

Description of *Hyalinocysta expilatoria* n. sp., a Microsporidian Parasite of the Blackfly *Odagmia ornata*

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Hyalinocysta expilatoria n. sp. is described from a larva of *Odagmia ornata* collected in Sweden. Infection was restricted to the adipose tissue which was transformed into a syncytium. The earliest stage observed was diplokaryotic merozoites, which mature directly into diplokaryotic sporonts. Each sporont produces a sporophorous vesicle (pansporoblast), which persists, also enclosing mature spores. Usually nuclear divisions result in a plasmodium with 8 nuclei, which fragments into 8 sporoblasts, each of which develops into a spore without further division. Occasionally an aberrant number of spores (2, 4, 6) is formed. The spores are pyriform with a flattened area at the posterior pole. Spores in sporophorous vesicles with 8 spores are 4.0-6.0 μm long, in vesicles with 4 spores 4.0-5.0 μm , and in vesicles with 2 spores 7.0-8.0 μm . In some vesicles the spores develop asynchronously, and 2, 4, or 6 mature spores are found together with 6, 4, or 2 immature. There was also a small number of vesicles with supernumerary spores, less than 8 normally developed. The 325-350 nm thick spore wall is composed of three layers. The polar filament is anisofilar with 7 coils in a single layer. The anterior 5-6 coils are wide, the posterior 2-1 thin. The angle of tilt of the anterior filament coil is approximately 50°. The spore has a single nucleus. The sporophorous vesicle is delimited by a thin membrane, also visible in haematoxylin stained preparations. Vesicles with mature spores are void of metabolic inclusions.

KEY WORDS: *Hyalinocysta expilatoria* n. sp.; *Odagmia ornata*; microsporidia; blackflies; cytology; ultrastructure; taxonomy.

INTRODUCTION

The genus *Hyalinocysta* was created by Hazard and Oldacre (1975) for a microsporidium with pyriform spores produced in octosporous pansporoblasts. It has a somewhat persistent pansporoblast membrane, which also surrounds mature spores, and it is clearly visible in smears stained with hematoxylin. The pansporoblast cavity is completely void of crystals, tubules, or granules, at least in pansporoblasts with mature spores, and the spores are distinctly seen against a clear background, whereas in most other microsporidian genera with octosporous pansporoblasts, metabolic products are usually prominent. In the genus *Hyalinocysta* the spores conform to the *Amblyospora* type, with an anisofilar polar filament. The type species, *H. chapmani*, a parasite of larvae of the mosquito *Culiseta melanura*, was collected in the USA. Infection was found in the adipose tissue. Ac-

cording to Sprague (1977), Hazard and Chapman intended to include another microsporidium in this genus. It was the parasite of *Aedes vexans* and *A. cantans*, which was depicted by Weiser (1969) in the first edition of "An Atlas of Insect Diseases" and there named *Thelohania barbata*. In the second edition of the atlas (Weiser, 1977), he transposed the species to the genus *Amblyospora*, based on ultrastructural findings. So far these are the only species with a suggested ranking into the genus *Hyalinocysta*.

In the autumn and winter of 1978, blackfly larvae were collected from different localities in the south of Sweden in the search for parasites. Infected larvae were only processed for light microscopy studies. It was found that one of the larvae was infected with a *Hyalinocysta* sp. clearly different from the species of mosquito larvae. Further search for infected material has so far been unsuccessful. Since ultrastructural

data are necessary in dealing with microsporidia of the families Thelohaniidae and Amblyosporidae, some paraffin sections were processed for electron microscopy. Although the results obtained by this method were inferior to what can be gained from material directly fixed for electron microscopy, they gave sufficient information to verify the generic assignment and provide characters for the identification of the species. The new species, which is named *H. expilatoria*, is described based on light microscopical and ultrastructural characteristics.

MATERIALS AND METHODS

The host was one larva of *Odagmia ornata* (Diptera, Simuliidae), collected in the small stream Klingavälsån, in the south of Sweden, on December 8, 1978. One smear was made before the larva was processed for paraffin sectioning. Fixations were performed in Bouin–Duboscq–Brasil solution overnight. After washing and dehydration in an ascending series of ethanol, the larva was embedded in paraplast. Sections were cut at 5 and 10 µm. The stains used were Giemsa solution and Heidenhain's iron haematoxylin (Romeis, 1968). Measurements were made with an eye piece micrometer at 1000× magnification.

Some paraffin sections were reprocessed for electron microscopy using a modification of the method described by Weiser and Žižka (1967). After removal of the paraffin and rehydration, the sections were covered with a thin layer of melted agar (1% in distilled water). When the solution was congealed, the agar layer with the adherent sections could be separated from the microscope slide, and small pieces of agar and parasite-filled tissue could be excised. These were fixed in 2.5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2, at 4°C overnight. After washing in cacodylate buffer and post fixation in 2% (w/v) osmium tetroxide in cacodylate buffer for 1 hr at 4°C, the pieces were dehydrated

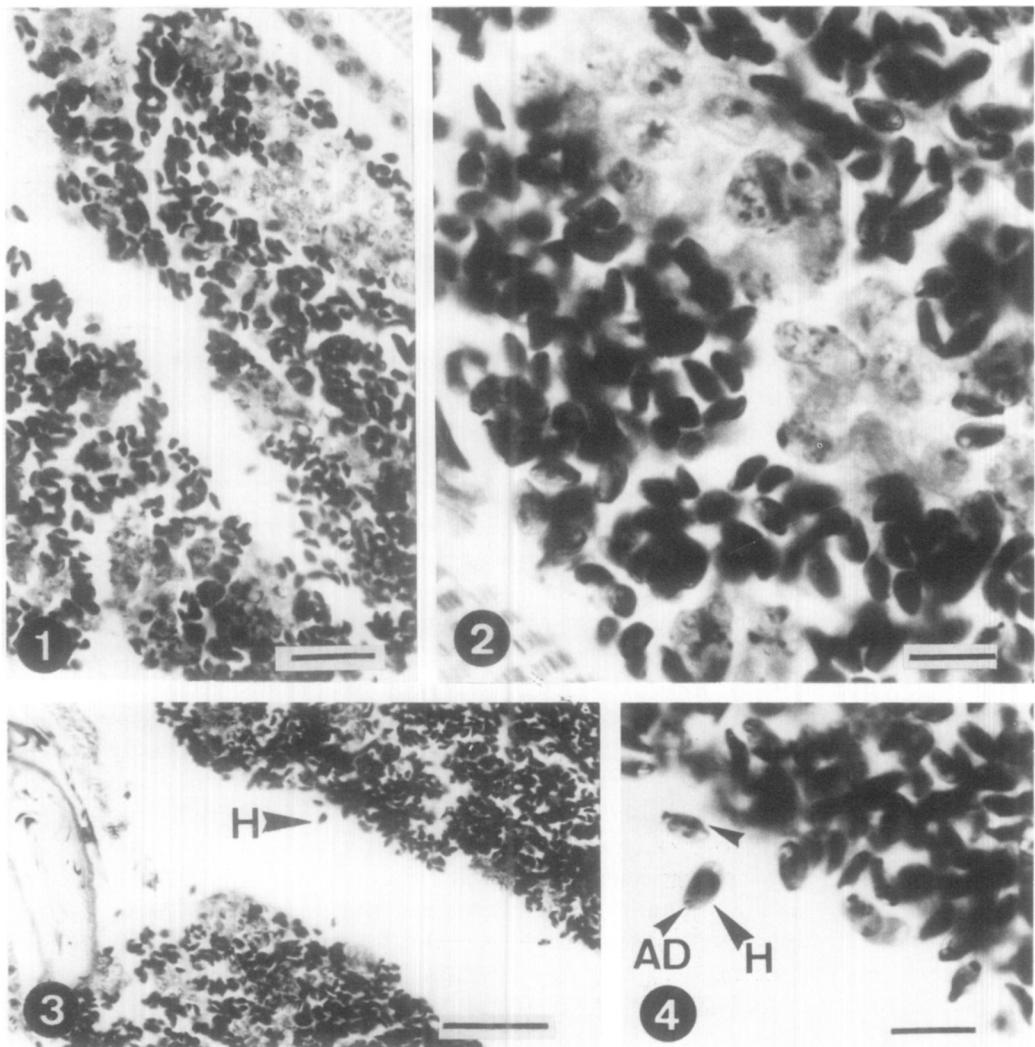
in an ascending series of buffer–acetone solutions to absolute acetone. They were embedded in epon, and sections were stained with uranyl acetate and lead citrate.

OBSERVATIONS

Light Microscopy

The larva, which had an apparent white color when alive, was heavily infected. All adipose tissue was invaded by microsporidia and degraded into a syncytium or, using the terminology by Weiser (1975), a xenoma of the syncytial type. The different sporogonial stages were floating among the nuclei of the fat cells (Figs. 1,2). Younger and older developmental stages of microsporidia occurred homogeneously dispersed, without any stratification. Parasites were not found outside the fat body.

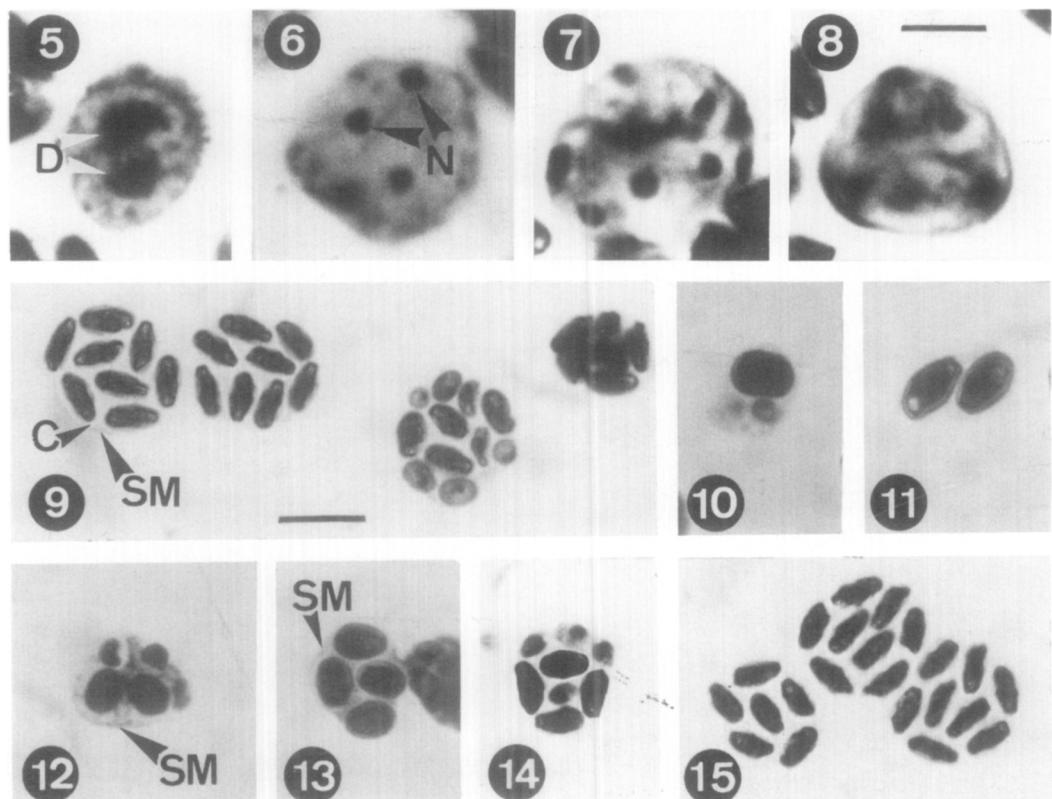
The youngest stages observed were diplokaryotic merozoites, measuring 8.0–10.5 µm, and with a nuclear diameter of approximately 3 µm (Fig. 5). Nuclear divisions resulted in plasmodia with 4 and finally 8 nuclei (Figs. 6,7). The plasmodium fragmented into 8 sporoblasts (Fig. 8), each of which matured into a spore without further division. Mature spores measured 4.0–6.0 × 2.0–2.5 µm. Their shape was pyriform with a characteristically flattened area at the posterior pole (Fig. 4). Each spore had a single nucleus. At the anterior pole the anchoring disk of the polar filament appeared as a dark spot (Fig. 4), and in some spores the straight part of the filament could be seen extending backwards from the disk. At the anterior end of the spore the polaroplast was seen as a faintly stained area, and at the opposite pole the posterior vacuole appeared as a clear spot. Fixed spores seemed to have a somewhat irregular surface (Fig. 9), but it is not clear if that was due to some kind of surface sculpture or if it was an artefact of the preparation. Pansporoblasts or, following the terminology suggested by Canning and Hazard (1982), sporophorous vesicles, normally with 8 spores, were of an oval shape (Fig. 9).



FIGS. 1–4. (1,2) Infection by *Hyalinocysta expilatoria* transforms the fat body of a blackfly larva into a syncytium. The cell walls are dissolved and the lobes filled with younger and older developmental stages of the microsporidium without any visible stratification. (3, 4) Localization of the holotype (H), (4) enlarged detail of (3). In the holotype (H) the anchoring disk (AD) is clearly visible. The flattened posterior pole of the mature spore is indicated by the arrow. Bars: (1) 25 μm ; (2) 10 μm ; (3) 50 μm ; (4) 10 μm .

There appeared to be plenty of space inside the membrane. Although the spores were overlayed in most of the vesicles seen in the smear, there were also numerous sporophorous vesicles where the spores were spread in one plane without disruption of the membrane. Vesicles with aggregated spores measured 10–12 \times 8–9 μm , and flat-

tened vesicles with the spores in one plane was a little bigger, 11–13 \times 10–11.5 μm . The membrane of the sporophorous vesicle was clearly seen in the hematoxylin-stained smear (Fig. 9). Each spore was surrounded by a clear space, probably a gelatinous capsule (Fig. 9). The vesicle appeared free from all types of metabolic inclusions.



FIGS. 5-15. Sporogony of the microsporidium. (5) Merozoite with a diplokaryon (D). (6, 7) Sporogonial plasmodia with four and 8 nuclei (N). (8) Sporophorous vesicle with sporoblasts. (9) Four sporophorous vesicles with mature spores. The membrane of the sporophorous vesicle (SM) and the supposed gelatinous capsule (C) are visible. All vesicles are void of metabolic inclusions (10-14) Sporophorous vesicles with one macrospore and one degenerated spore; two macrospores; two mature and two degenerated spores; four mature spores; and four mature and four immature or degenerated spores. (15) Vesicles with 6 or 8 mature spores. (Bars: (5-8) 5 μm ; (9-15) 10 μm .)

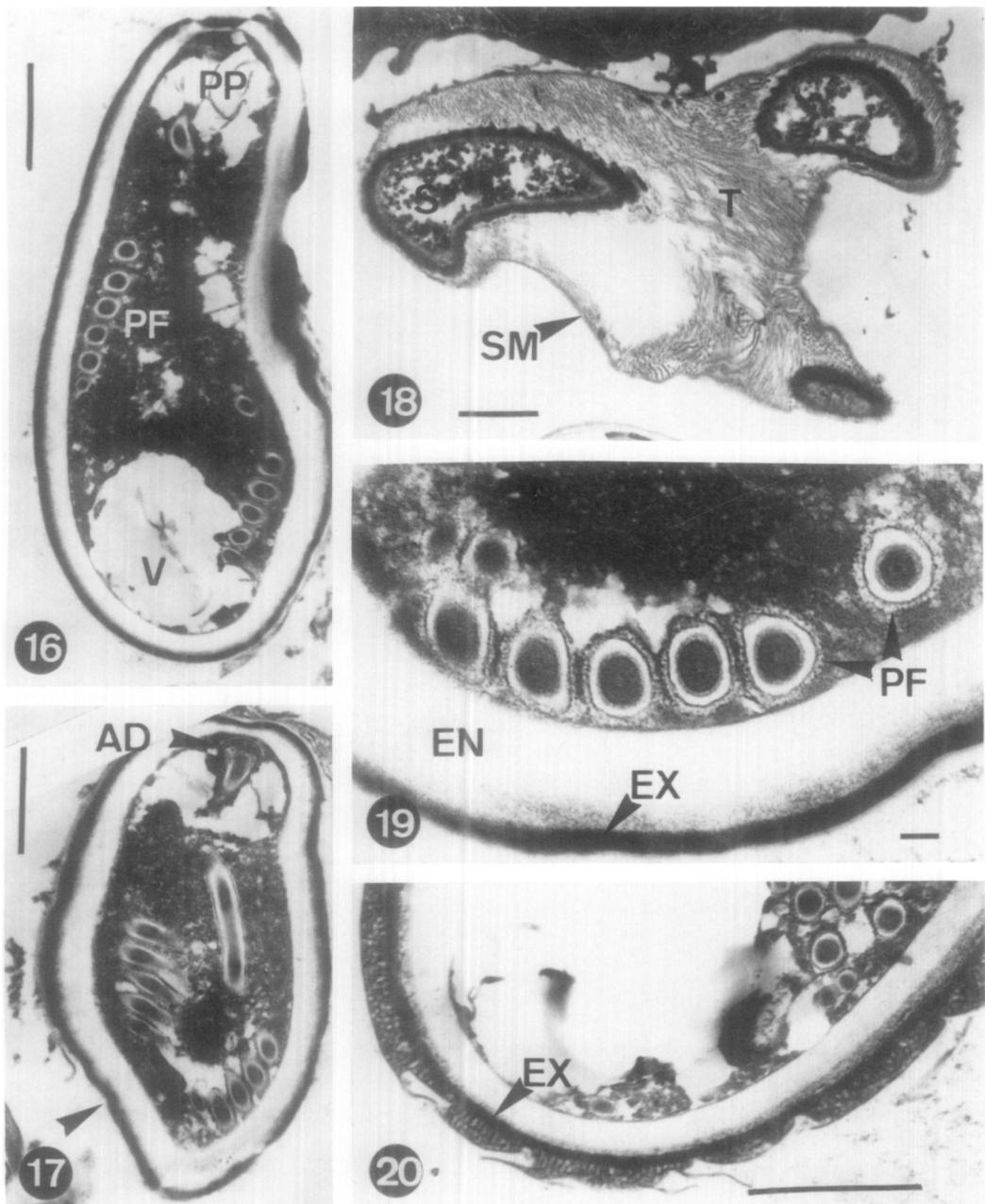
Sporophorous vesicles with an aberrant number of spores were not unusual (Figs. 10-15). Most of these contained 4 spores (Fig. 13), but there were also vesicles with 2 (Fig. 11) and with 6 spores (Fig. 15). The spores in vesicles with 4 spores were slightly wider than the octospores, measuring $4.0-5.0 \times 3.0-4.0 \mu\text{m}$. In vesicles with 2 spores they measured $7.0-8.0 \times 4.0-5.0 \mu\text{m}$. All spores of a vesicle were not always in synchronous development, and there were vesicles with 6 mature and 2 immature spores, 4 mature and 4 immature spores (Fig. 14), or with 1 mature and 1 immature spore (Fig. 10). Occasionally the number of

spores and sporoblasts in a vesicle exceeded 8, but less than 8 of these appeared to develop in the normal way (Fig. 9).

Ultrastructure of Spore and Sporophorous Vesicle

Since the microsporidia were not directly fixed for electron microscopy, all structures of the spore were not well preserved. However, the more solid structures appeared as they usually do in microsporidia and their dimensions conformed to the dimensions reported from related species.

Sporoblasts and young and mature spores were the only stages found. The spore wall



Figs. 16-20. Ultrastructure of *H. expilatoria*. (16,17) Longitudinally sectioned mature spores with 5 wide and 2 narrow coils of the polar filament (PF). The polaroplast (PP) appears as an empty space due to a fixation artefact. A vacuole (V) is found at the posterior pole and the anchoring disk (AD) is seen at the anterior end. The flattened posterior part is indicated by an arrow. (18) Sporophorous vesicle with sporoblasts. A tubular (T) or fibril-like material extends from the sporoblast wall to the membrane of the sporophorous vesicle (SM). (19) A detail of the spore wall with distinct exospore (EX) and endospore (EN) layers. The polar filament (PF) is anisofilar. (20) A spore wall with tubular exospore secretions. Bars: (16-18, 20) 1 μm ; (19) 100 nm.

of the mature spore was composed of the three layers usually found in microsporidia (Figs. 16,19). It was 325–350 nm thick. The inner layer was a plasmalemma, of unit membrane nature, about 8 nm thick. The structureless endospore comprised nearly three fourths of the wall thickness, but was considerably thinner above the anchoring disk. The exospore layer was electron dense, measured 55–80 nm, and was of nearly homogeneous thickness. The transitory zone between endo- and exospore was finely granular.

The anchoring disk, mushroom-like in sagittal sections, was composed of superimposed layers of different electron density (Fig. 17). Umbrella-like evaginations could be suspected but were not clearly seen. The polaroplast was not adequately preserved and this region appeared as an empty space traversed by a few electron-dense strands. The straight part of the polar filament reached over the anterior third of the spore length, and the posterior part was coiled in a single layer beneath the spore wall. The filament was anisofilar with 7 coils (Fig. 16), 5–6 wide and 2–1 thin. The diameter of the wider part was approximately 190 nm, and the thinner coils were about 140 nm thick. The filament was composed of concentrical layers of different electron density (Fig. 19), but they were not so well preserved as to allow a detailed comparison with the filaments of other microsporidia. The angle of tilt of the anterior filament coil was about 50°. The central part of the spore was electron dense and granular with the single nucleus visible in a few spores. Near the posterior pole was a voluminous vacuole (Fig. 16).

Sporophorous vesicles with mature spores were almost completely void of inclusions. Crystals were never seen, but there were occasionally a few fibrous structures. In vesicles with immature spores the cavity was filled with filaments or tubules (Fig. 18). They extended from the exospore to the membrane of the vesicle. The surface of some young spores was covered with a

tubular or reticulate layer of secretions (Fig. 20). The membrane of the sporophorous vesicle was apparently a single layer, approximately 25 nm thick (Fig. 18).

DISCUSSION

The microsporidium described here conforms to the definition of the genus *Hyalinocysta* given by Hazard and Oldacre (1975). The sporophorous vesicles (pan-sporoblasts) are oval, the thin membrane is visible both in hematoxylin-stained preparations and ultrathin sections, there are no metabolic inclusions in vesicles with mature spores, and the polar filament is anisofilar. Microsporidia of the genus *Amblyospora*, where the octospores also are anisofilar, have apparent crystal-like inclusions, visible both in Bouin-fixed and stained smears and in ultrathin sections, also when they are prepared by the unconventional method used here. The spore shape is further a character in common between this species and *H. chapmani*, the type species. The spores are pyriform with a characteristically flattened posterior part (Figs. 4, 17; see Hazard and Oldacre 1975, Fig. 45). However, the species is different from the two species of mosquito larvae, not only from the occurrence in different hosts, but also in the morphology of the spores, e.g., in the number and arrangement of the polar filament coils.

The numerous microsporidia with octosporous sporophorous vesicles, which have been described from simulid hosts, are grouped in the three genera *Amblyospora*, *Pegmatheca*, and *Thelohania*. Although some of these have been reported from different parts of the world, few are well known and so thoroughly described that they easily can be identified. The taxonomical problems and the synonymy of the different species are treated by Hazard and Oldacre (1975), Muu (1977), Sprague (1977), and Vávra and Undeen (1981), but there are still several difficulties concerning the identification of microsporidia from simulids.

Pegmatheca simulii Hazard and Oldacre, 1975, the only species of the genus, has been ultrastructurally investigated and is clearly defined. The polar filament is isofilar and the sporophorous vesicles, which are connected by cytoplasmic bridges, contain small metabolic granules. In the genus *Amblyospora* three species have been studied using electron microscopy: *A. canningae* (Gassouma, 1972) by Gassouma and Ellis (1973), *A. bracteata* (Strickland, 1913) by Hazard and Oldacre (1975), and *A. fibrata* (Strickland, 1913) by Maurand and Loubès (1978). Characteristic for this genus is the barrel-shaped spores and the prominent granules of the sporophorous vesicles. There are two other *Thelohania* species where ultrastructural details are known: *T. minispora* Sprague, 1977, studied by Gassouma and Ellis (1973), and *T. capillata* Larsson, 1982. In this genus the polar filament is isofilar. For these species the generic assignment is clear and they are undoubtedly different from the species of this paper.

Five simulid parasites of the genus *Thelohania* are only known by light microscopy, and their present generic attribution has not been verified: *T. avacuolata* Gassouma, 1972, *T. bertrami* Gassouma, 1972, *T. columbaczense* Weiser, 1960, *T. simulii* Gassouma, 1972, and *T. varians* (Léger, 1897). The two species *T. avacuolata* and *T. bertrami* have spores of about the same size as the species described here, but illustrations show that their spores are barrel-shaped with flattened poles, which is the spore shape usually found in *Amblyospora*. The spores of *T. simulii* are bigger and of a broadly oval shape. Muu (1977) considered this species to be a synonym for *A. fibrata*.

Thelohania columbaczense is also clearly different from the Swedish species. The spores and the sporophorous vesicles are smaller, in about 10% of the vesicles 12 or 16 spores are produced, and the most important difference, the spores have double nuclei. If the observation of the double nu-

clei is correct this is not a species of the genus *Thelohania*, but of a new and undescribed genus.

Thelohania varians cannot be identified. The description by Léger (1897) is very brief and there are no illustrations. The description of the developmental sequence of the species is so general that it could fit almost any microsporidium of the families Amblyosporidae and Thelohaniidae. The spores are of two types: microspores, 4–5 µm long, which are produced in octosporous sporophorous vesicles, and macrospores, 8 µm long, produced in vesicles with an indefinite number of spores. Both spore types are of an oval shape. Debaissieux (1919) claimed to have studied the same species as Léger (1897), and he depicted spores of a broadly elliptical type, different from the spores of the Swedish species.

The new genus *Pseudothelohania* was recently created by Codreanu and Codreanu-Balcescu (1982) for a microsporidium from simulids. They reported some ultrastructural data of the microsporidium, but they did not describe it as a new species, neither is there any indication of the genus being erected for a previously known species. However, the spore size reported is bigger than the size of the Swedish species, and there is no pansporoblast membrane, which is a further difference.

It must be concluded that the species described here is different from the previously known microsporidia from simulid hosts as well as it is different from the mosquito parasites of the genus *Hyalinocysta*.

Hyalinocysta expilatoria n. sp.

Host species: *Odagmia ornata* (Meigen, 1818) (Diptera, Simuliidae), larva.

Host tissues involved: The fat body, which develops into a syncytium.

Merogonial stages: Not recognized except for diplokaryotic merozoites, the final stage of merogony.

Sporogony: Only sporogony in sporophorous vesicles was observed. A dip-

lokaryotic sporont divides subsequently producing a plasmodium with 8 nuclei. From the plasmodium 8 sporoblasts are formed, each of which develops into a spore without further division. A small number of sporonts give rise to 2, 4, or 6 spores. Occasionally spore maturation is asynchronous, with 2, 4, or 6 mature spores found together with 6, 4, or 2 immature. Rarely vesicles with supernumerary spores are formed, but less than 8 of their spores are normally developed.

Spores: In fixed and stained preparations pyriform with a flattened area at the posterior pole. Dimensions of octospores: 4.0–6.0 × 2.0–2.5 µm. Spores in vesicles with 4 spores are slightly wider, 4.0–5.0 × 3.0–4.0 µm, and when only 2 spores are formed, these are considerably bigger, 7.0–8.0 × 4.0–5.0 µm. Spore wall 325–350 nm thick, thinner above the anchoring disk. The straight part of the polar filament about one third of the spore length, the posterior part coiled in a single layer beneath the spore wall. Polar filament anisofilar with 7 coils, 5–6 wide and 2–1 thin. The angle of tilt of the anterior filament coil about 50°. Spore with a single nucleus.

Sporophorous vesicle: The membrane is thin, but clearly seen both in hematoxylin stained preparations and in ultrathin sections. In vesicles with immature spores the cavity is filled with a tubular material, visible in ultrathin sections. Vesicles with mature spores usually void of metabolic inclusions. Crystal-like inclusions are never formed.

Type locality: Klingavälsån, Scania, Sweden.

Types: Holotype (Fig. 4) on slide No. 781208-A-11 RL, paratypes on slides No. 781208-A-(1-16) RL.

Deposition of types: The slide with the holotype in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, D.C. Paratypes in the collections of Dr. E. Hazard, Gainesville, Florida, Dr. J. Weiser, Prague, and in the collection of the author.

Etymology: *expilatoria* alluding to the consumptive activity of the species.

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