Europ. J. Protistol. 25, 200–208 (1990)
March 9, 1990

European Journal of
PROTISTOLOGY

Life Cycle of *Amblyospora weiseri* n.sp.: (Microsporidia) in *Aedes cantans* (Diptera, Culicidae)

Julius Lukeš
Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice, Czechoslovakia

Jiří Vávra
Department of Parasitology, Charles University, Prague, Czechoslovakia

SUMMARY

Amblyospora weiseri n.sp., parasite of the mosquito *Aedes cantans* in Czechoslovakia has two sporulation sequences: the octosporous sporulation sequence occurs in oenocytes and fat body tissue of larvae, involves meiosis and results in the formation of eight uninucleate spores (octospores) enclosed in a sporophorous vesicle and is fatal for the host. Octospores are not infectious to other larvae. The oenocytic sequence localized in oenocytes of larvae and adults of both sexes probably effects transovarial transmission to the next mosquito generation and involves the formation of isolated binucleate spores. The oenocytic development differs from the same sequence found in other mosquito *Amblyospora*, as it involves the development of uninucleate meronts and large plasmodia with single nuclei as well as binucleate stages. *A. weiseri* n.sp. is another member of the genus *Amblyospora* in which sporulation is not dependent upon the host taking a blood meal. Preliminary attemps to infect *Cyclops strenuus* and *Megacyclops gigas* with octospores failed.

Abbreviations

ad = adhesive disc
en = endospore
er = endoplasmatic reticulum
es = empty spores
ex = exospore
ga = Golgi apparatus
hn = host cell nucleus
m = muscle tissue
mg = metabolic granules
n = nucleus
nt = nervous tissue
nu = nucleolus
ov = ovarioles
p = polaroplast
pf = polar tube
pv = posterior vacuole
svm = sporophorous vesicle membrane

Introduction

According to present knowledge, the development of species of the trimorphic genus *Amblyospora* Hazard and Oldacre, 1975 is complex and distinct for individual species.

Species parasitizing mosquitoes have at least two developmental sequences in their life cycle. One in larvae produces diplokaryotic sporonts that undergo nuclear separation and meiotic division to form eight haploid spores enclosed in a sporophorous vesicle. This octosporous development starts in oenocytes, ends in fat body cells and is usually fatal for the host larvae. In some *Amblyospora* species the octosporous development is restricted to male larvae [3, 5, 6, 10, 17, 21]. In other species larvae of both sexes [2, 10, 13, 17, 18, 23, 24, 26] are infected.

The second developmental sequence, the purely oenocytic development, was thought to be restricted to the oenocytes of female larvae and female adults but recently its presence has been demonstrated also in male larvae and

0932-4739/90/0025-0200$3.50/0

adults [24]. It results in the formation of another morphological type of spore, binucleate and probably diploid, which is responsible for transovarial transmission to the next host generation. This complex dimorphic development has so far been documented for all species of the genus which have been studied in detail, except for *Amblyospora culicis* (see [26]).

It has been shown relatively recently that the complex life cycle of at least three *Amblyospora* species from mosquitoes is further complicated by transmission through an intermediate host. The octospores from larvae were found to be infectious for cyclopoid copepods, in which another type of uninucleate spore is formed. These spores are then infective to mosquito larvae [3, 7, 24, 25].

A microsporidian species, identified as *A. opacita* was reported in various mosquito species in Central Europe by Weiser [29]. The present paper provides data on its taxonomic position, life cycle and fine structure.

Material and Methods

The microsporidian parasite was found in larvae and adults of the univoltine mosquito *Aedes cantans* which occurs in spring in permanent and temporary pools in the inundation zone of the river Elbe near Poděbrady spa, Central Bohemia.

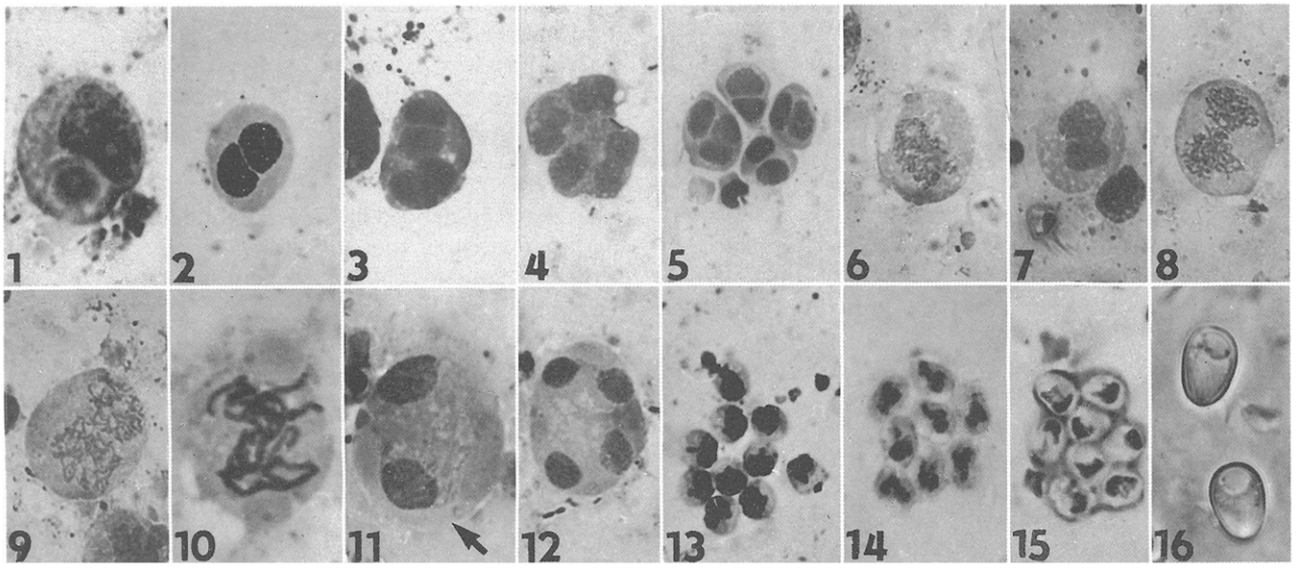
The second, third and fourth instar larvae collected in the field were reared in aquaria and were fed an aqueous suspension of dried yeast. Larvae showing symptoms of infection were smeared and stained with Giemsa's stain (without or after 1 N HCl hydrolysis at 60 °C for 8 minutes) or with Heidenhain's hematoxylin [15]. Spores immobilised in monolayers on agar were measured using a Vickers A. E. Image Splitting Eyepiece [28]. For electron microscopy the larvae were fixed using 3.2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at 4 °C for 1.5 h, postfixed in 2% OsO_4, at 4 °C for 1 h, dehydrated and embedded in Epon-Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in Jeol JEM 100 B electron microscope at 80 kW. Semithin sections 1 µm thick were stained with 1% toluidine blue at 60 °C for 10 min.

Larvae not exhibiting visible signs of infection were reared to adults. Hatched adults were allowed neither to mate nor to bloodfeed. They were smeared or fixed for electron microscopy 24 to 48 hours post-hatching.

Several experiments were conducted to ascertain the "per os" infectivity of octospores to *A. cantans* larvae and to all developmental stages of *Cyclops strenuus* and *Megacyclops gigas*, these copepod species being abundant in pools with the *Amblyospora*-infected mosquitoes. Twenty to fifty copepods of the same developmental stage (nauplius, copepodites or adults) were placed in pans containing filtered water from the breeding site along with octospores that were obtained by homogenisation of heavily infected IV. instar larvae. The spores were cleaned by centrifugation and preserved at 0–4 °C in distilled water with added penicillin G and dihydrostreptomycinsulphate. After cleaning they were fed to copepods immediately or were stored up to one year before use in transmission tests.

Results

Amblyospora weiseri n.sp. was found to have two developmental sequences. The octosporous developmental



Figs. 1–16. Octosporous development of *Amblyospora weiseri* sp. n. from larvae (light microscopy). – Fig. 1. Uninucleate meront in hemocyte (× 1500). – Fig. 2. Diplokaryotic meront from hypertrophic oenocyte (× 1000). – Fig. 3. Merogonial plasmodium with two diplokarya (× 1000). – Fig. 4. Merogonial plasmodium with four diplokarya (× 1000). – Fig. 5. Splitting of plasmodium into diplokaryotic meronts (× 1000). – Fig. 6. Early sporont with fused nuclei (× 1000). – Fig. 7. Diplokaryotic sporont (× 1000) (it differs from diplokaryotic meront in having less dense nuclei). – Fig. 8., Fig. 9. Stages in leptotene phase of meiosis (× 1000). – Fig. 10. Mingling of chromosomes during meiosis (× 1300). – Fig. 11. Binucleate sporont within arising sporophorous vesicle membrane (arrow) (× 1300). – Fig. 12. Quadrinucleate sporont (× 1000). – Fig. 13. Sporoblasts (× 1000). – Fig. 14. Young spores (× 1000). – Fig. 15. Mature spores (× 1000). – Fig. 16. Fresh mature spores released from sporophorous vesicle membrane (× 1500). – Figs. 1–12 from smears stained with Giemsa. Fig. 13 stained with Heidenhain's haematoxylin. Figs. 14, 15 stained with Giemsa after acid hydrolysis. Fig. 16 immobilised spores in agar monolayer.

sequence, involving meiosis, occurred in oenocytes and fat body of the larvae and resulted in eight uninucleate spores enclosed in a sporophorous vesicle ("pansporoblast"). The oenocytic developmental sequence began in larval oenocytes, continued in adults and resulted in isolated binucleate spores.

Octosporous Sequence

The part of the life cycle commencing in oenocytes and ending in fat body cells was found in II., III., and IV. instar larvae of unknown sex. The infected larvae had characteristic white spots in their bodies due to considerably enlarged fat tissue cells containing a great number of spores (Fig. 17). Infected individuals were unable to pupate and died when reaching III. or IV. instar.

The first stage of the octosporous sequence which we observed were rounded or oval diplokaryotic meronts (Fig. 2) in hypertrophic oenocytes of II. instar larvae. The meronts were limited by a thin unit type membrane. Large numbers of ribosomes and abundant endoplasmic reticulum were present in their cytoplasm. Their nuclei were in typical diplokaryotic arrangement (Fig. 18).

The nuclei of the diplokaryon divided at least twice and thus cells with four (Fig. 3) and later with eight (Fig. 4) (or many) diplokaryotic nuclei were formed. These multinucleate cells cleaved into meronts with one diplokaryon each (Fig. 5), which either continued the merogonial multiplication or entered the sporulation phase. Meronts were apparently released into the hemolymph by host cell rupture and invaded by an unknown mechanism the fat cells, where the development continued.

Young sporonts (Figs. 7, 8) differed from meronts in having less dense nuclei and especially in having the cell-limiting membrane thickened by an electron dense surface coat (Fig. 20). The sporophorous vesicle membrane appeared to separate from the cell membrane of the sporont (Figs. 11, 12). The space between the sporont and the sporophorous vesicle membrane (episporontal space) grew progressively larger and became filled with large patches of ill-defined granular material (Figs. 20, 21). At the beginning of sporulation the nuclei of the diplokaryon separated: meiosis as shown by the presence of meiotic configurations of chromosomes occurred in these nuclei (Figs. 8–10). The quadrinucleate sporont (Fig. 12) underwent a final mitotic division producing a sporogonial plasmodium with eight nuclei. Cytoplasmic fission then took place, giving rise to eight uninucleate sporoblasts within a sporophorous vesicle (Figs. 13, 21).

Mature haploid spores ("octospores") were broadly ovoid in shape with a large vacuole at the posterior end (Fig. 15). When fresh (Fig. 16) their size was 6.9 (6.48–7.2 μm) × 4.9 (4.69–5.35) μm (n = 100).

Octospores had a thick electron dense exospore (180 nm) and an underlying transparent endospore (90 nm) (of type III. C of Larsson [20]) both tapering towards the anterior end. The polar tube was of the anisofilar type, having in young spores a basal portion consisting of three thick coils and a distal portion of five to six thin coils (Fig. 22). In mature spores the tube was shorter, having two to three thick and four to five thin coils (Fig. 23).

Oenocytic Sequence

In Giemsa stained smears of larvae and adults we observed uninucleate (Fig. 28) and diplokaryotic meronts (Fig. 29), multinucleate plasmodia (Figs. 25, 26) with up to 16 isolated nuclei and their cleavage into uninucleate cells. There were also binucleate sporoblasts (Fig. 30) and spores (Figs. 31, 32). In addition, in smears from III. instar larvae, we have occasionally found small (5 × 4 μm) uninucleate cells of merogonial character, located singly in host hemocytes (Fig. 1). The possible succession of these stages in the life cycle is considered in the discussion.

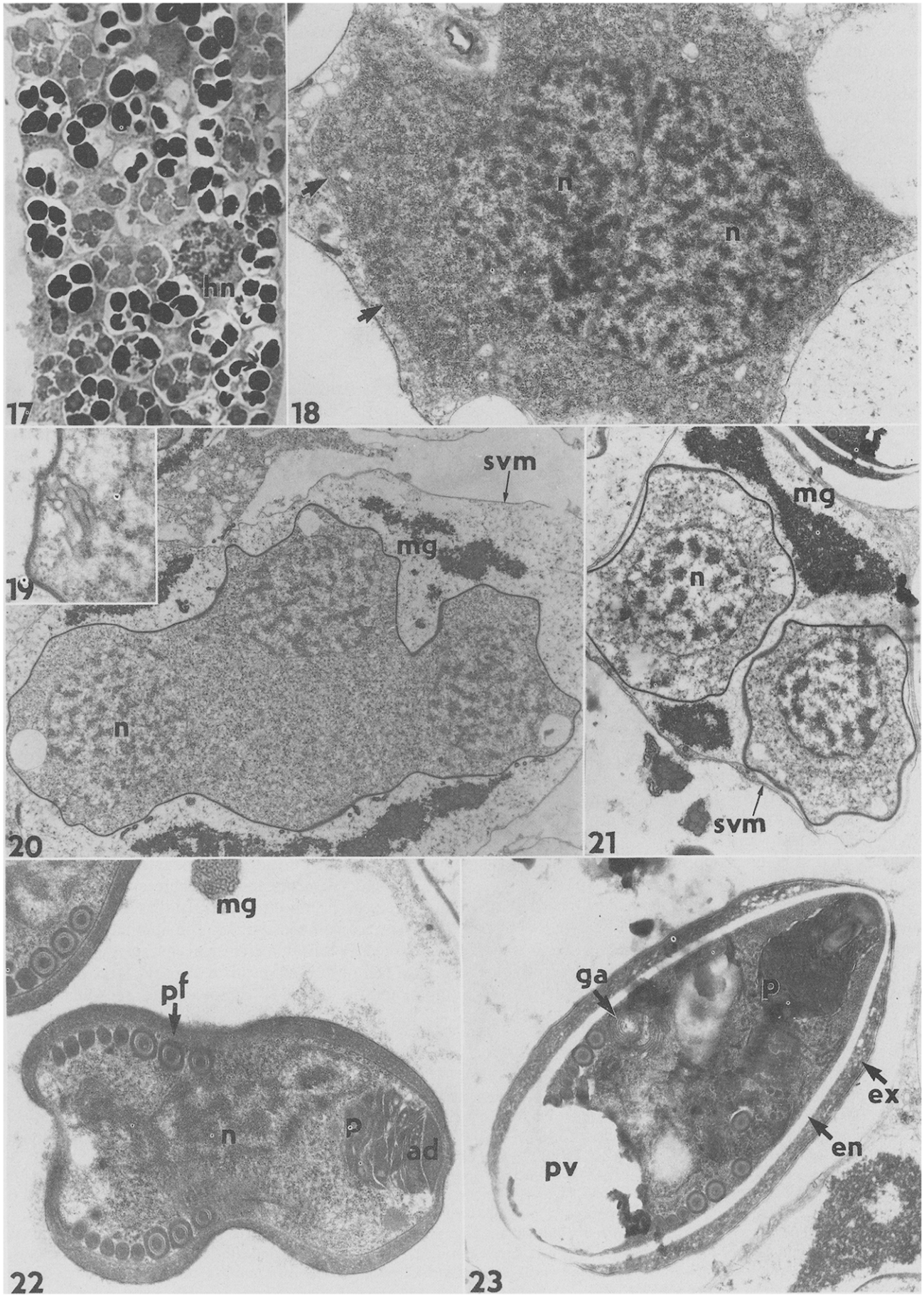
The sporulation phase was usually initiated when adults emerged, but spores were sometimes found in IV. instar larvae (Figs. 33, 34). In this part of the oenocytic sequence, the diplokaryotic cells became elongated (Fig. 29) and formed binucleate sporoblasts (Fig. 30) and later binucleate spores (Fig. 31, 32). Sporulation stages were found even in unmated and non blood-fed adults of both sexes.

However, spores from males and females were different. Those from females ("transovarial" spores) measured 13.0 (11.5–14.6) μm × 5.2 (4.8–5.8) μm (n = 20) whereas those from males measured 11.2 (10.3–11.9) μm × 4.9 (4.5–5.4) μm (n = 20) in smears. The majority of spores in males when observed under the light microscope seemed to be defective, partially extruded or not fully developed.

Spores were not well fixed in our material. The apical part of the spore was occupied by a finely lamellar polaroplast not divided into lamellar and vesicular part in contrast to that of the octospores. The polar tube was isofilar, making seven coils. The thin spore wall consisted of a transparent endospore (160 nm) and an electron dense exospore (40 nm), the outer limit of which was slightly undulated (Figs. 37, 39).

In larvae this developmental sequence was restricted to oenocytes (Figs. 33, 34). Parasitic stages filled the cytoplasm completely causing degenerative changes of the nucleus and considerable hypertrophy of the host cell. The development was not synchronous in individual oenocytes from the same specimen. The oenocytic infection was neither lethal to larvae nor to adults, as both males and females successfully emerged.

Figs. 17–23. Octosporous development of *A. weiseri* sp. n. from larvae (light and electron microscopy). – Fig. 17. Fat tissue filled with ▶ developmental stages and spores of the parasite (× 1200). – Fig. 18. Diplokaryotic meront, arrow = unit membrane surrounding the meront (× 11 600). – Fig. 19. Paramural body, "scindosome", in sporogonial plasmodium (× 35 000). – Fig. 20. Sporogonial plasmodium (× 12 200). – Fig. 21. Sporoblasts (× 13 000). – Fig. 22. Young spore (× 12 000). – Fig. 23. Mature spore (× 13 000). – Fig. 17 semithin section stained with toluidine blue. Figs. 18–23 electron micrographs.


17
hn
18
n
n
19
svm
mg
n
20
mg
n
svm
21
mg
pf
n
p
ad
22
ga
p
ex
en
pv
23

As far as the sporulation and the location of the infection in adult females are concerned, we found two types of development of nearly equal frequency: i) in some specimens clumps of oenocytes among the ovarioles contained a range of developmental stages (including spores) (Fig. 36). ii) in other specimens, large aggregates comprised of closely accumulated diplokaryotic cells only were present in unidentified tissue between the ovarioles. Other developmental stages were absent (Fig. 35).

Horizontal Transmission

Attempts to transmit infection by octospores from dead infected larvae to larvae of II. and III. instars were unsuccessful. Equally we obtained negative results when trying to infect various developmental stages of *Cyclops strenuus* and *Megacyclops gigas* using octospores either fresh or stored up to one year at 0–4 °C. The octospores were ingested by the copepods as deduced from the presence of spores in their guts. However, the spores were unextruded.

Discussion

Taxonomy

When shape and size of the octospore and its ultrastructure are considered, this species closely resembles *Amblyospora opacita,* as defined by Hazard and Oldacre [13]. Nevertheless, these authors restricted the name *A. opacita* to the microsporidium described from *Culex territans* by Kudo [19]. It is unlikely that the species found in *Aedes cantans* is the same, particularly in view of the strict host specificity found for *Amblyospora californica* in *C. tarsalis* (see [21]) and *A. dyxenoides* in *C. annulirostris* (see [24]). Moreover the thin portion of the polar tube of *A. opacita* is one coil shorter than that of our microsporidium. We therefore consider it to be a new species and propose the name *Amblyospora weiseri* n.sp. in honour of Dr. Jaroslav Weiser, an eminent insect pathologist, who first reported this microsporidian from Czechoslovak territory.

There is a seeming discrepancy in the host record of this microsporidium, which was reported by Weiser [29] from *Aedes vexans, A. annulipes, A. communis* and which was found by us in *A. cantans*. Weiser, however, recently in a personal communication confirmed the identity of our material with that reported by him under the name *Thelohania opacita*. He believes also that the earlier identifications of hosts of this parasite were not correct and that *A. cantans* was the true host and this was confirmed in old preserved material.

Another microsporidium was shown to exist in *Aedes cantans* larvae by Weiser, who published several micrographs labelled *A. barbata* [30, 31]. Unpublished observations by one of us (J.V.) confirmed the existence of this species. Its spores are, however, easily distinguished from those of *A. weiseri* n.sp. They are longer, pyriform, slightly asymmetrical and enclosed in a relatively thick gelatinous substance holding the spore octet quite firmly together. The taxonomic status of this species remains to be investigated.

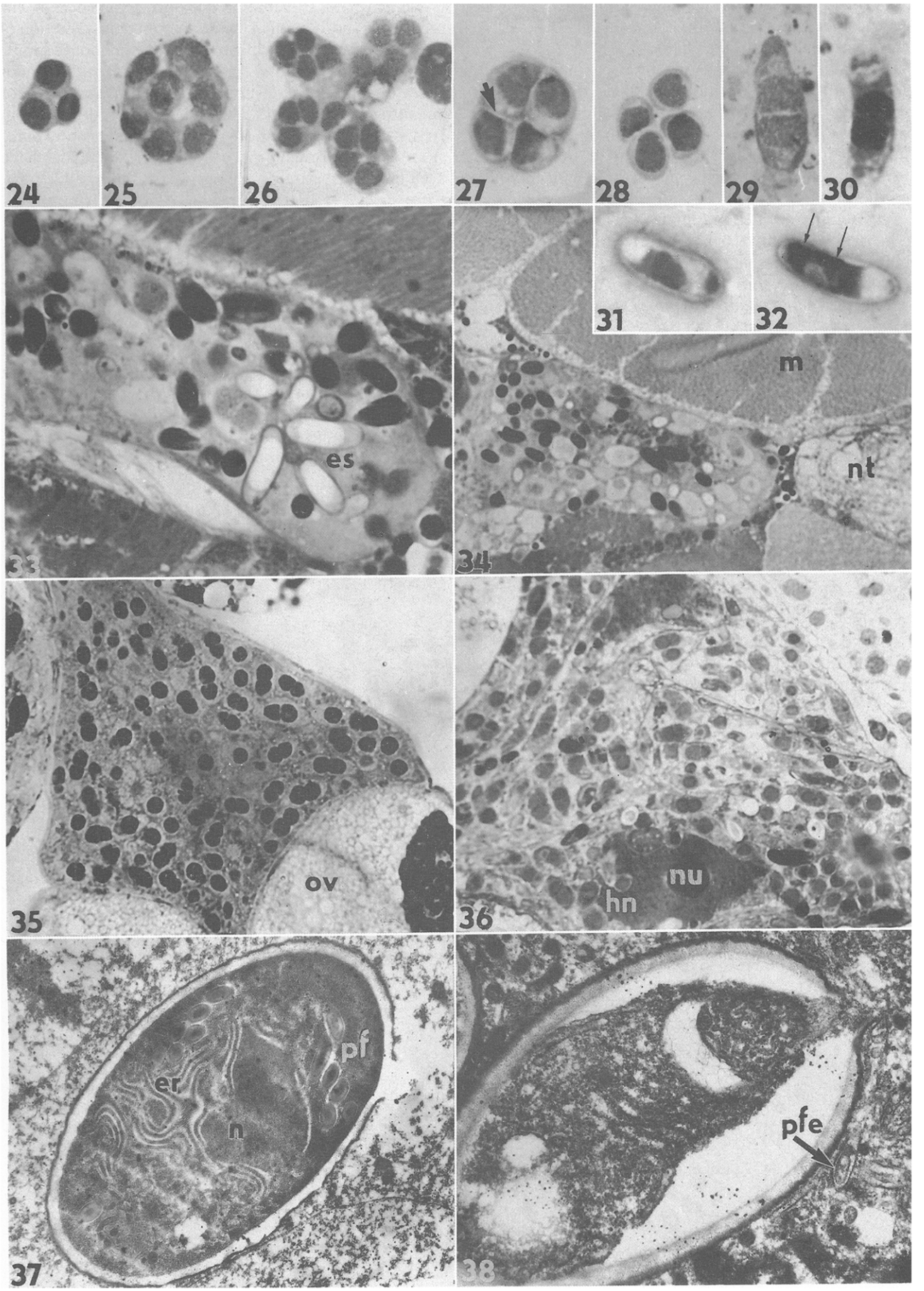
Life Cycle

Aedes cantans is a univoltine mosquito which does not mate in the laboratory. This makes it impossible to determine the function of the spores thought to be involved in the transovarial (= vertical) transmission of the parasite. We infer this function, however, from their morphological resemblance to spores of the same function in other *Amblyospora* species, as well as from their location in tissues [2, 5, 13, 23–25].

The developmental sequence leading to the transovarial transmission of *Amblyospora* spp. to the next mosquito generation is considered by the majority of authors to be a well known part of the life cycle in which an elaborate host-parasite relationship is reflected. Lord and Hall [22] assumed a slow multiplication of diplokaryotic meronts in hypertrophic oenocytes until the adult female takes its first blood meal. They further proposed that the acquisition of blood induces the secretion of ecdysone in ovaries, this hormone being then hydroxylated to 20-hydroxyecdysone. The increased level of this hormone in hemolymph was thought to trigger a very rapid sporulation giving rise to sporoblasts and spores within 24 and 48 h, respectively. Within the next 12 to 24 h the spores then extruded their sporoplasm which invaded host oocytes [5].

The oenocytic sequence of the life cycle of *A. weiseri* n.sp. seems to be more complicated that in other *Amblyospora* species from mosquitoes. The simultaneous presence of uninucleate cells of merogonial character located singly in host hemocytes, uninucleate meronts, large plasmodia, diplokaryotic meronts, diplokaryotic sporoblasts and

Figs. 24–38. Oenocytic development of A. weiseri sp.n. from larvae and adults of both sexes (light and electron microscopy). – ▶ Fig. 24. Meront with three nuclei from larvae (× 1500). – Fig. 25. Merogonial plasmodium with 7 nuclei (× 1500). – Fig. 26. Merogonial plasmodium with 16 nuclei (× 1400). – Fig. 27. Meront with 4 nuclei, arrow = arising cytoplasmic membranes (× 1800). – Fig. 28. Uninucleate meronts (× 1500). – Fig. 29. Lanceolate diplokaryotic meront from adults (× 1800). – Fig. 30. Binucleate sporoblast (× 1800). – Fig. 31. Binucleate spore from male adult (× 2000). – Fig. 32. Binucleate spore from female adult (× 2000) (arrows = nuclei). – Fig. 33, Fig. 34. Oenocytes from IV. instar larvae containing various developmental stages (× 1200, × 750). – Fig. 35. Group of diplokaryotic meronts localized in tissue between ovarioles of adult female (× 900). – Fig. 36. Group of oenocytes from the abdomen of adult female containing various developmental stages (× 800). – Fig. 37. Mature binucleate spore from adult female (× 11 300). – Fig. 38. Partly extruded spore from adult female (the extruded portion of the polar tube is labeled by pfe) (× 13 200). – Figs. 24–32 from smears stained with Giemsa. Figs. 33–36 semithin sections stained with toluidine blue. Figs. 37, 38 electron micrographs.


24
25
26
27
28
29
30
31
32
es
m
nt
34
ov
35
nu
hn
36
er
n
pf
37
pfe
38

spores makes the attempt to establish a logical life cycle sequence of these stages a difficult task.

In accordance with other authors [7, 16, 24, 25], we think that the eventual fusion of two uninucleate cells (plasmogamy) occurs in the early stages of infection in larvae. Nuclear division of the resulting binucleate stages is not followed by cytokinesis, and leads to the formation of multinucleate plasmodia. Nuclei of the plasmodia either form diplokarya and such plasmodia commence the octosporous development, or the nuclei remain isolated in the cytoplasm and such plasmodia start oenocytic development (in which the plasmodia cleave progressively into uninucleate cells (see Fig. 39)). The oenocytic development continues with the formation of lanceolate diplokaryotic meronts and subsequently of binucleate sporoblasts and spores.

At variance with the reports describing the "transovarial" spores from females only after blood acquisition, we have found them in IV. instar larvae, in unfed and unmated females and in males. Identical observations have been made recently by Sweeney et al. [24] in *A. dyxenoides* from *C. annulirostris*.

We have no explanation for the occurrence of the precocious sporulation in larvae. Spontaneous secretion of 20-hydroxyecdysone, which was thought to induce sporulation in *Amblyospora* sp. from *C. salinarius,* cannot operate in our host-parasite system, as *Aedes cantans* is not an autogenous species. The precocious sporulation in larvae is, however, not an isolated phenomenon. Binucleate spores normally occurring in adult females have been found very rarely in larvae parasitized by *Parathelohania legeri* [14]. Recently, an oenocytic sporulation sequence of *Culicospora magna* was also reported to be completed occasionally in the larvae [9].

Interestingly, in some females sporogony does not take place neither in larvae or within 48 to 72 h of adult emergence. In these females large clumps of closely adjacent diplokaryotic cells develop in an undetermined tissue between the oocytes (Fig. 35). For *Amblyospora* species, stages involved in "transovarial" infection were hitherto described only from oenocytes [2, 3, 21, 23–25] whereas a similar location of the parasite vaguely described as "in the fluid between the ovarioles" was published from *Parathelohania legeri* by Hazard and

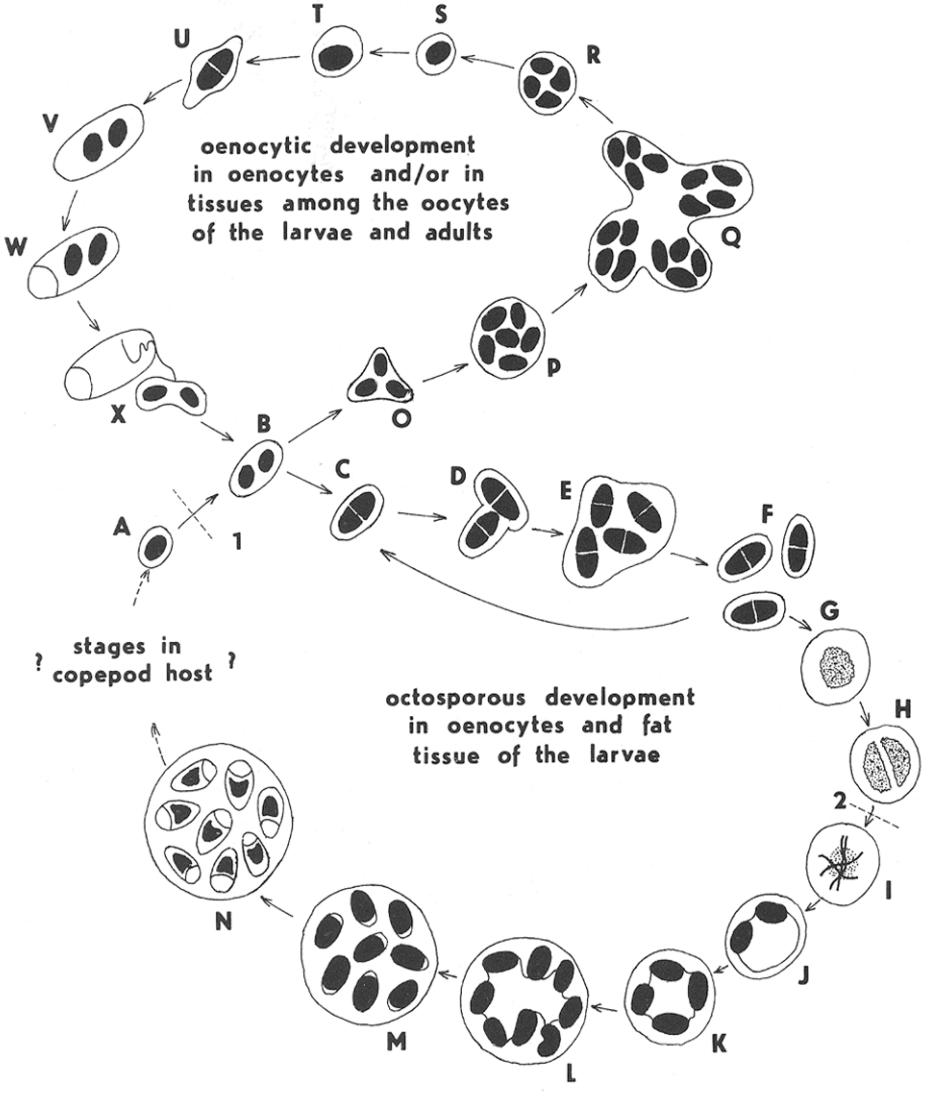


Fig. 39. Proposed life cycle of *Amblyospora weiseri* sp.n. in *Aedes cantans*.

A	= uninucleate meront (in hemocyte)
B	= binucleate meront
C, F	= diplokaryotic meronts
D, E	= merogonial plasmodia with diplokarya
G	= early sporont with fused nuclei
H	= diplokaryotic sporont
I	= mingling of chromosomes during meiosis
J	= binucleate sporont
K	= quadrinucleate sporont
L	= octonucleate sporont
M	= sporoblasts
N	= mature octospores
O, P, Q	= multinucleate plasmodia
R	= quadrinucleate meront
S, T	= uninucleate meront
U	= lanceolate diplokaryotic meront
V	= binucleate sporoblast
W	= mature transovarial spores
X	= extruded binucleate sporoplasm
1	= probably plasmogamy
2	= meiotic division

Weiser [14]. The factor responsible for the delay of sporulation is unknown.

Sporogenesis, not entirely dependent upon a host blood meal, has also been reported. It occurs in adult females of autogenous *C. tarsalis* infected with *Amblyospora californica* (see [11]), in adults of both sexes of *Aedes triseriatus* parasitized by *Pilosporella chapmani* (see [8]), *C. restuans* infected with *Culicospora magna* (see [9]) and *C. annulirostris* infected with *Amblyospora dyxenoides* (see [24]).

The function of spores found in male adults remains obscure. Previously, they were thought to be involved in the paternal transmission of infection [2]. However, the discovery of the transmission through the copepod host enfeebles the necessity for the concept of paternal transmission.

Our results support the claims of several authors [5, 6, 12, 23–25] that octospores of *Amblyospora spp.* from mosquito larvae are not directly infectious to other larvae. In fact, only three authors [1, 27] postulate such direct infectivity. A few authors have proved that the octospores are infectious for copepods, which serve as intermediate hosts of *Amblyospora* spp. [4, 7, 24, 25]. So far, however, we have not been able to demonstrate the infectivity of octospores of *A. weiseri* n.sp. to the copepods *Cyclops strenuus* and *Megacyclops gigas*. These two species occur regularly in habitats with infected mosquito larvae and have their ovaries infected with undescribed microsporidia, the spores of which are structurally similar to those described as copepod stages of other *Amblyospora* (Vávra, unpublished).

Diagnosis

Amblyospora weiseri, n.sp.
Host: *Aedes cantans* Meigen, 1818
Type locality: temporary pools, Poděbrady spa, Central Bohemia
Site of infection: fat body of larvae; oenocytes of larvae and adults of both sexes
Developmental stages: oenocytic development involves multinucleate plasmodia with isolated nuclei ending in isolated binucleate spores. Octosporous sequence involves multinucleate plasmodia with diplokaryotic nuclei resulting in uninucleate octospores.
Spores: Octospores: broadly ovoid 6.9 × 4.9 μm. Spores from adult females 13.0 × 5.2 μm. Spores from adult males 11.2 × 4.9 μm.
Type slides: H-PI-032, H-PI-033
Institute of Parasitology, Czechoslovak Academy of Sciences.

Acknowledgements

Thanks are expressed to Prof. E. U. Canning (Imperial College, London) for her kind help and comments during the preparation of the manuscript.

References

1 Alichanov S. G. (1973): Izmenenie v sootnoshenii polov u *Aëdes caspius caspius* pri zarazhenii poluliacii microsporidiei *Thelohania opacita*. Parazitologia, *7*, 175–179.
2 Andreadis T. G. (1983): Life cycle and epizootology of *Amblyospora* sp. (Microspora: Amblyosporidae) in the mosquito, *Aëdes cantator*. J. Protozool., *30*, 509–518.
3 Andreadis T. G. (1985a): Life cycle, epizootiology and horizontal transmission of *Amblyospora* (Microspora: Amblyosporidae) in a univoltine mosquito, *Aëdes stimulans*. J. Invertebr. Pathol., *46*, 31–46.
4 Andreadis T. G. (1985b): Experimental transmission of a microsporidian pathogen from mosquitoes to an alternate copepod host. Proc. Natl. Acad. Sci. USA, *82*, 5574–5577.
5 Andreadis T. G. and Hall D. W. (1979a): Development, ultrastructure, and mode of transmission of *Amblyospora* sp. (Microspora) in the mosquito. J. Protozool., *26*, 444–453.
6 Andreadis T. G. and Hall D. W. (1979b): Significance of transovarial infections of *Amblyospora* sp. (Microspora: Thelohaniidae) in relation to parasite maintenance in the mosquito *Culex salinarius*. J. Invertebr. Pathol., *34*, 152–157.
7 Becnel J. J. (1986): Microsporidian sexuality in culicine mosquitoes. In: Samson R. A., Vlak J. M. and Peters D. (eds.): Fundamental and applied aspects of invertebrate pathology, pp. 331–334. IVth International Colloquium Invertebr. Pathol., Veldhoven, The Netherlands.
8 Becnel J. J., Hazard E. I. and Fukuda T. (1986): Fine structure and development of *Pilosporella chapmani* (Microspora: Thelohaniidae) in the mosquito *Aëdes triseriatus* (Say). J. Protozool., *33*, 60–67.
9 Becnel J. J., Hazard E. I., Fukuda T. and Sprague V. (1987): Life cycle of *Culicospora magna* (Kudo 1920) (Microsporida: *Culicosporidae*) in *Culex restuans* Theobald with special reference to sexuality. J. Protozool., *34*, 313–322.
10 Chapman H. C., Woodard D. B., Kellen W. R. and Clark T. B. (1966): Host-parasite relationships of *Thelohania* associated with mosquitoes in Louisiana (Nosematidae: Microsporidia). J. Invertebr. Pathol., *8*, 452–456.
11 Hall D. W. and Washino R. K. (1986): Sporulation of *Amblyospora californica* (Microspora: Amblyosporidae) in autogenous female *Culex tarsalis*. J. Invertebr. Pathol., *47*, 214–218.
12 Hazard E. I. and Brookbank J. W. (1984): Karyogamy and meiosis in an *Amblyospora* sp. in the mosquito *Culex salinarius*. J. Invertebr. Pathol., *44*, 3–11.
13 Hazard E. I. and Oldacre S. W. (1975): Revision of Microsporida (Protozoa) close to *Thelohania*, with description of one new family, eight new genera, and thirteen new species. U.S. Dept. Agric. Techn. Bull., *1530*, 1–104.
14 Hazard E. I. and Weiser J. (1968): Spores of *Thelohania* in adult female *Anopheles:* development and transovarial transmission, and redescription of *T. legeri* Hesse and *T. obesa* Kudo. J. Protozool., *15*, 817–823.
15 Hazard E. I., Ellis E. A. and Joslyn D. J. (1981): Identification of microsporidia. In: Burges H. (ed.): Microbial control of pests and plant diseases, pp. 163–182. Academic Press, London.
16 Hazard E. I., Fukuda T. and Becnel J. J. (1985): Gametogenesis and plasmogamy in certain species of microspora. J. Invertebr. Pathol., *46*, 136–142.
17 Kellen W. R., Chapman H. C., Clark T. B. and Lindegren J. E. (1965): Host-parasite relationships of some *Thelohania* from mosquitoes (Nosematidae: Microsporidia). J. Invertebr. Pathol., *7*, 161–166.

18 Kellen W. R., Chapman H. C., Clark T. B. and Lindegren J. E. (1966): Transovarian transmission of some *Thelohania* (Nosematidae: Microsporidia) in mosquitoes of California and Louisiana. J. Invertebr. Pathol., *8,* 355–359.
19 Kudo R. (1924): Biology and taxonomic study of the microsporidia. Ill. Biol. Monogr., *9* (2–3), 268 pp.
20 Larsson R. (1986): Ultrastructure, function and classification of microsporidia. Progr. Protistol., *1,* 325–390.
21 Lipa J. J. and Bartkowski J. (1981): Light and electron microscope study of *Amblyospora (Thelohania) californica* (Kellen et Lipa) (Microsporidia) in larvae of *Culex tarsalis* Coq. (Culicidae). Acta Protozool., *20,* 209–213.
22 Lord J. C. and Hall D. W. (1983): Sporulation of *Amblyospora* (Microspora) in female *Culex salinarius:* induction by 20-hydroxyecdysone. Parasitology, *87,* 377–383.
23 Lord J. C., Hall D. W. and Ellis E. A. (1981): Life cycle of a new species of *Amblyospora* (Microspora: Amblyosporidae) in the mosquito *Aëdes taeniorhynchus.* J. Invertebr. Pathol., *37,* 66–72.
24 Sweeney A. W., Graham M. F. and Hazard E. I. (1988): Life cycle of *Amblyospora dyxenoides* sp. nov., in the mosquito *Culex annulirostris* and the copepod *Mesocyclops albicans.* J. Invertebr. Pathol., *51,* 46–57.
25 Sweeney A. W., Hazard E. I. and Graham M. F. (1985): Intermediate host for an *Amblyospora* sp. (Microspora) infecting the mosquito, *Culex annulirostris.* J. Invertebr. Pathol., *46,* 98–102.
26 Toguebaye B. S. et Marchand B. (1985): Pathogénie, cycle de développement et ultrastructure d'*Amblyospora culicis* n.sp. (Protozoa, Microspora), parasite du moustique *Culex quinquefasciatus* Say, 1823 (Diptera, Culicidae). Can. J. Zool., *63,* 1797–1809.
27 Toguebaye B. S. et Marchand B. (1986): Fusion entre deux cellules uninucléées au cours du cycle de développement sexué d'*Amblyospora culicis* (Protozoa, Microspora): un argument en faveur d'une fécondation chez des Microsporidies. Protistologica, *22,* 359–367.
28 Vávra J. and Maddox J. V. (1976): Methods in microsporidiology. In: Bulla L. A. and Jr. Cheng T. C. (eds.): Comparative pathobiology of the microsporidia, pp. 341–365. Plenum Press, New York.
29 Weiser J. (1961): Die Mikrosporidien als Parasiten der Insekten. Monographie zur Angew. Entomologie, Nr. 17, pp. 1–149. Paul Parey, Hamburg.
30 Weiser J. (1969): An atlas of insect diseases, pp. 43–292. Academia, Prague.
31 Weiser J. (1977): An atlas of insect diseases, pp. 72–240. Academia, Prague.

Key words: Amblyospora – Aedes – Life cycle – Ultrastructure – Microsporidia

Julius Lukeš, Institute of Parasitology, Czechoslovak Academy of Sciences, Branišovská 31, 370 05 České Budějovice, Czechoslovakia