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FOUR NEW MICROSPORIDIA FOUND IN THE MOSQUITOES ANOPHELES GAMBIAE AND CULEX PIPIENS QUINQUEFASCIATUS FROM NIGERIA

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Abstract. In the salivary glands of adult mosquitoes *Anopheles gambiae* from Central and Northern Nigeria the microsporidia *Parathelohania africana* and *Amblyospora coluzzii* sp.n. with thick-walled spores in mucous envelopes were found, respectively. The corresponding supposed thin-walled form had kidney-shaped elongated spores. *Nosema salivaria* sp.n., forming large clusters of oval spores in the salivary glands, parasitized the same mosquito population. Two microsporidia, *Amblyospora nigeriana* sp.n. and *A. kadunae* sp.n., infecting the fat body were found in larvae of *Culex pipiens quinquefasciatus* from the environs of Kaduna.

Microsporidia play an important role in the reduction of mosquito numbers in nature. They are well adapted to this role, as some species are currently transmitted from one host generation to the next, without producing high kills, but infecting a certain percentage of mosquito larvae which is approximately at the same level of frequency in mosquito hatching sites all over a vast territory. Such infections are caused by microsporidia of the genera *Amblyospora* and *Parathelohania*, transmitted transovarially; acute infections develop mainly in male larvae, while in female larvae the infection remains latent developing as late as in pupae and during the life of imagoes. In adult female mosquitoes the microsporidia invade ovaries infecting the progeny (Hazard and Weiser 1968, Weiser 1976a). Because the infected males die prior to pupation the infected generations become sterilized having only female adults. In nature the infected and non-infected lines are complementing one another, resulting in an average 2—10 % infection rate. The mode of transmission through eggs reduces the influence of the type of mosquito hatching site decreasing the local fluctuation of the infection rate percentage, because the infected and non-infected females oviposit in different places all over a relatively vast area and the infected and non-infected egg batches are overlapping. The development of microsporidia in larvae and adult mosquitoes is different; in larvae there are distinct octosporous pansporoblasts with thick-walled uninucleate spores, while in adult mosquitoes a variable number of thin-walled, usually binucleate spores, is forming. This general pattern has a number of exceptions in particular species and hosts.

Apart from these infections showing a low fluctuation in the general representation, there are other microsporidian infections in mosquitoes which are transmitted with food and whose spread in the mosquito populations depends on the density of the host populations, on their mutual contacts. The infections rates vary from the minimum to the massive infections with unexpected die-off of entire mosquito populations. Typical examples of such microsporidia are *Nosema stegomyiae*, *N. algerae* or *Vavraia culicis*. All three are transmitted with food, partly transovarially. Their development is not associated with a certain sex or a certain instar of mosquito and they occur in local mass outbreaks of mosquitoes. They depend on the type of hatching site where their spores

accumulate and where the infection is transmitted with food. Their effect is most apparent in laboratory colonies of mosquitoes, where the intensity of transmission and consequently the infection rate as well periodically vary until the colonies die off. The shape of spores in adult mosquitoes and their arrangement do not differ from the morphology of similar stages in larvae.

Both types of infection occur in all the savannah territory of West Africa. In papers of Hazard and Anthony (1974) and Hazard and Oldacre (1975) the following species are reported from Nigeria:

Parathelohania sp.	in *Anopheles funestus*
Parathelohania africana	in *Anopheles gambiae*
Parathelohania sp.	in *Anopheles nili*
Parathelohania sp.	in *Anopheles pharoensis*
Parathelohania octolagenella	in *Anopheles pretoriensis*
Pleistophora sp.	in *Anopheles gambiae*
Pleistophora sp.	in *Anopheles funestus*

From the same region, the occurrence of *Nosema* cf. *stegomyiae* (Fox and Weiser 1959) in *Anopheles gambiae* is known from Liberia; it is now considered to belong to the group of *N. algerae.*

The present paper deals with microsporidia which infect two important mosquito species: *Anopheles gambiae* Giles and *Culex pipiens quinquefasciatus* Say.

MATERIAL AND METHODS

The first part od material from adult *A. gambiae* females comes from smears which have originally been the salivary gland material used for studying chromosomes of *A. gambiae* complex prepared by M. Coluzzi in Northern Nigeria. Adult mosquitoes were dissected, their salivary glands removed from the body and stained after fixation in Carnoy by orcein-lactic acid method. Material, in which spores of microsporidia were observed in the crushed salivary gland, was sent to us for determination. After removing the cover slip the material was thoroughly washed in distilled water and refixed in methanol to be ready for Giemsa staining. The lactic acid damaged the vegetative stages which showed no staining. After HCl hydrolysis (Weiser 1976) the nuclei inside the spores could be partly stained. Further data on the material are missing. It had been sent for identification to the WHO-Collaborating Centre, Praha, under No. CCBC 1868 in May 1976.

A. gambiae larvae in the second part of material were collected in temporary hatching sites in the Kaduna environs during the 1979 rainy season and were treated as dry Giemsa-stained smears. *C.p. quinquefasciatus* larvae were collected in moderately polluted waters devoid of vegetation in the Kaduna environs, and in the marshy waters overgrown with reeds below the Tiga Dam in the dry season in April 1980. Living larvae were transferred to laboratory and the infections were studied on Giemsa-stained dry smears after fixation in methanol and in wet smears fixed in Bouin's fluid and stained with Heidenhain's iron hematoxylin. In the same way were stained histological sections 4—6 μm thick from larvae fixed in Bouin's fluid. For staining the nuclei the dry smears were hydrolyzed after Weiser (1976b) and stained with Giemsa.

The material was compared with paratypes of other microsporidia described from the same hosts and deposited in our microsporidian collection. The type preparations, on which the descriptions of the species mentioned below are based, are included in our collection in Prague (Weiser 1979).

RESULTS

The following five microsporidia were found in the material studied.

1. *Parathelohania africana* Hazard et Anthony, 1974

Host: *Anopheles gambiae* Giles, larva, fat body, oenocytes
Locality: Kaduna, Agabe, Northern Nigeria

Sporadic cases of infection detected do not differ in morphology and dimensions of spores from descriptions by the authors who studied material from the same region. Fresh spores are oval, measuring 4×2—2.5 μm, in dry smears 3.5—3.7×2—2.3 μm. The thickened wall of the posterior end of spore is about three times as thick as the wall at the anterior end. One round, relatively big nucleus in the centre of spore is stained after hydrolysis in hydrochloric acid. After desiccation the spores are jug-shaped, similar to *P. legeri*.

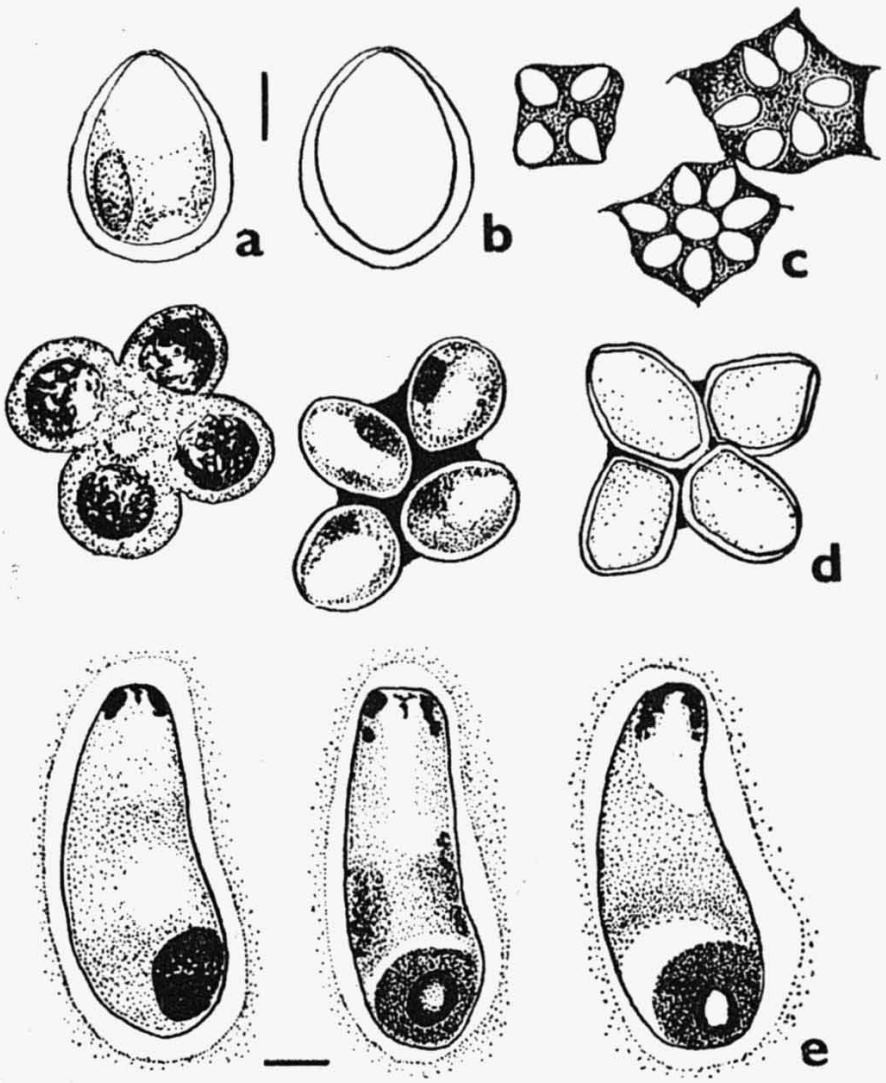


Fig. 1. *Amblyospora coluzzii*. a — thick-walled spore with distinct nucleus, b — thick-walled spore in the smear of mosquito salivary gland, c — groups of spores in mucous envelopes, d — tetrasporous group and rare stage of tetranucleate sporont, four sporoblasts and spores, e — thin-walled spores in *A. gambiae* salivary gland. Distinct granule on the anterior end at the termination o polar filament and germ with nucleus and posterosome at the end of spore. Scale bar = 1 μm.

2. *Amblyospora coluzzii* sp. n. Figs. 1, 5a—c

Host: *Anopheles gambiae* Giles, salivary glands and further tissues of adult mosquitoes
Locality: Northern Nigeria

The spores of this microsporidian are oval, slightly tapering towards the anterior end. After fixation and treatment with lactic acid they measure 3—4×2.4—3 μm. Endospore is rather thick. The spores are enclosed in a mucous envelope of relatively solid consistence and they form groups of four or more. Tetrasporous sporogony is most

common. The groups are not morphologically defined as pansporoblasts. The pansporoblast membrane is not developed or it is indistinct. The vegetative stages showed no staining in the material. The spores form several (3—5) small groups in the salivary gland. The main seat of the microsporidian was apparently in other tissues of the host too. The infection was not common and two positive mosquitoes were used for this description.

From the material analyzed another microsporidium was isolated, probably representing thin-walled forms of this dimorphic microsporidian; this assumption must be confirmed in further material. An elongate pear-shaped, arched or kidney-shaped spore 4.5×2 μm large is enclosed in kidney-shaped or pear-shaped vacuoles in the tissue, measuring 6—7×3—3.5 μm. It is not clear whether the unstained space in which the spore is enclosed, is a vacuole or exosporium. Other microsporidia prepared in the same way do not show such vacuoles. In the Giemsa-stained smears two small granules are stained red on the anterior narrowed and truncate end of the spore. On its opposite end is the germ in which a rounded nucleus and metachromatically coloured posterosome (Fig. 1e) are stained. The spores are located separately in a scattered group (Fig. 5), without any distinct wall of pansporoblast or cyst. The vegetative stages showed no staining. Due to the coincidence of host and locality, the thin walls and their free arrangement the author considers these spores together with the preceding thick-walled ones to be one species. Their occurrence in salivary glands distorts the dimorphic picture because nothing is known about the occurrence of thin-walled spores of the genus *Amblyospora* in salivary glands and about their morphology there. The staining of nucleus, after maceration in lactic acid, is not sufficiently distinct to rule out the hidden binucleate condition when two nuclei are closely attached to one another. This microsporidian distinctly differs from other mosquito microsporidia in morphology and dimensions and we propose to name it *Amblyospora coluzzii* sp.n. after Dr. Mario Coluzzi, head of the group who detected the material.

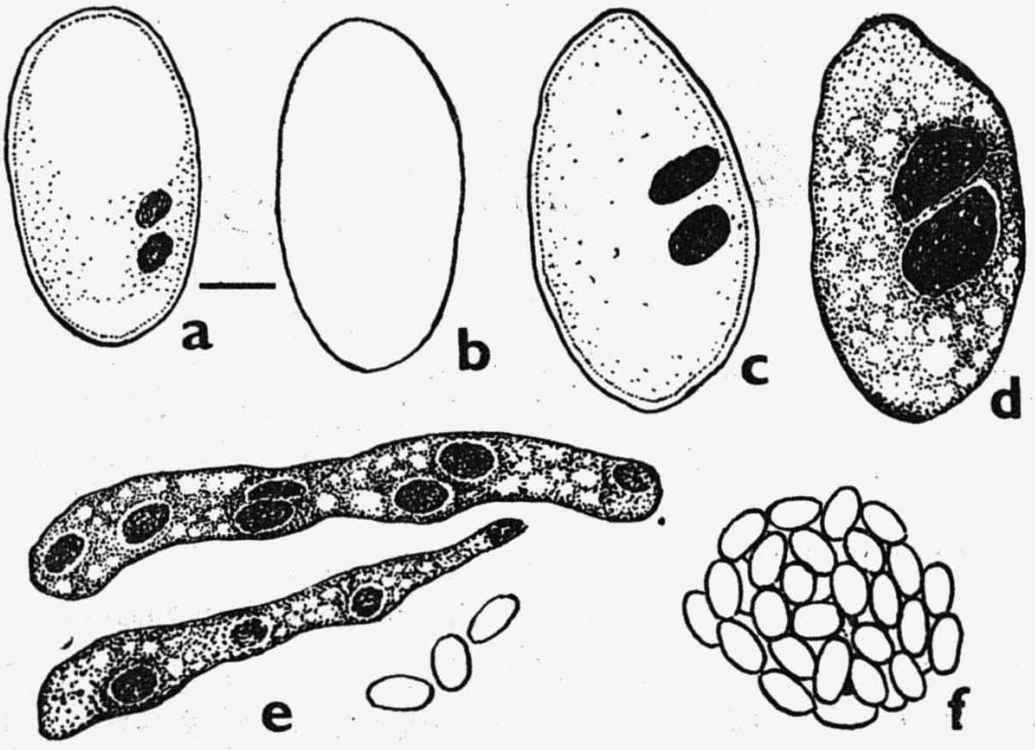


Fig. 2. *Nosema salivaria.* a, b — spores, c, d — sporoblasts from *A. gambiae* salivary gland, e—ribbon-like schizonts, f — portion of spore mass. Scale bar = 1 μm.

3. *Nosema salivaria* sp.n. Figs. 2, 5d

Host: *Anopheles gambiae* Giles; salivary gland of adult mosquito
Locality: Northern Nigeria

Regularly oval, thin-walled spores with both ends rounded measure 4.5×2.5—3 μm. They are present in salivary glands as clumps of irregular size which are not surrounded with distinct persistent pansporoblastic membrane. Two spherical nuclei are located one after the other in the posterior half of the spore. Polaroplast forms a visible unstained anterior vacuole, while the second vacuole is in place of posterosome. Sporadically scattered sporoblasts are binucleate, poorly stained. From some parts of the spore clump ribbon-like stages, poorly stained, are projecting, reminding of ribbon-like stages of the second schizogony typical of other nosemas, and from which autogametes and sporoblasts are formed. Information on the presence of infection in other tissues is not available. In localization and morphology this microsporidian differs from hitherto known mosquito microsporidia. We propose to name it *Nosema salivaria* sp.n.

4. *Amblyospora nigeriana* sp.n. Figs. 3, 5e, f

Host: *Culex pipiens quinquefasciatus* Say, fat body, oenocytes
Locality: Nasarawa bridge, Kaduna, Tiga Dam, north of Kaduna, Nigeria, April 1980

Infected larvae were present in 5—15 % of larvae of 4th instar collected in different moderately polluted waters near the highway running at Nasarawa Bridge, not far from Kaduna, and in densely overgrown pools below the Tiga Dam north of Kaduna. In different collection sites the frequency of infection varied only slightly. The infected larvae showed a distinct opaque body content and whitish cysts in the invaded fat body of thorax and abdominal segments. Unlike the infections in the genus *Aedes*, the parasite's masses were not prominent not enlarging the infected body segments.

In the body of *C.p. quinquefasciatus* larvae only the sporogony is present starting from the "autogamete" stages. They are spherical stages with gradually dividing nuclei, producing plasmodia 14—20 μm in diameter, with 2, 4 and 8 nuclei. With the gradual division of nuclei secretion granules appear in the cytoplasm, filling up the space between nuclei. In Giemsa-stained smears they stain grayish. Following the second nuclear division, plasma concentrates around the nuclei in the tetranucleate plasmodium, after the subsequent nuclear division prosporoblastic walls begin to form around the nuclei, and simultaneously secretion bodies are resorbed from the cytoplasm. The wall of the initial sporont is preserved, temporarily forming the pansporoblastic membrane. In the material fixed in Bouin's fluid in wet smears or in sections well formed chromosomes are observed during divisions of autogametes and during subsequent divisions of sporonts. Mature spores are stained with Giemsa in the usual way, with a vacuole on both ends. Only sporadically the coils of polar filament can be distinguished. The posterior vacuole contains a rounded posterosome. After hydrolysis one elongated nucleus is coloured by the stain. In sections and wet smears the fixed spores are deformed in shapes characteristic of the genus *Amblyospora*, with depressed anterior and posterior ends. Fresh spores are broadly oval, regular, with both ends equally rounded, measuring 6—7×3.5—4 μm. Pansporoblasts measure 14—16 μm in diameter.

During the sporogony some teratologies are formed when a nucleus is halted during division, when instead of 4 only 3 nuclei or instead of 8 spores only 4 or 3 are formed. This is resulting in spores with 3, 6 or 4 nuclei. The retarded sporoblasts are double the size of the others. Teratospores measure 8×5 μm, the visible organelles retaining the

number and shape as in normal spores. During the division in sporogony more anomalies occur than the number of teratospores. During the last phase of the third division nuclei catch up with the retarded divisions.

Although *Culex pipiens quinquefasciatus* is distributed in large areas of the world and is used for rearings in many laboratories, there is no mention in literature of any *Amblyospora* from this host. Our material, in contrary, does not allow any doubt of its

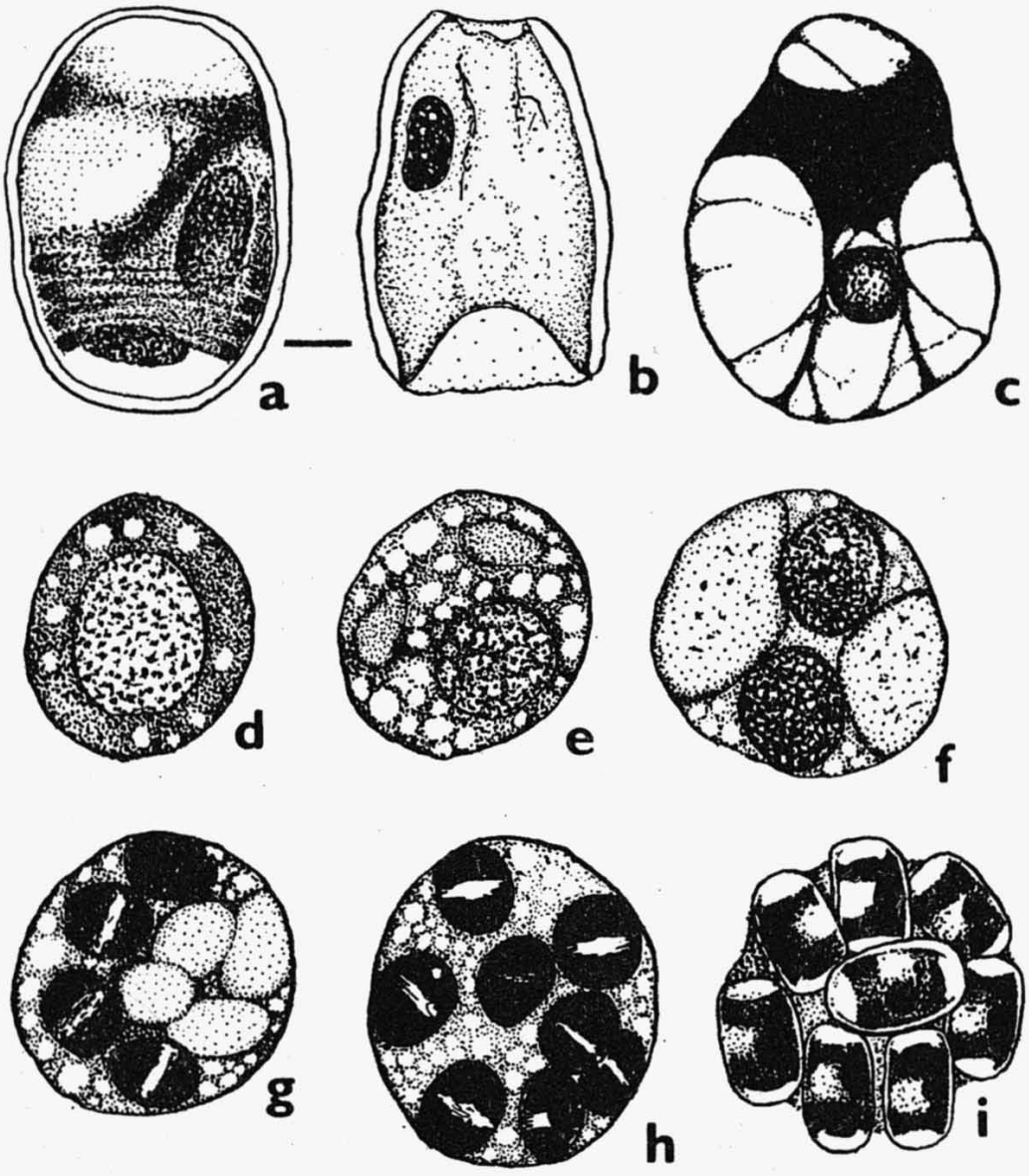


Fig. 3. *Amblyospora nigeriana.* a — spore from dry Giemsa-stained smear, b — fixed spore stained with Heidenhain's hematoxylin, with distinct nucleus and thin endospore on the anterior and posterior ends, c — Heidenhain's hematoxylin-stained immature spore, d—g — first nuclear division of sporont, vacuolized cytoplasm with distinct secretion granules, h, i — maturing sporoblasts in pansporoblast. Scale bar = 1 μm.

affiliation to *Amblyospora.* Artificial infections of *C. p. quinquefasciatus* with *Nosema algerae* and *Pleistophora culicis* (Hazard and Lofgren 1971, Reynolds 1966) were published. From field collected materials a *Pleistophora* was mentioned from the gut of larvae in the USA (Hazard and Chapman 1977) and this mosquito belongs also to the host range of the microsporidian *Hazardia milleri* in Florida (Hazard and Fukuda 1974), both microsporidia being different in morphology from our material. There is no

Table 1. Microsporidia of the genus *Amblyospora* in mosquitoes

Microsporidian	Mosquito	Fixed spores, μm	Origin
A. benigna	*Culex apicalis*	4 × 3	USA
A. californica	*Culex tarsalis*	5.5—8.7 × 4.3—5.4 12.7—13 × 3.7	USA
A. gigantea	*Culex erythrothorax*	6.7 × 5.4	USA
A. minuta	*Culex erraticus*	2.5—3.5 × 1.5—2.0 4.6 × 2.4	USA
A. nigeriana	*Culex pipiens quinquefasciatus*	6—7 × 3.5—4	Nigeria
A. kadunae	*Culex pipiens quinquefasciatus*	4 × 2.5—2.8	Nigeria
A. opacita	*Culex territans*	5.8 × 4.2	USA
A. campbelli	*Culiseta incidens*	5.5 × 3.9	USA
A. inimica	*Culiseta inornata*	5.4 × 4.1	USA
A. bolinasae	*Aedes squamiger*	5.7 × 4.5	USA
A. canadensis	*Aedes canadensis*	4.9—5.5 × 4.5—5	USA
A. barbata	*Aedes cantans*	6.5—7 × 3.5—4	Czechoslovakia
A. keenani	*Aedomyia squamipennis*	2.8—3.3 × 2.7—3.2	Panama
A. khaliulini	*Aedes communis*	5.6—7.3 × 4.2—4.8 7.4 × 5.6	USSR
A. unica	*Aedes melanimon*	5.6 × 4.5	USA
A. mojingensis	*Anopheles eiseni*	2.6—3.5 × 2.4—2.6	Panama
A. coluzzii	*Anopheles gambiae*	3—4 × 2.4—3	Nigeria

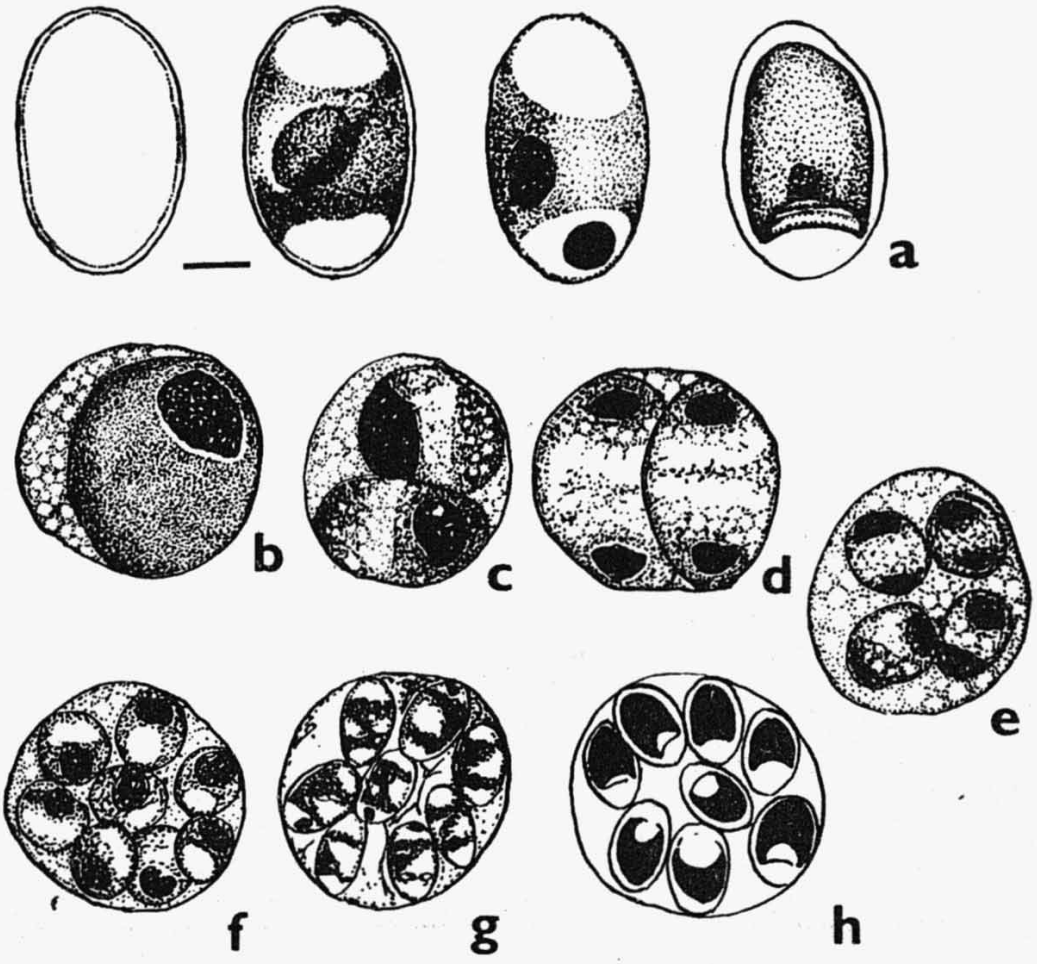
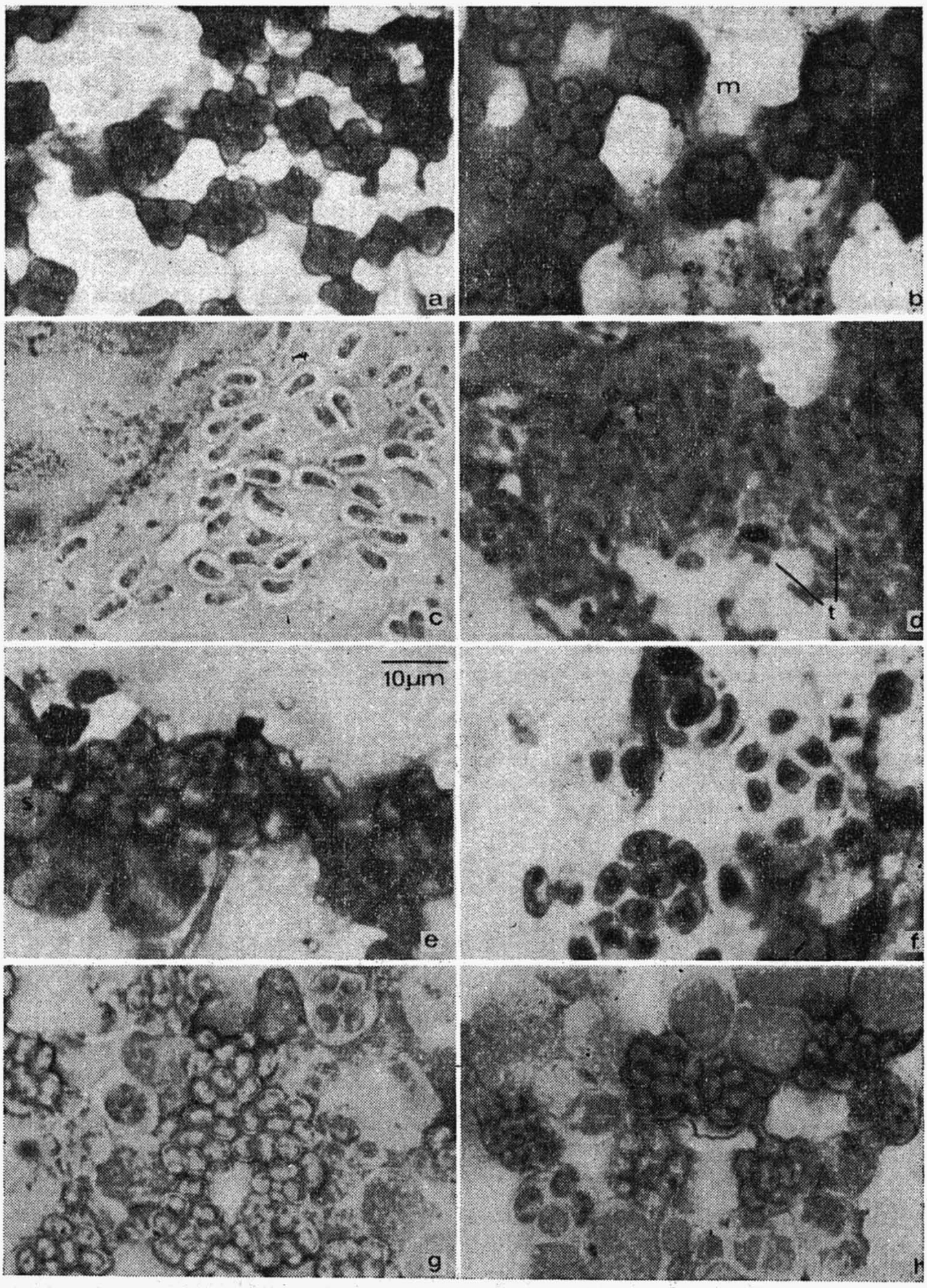


Fig. 4. *Amblyospora kadunae.* a — water-mounted spores in Giemsa-stained smears and fixed material, b—e — division inside sporoblast and development of sporoblasts, f — group of eight sporoblasts in pansporoblast, g—h — mature pansporoblasts. Scale bar = 1 μm.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publication of any *Amblyospora* in mosquitoes from Nigeria. Therefore we believe this microsporidian, which differs in morphology from other known species (see Table 1) to be a new species and propose for it the name *Amblyospora nigeriana* sp.n.

5. *Amblyospora kadunae* sp.n. Figs. 4, 5g, h

Host: *Culex pipiens quiquefasciatus* Say, fat body
Locality: Tiga Dam, north of Kaduna, Nigeria, April 1980

Sporadic cases of this infection were present among specimens infected with the preceding microsporidian, having the same external symptoms, with white opaque cysts in the fat body of thoracic and abdominal segments. Only two larvae were found to be in fected with this microsporidian. The main differences are in the size of the spores. The oval spores broadly rounded on their poles are 4 μm long and 2,5 to 2.8 μm in diameter on dry smears. They are formed in groups of eight in relatively persistent pansporoblasts, without any formation of teratospores or abnormities. The pansporoblastic membrane is well visible on wet smears stained with Heidenhain's iron hematoxylin. The only one broad elyptic nucleus is located in the second half of the spore, the posterosome is sometimes stained red. In the development of the sporoblasts we find distinct differences from *A. nigeriana*. From the beginning around the active nuclei developing germs are differentiated in the cytoplasm closed in the pansporoblastic membrane and with each subsequent division the prosporoblasts and sporoblasts are more and more isolated from the vacuolized remains of the cytoplasm. With the formation of the eight oval spores this process is finished and the remains of cytoplasm around the spores disappear. In fixed spores the endospore is less attenuated than in *A. nigeriana*. The spore content is darkly stained, with a deep vacuole in the posterior pole. The posterior end of the spore is not heavily depressed. During the whole sporogony no secretion granules, so typical of *A. nigeriana*, are formed. Differential diagnostics is based mainly on spore size and shape (see Table 1). We propose for this microsporidian the name *Amblyospora kadunae* sp.n.

DISCUSSION

The taxonomy of microsporidia parasitic in mosquitoes is rather complicated and requires to compare the species under study with type materials from the same host or with materials of microsporidia which have a similar morphology. It is sometimes difficult to make a decision, the more so because no method is available so far which would allow a successful experimental transmission of microsporidia to specimens of their initial host or to other hosts, so that we cannot verify whether the deviations in morphology of different materials are caused by different hosts or whether they are symptoms of distinct species. For this precarious reason and pending later evidence the proposal of the genus *Amblyospora* (Hazard and Oldacre 1975) has been accepted to replace the older concept of a "*Thelohania opacita*-group" and the genus *Parathelohania* (Hazard

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Fig. 5. a, b — *Amblyospora coluzzii*, thick-walled spores in mucus (m), c — thin-walled spores from *A. gambiae* salivary gland, d — *Nosema salivaria*, spores and indistinct ribbon-like schizonts(t), e, f — *Amblyospora nigeriana*, Giemsa-stained smear and hematoxylin-stained wet smear, secretion granules(s) and stages with large nucleus, autogametes, g, h — *Amblyospora kadunae*, Giemsa-stained dry smear, and fixed hematoxylin-stained wet smear. Distinct sporoblasts inside the pansporoblast wall. All photographs were taken with equal magnification.

and Anthony 1974) to replace the concept of "*Thelohania legeri*-group". While most old descriptions are based on morphology seen in optical microscope, revisions of 1974 and 1975 are proposed on basis of details of spore ultrastructures. A brief definition of the genera involved was proposed by Weiser (1977). Of the genus *Amblyospora* in optical microscope it is characteristic that after fixation the spores are depressed on both ends and their wall on the posterior end is much thinner than on the sides. Their developmental stages in the mosquito larva are only stages of sporogony and stages of "autogamy" with prominent enlarged nuclei containing chromosomes. During the first division of sporont nucleus usually secretion granules are formed in the cytoplasm. "Anlagen" of gonads in infected larvae have characters of testes. The remaining part of the development, including the second type of sporogony in imagoes, can be demonstrated in pupae and adult mosquitoes cultured in laboratory. The narrow host specificity is supposed to exist, but it has not been proved as yet.

In the new material studied the *C.p. quinquefasciatus* mosquitoes were infected with microsporidia evidently of the genus *Amblyospora*. Less distinct is the generic affiliation of microsporidia found in the salivary glands of *A. gambiae*. This is due to the fact that the salivary glands are not an organ currently examined in insects infected with the genus *Amblyospora*, and certain anomalies may appear in the development of the "thin-walled" spores in the salivary glands. In our case, the preceding treatment of material has influenced the atypical appearance of the regenerated material. It is therefore necessary to pay further attention to the occurrence of these microsporidia in *A. gambiae* and complete their description on fresh material.

The studied infections are not important factors in reducing the numbers of the two mosquito species and on the average do not cause more than 15 % reduction in population. However, *A. gambiae* and *C.p. quinquefasciatus* (*C. fatigans*) belong to important vectors of malaria or Bancroft's filariasis and various viruses, and the knowledge of additional factors reducing their numbers or ability of transmitting infections, or deforming the sex ratio, may be used as a tool for long-term control of mosquito populations.

This investigation received financial support from UNDR/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. At the same time the authors are grateful to Dr. Mario Coluzzi who supplied us with the material and to the staff of WHO Research Unit 1, Kaduna, for assistance in collecting mosquitoes in the field.

ЧЕТЫРЕ НОВЫЕ МИКРОСПОРИДИИ ОТ КОМАРОВ *ANOPHELES GAMBIAE* И *CULEX PIPIENS QUINQUEFASCIATUS* ИЗ НИГЕРИИ

Я. Вайзер и С. Празертфон

Резюме. В слюнной железе половозрелых комаров *Anopheles gambiae* из средней и северной Нигерии обнаружены соответственно микроспоридии *Parathelohania africana* и *Amblyospora coluzzii* sp. n. с толстостенными спорами в слизистой оболочке. Соответствующая предполагаемая тонкостенная форма отличается продолговатыми, прогнутыми в виде почек спорами. В той же популяции паразитирует *Nosema salivaria* sp. n., образующая в слюнной железе большие скопления овальных спор. В личинках *Culex pipiens quinquefasciatus* из окрестности г. Кадуны обнаружены микроспоридии *Amblyospora nigeriana* sp. n. и *A. kadunae* sp. n., поражающие жировое тело личинок.

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50th birthday of Dr. Jiří Lom, D.Sc.

Dr. Jiří Lom, D.Sc., a prominent Czechoslovak parasitologist and protozoologist was born on 24th October 1931 in Prague as son of a professor known by his pioneering work in economy of agriculture. Already during his studies at the secondary school he showed an interest in nature, particularly in birds. Having passed the secondary, he enrolled the Faculty of Sciences of the Charles University in Prague which he finished in 1954. At that time he was engaged in the study of the biology of ciliates of the genera *Balantidium* and *Nyctotherus*. In 1958, Dr. Lom was awarded the scientific degree of C.Sc. (= Ph.D.) on the basis of his thesis "A study of parasitic ciliates of the order Astomata" and 16 years later he won the degree of D.Sc. for the thesis "Electron-optical study of protozoans infecting body surface of fishes".

He begun his scientific career as teaching assistant at the Department of Parasitology of the Faculty of Sciences, Charles University (1953—1954); then he started to work as a scientific worker in the newly established Protozoological Laboratory of the Czechoslovak Academy of Sciences. Since the foundation of the Institute of Parasitology of the Czechoslovak Academy of Sciences, into which the Protozoological Laboratory was included, Dr. Lom started to work in the Institute's Department of Protozoology. At the Institute of Parasitology,