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Microsporidia infecting *Simulium damnosum* in Nigeria

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Abstract

The microsporidia *Amblyospora bracteata, A. varians, Thelohania fibrata* and *Vavraia multispora* are recorded from larvae of *Simulium damnosum* in Northern Nigeria. A new microsporidian, *Culicospora damnosi* sp. n. is described. Morphological details of the sporogonial stages of the microsporidia in *S. damnosum* are presented. Listed are also microsporidia in *Simulium ruficorne, S. hargreavesi* and *S. wellmanni.* The fungus *Coelomycidium simulii* was found only in *S. ruficorne.*

1 Introduction

The West coast of tropical Africa is an area with large seasonal outbreaks of blackflies breeding in the many fast flowing rivers of the region. The adult insects are carried by winds all over the area. If only diseases transmitted by adults to their progeny would be involved we may expect that the distribution of their diseases in the whole area would be similar or identical, with some quantitative changes during the year. Of the many species which are present in this area, *Simulium damnosum* is far the most dangerous for man with its strict anthropophily and ability to transmit river blindness in the area. Any natural factor which is able to reduce the number of these vector insects is worth of study.

Despite the numerous papers on blackflies in West Africa, the data on

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distribution of dieseases in natural populations are only scarce. One of the rare old sources of information is the report by HENRARD (1930) who was able to identify in blackfly larvae collected at Kisantu, Inkisis (Congo) the microsporidia *Thelohania fibrata, Thelohania bracteata* and *Thelohania multispora.* In other reports infections with *Thelohania* were mentioned from Ghana by BRIGGS (1969) in *Simulium adersi* and from the same host JAMNBACK (1970) reported a new microsporidian *Caudospora nasiae.* LEWIS (1965) mentioned a *Haplosporidium* infecting *Simulium damnosum* in the Cameroons. Later EZENWA et al. (1974) reported *Thelohania fibrata* and *Pleistophora multispora* among the pathogens of *Simulium damnosum.*

This study is based on fresh material collected at the end of the dry season in April 1980 in Northern Nigeria and on fixed larvae collected during ecological studies of the seasonal distribution of blackfly larvae in Nigeria by the staff of the WHO Research Unit 1, Kaduna.

2 Material and methods

Fresh material of larvae, pupae and adults was collected by the authors on the Kaduna River around Kaduna mostly fixed to submerged plants. The material was brought to the laboratory in cooled boxes in plastic bags and here it was sorted, inspected and the blackflies were identified with the help of the staff of the Unit. Infected larvae were separated and the microsporidia were identified first on dry smears stained with Giemsa, later the rest of the material was fixed with Bouin's solution as wet smears or histological material. Adults were collected from plastic bags with pupae and inspected on smears. The animals were brought to heat-sterilized slides and the crushed adult was also used for bacteriological evaluation on nutrient agar plates. The rest of the smear was used after staining with Giemsa for evaluation of eventual other infections in their tissues. In some cases a suspension of spores in India ink was used in search for mucose envelopes and in all cases one part of the smear was hydrolysed according the method proposed by WEISER (1976a) and stained for demonstration of nuclei in spores.

Fixed samples of larvae collected in different localities were stored in Carnoy's liquid. Infected animals were diagnosed under the dissection microscope. The cysts with the parasite being well visible in the transparent posterior segments. Infected larvae were washed in tap water and one part of the cyst was used for preparation of a smear for identification. In interesting or difficult to identify cases, the rest of the body was refixed in Bouin's and brought to parafin for histology.

The estimate of the frequency of infections was based on the count of infected larvae in the total of the collected larvae at the given date. During the end of the dry season most streams were dry and current water was only on outlets of dams and the streams which were maintained for irigation. During the time of visit in mid April, the dry period was at its peak and in the still active streams an intensive oviposition was in progress whereas last larvae and pupae of the old generation were still present. Early 1st and 2nd larvae were present, but these were not included in the calculation of the frequency of diseases.

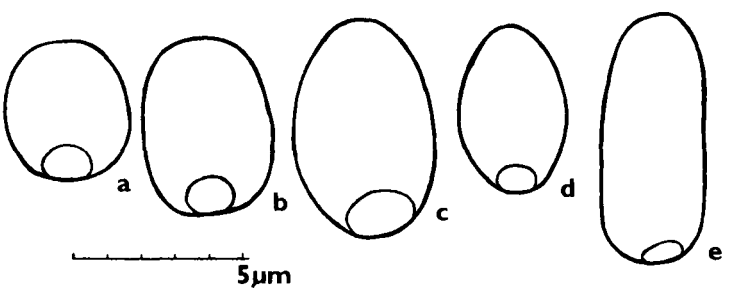

Fig. 1. Shape of fresh spores of microsporidia attacking *S. damnosum* in Northern Nigeria. a = *Amblyospora bracteata*, b = *A. varians*, c = *Thelohania fibrata*, d = *Vavraia multispora*, e = *Culicospora damnosi*

3 Results

The results of microbiological evaluations of adult blackflies were not included in this report. All 230 smears of adults inspected under the microscope were free of any microsporidian or other infection with a protozoan pathogen. In the material of larval blackflies we inspected 1646 fixed *Simulium damnosum*, 582 *S. ruficorne*, 146 *S. hargreavesi* and 25 *S. wellmanni*. Another 670 *S. damnosum* and 137 *S. ruficorne* were collected living. Only 243 of the total of 3206 larvae had microsporidian infections. Another 10 larvae had *Coelomycidium simulii* and in two cases we found mermithids. Of the recorded microsporidia most common were *Pleistophora multispora* in 132 cases, and *Thelohania fibrata* in 107 cases. Relatively rare were *Amblyospora bracteata* (2), *A. varians* (1) and *Culicospora* n. sp. (1 case). The distribution of the infections in different samples is given in tables 1 and 2. The larvae were inspected also for eventual occurrence of the cytoplasmic polyhedrosis virus, different other fungus- and protozoan infections, but only the pathogen species listed above were found.

Table 1. Distribution of infected *Simulium damnosum* larvae in different localities in N. Nigeria

Locality	Date	No. total collected	*Vavraia multispora*	*Thelohania fibrata*	*Amblyospora bracteata*	*Amblyospora varians*	*Culicospora damnosi*
Malabi River	4. 3. 80	300	11	–	–	–	–
Zagun River	11. 12. 79	480	–	55	–	1	–
Zagun River 3	11. 12. 79	320	33	24	–	–	–
Zagun River 2	3. 10. 79	150	2	–	–	–	–
Roro River	21. 11. 79	72	6	–	–	–	1
Bwashishi stream	13. 2. 80	4	–	–	–	–	–
Kan Gimi Dam	2. 11. 79	300	1	20	–	–	–
Zungeru River	11. 3. 80	20	2	–	–	–	–
Kaduna River	11. 4. 80	629	49	8	1	1	–
Kan Gimi Dam	11. 4. 80	28	1	–	–	–	–
Tiga Dam	15. 4. 80	78	3	1	–	–	–
Sarkin Pawa River	17. 4. 80	8	–	1	–	–	–

3.1 *Amblyospora bracteata* (Strickland, 1913)

This microsporidian was found only in one L_4 of *Simulium damnosum* from the Kaduna River in Kaduna. The microsporidian in this host causes the same deformation of the abdomen of the infected larva as is the case in infections of *Odagmia ornata* in Europe. White bilateral cysts with a broad lobe filling each segment are formed in the fat body. This cyst is during the development of the infected fat body changed into a syncytial xenoma (Weiser 1976b). Inside the cyst there were only sporoblasts and spores. Most spores were released from the temporary membrane of the pansporoblast. The pansporoblasts while still intact, measured 12 µm in diameter and were spherical. In the optical microscope spores seem to be packed together without any interposed material. The bluish secretion granules which were present in early sporonts during the second and third division of the nuclei disappeared during the prosporoblast and sporoblast stages of the sporonts.

Fresh spores in the material from Kaduna are almost spherical, or broadly elliptical, with broadly rounded ends (fig. 1).

A lenticular vacuole fills the posterior end region of the posterosome. The spores have no mucose coat (mucocalyx) and are 3–4 × 3–3.5 μm in size. When fixed or dried in smears the spores are sometime barrel-like, in scanning EM pictures we find nibbles (fig. 4) on both ends of the spore, characterizing the region where only a thin endospore is formed on both poles. In Giemsa-stained spores during the stage of sporoblast a bright red posterosome is stained. The nucleus is revealed as minute spherical lateral body situated in the second half of the spore.

3.2 *Amblyospora varians* (Leger, 1897)

A. varians is another rare microsporidian. It was recorded only in 2 cases in the studied material, one from the Zagun River, the other from fresh material collected in the Kaduna River. The cyst of this microsporidian has the same morphology as that of *A. bracteata* and the two infections could not be differentiated on basis of their gross morphology.

In fresh material, pansporoblasts were less persistent than in other species. Secretion granules during the first nuclear divisions of the sporonts were large, diminishing subsequently and disappearing before spore formation. Fresh spores had their both ends equal, broad and blunt, in fixed material they were more rounded. The exospore is well visible in fresh spores as a double contour. The spore size was 4–5 × 3.5–4 μm and these dimensions correspond with the original description by LEGER. DEBAISIEUX (1919) included in this spec. microsporidia with spores ranging from 6.5 to 8 μm in length and 4.5–5.5 μm in width, but at that time the larger form, *Pleistophora debaisieuxi* Jírovec was not yet differentiated and this material could be mixed up in his specimens. The internal organization of the spore (on smears) is identical with *A. bracteata.* In scanning pictures, the posterior nibble is not prominent.

3.3 *Thelohania fibrata* (Strickland, 1913)

This microsporidian causes in *Simulium damnosum* a rather frequent infection of old larvae. It changes the fat body in a white broadly lobate system of cysts and the abdominal segments are visibly inflated. In fresh water-mounts we find the usual mixture of spherical sporogonial stages and pansporoblasts with mature spores. Only later the pansporoblasts release the spores. "Autogamets" with well formed chromosomes are rather rare. Pansporoblasts are spherical, usually 15 μm in diamter and the regular elliptical spores are held together in regular groups of eight by a thin and rather persistent pansporoblastic membrane. Binucleate and tetranucleate sporonts have their periphery filled with a granular light scattering material of secretions, the granules contrary to what we know in *A. bracteata* or *A. varians* not surpassing the size of the nuclei. This material disappears during the spore formation. Fresh spores are elliptical, with both ends equal, with a relatively thin and uniform membrane. A round or oval vacuole fills the posterior end of the spore. The spores measured 6 × 4.5 μm when fresh and on dry smears 5–5.5 by 3.5 μm. India ink reveals only a thin, non-persistent mucus layer around each spore at the moment when these are released from the pansporoblast.

On Giemsa stained dry smears, one round bright red posterosome is stained

in the posterior end of the spore. The hydrolysis during the staining of nuclei changes in early sporonts the size and distribution of secretion granules and in mature spores a large oval nucleus is stained in each. In the material from Kaduna there were no teratospores with two nuclei and double the size of normal spores as it is often the case with this microsporidian in *Odagmia ornata.*

This infection was present in all larger samples from different localities of *Simulium damnosum.* It was not present in *Simulium ruficorne*, *S. hargreavesi* and *S. wellmanni* collected during the same period of time.

3.4 *Vavraia multispora* (Strickland, 1913)

With the deepening understanding of the complicated development of microsporidia, this species changed several times its taxonomical afiliation. Formerly identified by DEBAISIEUX et GASTALDI (1919) as *Thelohania multispora* it was later changed by WEISER (1947) to *Pleistophora multispora* and in 1961 discussed by WEISER due to only minute differences in the spore morphology, as possibly only a form of *Pleistophora simulii.* More experience with sporogonial development in microsporidia brought back again original decision to separate this *Pleistophora* and on basis of its morphology during sporogony it was included in the genus *Vavraia* Weiser, 1977, defined as a genus where sporonts divide 5 times and produce plasmodia with 32 nuclei and pansporoblasts with persistent or subpersistent membrane and 32 spores. Spores are uninucleate, divisions synchronous, resulting in subsequent 2, 4, 8, 16 and 32 nuclei, 32 sporoblasts and 32 spores. The polar filament presents uniform thickness in its whole length. This general definition has to be complemented in the case of *Vavraia multispora* where the spore number in pansporoblasts is up to 140 corresponding to 7 subsequent divisions.

In *Vavraia multispora* we do not distinguish stages of schizogony and of autogamy in the hosts with visible cysts. Stages of autogamy in microsporidia are characterized by cells with a giant nucleus with well formed chromosomes. Immediately before the series of stages of the autogamets in other microsporidia diplokarya are formed with nuclei flattened in a specific way in the area of contacts (coffee bean like arrangement). This stage is absent among the stages distinguished in *S. damnosum.* On the other hand, during the subsequent divisions of nuclei, pairs of nuclei can be found in binucleate, tetranucleate and all kinds of subsequent stages of division, but not in the coffee bean position, the diplokarya.

The cysts inside the abdomen of *S. damnosum* are not confluent in two lateral lobate masses but are formed of a group of spherical or oval white cysts 0.2–0.1 mm in diameter. When the larvae are dissected the individual cysts are liberated into saline and under the cover slip they liberate under pressure their content which is a mass of sporonts and pansporoblasts. In a suspension of India ink it is evident that sporonts and pansporoblasts were closed in a rather persistent membrane which first closed up the plasmodium. The first four divisions of nuclei produced spherical plasmodia with rather large nuclei, their division showing chromosomal figures. During the divisons with approximately 30, 60 and 120 nuclei, the body of the plasmodium, now elongated, twisted and formed broad lobes. This seems to be necessary to accomodate the increasing number of nuclei which were formed in dense rows under the surface of the lobes. Cleavage splits appear soon and individual nuclei are

closed in concentrated oval plasmatic masses and around each mass a sporoblastic membrane is formed. The remains of the original cytoplasm are absorbed by the maturating spores.

The divisions of nuclei are proceeding synchronously and at the moment when spores were all mature, only some 18 to 20 spores were remaining behind, with their content stained more intensively. Fresh spores are oval, with the anterior end slightly more tapering than the posterior end. The spore size varies in the material from *S. damnosum* from Nigeria in the range of 4–5 × 2.5–3 µm. Teratospores are formed in some pansporoblasts, 5–6 µm in

Table 2. Distribution of infected *Simulium* larvae in different localities in Northern Nigeria

Locality	Date	No. total collected	*Vavraia multispora*	*Thelohania fibrata*	*Amblyospora bracteata*	*Amblyospora varians*	*Culicospora damnosi*	*Coelomycidium simul.*
Simulium ruficorne								
Kan Gimi Dam	11. 3. 80	462	11	–	–	–	–	10
Zagun River	11. 12. 79	75	2	–	–	–	–	–
Kadaru River		15	–	–	–	–	–	–
Zagun River	11. 12. 79	30	–	–	–	–	–	–
Simulium hargreavesi								
Bwashishi stream	13. 2. 80	146	12	–	–	–	–	–
Simulium wellmanni								
Kulaji River	11. 8. 79	25	–	–	–	–	–	–

length. The single nucleus of the spore stains after hydrolysis only with difficulty. There were no secretion granules present during sporogony. Before formation of sporoblasts the cytoplasm is filled with irregular granulations staining dark with Heidenhain.

Vavraia multispora is a rather common pathogen of *S. damnosum* in Nigeria and it seems to be common also in other blackflies in this region, *Simulium hargreavesi* and *S. ruficorne.* (table 2.)

3.5 *Culicospora damnosi* sp. n.

In one of 72 late larvae of *S. damnosum* collected on November 21, 1979 in the Roro River were large white cysts in the posterior segments connected with the muscles and tearing them aside. Spores inside the cysts were oval to sphenoidal, flattened and bent unilaterally, measuring 7–8 × 3 µm. Only fixed material was at hand in Carnoy's liquid. Spores had a distinct thin, easily folded wall. At the posterior end, the posterosome stained as a metachromatic particle. The area of the polaroplast protruded through two thirds of the spore length (fig. 2), in apearance similar to *Culicospora magna.* The germ plasm is a narrow ring between the posterior vacuole and the polaroplast. Vegetative stages were present as uni- and binucleate round or piriform bodies with a broad nucleus. No pansporoblasts with sporoblasts sticking together in a membrane were observed. On basis of the darkly stained long polaroplast and the spore morphology we range it in the genus *Culicospora* (Weiser 1977). It differs in dimensions of its spores from other microsporidia known in blackflies and we propose for it the name *Culicospora damnosi* sp. n.

In the studied material the only other infection was with *Coelomycidium simulii* Debaisieux in larvae of *Simulium ruficorne*. The infection was not present in *S. damnosum* from the same locality.

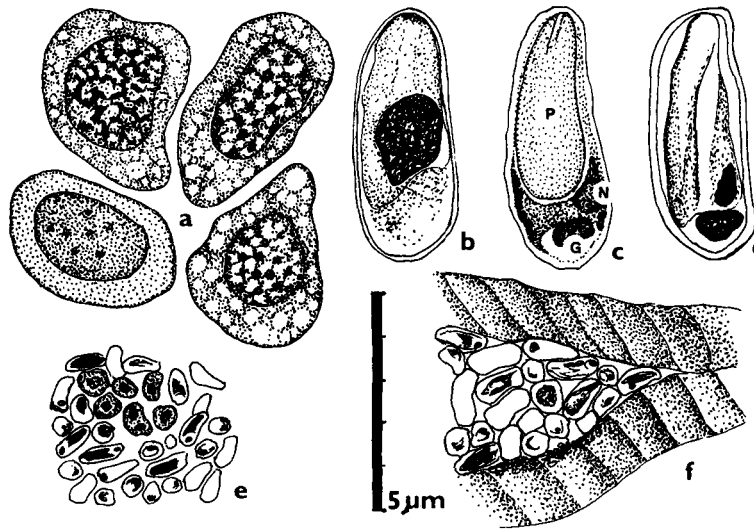

Fig. 2. Culicospora damnosi sp.n., stages present in the studied material. a = late sporonts (sporoblasts), b = sporoblast with well formed spore membrane but with visible large nucleus, c = young spore with visible polaroplast (P), germ with nucleus (N) and posterior vacuole with posterosome (G), d = mature spore. e, f = views of spores in cysts. Magnification indicated for a–d only

4 Discussion

The study of the protozoan diseases of *S. damnosum* in Northern Nigeria brought evidence of six microsporidia, except one all identical with the species present in blackflies of the mild climate. The frequency of the diseases was not very high, not more than 10 % in most cases. Different infection rates in different localities show that there is a visible impact of ecological conditions of the locality and not a standard distribution all over the area. There was no recovery of any microsporidian from adult blackflies collected in localities with visibly infected larvae.

All collected data show the limited role of microsporidian infections in reduction of blackflies in the streams of Nigeria. The specific host, *S. damnosum*, did not show a great specificity for pathogens and the very specific conditions of fast changing currents of African rivers did not select a well adapted microsporidian. The speed of the development of the infections was adapted to the general speed of development of blackfly larvae under condition of the local climate. Visibly infected larvae did not survive pupation, but all mortality concentrated in last larvae or prepupae. In HAZARD and OLDACRE (1975) the definition of the genus *Amblyospora* was not formulated in a diagnosis. WEISER (1976b) formulated a definition based in principle on the above publication, but due to a fragmentous knowledge of other species which may belong in this genus, the range of variability of individual characteristics is

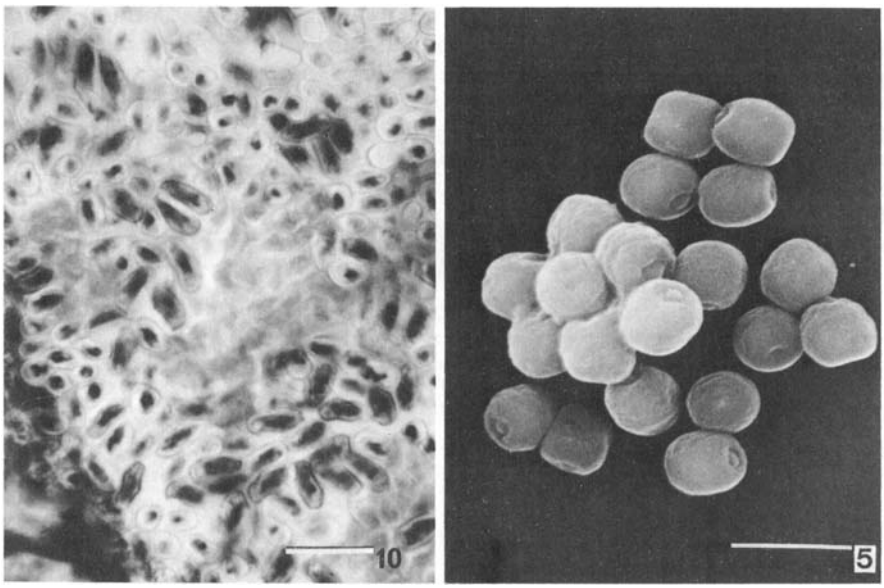

Fig. 3 (left). Culicospora damnosi, section of a cyst with spores and vegetative stages and with border of a muscle strand. – *Fig. 4 (right). Amblyospora bracteata* spores in scanning EM, showing the nibbles on poles (court. Z. Žižka)

not yet known. Therefore it is difficult every time a new species has to be included, to interpret the definition, especially if ultrastructures and surface patterns vary due to different procedures of preparation of the material. HAZARD and OLDACRE (1975) proposed *Thelohania bracteata* to be included in the genus *Amblyospora*. For the same morphological reasons we propose in this paper to include also. *T. varians* in the genus *Amblyospora*. It remains a question how far the absence of schizogonial stages and "autogamets" in visibly infected larvae is a typical feature of *Amblyospora* or *Parathelohania*. In larvae with *Vavraia multispora* we find only stages in sporogony whereas stages in schizogony and "autogamets" starting with the diplokaryon stage are absent.

The description of the new microsporidian, *Culicospora damnosi* sp.n. is based only on limited, fixed material and its description is mainly based on comparison with other materials of *Culicospora* in mosquitoes. The closer adhesion of the cysts to muscle strands is new for parasites of blackflies. There is no sign of any plasmodial stage in sporogony and the number of sporogonial divisions can be judged as five or six. After each nuclear division the stages divide. There is no sign of any pansporoblastic membrane and fingerlike protrusions which are present in other pathogens of blackflies such as *P. debaisieuxi* or *Stempellia simulii*. In smears of spores from fragments of the cyst, we find the long cone-shaped, stained polaroplast of *Culicospora*, where in other genera there is an empty vacuole. This infection needs further investigation of fresh material from the same locality (fig. 3).

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Zusammenfassung

Mikrosporidien als Parasiten von Simulium damnosum in Nigeria

Die Mikrosporidien *Amblyospora bracteate, A. varians, Thelohania fibrata* und *Vavraia multispora* wurden in Larven der Kriebelmücke, *Simulium damnosum*, in Nigeria gefunden. Eine weitere Mikrosporidae, *Culicospora damnosi* sp. n. wird beschrieben. Morphologische Details der Sporogonie-Stadien der Mikrosporidien in *S. damnosum* werden dargestellt. Weiterhin werden Mikrosporidien-Arten von *Simulium ruficorne, S. hargreavesi* und *S. wellmanni* genannt. In *S. ruficorne* wurde der Pilz *Coelomycidium simulii* festgestellt.

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