt, polar tube; pp, polaroplast; ex, exospore; en, endospore; n, nucleus.

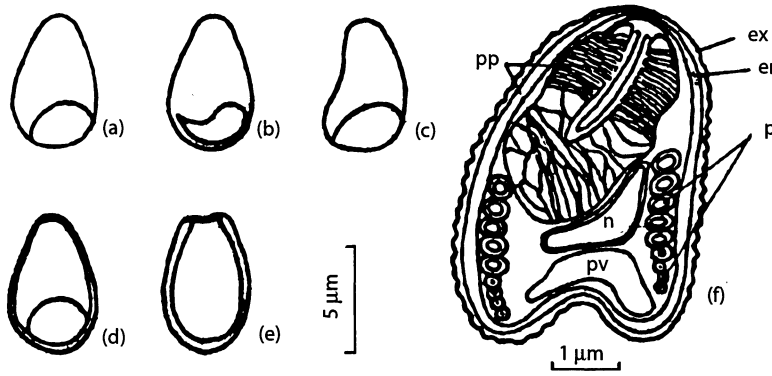

Fig. 3. Morphology and ultrastructure of *A. rustica* spores: (a–e) native spores; (f) ultrathin section of a mature spore; pv, posterior vacuole; pt, polar tube; pp, polaroplast; ex, exospore; en, endospore; n, nucleus.

Amblyospora (*Lanicysta*) *rustica*† **Kilotchitskyj, sub. gen. et sp. n.**

Synonyms: *T. opacita* Kudo, 1992 [6]; Amblyospora sp. [7].

Type material: hapantotype (specimen No. 71-0) and paratypes are stored in the collection of the Laboratory of Ecology and Toxicology, Kiev State University.

Hosts: bloodsucking mosquitoes *Ae. c. dorsalis* (typical host) and *Ae. c. caspius*; male and female larvae of the forth instar.

Histotropism: fat body.

Type locality: Chernigovskaya oblast (Desna basin), Ukraine; small nonshaded temporary pools.

Species description. Of all developmental stages, electron microscopy revealed diplokaryons (Fig. 10b) and mono- to octonuclear sporonts of 12.5–13.8 μm. Sporogony results in the formation of octospore sporophore cysts of 11.3–12.5 μm (when fixed). The free space within tetra- and octonuclear sporonts and sporophore cysts was filled with fibrillar mass with a medium electron density. Under a light microscope, this mass seemed transparent, negatively stained with Romanovsky-Giemsa stain.

Native spores were ellipsoidal, pointed at one end and sometimes had a lateral bend (Figs. 3a–3d). The posterior vacuole was large. In ink suspension, spores were ellipsoidal and the posterior vacuole was undetectable (Fig. 3e). Mucocalyx was absent. Native spores significantly varied in size (Table 1). Ellipsoidal macrospores (some being pointed at one end) of 8.8–10.0×6.3–7.5 μm were the most common. When spontaneously extruded in distilled water, the polar tube was 50–94 μm long. The size of fixed spores (on electron microphotographs) varied only slightly. Exospore had the same or lower thickness as endospore. The laminar polaroplast consisted of two regions: one including 35–37 closely packed laminae and the other with loosely packed laminae. The polar tube consisted of three regions differing in diameter (Table 2). Tube twists were inclined at an angle of 85–90 degrees to the longitudinal axis of a spore. The large nucleus was in the central region of a spore, above the posterior vacuole (Fig. 3f).

Up to 10% forth-instar larvae were infested; infestion was generalized over the entire fat body.

Differential diagnosis. With respect to the number and size ratio of polar tube twists, the species is similar to two microsporidia species, *A. inimica* Hazard et Oldacre, 1975 and *A. culicis* Toguebaye et Marchand, 1985, found in mosquitoes from the North America. The major difference is that the described species display fibrillar inclusions in sporonts and sporophore cysts, heterophyllary structure of the polar tube, the absence of mucocalyx, and different spore size and thickness of tube twists.

Distribution. Microsporidia were found in Poles'e in autumn.

† *Rustica* is Latin for rural.

Amblyospora (*Lanicysta*) *certa*† **Kilotchitskyj, sub. gen. et sp. n.**

Type material: hapantotype (specimen No. 78-0) and paratypes are stored in the collection of the Laboratory of Ecology and Toxicology, Kiev State University.

Host: bloodsucking mosquito *Ae. c. cinereus* (typical host); female larvae of the forth instar.

Histotropism: fat body.

Type locality: forest-steppe zone, Kievskaya oblast (Dnieper basin), Ukraine; small nonshaded semipermanent pools.

Species description. Analysis of preparations stained with Romanovsky-Giemsa stain revealed mature spores, mono- to octonuclear sporonts of 12.5–13.0 μm in diameter, and octospore cysts of 12.5 μm in diameter with a relatively stable membrane. The diameter was 8.3–9.0 μm in sporonts and 9.0 μm in sporophore cysts on electron microphotographs. Dividing sporonts contained four or five large metabolic granules containing microgranular mass with a medium electron density. During spore maturation, the granules were transformed into fibrillar mass filling the space between spores in sporophore cysts (Fig. 10d).

Native spores were ellipsoidal, more or less pointed at one end (Figs. 4a–4c). Their shape and size varied only slightly (Table 1). No macrospores were found. Mucocalyx was absent. Ellipsoidal spores of 4.5–4.8×2.3–2.4 μm were observed on preparations stained with Romanovsky-Giemsa stain. The size of spores on electron microphotographs was similar (Table 2). Thin spore wall

† *Certa* is Latin for accurate, reliable.

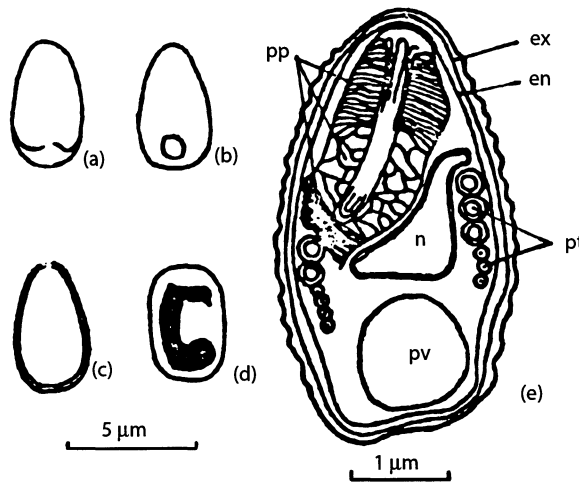

Fig. 4. Morphology and ultrastructure of *A. certa* spores: (a–c) native spores; (d) Romanovsky-Giemsa staining; (e) ultrathin section of a mature spore; pv, posterior vacuole; pt, polar tube; pp, polaroplast; ex, exospore; en, endospore; n, nucleus.

included wrinkled exospore and thicker endospore. The large polaroplast included one or two regions with 40–50 closely packed laminae and one region with loosely packed laminae (Fig. 4e). Twists of the anisophyllary polar tube were inclined at an angle of 50 degrees to the longitudinal axis of a spore. Diameter of its basal region was nearly twice as large as that of the distal region. The nucleus is in the central region of a spore, above the large posterior vacuole.

Up to 15% of the fourth-instar larvae were infested.

Differential diagnosis. With respect to its morphometric parameters, the described species has no analogues among known species of mosquito microsporidia.

Amblyospora (*Amblyocysta*) *aestiva*† Kilotchitskyj, sub. gen. et sp. n.

Synonyms: Thelohania obesa Kudo, 1924 [3, 6]; Amblyospora sp. [7].

Type material: hapantotype (specimen No. 63-0) and paratypes are stored in the collection of the Laboratory of Ecology and Toxicology, Kiev State University.

Hosts: bloodsucking mosquitoes *Ae. c. dorsalis* (typical host) and *Ae. c. caspius*; female and male larvae of the forth instar.

Histotropism: fat body.

Type locality: Chernigovskaya oblast, Ukraine; small nonshaded temporary pools.

Species description. The early developmental stages were represented by lines of ellipsoidal diplokaryons on electron microphotographs (Fig. 10a). Mono- to octonuclear sporonts of 8.0–12.5 μm in diameter corresponded to various stages of sporogony. Sporonts contained several large metabolic granules. Sporogony results in the formation of octospore sporophore cysts with diameter of 8–10 μm (native), 10 μm (Romanovsky-Giemsa staining), or 7–8 μm (electron microscopy).

Native spores were ellipsoidal and had the small posterior vacuole (Figs. 5a, 5b; Table 1). Fixation affected the spore size only slightly (Table 2). Spores were 3.8–4.0×2.8–3.1 μm on preparations stained with Romanovsky-Giemsa stain. Macrospores of 6.3×3.8 μm (native) or 5.7×4.8 μm (electron microscopy) were rare. Most sporophore cysts with mature spores were filled with microgranular mass of a medium electron density rather than had distinctly separated metabolic granules (Fig. 10c). The thick wall included wrinkled exospore and relatively thin endospore (Figs. 5c, 10c; Table 2). The large polaroplast included about 50 closely and loosely packed laminae. The large fungiform anchor disk was observed (Fig. 5c). The polar tube commonly formed five twists, two of them corresponding to the basal region (Table 2). Tube twists were inclined at an angle of 50 degrees to the longitudinal axis of a spore. The basal region of the tube was slightly larger in diameter than the distal one. The large nucleus was in the central region of a spore, at the side of the polaroplast, above the small posterior vacuole (Fig. 5c).

† *Aestiva* is Latin for summer.

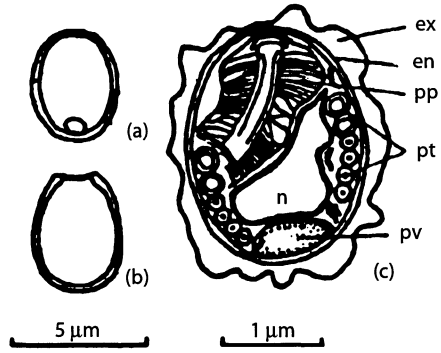

Fig. 5. Morphology and ultrastructure of *A. aestiva* spores: (a, b) native spores; (c) ultrathin section of a mature spore; pv, posterior vacuole; pt, polar tube; pp, polaroplast; ex, exospore; en, endospore; n, nucleus.

Up to 5% fourth-instar larvae were infested; infestion of the host fat body was generalized.

Differential diagnosis. This species is similar in the total twist number in polar tube to *A.* (*L.*) *certa* described above. They can be distinguished by the shape and size of spores, structure and thickness of the spore wall, and the character of inclusions in sporonts and sporophore cysts.

Distribution. These microsporidia were found in Poles'e and forest-steppe and steppe zones of Ukraine. They were observed mostly in summer and, more rarely, in early autumn.

Amblyospora (*Amblyocysta*) *media*† Kilotchitskyj, sub. gen. et sp. n.

Synonyms: *T. opacita* Kudo, 1922 [6]; Amblyospora sp. [7].

Type material: hapantotype (specimen No. 41-0) and paratypes are stored in the collection of the Laboratory of Ecology and Toxicology, Kiev State University.

Host: bloodsucking mosquito *Ae. c. dorsalis* (typical host); male larvae of the forth instar.

Histotropism: fat body.

Type locality: forest-steppe zone, Kievskaya oblast, Ukraine; small nonshaded temporary pools.

Species description. Synchronized in time sporogony is characteristic of this species. Only mature spores were found on preparations. In water suspension, native spores were ellipsoidal, pointed at one end and had the large posterior vacuole. In ink suspension, spores were ellipsoidal,

† *Media* is Latin for medium.

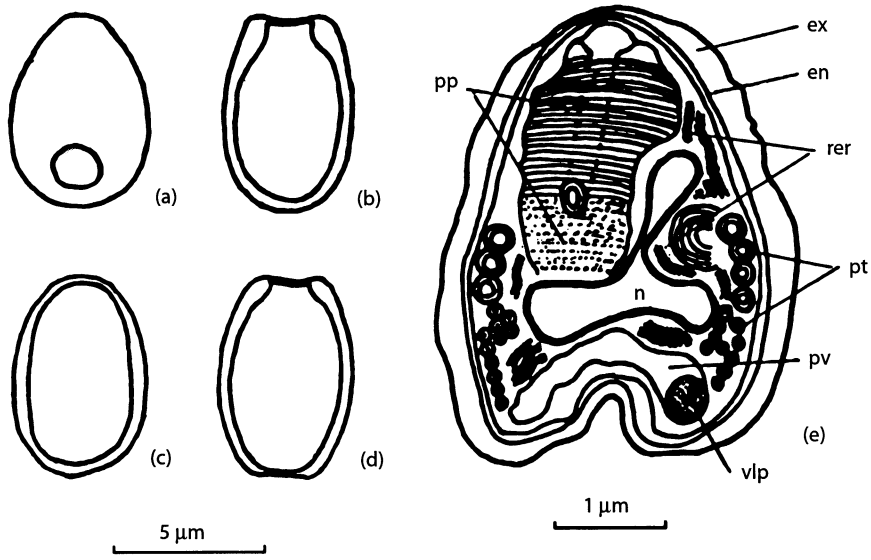

Fig. 6. Morphology and ultrastructure of *A. media* spores: (a–d) native spores; (e) ultrathin section of a mature spore; vlp, virus-like particles; pv, posterior vacuole; pt, polar tube; pp, polaroplast; ex, exospore; en, endospore; rer, rough endoplasmic reticulum; n, nucleus.

flattened at one or both poles; the vacuole was undetectable (Figs. 6a–6d, Table 1). Mucocalyx was absent. Native macrospores were 8.8–10.0×5.8–6.3 μm, teratospores were 11.7–12.7×7.5–8.8 μm. Spore size was markedly reduced after fixation (Table 2). When stained with Romanovsky-Giemsa stain, spores were 4.9–5.3×3.8–4.2 μm. The spore wall was thick. Wrinkled exospore ware 2–3 times thicker than endospore. The polar tube consisted of three regions and commonly formed 14 twists (Table 2). The nucleus was in the central region of a spore, near the large laminar polaroplast (Fig. 6e). In some spores, a small capsule containing smaller (virus-like?) particles was detected within the posterior vacuole.

Up to 15% fourth-instar larvae were infested; infestion of the host fat body was generalized.

Differential diagnosis. This species is intermediate in total twist number in the polar tube between *A. verna* (14–17 twists) and *A. firma* (10–13 twists) which were described above. They are distinguished by the absence of mucocalyx, size of fixed spores, and twist number (total and in each individual region of the polar tube) (Table 2).

Amblyospora (*Amblyocysta*) *ukrainica* Kilotchitskyj, sub. gen. et sp. n.

Synonyms: *T. opacita* Kudo, 1922 [3, 6]; Amblyospora sp. [7].

Type material: hapantotype (specimen No. 77-0) and paratypes are stored in the collection of the Laboratory of Ecology and Toxicology, Kiev State University.

Hosts: bloodsucking mosquitoes *Ae. c. caspius* (typical host) and *Ae. c. dorsalis*; male and female larvae of the forth instar.

Histotropism: fat body.

Type locality: Kievskaya oblast (Dnieper basin), Ukraine; small nonshaded temporary pools.

Species description. The observed stages of sporogony involved mono- to octonuclear sporonts of 12.5–15.0 μm in diameter and octospore sporophore cysts of 10.0–11.3 μm in diameter.

Native spores were ellipsoidal, pointed at one end and had the small, nearly spherical posterior vacuole. In ink suspension, spores were ellipsoidal with less pronounced pointing. Mucocalyx was absent. A thin membrane (membrane vesicle) enclosing one or two spores was occasionally observed in water suspension (Figs. 7a–7d). Similar structures were previously found in microsporidia of this genus [5]. Fixed spores were ellipsoidal. Spores were 5.0–6.3×3.5–4.8 μm when stained with Romanovsky-Giemsa stain (Table 2). The spore wall was thick. Exospore was 1.5–3 times thicker that endospore.

Electron microscopy revealed several layers in exospore. The layer immediately adjacent to endospore (0.25–0.5 of the exsospore thickness) consisted of electron-dense mass displaying no special structure. This layer was consecutively overlain by an electron-dense stratified layer (0.4–0.5 of the exospore thickness), a highly electron-dense layer (0.1–0.2 to the total thickness), an

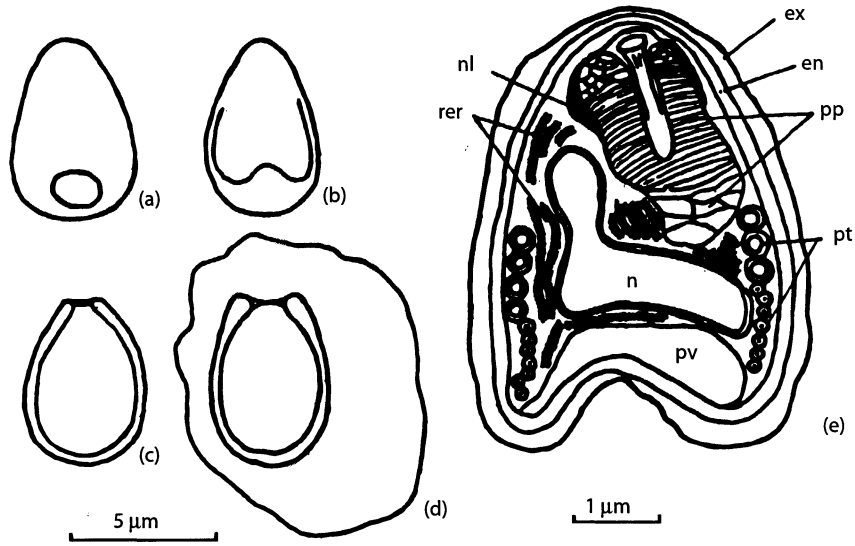

Fig. 7. Morphology and ultrastructure of *A. ukrainica* spores: (a–d) native spores; (e) ultrathin section of a mature spore; pv, posterior vacuole; pp, polaroplast; pt, polar tube; nl, polaroplast containing narrow laminae; ex, exospore; en, endospore; rer, rough endoplasmic reticulum; n, nucleus.

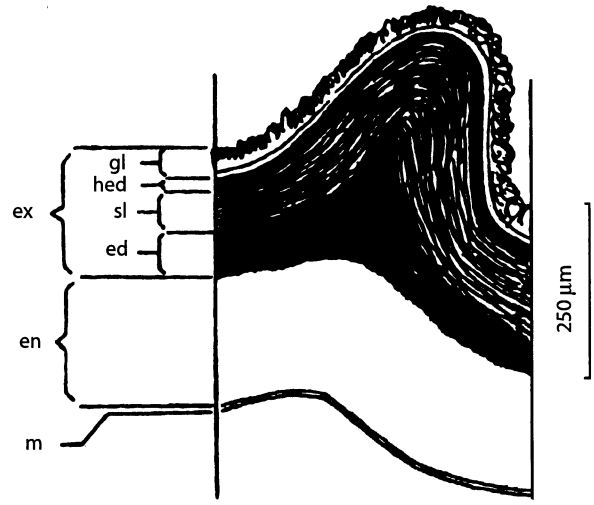

Fig. 8. Ultrastructure of *A. ukrainica* spore wall: ed, electron-dense layer of exospore; m; spore membrane; gl, granular layer; sl, stratified layer; ex, exospore; en, endospore; hed, highly electron-dense layer.

extremely thin membrane, and a granular layer of a medium electron density (Fig. 8). This structure was similar to a certain extent to that of insect cuticle.

The laminar polaroplast had a complex structure. Closely or loosely packed laminae comprised its major part. However, it had one more layer (as though it were an extension of the polar sac) consisting of closely packed narrow laminae (Figs. 7e, 10e). The polar tube consisted of three regions and commonly formed ten twists in a spore. The large nucleus was in the central region of a spore, above the posterior vacuole.

Up to 15% fourth-instar larvae were infested; infestion of the host fat body was generalized.

Differential diagnosis. In twist number of the polar tube, this species is similar to five known species of mosquito microsporidia: *Amblyospora canadensis*, *A. inimica* (Hazard, Oldacre, 1975), *A. connecticus* (Andreadis, 1988), *A. culicis* (Toguebaye, Marchand, 1985), and *A. conopsa* [5]. They are distinguished by the size of native and fixed spores, the absence of mucocalyx, structure of the polaroplast, and morphology of the polar tube.

Distribution. These microsporidia were found in Poles'e and forest-steppe and steppe zones of Ukraine. Phenology of the parasite was the same as that of the host.

Conclusion

Light and electron microscopic analysis of specimens from bloodsucking mosquitoes collected in Ukraine allowed identification of three new subgenera and seven new species of microsporidia of the genus *Amblyospora*.

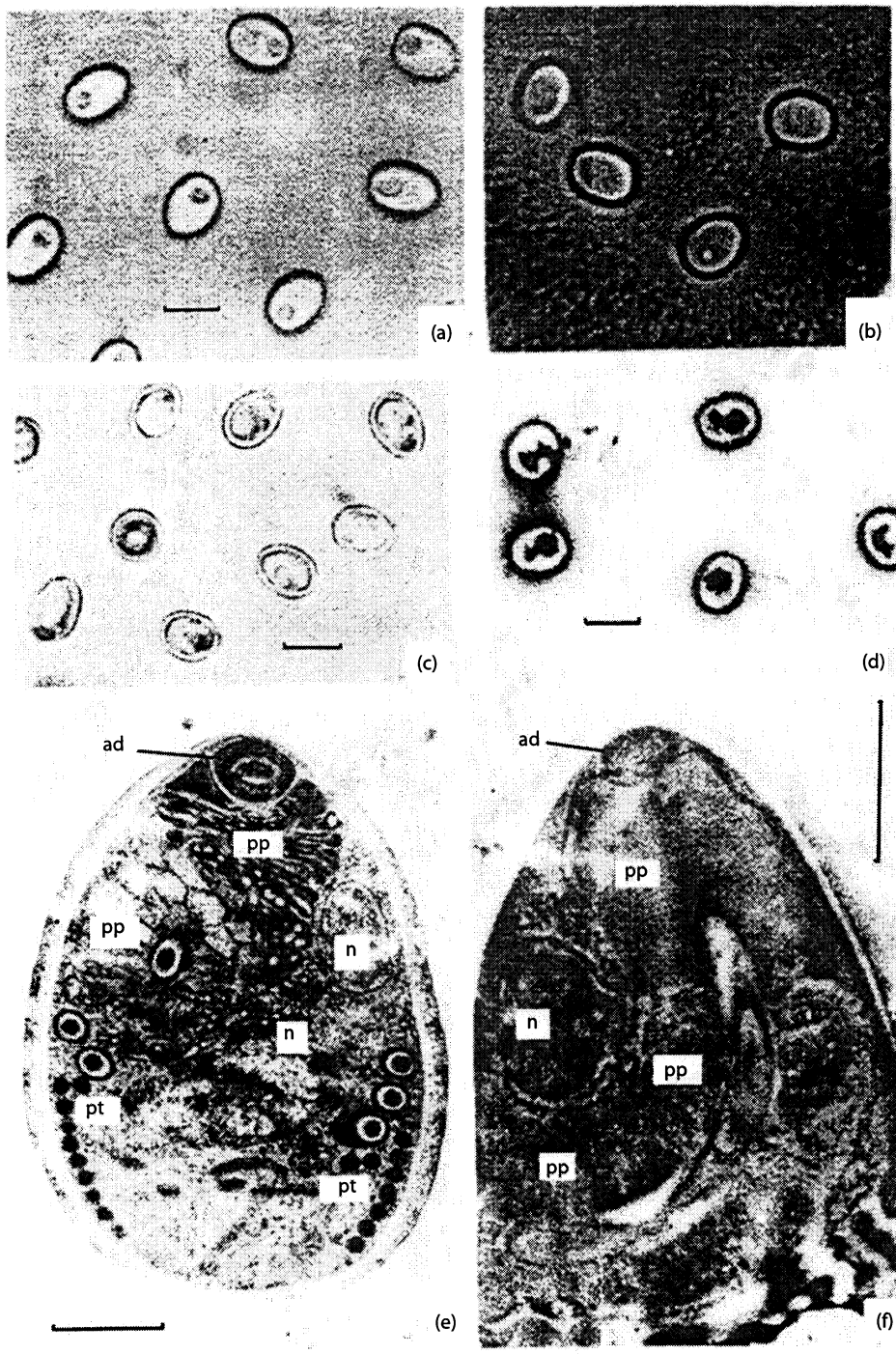

Fig. 9. Morphology and ultrastructure of microsporidia spores: (a) native *A. verna* spores; (b) *A. verna* spores in ink suspension; (c) native *A. firma* spores; (d) *A. firma* spores stained with Romanovsky-Giemsa stain; (e) ultrathin section of an immature *A. verna* spore; (f) ultrathin section of a mature *A. firma* spore; pp, polaroplast; pt, polar tube; n, nucleus; ad, anchor disk. Scale lines correspond to 5 μm (a–d) or 1 μm (e, f).

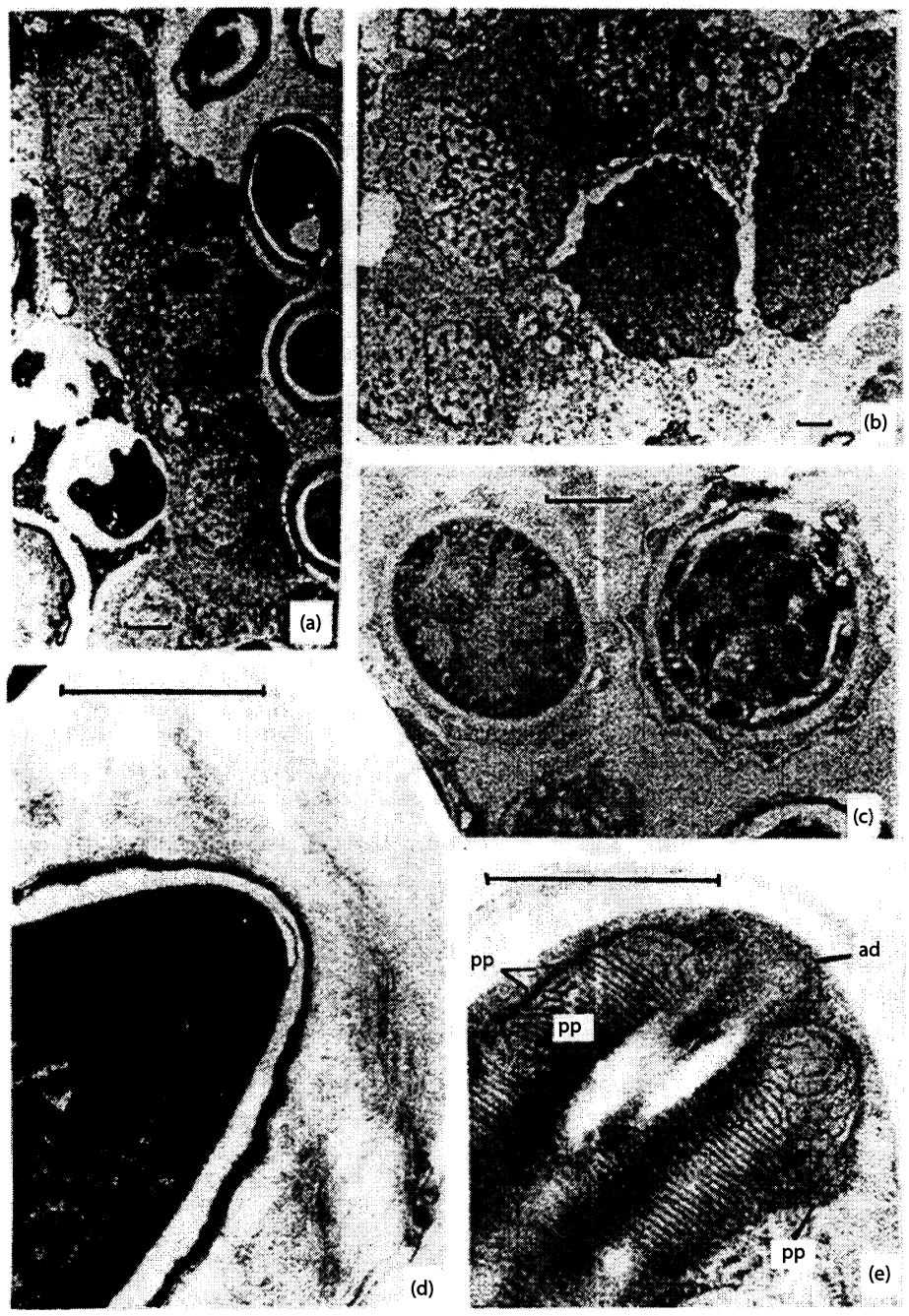

Fig. 10. Ultrastructure of microsporidia at various stages of development: (a) *A. aestiva* line of diplokaryons; (b) *A. rustica* diplokaryons; (c) *A. aestiva* spores; (d) fibrillar structures in *A. certa* sporophore cysts; (e) fragment of *A. ukrainica* polaroplast; pp, polaroplast; ad, anchor disk. Scale lines correspond to 1 μm.

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