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On *Amblyospora styriaca* sp. nov. (Microspora, Amblyosporidae) – a microsporidian of the blackfly *Eusimulium costatum* (Diptera, Simuliidae)

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Abstract. An *Amblyospora* species (Microspora, Amblyosporidae) collected in the southeast region of Austria was examined using light and electron microscopic methods. The hosts were larvae of *Eusimulium costatum* (Diptera, Simuliidae). The merogonial stages were found to be diplocaryotic. During sporogony, eight monocaryotic spores developed in a sporophorous vesicle. The truncate spores measured between 2.9 × 2.3 and 4.1 × 3.2 μm and had an anisofilar polar tube, the narrow part of which was rather short. Besides tubules and homogeneous, electron-dense inclusions, unique filamentous structures were observed in the episporontal space. The microsporidian was compared with other species showing similar characteristics. Because of the variations observed in its ultrastructure as compared with that of other species, we consider this microsporidian to be a new species and have named it *Amblyospora styriaca*.

Since 1977, when Sprague described about 30 species of blackfly microsporidia, a number of papers dealing with this subject have been published (e.g., Vávra and Undeen 1981; Larsson 1983). Many of these microsporidia are not restricted to one host species. To date, no microsporidian has been reported from *Eusimulium costatum*. The present report describes the ultrastructure of the developmental stages as well as the differing size of spores from different specimens of the same locality and compares this new microsporidian with previously described species showing similar characteristics.

Materials and methods

Four infected *Eusimulium* larvae were found between 1988 and 1990 in a brook in the north of Graz (capital of Styria) in the southeast region of Austria. Pieces of infected tissue were smeared on microscope slides, air-dried, fixed in methanol and stained in Giemsa solution. Spore measurements were taken of the smears of three larvae using an ocular micrometer. Mean measurements of 50 spores from each larva were obtained. One entire specimen was fixed in Heidenhain's Susa, dehydrated and embedded in Paraplast. Sections were stained in Mayer's hemalum solution and eosin or light green.

For ultrastructural studies, small pieces of infected tissue were fixed in 4% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 24 h. After being washed in phosphate buffer and post-fixed in 1% OsO_4 in the same buffer, the pieces were dehydrated in increasing concentrations of ethanol, transferred first to propylene oxide and then to mixtures of propylene oxide and Epon and finally embedded in Epon. Ultrathin sections were stained in uranyl acetate and lead citrate and then examined with Zeiss EM9 and Philips 300 microscopes.

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Results

Pathology and light microscopic appearance

The infection was restricted to the adipose tissue. Only 0.6% of the collected specimens were parasitised. These showed a characteristic yellowish colour, but no enlargement of the body was caused by the parasite. In the smears, sporogonial plasmodia with two, four or eight nuclei, sporoblasts and spores could be identified. Sporoblasts and young spores were oval, whereas mature spores were truncate (Fig. 1). The size of spores differed from one host specimen to another, measuring 2.9 × 2.3, 3.3 × 2.7 and 4.1 × 3.2 μm. In most cases the sporophorous vesicles, which measured up to 10.4 μm in diameter, contained eight spores; some sporophorous vesicles also enclosed pairs of macrospores.

Ultrastructure

Two different merogonial stages were found. Diplocaryotic plasmodia were hardly stained and could be discerned only with difficulty. The other developmental stages of merogony were cells with a single diplocaryon (Fig. 2); their cytoplasm contained numerous ribosomes and endoplasmic reticulum.

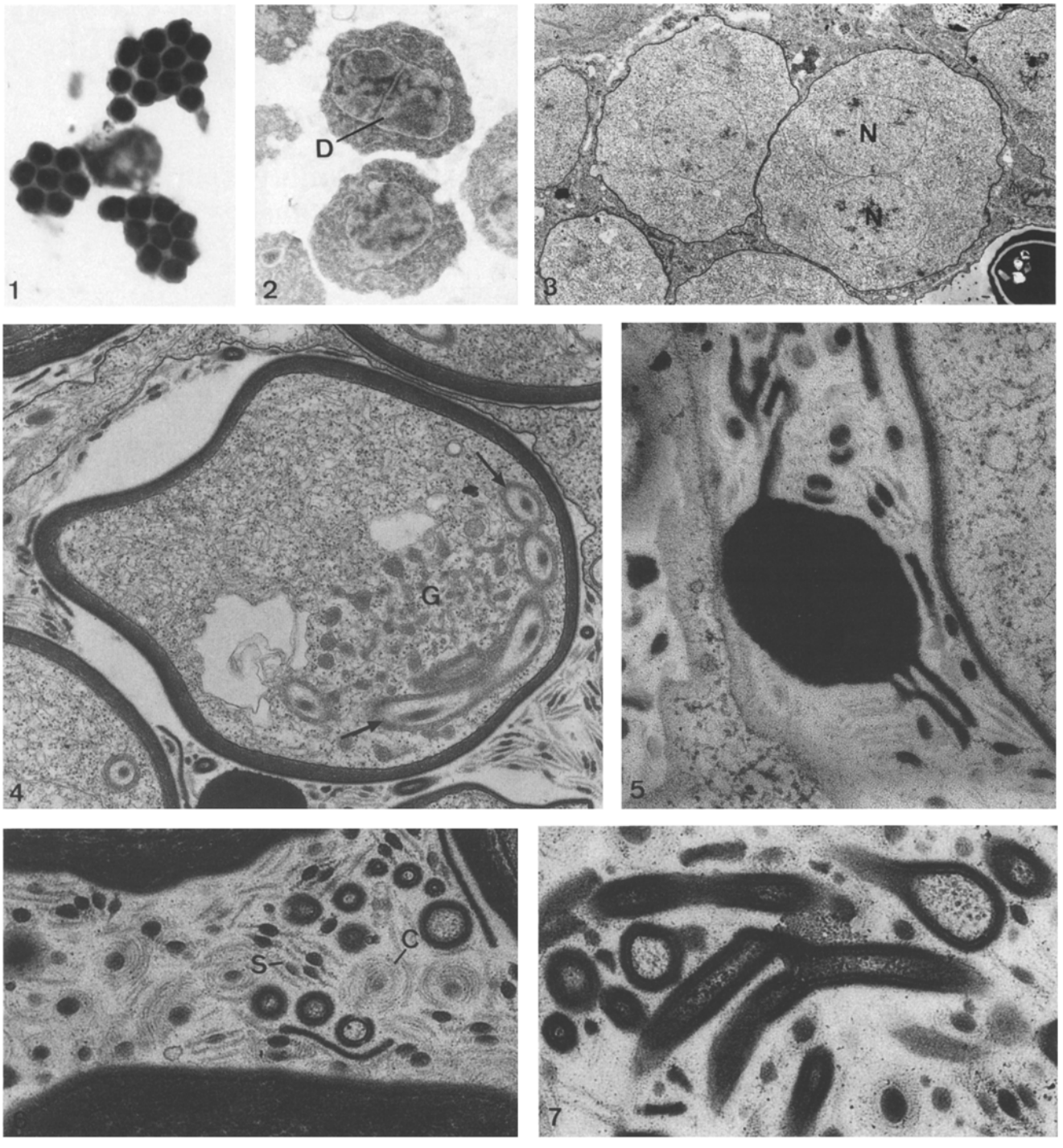
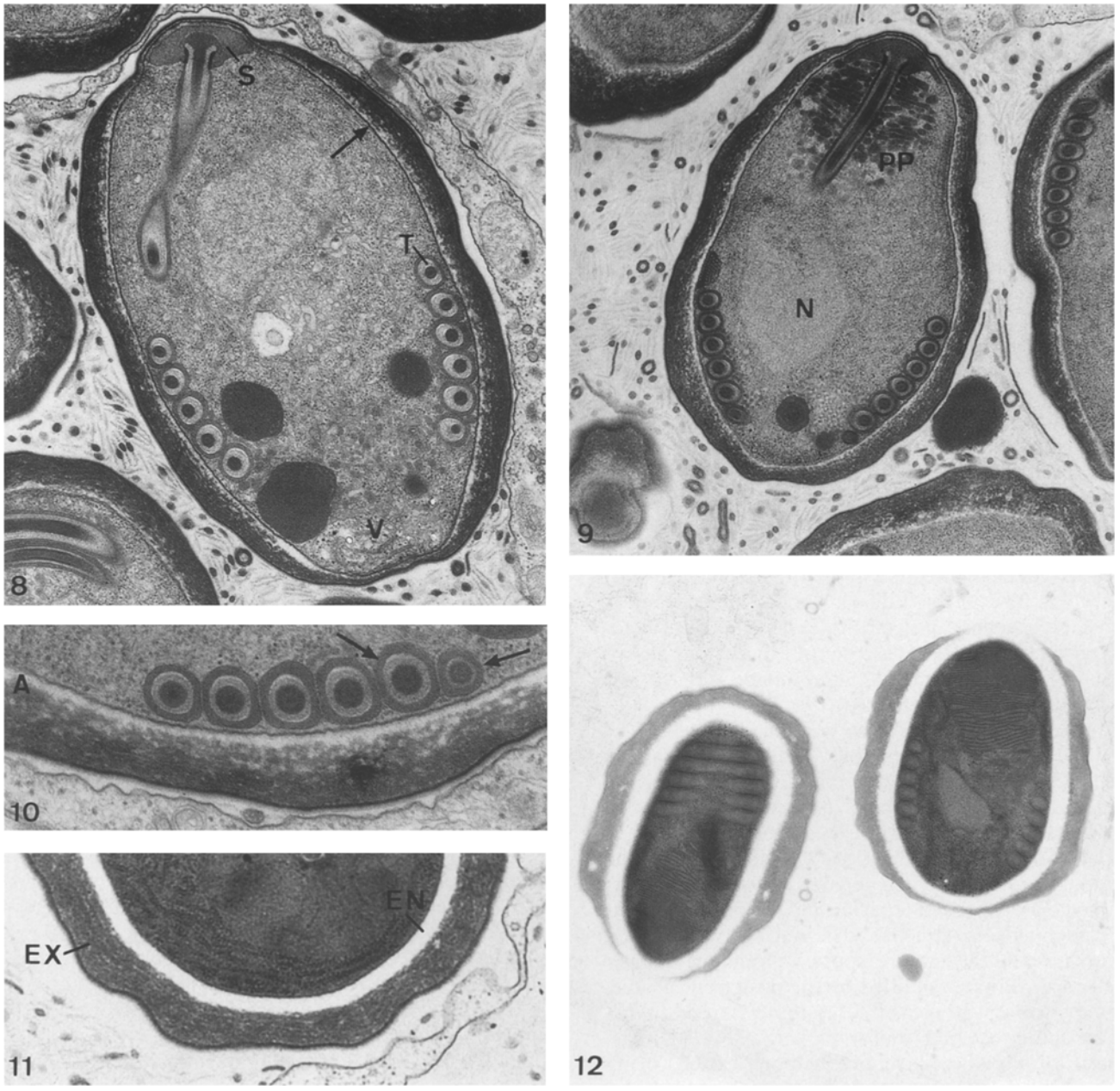


Fig. 1. Light microscopic appearance of *Amblyospora styriaca* sp. nov. × 1700. **Figs. 2–7.** Ultrastructure of some developmental stages of *A. styriaca*. **Fig. 2.** Merogonial stages with diplocaryon (*D*). × 8700. **Fig. 3.** Sporonts. The two nuclei (*N*) of the diplocaryon are separated. × 5000. **Fig. 4.** Sporoblast with Golgi apparatus (*G*) and polar tube (*arrows*). × 22000. **Fig. 5.** Electron-dense inclusions with filamentous projections from the episporontal space. × 41100. **Fig. 6.** Concentrically arranged filamentous structures (*C*) and stacks of filamentous structures (*S*) from the episporontal space. × 36100. **Fig. 7.** Tubules of varying diameters from the episporontal space. × 50000

At the beginning of sporogony an electron-dense layer accumulated on the plasma membrane. Young sporonts were recognised by the detachment of a membrane, the sporophorous vesicle, from the cell surface. This began at specific points before the separation of the entire vesicle; at these places, metabolic products in the form of electron-dense material appeared. Also during this time the two nuclei of the diplocaryon separated (Fig. 3) and the endoplasmic reticulum became more apparent. Within the vesicle we observed a gradual



Figs. 8–12. Ultrastructure of spores of *Amblyospora styriaca* sp.nov. **Figs. 8, 9.** Immature spores with nucleus (*N*), polar tube (*T*), polaroplast (*PP*), polar sac (*S*) and posterior vacuole (*V*). The spore wall consists of the plasma membrane (*arrow*) and, outside of it, the electron-translucent endospore and the electron-dense and laminated exospore. Fig. 8, ×17500; Fig. 9, ×15100. **Fig. 10.** The coils of the polar tube have different diameters (*arrows*). Anterior end (*A*) of the figure, ×43400. **Fig. 11.** Wall of a mature spore. *EX*, exospore; *EN*, endospore. ×25100. **Fig. 12.** Mature spores with anisofilar polar tubes. ×12100

thickening of the plasma membrane of the sporogonial plasmodium. More advanced stages contained tubular material and non-tubular structures in the cavity between the cell surface and the envelope. The tubules (Fig. 7) had diameters of 120–250 nm. The non-tubular structures were of three kinds. The first type consisted of large, homogeneous, electron-dense, bulbous inclusions with filamentous projections (Fig. 5). The second type comprised filamentous structures that were expanded in the centre by an electron-dense accumulation; these structures occurred in stacks (Fig. 6). The third type consisted of filamentous structures concentrically arranged around an electron-dense axis (Fig. 6).

After division of the plasmodia, the Golgi apparatus, a network-like system consisting of vesicles of moderate density, began to produce the polar tube in the young monocaryotic sporoblasts. They were irregular in shape and were surrounded by a stratified membrane of the same thickness as that of sporogonial plasmodia. During further maturation the sporoblasts gradually became more oval. Electron-lucent vacuoles arose close to the Golgi apparatus and the first coils of the polar tube.

The exospore, composed of an exterior amorphous layer, a clearly separated, electron-dense layer internal to this and an interior laminated layer, became thicker (Fig. 4).

Further thickening of the wall of young spores initially involved only the exospore. The endospore was later interpolated between the exospore and the plasma membrane. The exterior layer of the exospore was about 20 nm thick, amorphous and hardly discernible. Internal to the exterior layer was a narrow, clearly separated, electron-dense layer, and internal to this was a thick, laminated layer, which seemed to become less electron-dense near the endospore. The nucleus was situated in either the anterior or the posterior half of young spores. Each of these spores (Figs. 8, 9) had five to seven closely neighbouring coils of the anisofilar polar tube; up to seven layers could be seen in transverse-sections. About one half coil, which was not always visible, had at most two-thirds of the diameter of the other coils (Fig. 10). A posterior vacuole filled with granular material and the remains of the network-like Golgi apparatus were located at the posterior pole. The polaroplast was formed as vesicular protuberances of the uncoiled part of the polar tube in the anterior half of the spore near the polar sac. Nearly all electron-dense inclusions (metabolic products) of the episporontal space had disappeared in this developmental stage; some tubules and filamentous structures remained.

Mature spores (Fig. 12) also contained an anisofilar polar tube with up to seven coils, which were arranged in a single layer in the posterior half of the spore. The diameter of coils in the short, narrow part measured about three-quarters of that of the other coils (Fig. 12). The polaroplast nearly filled the anterior half of the spore and consisted of two regions. The anterior part contained closely packed lamellae and the posterior part, more widely spaced lamellae. The nucleus was located in the centre of the mature spore. About one-quarter of the spore volume was filled by the posterior vacuole. The whole spore wall measured up to 470 nm in diameter. The endospore was uniformly thick (Fig. 11). The thickness of the three-layered exospore varied, being thinner at the anterior and posterior ends. Some tubules and filamentous structures remained in the space between the mature spores and the 10-nm-thick membrane of the sporophorous vesicle. Another type of spore, which is known from other *Amblyospora* species, was not observed.

Table 1. Differing distribution of coils in anisofilar polar tubes in parts of wide and narrow diameter according to species

Species	Number of coils	
	Wide diameter	Narrow diameter
Amblyospora canadensis	3	8
A. inimica	5	5
A. keenani	6	2
A. styriaca sp.nov.	4.5–6.5	0.5
A. capillata	4–5	0
Napamichum aequifilum	6–7	0
Hrabyeia xerkophora	6–9.5	0.5–1

Discussion

The diplocaryotic merogonial stages; the homogeneous, electron-dense granules in the episporontal space; the ultrastructure of the spore wall; and the shape of the octospores are reasons why this species belongs to the genus *Amblyospora* (Larsson 1988).

The difference in the size of spores from various host specimens of the same locality described in this study may be related to the extended period of collection. Vávra and Undeen (1981) reported a difference in the spore sizes of *A. bracteata* collected from different localities, which included the data of Weiser (1961). According to Maddox and Luckmann (1966), spore sizes may depend on the environmental temperature.

The number of coils in the polar tube and their distribution in parts of wide and narrow diameter differs considerably from species to species (Table 1). Apart from the special construction of the anisofilar polar tube of *Jirovecia involuta* (Larsson 1989), which shows a successive reduction in the diameter of the coils, anisofilar polar tubes are abruptly constricted. Hazard and Oldacre (1975) mentioned, among others, the following examples: the polar tube of *A. inimica* has five wide and five narrow coils. *A. canadensis* shows more narrow coils (eight) than wide ones (three). More wide coils (six) than narrow ones (two) were found in octospores of *A. keenani*. The microsporidian described in this report shows only about one half coil with a narrower diameter similar to that of *Hrabyeia xerkophora* (Lom and Dykova 1990), which has one full turn or only a half turn of the narrow polar tube comprising seven to ten turns. The narrow part of the polar tube may be completely reduced, as has been observed in *A. capillata*, a microsporidian originally described as a *Thelohania* species by Larsson (1983). *Napamichum aequifilum* (Larsson 1990) also has a uniformly thick polar tube.

The sporophorous vesicle frequently contains different inclusions in addition to the sporogonial stages; there may be tubules, electron-dense material and some specific structures. Some tubules of the sporophorous vesicles containing mature spores of *Polydispyrenia simulii* (Larsson 1986) have a characteristic coil-spring-like structure. For a period of its development the cavity of the sporophorous vesicle of *Duboscquia sidae* (Larsson and Yan 1988) shows inclusions with the appearance of sectors of a spherical body with concentric, alternating electron-dense and electron-translucent layers. The filamentous structures described in the present paper also seem to be unique.

The microsporidia listed by Sprague (1977) include only one species of the genus *Amblyospora* from blackflies and none from *Eusimulium costatum*. Except for *A. capillata* (*T. capillata*, Larsson 1983), no microsporidian of blackflies has been reported to have a reduced polar tube similar to that of the present species. The polaroplast of spores of *A. capillata* is more voluminous

than that of the species described herein. In contrast to the present species, no tubule was observed in the episporontal space of *A. capillata*. The filamentous structures arranged in stacks or lying concentrically around an electron-dense axis in the new species also differ from the fibrillar material found in *A. capillata*. We consider the present species to be new and have named it *Amblyospora styriaca* sp.nov.

Description: Amblyospora styriaca sp.nov.

Presporal stages: Diplocaryotic merogonial stages; sporonts normally develop into sporogonial plasmodia with eight single nuclei; sporogonial plasmodia produce eight monocaryotic sporoblasts.

Spores: Truncate and monocaryotic. Dimensions (fixed and stained), from 2.9×2.3 to 4.1×3.2 µm; spore wall, up to 470 nm in diameter, three-layered exospore; polar tube, anisofilar with a very short narrow part consisting of a half coil; polaroplast with two lamellar regions; nucleus in the centre of the mature spore; large posterior vacuole. Two large spores were observed by light microscopy.

Sporophorous vesicle: Mostly eight, sometimes two spores; episporontal space with tubules, homogeneous electron-dense bulbuous inclusions, stacks of filamentous structures with an electron-dense centre and filamentous structures concentrically arranged around an electron-dense axis.

Host: *Eusimulium costatum* Friedrich, 1920 (Diptera, Simuliidae).

Affected host tissue: Adipose tissue.

Locality: Graz, Styria, Austria.

Etymology: *styriaca*, in allusion to Styria, the country in which the infected host was found.

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