

body. There is no visible sub-stieda body. The sporozoites measure about 6.5×2.0 and are slightly curved about a modest sporocyst residuum of some 10–20 spherical granules. Sporozoite refractile bodies were not discernable.

Type host. *Tamandua tetradactyla* (Linn.). The "lesser anteater" (Mammalia: Xenarthra: Myrmecophagidae).

Location in the host. The ileum.

Sporulation. Exogenous: at 24–26°C a few oocysts were mature at 7 days, and almost all at 8 days.

Type material. Oocysts from the feces and developmental stages in the ileum, fixed in 10.0% buffered formalin and held in the Parasitology Department of the Instituto Evandro Chagas, Belém, Pará, Brazil. Repository number M-12823.

Type locality. Ponta de Pedras, Island of Marajó, Pará State, northern Brazil.

Prevalence. No information.

Pathology. The infected animal showed no signs of illness and the feces were of normal consistence. Histology of the ileum remains to be studied.

Etymology. The specific name is derived from the geographic locality (Marajó) where the infected animal was captured.

DISCUSSION

E. tamanduae and *E. corticulata* are readily distinguished from *E. marajoensis* by their larger oocysts and sporocysts (Fig. 1–5), thick striated and brown-yellow oocyst wall, possession of a conspicuous oocyst residuum, and the absence of a polar granule. The sporulation time of the latter parasite is 7–8 days

at 24–26°C, while the sporulation times of *E. tamanduae* and *E. corticulata* are 9–10 days and 14 days respectively, under exactly the same conditions. The site of infection and similar sporulation time were given in the first description of *E. tamanduae*, but such information was not available when we first described *E. corticulata*.

Finally, the comparative morphology of the oocysts of *Eimeria* species from other xenarthrans (sloths and armadillos) has been discussed elsewhere [2]. None of them resemble *E. marajoensis*.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Lainson, R. 1968. Parasitological studies in British Honduras. III - Some coccidial parasites of mammals. *Ann. Trop. Med. Parasitol.*, **62**:252–259.
2. Lainson, R. & Shaw, J. J. 1990. Coccidia of Brazilian mammals: *Eimeria corticulata* N. sp. (Apicomplexa: Eimeriidae) from the anteater *Tamandua tetradactyla* (Xenarthra: Myrmecophagidae) and *Eimeria zygodontomyis* N. sp. from the cane mouse *Zygodontomys lasiurus* (Rodentia: Cricetidae). *J. Protozool.*, **37**:51–54.

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***Microfilm lutjani* N. G. N. Sp. (Protozoa Microsporidia), a Gill Parasite of the Golden African Snapper *Lutjanus fulgens* (Valenciennes, 1830) (Teleost Lutjanidae): Developmental Cycle and Ultrastructure**

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ABSTRACT. *Microfilm lutjani* n. g., n. sp. (Microsporidia) was found on the gill filaments of *Lutjanus fulgens* (Teleost) inhabiting the coasts of Senegal. This microsporidium forms xenomas distinguished by the microvilli covering the plasma membrane. At all stages of development individuals have isolated nuclei and are in direct contact with the host cytoplasm. Merogony is binary and sporogony is tetrasporoblastic. The spore ($4.75 \times 2.60 \mu\text{m}$) is characterized by a manubrium inserted on a laterally offset anchoring disc and extending into a very short, noncoiled polar filament (no longer than 500 nm) in the form of a hook. This type of polar filament has not been described previously in the Microsporidia.

Key words. Africa, *Microfilm lutjani*, Microsporidia, Teleost, ultrastructure.

MANY Microsporidia have been described in fish [5, 28, 29, 30] and are classified in twelve genera: *Glugea* The-lohan, 1891; *Thelohania* Henneguy, 1892; *Pleistophora* Gurley, 1893; *Ichthyosporidium* Caullery & Mesnil, 1905; *Mrazekia* Léger & Hesse, 1916; *Encephalitozoon* Levaditi, Nicolau & Schoen, 1923; *Heterosporis* Schubert, 1969; *Nosemoides* Vinckier, 1975; *Spraguea*, Weissenberg, 1975; *Tetramicra* Matthews & Matthews, 1980; *Loma* Morrison & Sprague, 1981, and *Microgemma* Ralphs & Matthews, 1986. Species not sufficiently described currently are classified in the collective group *Microsporidium* Balbiani, 1884.

These Microsporidia infect different tissues and organs and frequently cause the formation of xenomas. Some Microsporidia are pathogenic and are responsible for considerable mortality both in the natural environment and in aquaculture. *Glugea*

hertwigi Weissenberg, 1911, causes the death of young smelt of the species *Osmerus eperlanus* & *Osmerus mordax* [7, 24]. *Glugea stephani* (Hagenmüller, 1899) reduces intestinal absorption in young specimens of *Parophrys vetulus* and causes death [25]. *Glugea* sp. forms xenomas that cause displacement and atrophy of intestinal organs in *Gambusia affinis* [10]. *Loma salmonae* (Putz, Hoffman & Dunbar, 1965) causes inflammatory lesions in *Oncorhynchus kisutch* after transfer to sea water; these occur mainly in the heart, resulting in high mortality in the summer [13]. *Heterosporis finki* Schubert 1969, intensively parasitizes young individuals of *Pterophyllum scalare*, causing millessness of skeletal muscles [21]. During research on microsporidia of fish inhabiting the coasts of Senegal, we found a species on the gills of *Lutjanus fulgens* (Lutjanidae), a fish that is widely eaten in Senegal. Further ultrastructural and pathological studies were

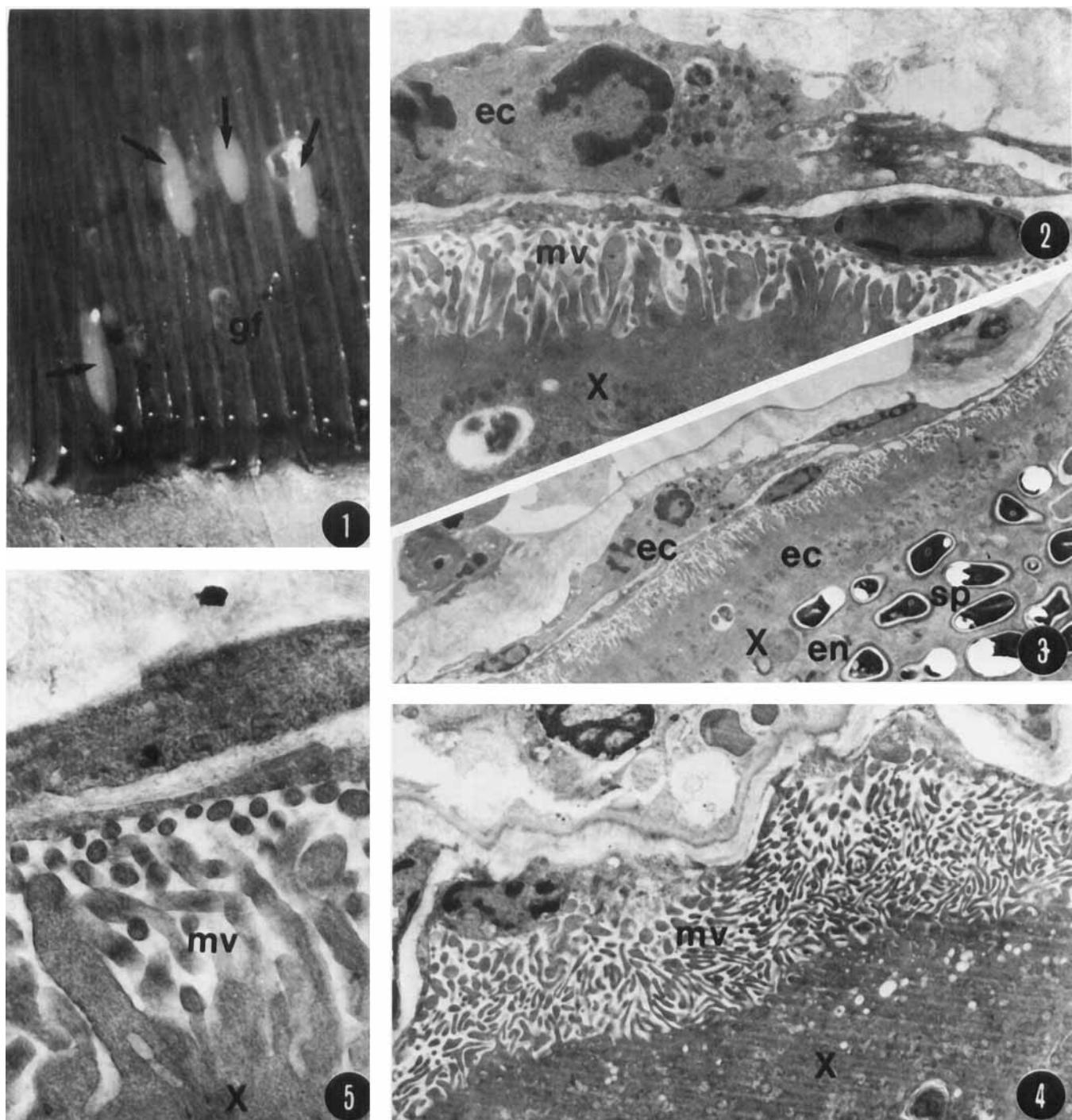


Fig. 1–5. Xenoma of *Microfilum lutjani*. 1. Four xenomas (arrows) on gill filaments (gf) ($\times 20$). 2, 4. Several aspects of periphery of xenomas (x), with numerous microvilli (mv) on plasma membrane. Fig. 2, $\times 8,200$. Fig. 3, $\times 2,800$. Fig. 4, $\times 5,4000$. 3. On Fig. 3 note the difference between ectoplasmic zone (ec) and endoplasmic zone (en), ec: endothelial cells, sp: spores. 5. Detail on the microvilli (mv) of xenomas (x), $\times 28,000$.

carried out on the parasite and our initial results are reported in this paper.

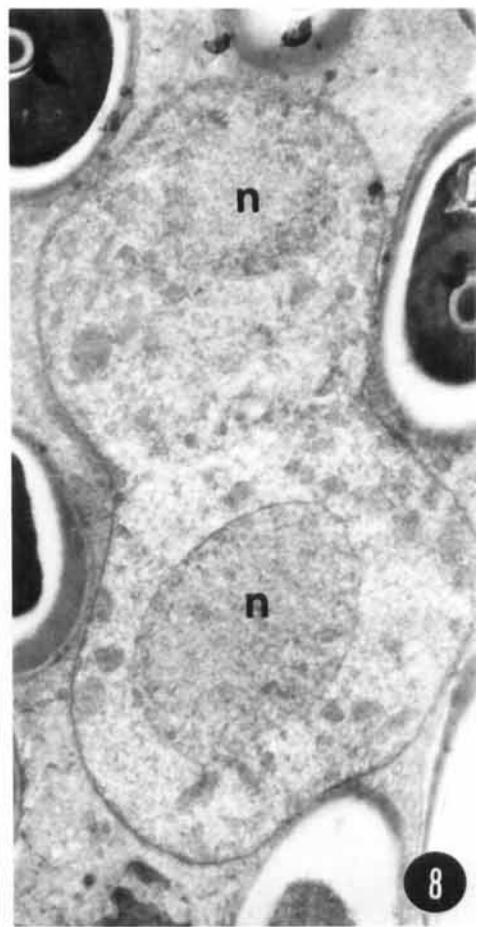
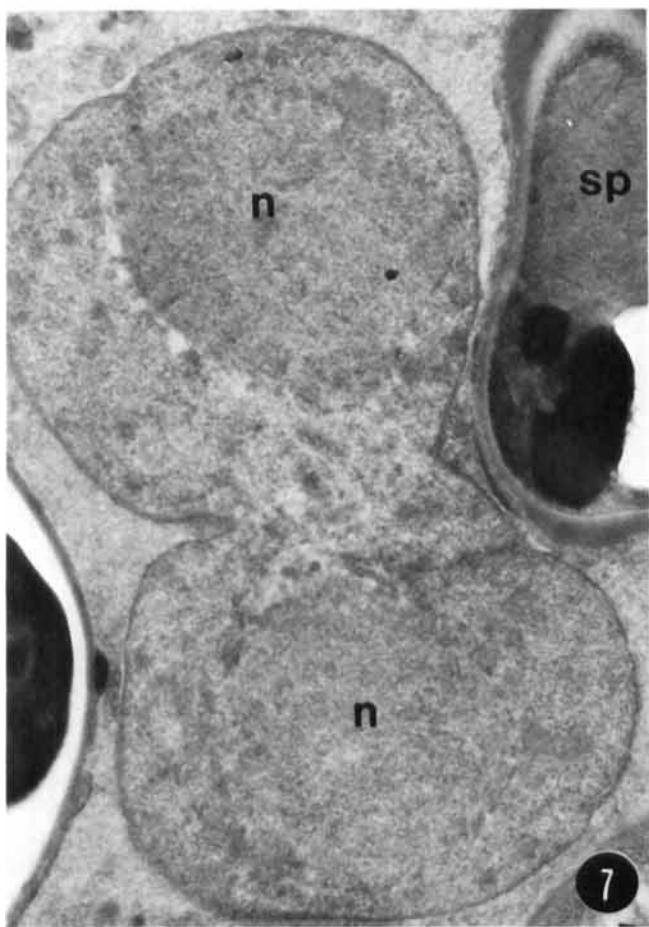
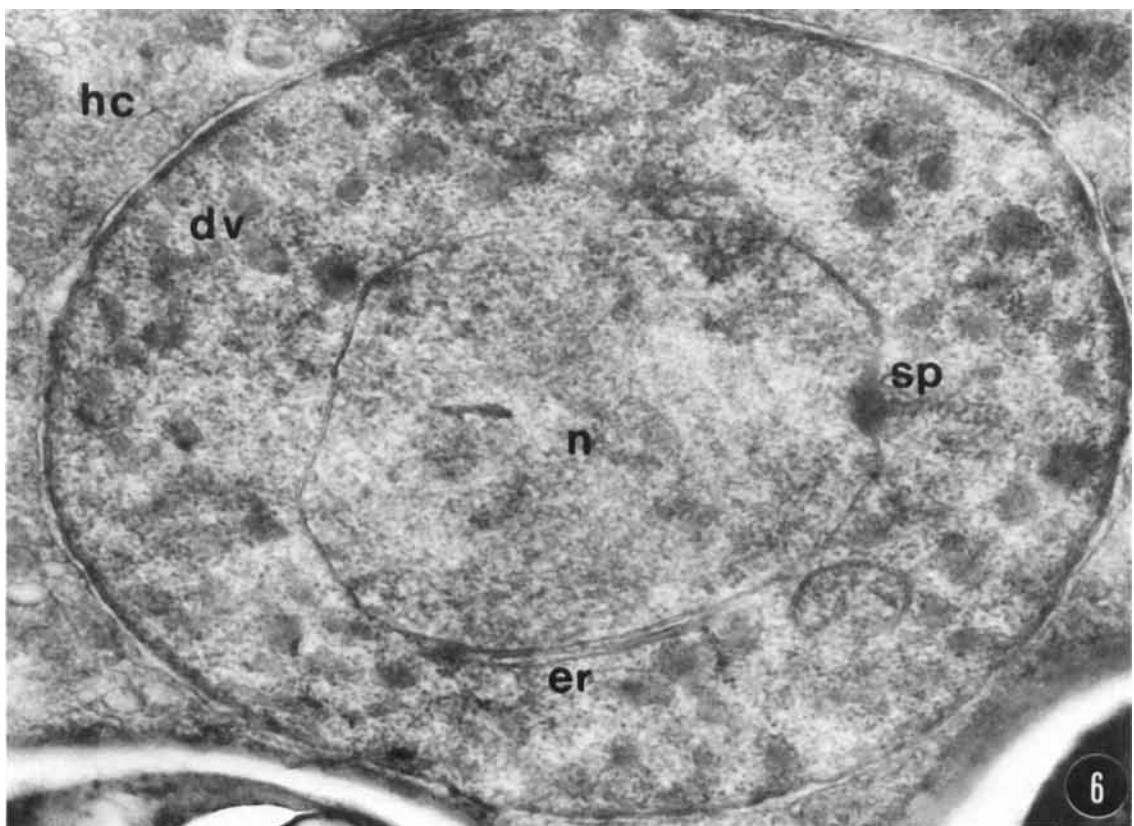
MATERIALS AND METHODS

Fish were caught on the beaches of Dakar. Fifteen of the 42 individuals examined were infested with the parasite, amounting to a prevalence of 36%.

Light microscopy. Smears of parasitized gill filaments were

examined by phase-contrast microscopy or stained by the May-Grunwald-Giemsa method. Samples subjected to histological studies were fixed in Carnoy's solution. Sections were stained with Heidenhain's azan or Masson's trichrome.

Transmission electron microscopy. Gill fragments were fixed for 1 h with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2 and then for 1 h with 2% osmium tetroxide in the same buffer. After dehydration in ethanol and propylene



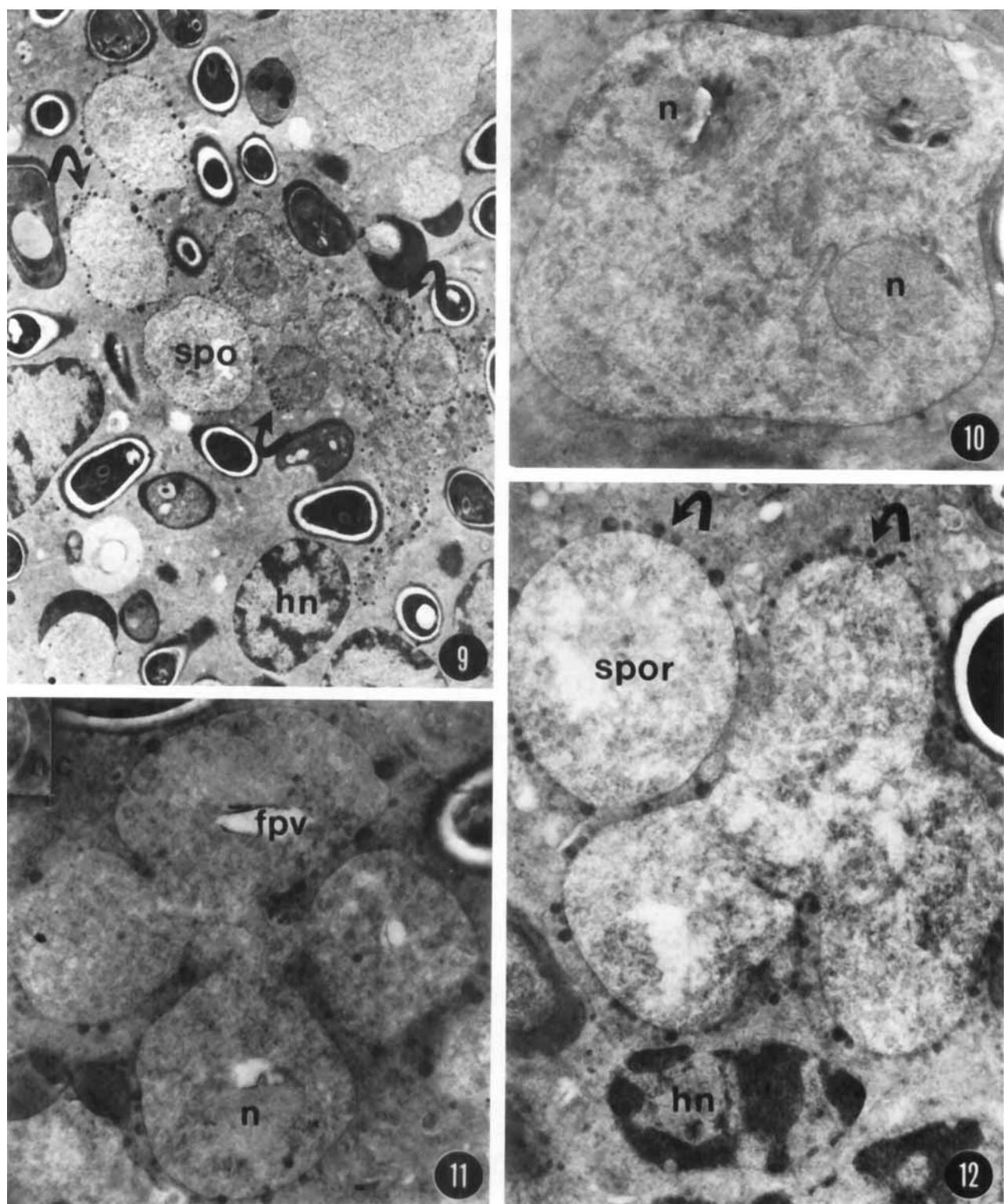


Fig. 9–12. Sporogony of *Microfilum lutjani*. 9. Overview of sporogony showing the formation of dense vesicles (curved arrows) around the sporonts (spo); hn: host nucleus, $\times 5,400$. 10. Sporogonial plasmodium with four nuclei (n) (only three are visible), $\times 16,400$. 11, 12. Sporogonial rosettes exhibiting the beginning of separation of sporoblasts (spor.). Dense vesicles of the wall are designed by curved arrows. hc: host cytoplasm, hn: host nucleus, fpv: filament primary vacuole, n:nucleus. Fig. 11, $\times 16,400$. Fig. 12, $\times 16,000$.

← Fig. 6–8. Meronts and merogony of *Microfilum lutjani*. 6. Young meront, exhibiting a spindle plaque (sp), dv: dense vesicles; hc: host cytoplasm, er: endoplasmic reticulum; n: nucleus, $\times 35,000$. 7, 8. Dividing meronts; n: nucleus; sp: spore. Fig. 7, $\times 20,000$. Fig. 8, $\times 16,400$.

oxide, gill fragments were embedded in Spurr resin and sectioned with a Reichert OM U2 microtome. Sections were contrasted with uranyl acetate and lead citrate and observed with a JEOL 200 CX (Central Electron Microscopy Laboratory, University of Montpellier II) and a Siemens Elmiskop 101 or JEOL JEM microscope (Department of Biology, University Diop, Dakar).

Scanning electron microscopy. Preparation was the same as above. Xenomas were then sectioned, subjected to the CO₂ critical point method, coated with gold-palladium and observed with a JEOL JSM 35 (CEML, University of Montpellier II).

RESULTS

Implantation and structure of xenomas. *Microfilum lutjani* produced small whitish xenomas in the gill filaments (Fig. 1). They were oval, measuring 785 × 252 µm (mean values), and were located within blood vessels or lacunae of the pillar system of secondary filaments. The host cell from which xenomas developed was a blood cell, probably a monocyte or a polymorphonuclear granulocyte, as indicated by the structure of the granules in the cytoplasm. Host nuclei were multiple and were distributed throughout the cytoplasm (Fig. 9, 12).

The wall of the xenoma showed no thickening, whereas the surrounding plasma membrane formed an extremely dense covering of microvilli without apparent order but abundantly ramified (Fig. 2-5). Two zones could be distinguished within the xenoma (Fig. 3, 4), a peripheral ectoplasmic zone containing abundant organelles (especially mitochondria) but no parasite was distinguished and an internal endoplasmic zone where the microsporidium developed without particular stratification.

Developmental cycle and ultrastructure. The parasite was in direct contact with host cell cytoplasm in all stages of the cycle (merogony and sporogony) (Fig. 6-27).

Meronts and merogony. The meronts had single isolated nuclei (Fig. 6) surrounded by a double membrane. Their cytoplasm showed many electron-dense aggregated granules (clusters of ribosomes ?) and rare saccules of endoplasmic reticulum (Fig. 6, 7, 8). They were spherical or oval in shape. A spindle plaque, in the form of stacked vesicles, often appeared in a depression on their nuclear envelope, marking the onset of division (Fig. 6). Merogony was binary; two nuclei were first produced, then a stretching process yielded two new uninucleate meronts (Fig. 7, 8).

Sporonts and sporogony. The beginning of sporogony was marked by the structural transformation of the membrane surrounding the meront. As the meront became a sporont, it underwent two successive nuclear divisions, yielding a tetranucleate sporogonic plasmodium (Fig. 10). Membrane transformation began during this development; small dense vesicles appeared on the outer surface of the sporont (Fig. 9) and plasmodium (Fig. 10, 12) membranes. The spore wall originated from these vesicles. As the membrane changed and greater numbers of larger vesicles accumulated (by coalescence), the sporogonic plasmodium underwent division in the form of a rosette which isolated four uninucleate sporoblasts (Fig. 11, 12). These images correspond to what Larsson [15] called schizogony.

A large vacuole that was in contact with the nucleus (primary

vacuole of the filament) appeared in the cytoplasm of the sporoblasts before their isolation (Fig. 11, 13).

Sporoblasts. The sporoblasts were uninucleate (Fig. 13). The youngest were more or less spherical and in full development. The external dense vesicles merged in large masses of amorphous material (Fig. 13) which gradually covered them (Fig. 14, 16). The cytoplasm often contained saccules of endoplasmic reticulum that joined and dilated into a single vacuole near the nucleus. This was the primary vacuole of the filament, which individualized before separation of the sporoblasts (Fig. 13).

The older sporoblasts (Fig. 14-16) were elongated. The wall then consisted of two layers of opaque material of variable thickness (Fig. 17), i.e. an inner layer that was thick and dense, and an outer layer that was granular and less dense. The polar filament differentiated in the cytoplasm, forming an anchoring disc that was first globular (Fig. 14, 15), and then spread laterally like an open umbrella, finally covering the anterior end of the filament (Fig. 16). At this stage, the polar filament had a dense central axis, a clear intermediate zone, and an opaque peripheral layer (Fig. 15, 16).

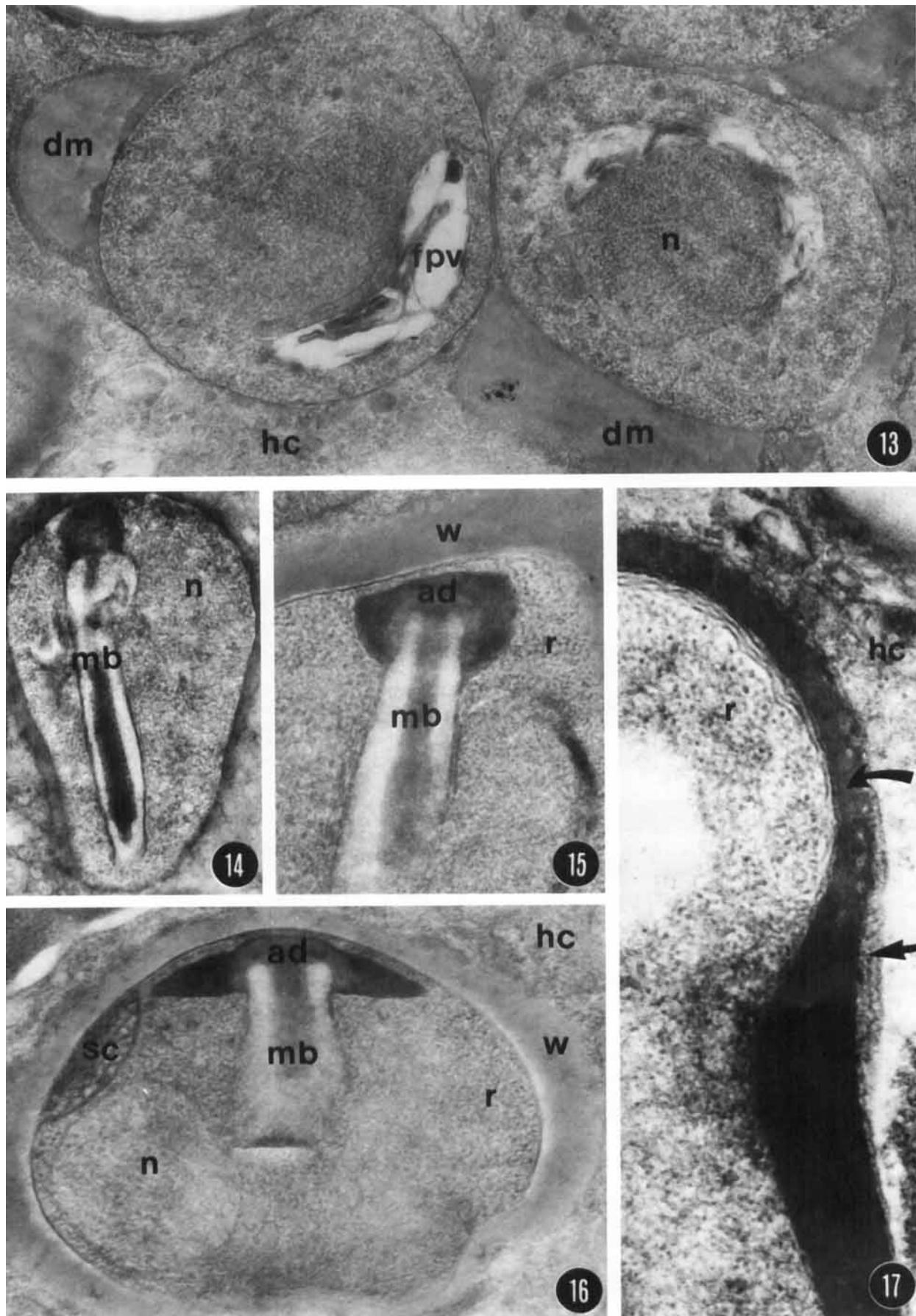
Spores. Live spores (Fig. 18) were usually pyriform, with a flat or sometimes curved anterior end. Their length was 4.57 ± 0.11 µm and their largest width was 2.60 ± 0.08 µm. Transmission electron microscopy showed them to be surrounded by a wall consisting of an exospore of variable thickness, whose surface was spongy (Fig. 25), and a clear endospore that was 100-110 nm thick, except at the anchoring disc where it was about half as thick (Fig. 23, 24). The anterior half of the exospore was always larger (Fig. 24) and scanning electron microscopy confirmed its spongy surface (Fig. 20).

The most original structure of the *Microfilum lutjani* spore was the polar filament with an axial portion corresponding to a typical manubrium 300-500 nm thick inserted on the laterally displaced anchoring disc, giving the spore bilateral symmetry (Fig. 23, 28). The manubrium then crossed the whole length of the spore cytoplasm, underwent an abrupt posterior constriction, forming a very short and curved appendix (no longer than 500 nm), that represented the actual polar filament (Fig. 22, 26, 27) and ranged in diameter from 70 to 80 nm. On transverse sections, the manubrium showed a central axis consisting of several layers of regularly arranged material that varied in density; a clear intermediate layer, and a thin dense periphery surrounded by plasma membrane (Fig. 21). On longitudinal sections, the innermost layers of the central axis of the manubrium separated posteriorly to form a basal swelling that was always clearly visible (Fig. 23, 26, 28). The terminal polar filament was formed by the extension of the central axis of the manubrium (Fig. 23, 26).

The other structures were more classical: the single nucleus was always clearly visible on transverse (Fig. 19) and longitudinal sections (Fig. 23), the polaroplast was entirely lamellar (Fig. 25), the posterior vacuole sometimes was visible in young spores (Fig. 22), and polyribosomes were very clearly defined (Fig. 25). Scindosomes (Fig. 16) may be found near the surface of several young spores.

In older spores, two electron-clear masses were sometimes visible on either side of the manubrium behind the polaroplast (Fig. 24).

Fig. 13-17. Sporoblasts and young spores of *Microfilum lutjani*. 13. Two sporoblasts, with dm: dense material, hc: host cytoplasm, fpv: filament primär vacuole, n: nucleus, $\times 28,000$. 14, 15. Two stages of anchoring disc formation and manubrium in very young spores; ad: anchoring disc, mb: manubrium, n: nucleus, r: ribosomes, w: spore wall. Fig. 14, $\times 20,000$. Fig. 15, $\times 14,000$. 16. Young spore in longitudinal section, with ad: anchoring disc, hc: host cytoplasm, mb: manubrium, n: nucleus, r: ribosomes, sc: scindosome, w: spore wall, $\times 40,000$. 17. Wall structure in a young spore, with the two zones in exospore (arrows), $\times 60,000$.



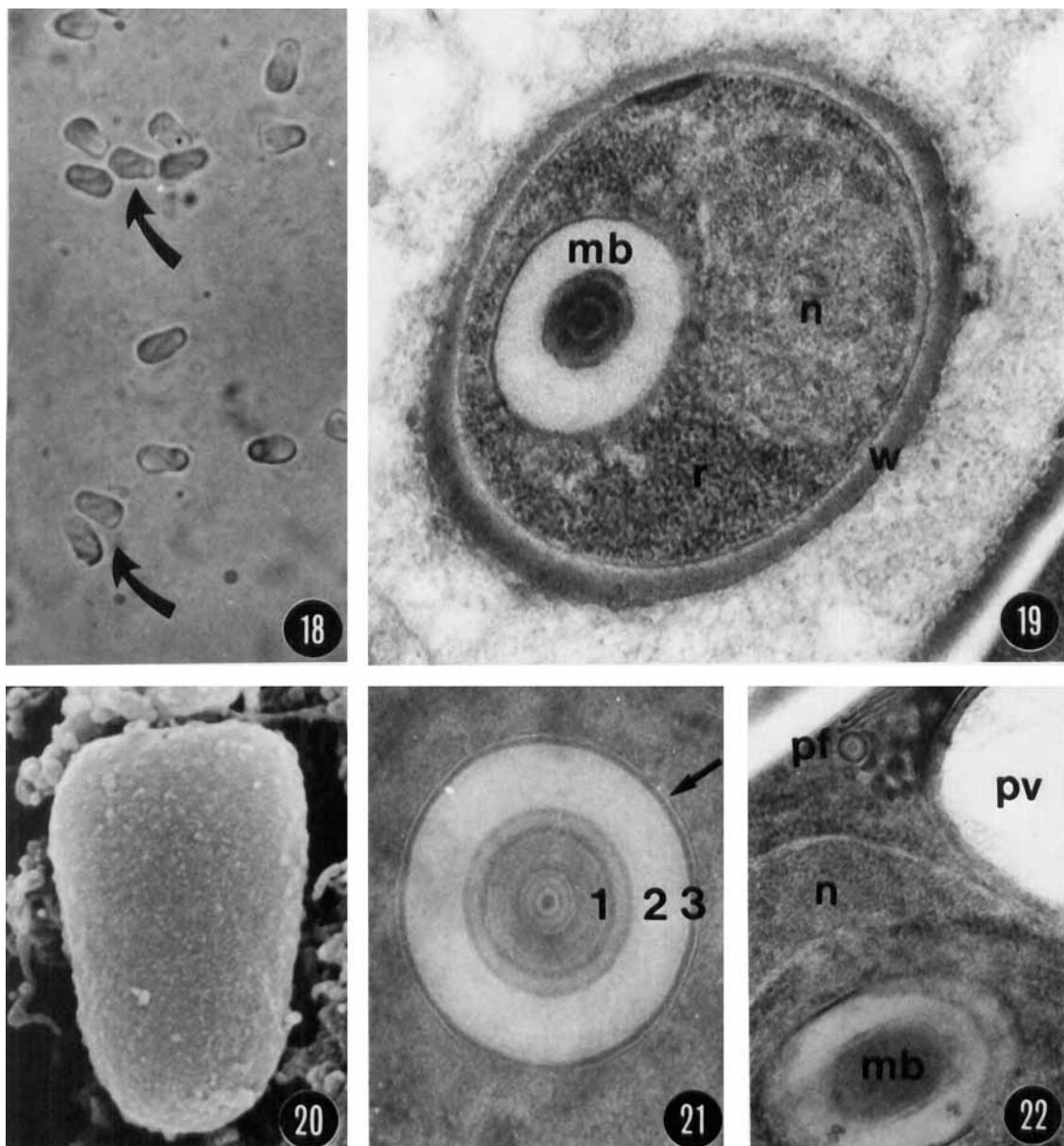


Fig. 18-22. Spores of *Microfilum lutjani*. 18. Fresh mature spores (arrows) in unstained smears, $\times 100$. 19. Transverse section of a mature spore showing mb: manubrium, n: nucleus, r: ribosomes, w: spore wall, $\times 54,000$. 20. Mature spore in scanning electron microscope, $\times 8,000$. 21. Detail of transverse section of the manubrium, showing different layers; central dense (1), intermediate clear (2), peripheral dense (3) and plasma membrane (arrow), $\times 120,000$. 22. Detail of oblique section of the manubrium (mb) and transverse section of the polar filament (pf), n: nucleus, pv: posterior vacuole, $\times 54,000$.

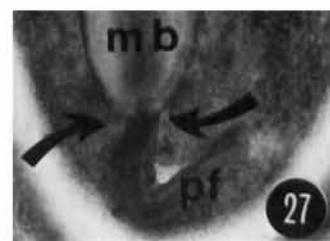
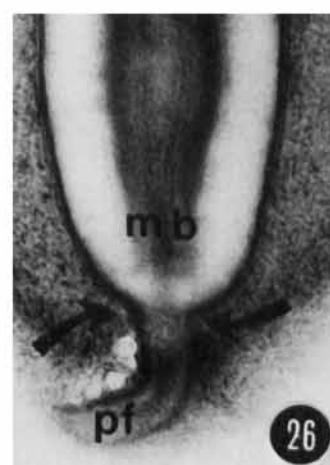
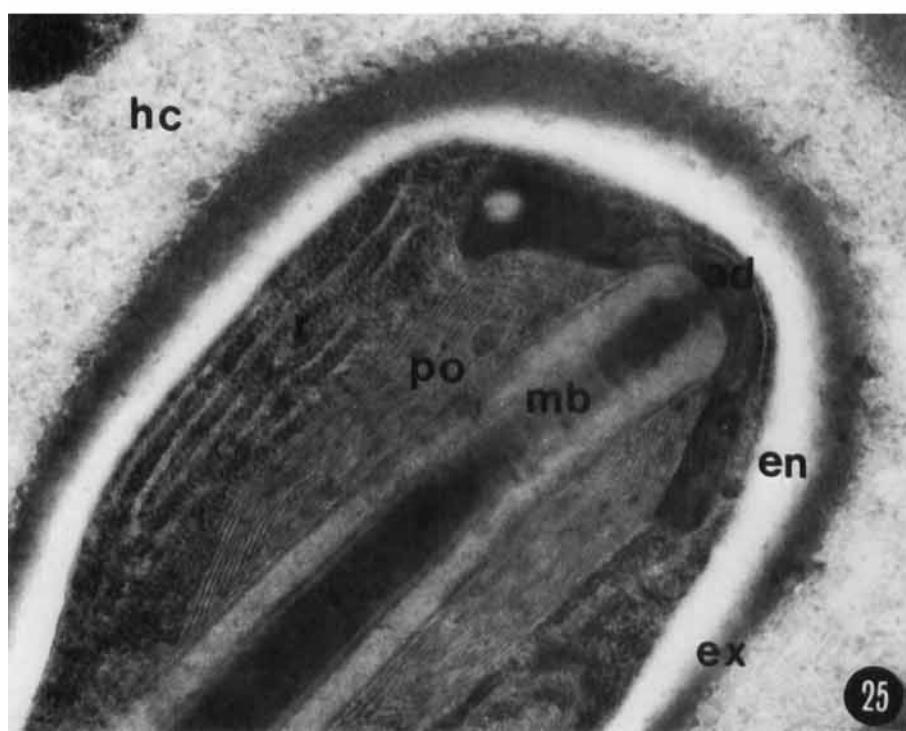
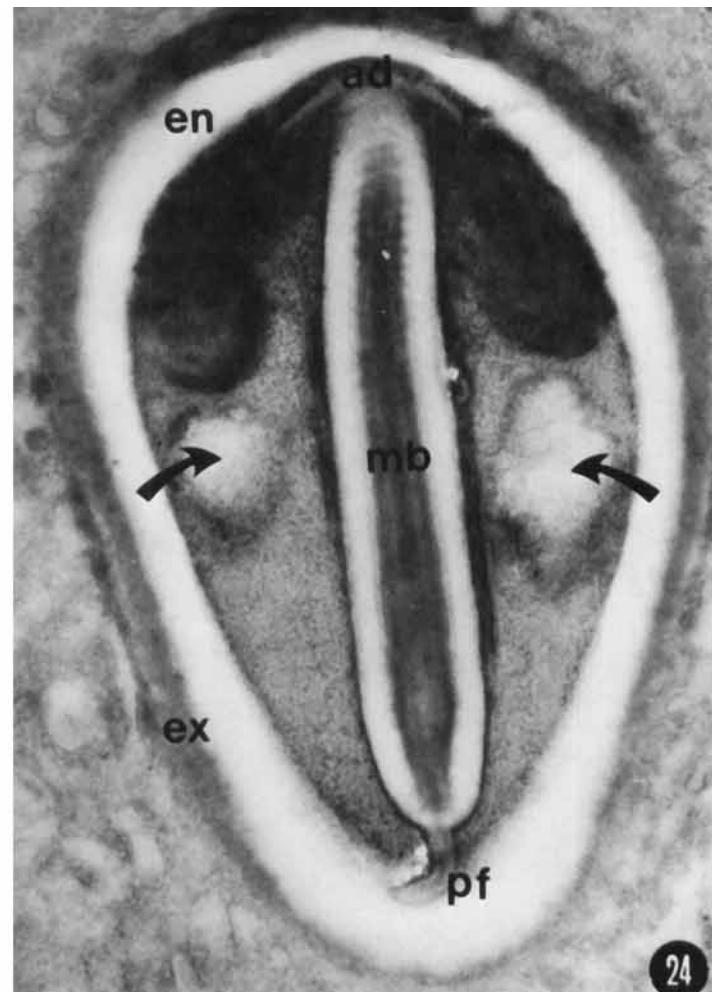
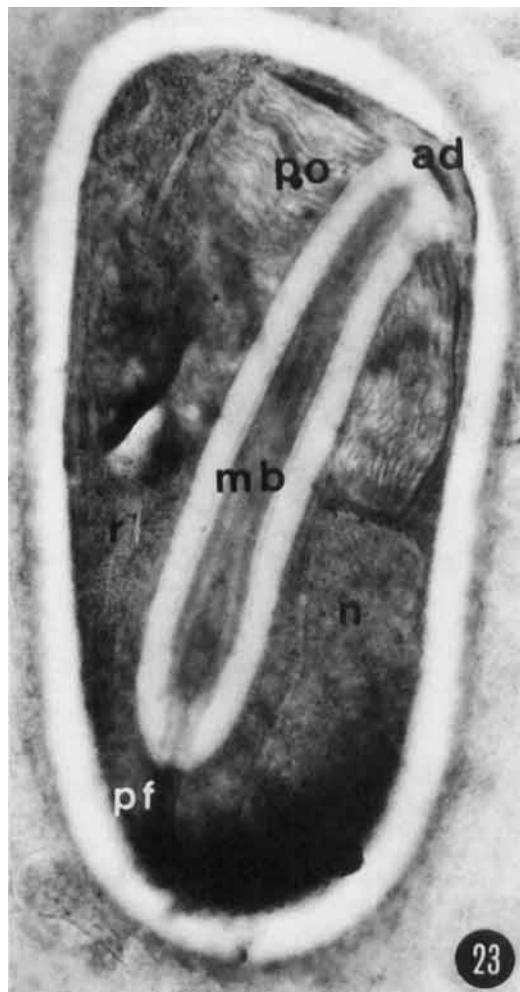
DISCUSSION

Implantation and structure of the xenoma. In the species found in *Lutjanus fulgens*, xenomas had developed in the gill circulatory system. This implantation is classic, resembling xe-

nomas in *Loma salmonae* (Putz, Hoffman & Dunbar, 1965) and *Loma diploidae* (Bekhti & Bouix, 1985) [2, 23].

The host cell of certain Microsporidia in fish can be determined by identifying specific granules in the cytoplasm. For

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Fig. 23-27. Spores of *Microfilum lutjani*. (longitudinal sections). 23. Sagittal section of an immature spore; ad: anchoring disc, mb: manubrium, n: nucleus, pf: polar filament, po: polaroplast, r: ribosomes, $\times 40,000$. 24. Longitudinal section of a mature spore (perpendicular at the previous); ad: anchoring disc, en: endospore, ex: exospore, mb: manubrium, pf: polar filament. The arrows show the two clear masses under the polaroplast, $\times 56,000$. 25. Detail of anterior part of a mature spore; ad: anchoring disc, en: endospore, ex: exospore, hc: host cytoplasm, mb: manubrium, po: polaroplast, r: ribosomes, $\times 54,000$. 26, 27. Two aspects of the passage from the manubrium to the polar filament (indicated by the arrow); mb: manubrium, pf: polar filament. Fig. 26, $\times 80,000$. Fig. 27, $\times 54,000$.



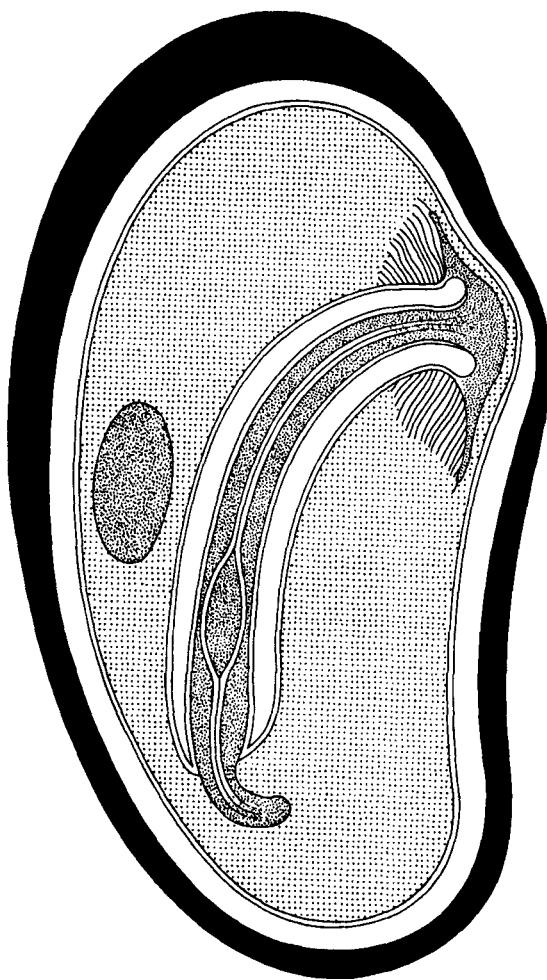


Fig. 28. Schematic drawing summarizing the structure of the mature spore of *Microfilum lutjani* (sagittal section).

instance, in the case of *Glugea stephani* (Hagenmüller, 1899), a parasite of the flounder *Platichthys flesus*, polymorphonuclear neutrophils support the development of Microsporidia [3]. The host cell in *Lutjanus fulgens* is probably a monocyte or a polymorphonuclear granulocyte. The xenoma of *M. lutjani* is distinguished mostly by the structure of its wall, i.e. an extremely dense and irregular covering of microvilli surrounds the host cell and presses against the endothelial cells of the gill vessel. A brush border has previously been described at the periphery of *Microsporidium cotti* (Chatton & Courrier, 1923), a parasite of the testicular tissue of *Taurus bubalis* [5]. In this case, however, the nuclei of the host cell occupy the outer layer, whereas in the parasite of *Lutjanus fulgens* they are disseminated among the developing stages and spores. In *Loma branchialis* (Nemeczek, 1911), the plasma membrane of the host cell is covered with irregular microvilli that overlap in the basal membrane of the pillar system, together forming a wall that is thicker than 1.5 μm [22].

Systematic position. During its whole developmental cycle, the Microsporidium of *Lutjanus fulgens* remains in direct contact with the host cytoplasm, while the nuclei are isolated. In addition, sporogony is tetrasporoblastic. These are fundamental systematic characteristics allowing comparison with the genera *Unikaryon* Canning, Lai & Lie, 1974, *Nosemoides* Vinckier,

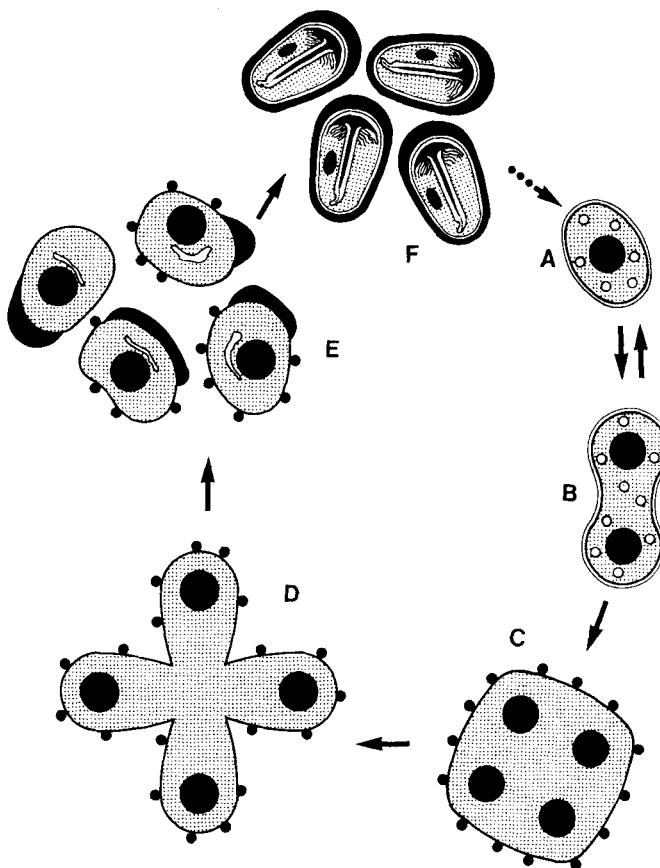


Fig. 29. Schematic drawing summarizing the development cycle of *Microfilum lutjani*; A: meront; B: merogony; C: sporogonial plasmodium, D: sporogonial rosette, E: sporoblasts, F: spores.

1975, *Microgemma* Ralphs & Matthews, 1986 and *Lanatospora* Voronin, 1986.

Unikaryon species are all parasites of trematodes and insects. Moreover, they are disporoblastic and produce spores with a classic spiral polar filament [4, 6, 32]. Recently, Azevedo and Canning [1] evidenced a sporophorous vesicle in *Unikaryon legeri* where sporogony occurs, which calls into question its systematic classification in the genus *Unikaryon*.

Microsporidia of the genus *Nosemoides* are found in gregarines, crustaceans and fish. Their sporogony is polysporoblastic and the spores have a classic spiral polar filament. The formation of xenomas has only been described in fishes [18, 28, 33].

Microgemma hepaticus Ralphs & Matthews, 1986, is the only species of this genus found in a fish, i.e. *Chelon labrosus* [27]. As above, sporogony is polysporoblastic, the spores have a classic spiral polar filament and xenomas are induced in the liver.

Finally, in the genus *Lanatospora* [36] the sporogony is polysporous with rosette-like exogenous budding producing 6–16 spores with a woolskin-like exosporal coat. The polar filament is classic and isofilar.

The sporogony and spore structure of *Microfilum* clearly distinguish it from four preceding genera. Tetrasporoblastic sporogony is also found in the genus *Tetramicra* Matthews & Matthews, 1980. However, *T. brevifilum*, the only species of the genus found in a fish (i.e. *Scophthalmus maximus*), produces spores with a classic spiral polar filament and the young sporogonic stages ("sporont mother cells") have diplokarya [20].

In Sprague system [29], the genera *Unikaryon* and *Nosemoides* are classified in the family of Unikaryonidae Sprague, 1977. This family does not appear in the systems of Weiser [37, 38] and Issi [12]. Ralphs and Matthews [27] consider that the genus *Microgemma* also resembles the Unikaryonidae but for reasons of prudence they do not classify it in this family.

Recently, Voronin [36] suggested dividing the microsporidia that have isolated nuclei during sporogony and develop without sporophorous vesicle into three families: Encephalitozoonidae Voronin, 1989 (synonym Unikaryonidae Sprague, 1977) with disporoblastic sporogony; Tetramicridae (Matthews & Matthews, 1980), Ralphs & Matthews, 1986 with polysporoblastic sporogony (rosette-like exogenous budding); and Pereziidae Loubès, Maurand, Comps & Campillo, 1977 with diplokarya at the end of merogony and polysporoblastic sporogony (moniliform sporogonial plasmodium).

We consider that the distinctive and original characters of the microsporidium studied here justify the creation of a new genus *Microfilum* (which refers to the shortness of the polar filament) and a new species *M. lutjani* (after the host fish). In the Voronin system [36], *M. lutjani* would be classified in the family of Tetramicridae. But, retaining in the same family genera with isolated nuclei in all developmental stages (*Nosemoides*, *Microgemma* or *Microfilum*) and another one with paired nuclei in the youngest sporogonic stages (*Tetramicra*) seems artificial to us.

DIAGNOSIS

Microfilum n. g.

Description. Direct contact with the cytoplasm of the host cell in all stages of development. Unikaryotic (isolated nucleus) during the whole cycle. Merogony by binary division. Tetrasporoblastic sporgony. Spore with a manubrium terminating in a very short polar filament in the form of a curved appendix.

Microfilum lutjani n. sp., type species

Description. Shows the characters of the genus. Meront cytoplasm contains many aggregated granules. Membrane of the sporonts and sporoblasts covered with dense vesicles which develop into the spore envelope. Uninucleate pyriform or oval spores measuring $4.57 \pm 0.11 \times 2.60 \pm 0.09 \mu\text{m}$; exospore with a spongy surface, thicker at the anterior pole; entirely lamellar polaroplast; manubrium 300–350 nm in diameter, very short polar filament no more than 500 nm long, 70–80 nm wide.

Xenoma. Present in the gill circulatory system, oval (785 × 252 μm), with abundant microvilli on its wall.

Infestation site. Gills.

Type host. *Lutjanus fulgens* (Valenciennes, 1830) (Teleost, Lutjanidae).

Type locality. Dakar (Senegal, Africa).

Deposition of types. The slides with the holotype in the International Protozoan Type Slide Collection, National Museum of Natural History, Smithsonian Institution, Washington, USA. Paratypes are kept in the collection of the authors.

The polar filament. The originality of the polar filament in *M. lutjani* spores, with its axial manubrium, deserves particular attention. Loubès [17] has defined the following four types of polar filaments in the Microsporidia. Type I filaments are spiral and their diameter is about the same over the whole length. These are the isofilar filaments found in most genera, for example in *Nosema* and *Unikaryon* [31, 32]. In type II filaments, the straight and the first turns of the spiral are larger in diameter than the last turns of the spiral. These are the anisofilar filaments found in *Amblyospora* [11], among other genera. Type III filaments consist of a straight, thick proximal portion, which is

called the manubrium, and a short distal portion that is recurrent or spiral. Microsporidia of the genera *Ormieresia* and *Mrazekia* have this type of structure [9, 34]. Lastly, type IV filaments are limited to a single proximal portion, i.e. the manubrium. This type of filament is found in *Metchnikovella* [35], *Amphiambllys* [8, 26], *Baculea* [19], *Helmichia* [14], *Cylindrospora* [16] and *Striatospora* [12].

The filament of *Microfilum lutjani*, with its typical manubrium and a distal portion in the form of a short appendix or hook, does not conform to any of the above categories. It is original and constitutes a 5th type, between types III and IV. Hence, *Microfilum lutjani* has a set of features that justify the creation of a new genus. Its developmental cycle and the morphology of its polar filament are the determining elements.

LITERATURE CITED

1. Azevedo, C. & Canning, E. U. 1987. Ultrastructure of a microsporidian hyperparasite, *Unikaryon legeri* (Microsporida) of Trematode. *J. Parasitol.*, **73**:214–223.
2. Bekhti, M. & Bouix, G. 1985. *Loma salmonae* (Putz, Hoffman & Dunbar, 1965) et *Loma diplodae* n. sp., Microsporidies parasites de branchies de poissons téléostéens: implantation et données ultrastructurales. *Protistologica*, **21**:47–59.
3. Bekhti, M. & Bouix, G. 1985. Sur l'évolution des xénomes et le double rôle des polynucléaires neutrophiles dans la microsporidiose à *Glugea stephani* (Hagenmuller, 1899) chez le Flet *Platichthys flesus* (Linné, 1758). *Annls Parasit. Hum. Comp.*, **60**:509–522.
4. Canning, E. U., Lai, P. F. & Lie, K. J. 1974. Microsporidian parasites of trematode larvae from aquatic snails in West Malaysia. *J. Protozool.*, **21**:19–25.
5. Canning, E. U. & Lom, J. 1986. The Microsporidia of Vertebrates. Academic Press, Orlando, Florida, pp. 1–289.
6. Canning, E. U. & Nicholas, J. P. 1974. Light and electron microscope observations on *Unikaryon legeri* (Microsporida, Nosematidae), a parasite of the metacercaria of *Meigymnophallus minutus* in *Cardium edulis*. *J. Invertebr. Pathol.*, **23**:92–100.
7. Delisle, C. 1972. Variations mensuelles de *Glugea hertwigi* (Sporozoa: Microsporida) chez différents tissus et organes de l'Eperlan adulte dulcicole et conséquences de cette infection sur une mortalité massive annuelle de ce poisson. *Can. J. Zool.*, **50**:1589–1600.
8. Desportes, I. & Théodorides, J. 1979. Etude ultrastructurale d'*Amphiambllys laubieri* n. sp. (Microsporidie, Metchnikovellidae), parasite d'une Grégarine (*Lecudina* sp.) d'un Echiurien abyssal. *Protistologica*, **15**:435–457.
9. Götz, P. 1981. Homology of the manubrium of *Mrazekia brevicauda* and the polar filament of other Microsporida. *Z. Parasitenkd.*, **64**:321–333.
10. Grandall, T. A. & Bowser, P. R. 1981. A microsporidian infection in a natural population of mosquitofish, *Gambusia affinis* (Baird and Gerard). *J. Fish Dis.*, **4**:317–324.
11. Hazard, E. I. & Oldacre, S. W. 1975. Revision of Microsporida (Protozoa) close to *Thelohania*, with descriptions of one new family, eight new genera and thirteen new species. *U.S. Dep. Agric. Techn. Bull.*, **1530**:1–104.
12. Issi, I. V. 1986. Microsporidia as a phylum of parasitic protozoan. In: Beyer, T. V. & Issi, I. V. (ed.), Microsporidia. *Protozoology*, Leningrad, Russia, **10**:6–136.
13. Kent, M. L., Elliott, D. G., Graff, J. M. & Hedrick, R. P. 1989. *Loma salmonae* (Protozoa: Microspora) infections in seawater reared coho salmon *Oncorhynchus kisutch*. *Aquaculture*, **80**:211–222.
14. Larsson, R. 1982. Cytology and taxonomy of *Helmichia aggregata* gen. and sp. nov. (Microspora Thelohaniidae), a parasite of *Endochironomus larva* (Diptera Chironomidae). *Protistologica*, **28**:355–370.
15. Larsson, R. 1986a. Ultrastructure, function and classification of Microsporida. In: Corliss, J. O. & Patterson, D. J. (ed.), *Progress in Protistology*, Vol. I, Biopress Ltd, England, pp. 325–390.
16. Larsson, R. 1986b. Ultrastructural investigation of two microsporidia with rod shaped spores, with description of *Cylindrospora fasciculata* sp. nov. and *Resiomeria odonatae* gen. and sp. nov. (Microspora Thelohaniidae). *Protistologica*, **22**:379–398.

17. Loubès, C. 1979. Ultrastructure, sexualité, dimorphisme sporogonique des Microsporidies (Protozoaires). Incidences taxonomiques et biologiques. Thèse Doct. Etat, U.S.T.L., Montpellier, France, pp. 1-186.
18. Loubès, C. & Akbarieh, M. 1977. Etude ultrastructurale de *Nosemoides simocephali* n. sp. (Microsporidie), parasite intestinal de la Daphnie *Simocephalus vetulus* Muller, 1776. *Z. Parasitenkde*, **54**:125-137.
19. Loubès, C. & Akbarieh, M. 1978. Etude ultrastructurale de la Microsporidie *Baculea daphniae* n. g., n. sp., parasite de l'épithélium intestinal de *Daphnia pulex* Leydig, 1860 (Crustacé, Cladocère). *Protistologica*, **14**:23-38.
20. Matthews, R. A. & Matthews, R. P. 1980. Cell and tissue reactions of turbot *Scophthalmus maximus* (L.) to *Tetramicra brevifilum* gen. n., sp. n. (Microspora). *J. Fish. Diseases*, **3**:495-515.
21. Michel, C., Maurand, J., Loubès, C., Chilmonczyk, S. & De Kinkelin, P. 1989. *Heterosporis funki*, a microsporidian parasite of the angel fish *Pterophyllum scalare*: pathology and ultrastructure. *Dis. Aqua. Org.*, **7**:103-109.
22. Morrison, C. M. & Sprague, V. 1981a. Electron microscopical study of a new genus and new species of microsporidia in the gills of atlantic Cod *Gadus morhua* L. *J. Fish Dis.*, **4**:15-32.
23. Morrison, C. M. & Sprague, V. 1981b. Microsporidian parasites in the gills of salmonid fishes. *J. Fish Dis.*, **4**:371-386.
24. Nepszy, S. J., Budd, J. & Dechtiar, A. O. 1978. A mortality of young of the year rainbow smelt (*Osmerus mordax*) in Lake Erie associated with the occurrence of *Glugea hertwigi*. *J. Wildlife Dis.*, **14**: 233-239.
25. Olson, R. E. 1976. Laboratory field studies on *Glugea stephani* (Hagenmuller), a microsporidian parasite of pleuronectid flatfishes. *J. Protozool.*, **23**:158-164.
26. Ormières, R., Loubès, C. & Maurand, J. 1981. *Amphiambylops bhatiellae* n. sp., microsporidie parasite de *Bhatiella morphysae* Setna, 1931, Eugregarine d'Annélide Polychète. *Protistologica*, **17**:273-280.
27. Ralphs, J. R. & Matthews, R. A. 1986. Hepatic microsporidiosis due to *Microgemma hepaticus* n. gen., n. sp. in juvenile grey mullet *Chelon labrosus*. *J. Fish. Dis.*, **9**:225-242.
28. Sakiti, N. G. & Bouix, G. 1987. *Nosemoides tilapiae* n. sp., Microsporidie parasite des Poissons Cichlidae des eaux saumâtres du Bénin: implantation et caractères ultrastructuraux. *Parasitol. Res.*, **73**: 203-212.
29. Sprague, V. 1977. Systematics of Microsporidia. In: Bulla, L. A. & Cheng, T. C. (ed.), Comparative Pathobiology, Vol. II. Plenum Press, New York, New York, pp. 1-510.
30. Sprague, V. 1982. Microspora. In: Parker, S. P. (ed.), Synopsis and Classification of Living Organisms, Vol. I. McGraw-Hill Book Company, New York, New York, pp. 589-594.
31. Toguebaye, B. S. & Bouix, G. 1983. *Nosema maniera* n. sp., Microsporidie parasite de *Chilo zacconius* Blezenski, 1970 (Lepidoptera Pyralidae), hôte naturel, et *Heliothis armigera* (Hübner, 1808) (Lepidoptera, Noctuidae), hôte expérimental: cycle évolutif et ultrastructure. *Z. Parasitenkde*, **69**:191-205.
32. Toguebaye, B. S. & Marchand, B. 1983. Développement d'une microsporidie du genre *Unikaryon* Canning, Lai & Lie, 1974, chez un Coléoptère Chrysomelidae *Euryope rubra* (Latreille, 1807): étude ultrastructurale. *Protistologica*, **19**:371-383.
33. Vinckier, D. 1975. *Nosemoides* gen. n., *N. vivieri* (Vincker, Devauchelle & Prensier, 1970) comb. nov. (Microsporidia): étude de la différenciation sporoblastique et genèse des différentes structures de la spore. *J. Protozool.*, **22**:170-184.
34. Vivarès, C. P., Bouix, G. & Manier, J. F. 1977. *Ormieresia carcinii* gen. n., sp. n., Microsporidie du Crabe méditerranéen *Carcinus mediterraneus* Czerniavsky, 1884: cycle évolutif et étude ultrastructurale. *J. Protozool.*, **24**:83-94.
35. Vivier, E. 1965. Étude en microscopie électronique de la spore de *Metchnikovella hovassei* n. sp.; appartenance des Metchnikovellidae aux Microsporidies. *C. R. Acad. Sci., Paris*, **260**:6982-6984.
36. Voronin, N. N. 1989. The ultrastructure *Lanatospora macrocyclops* (Protozoa Microsporida) from the Cyclope *Macrocyclops albidus* (Jur.) (Crustacea Copepoda). *Arch. Protistenkde*, **137**:357-366.
37. Weiser, J. 1977. Contribution to the classification of Microsporidia. *Vestn. Cs Spol. Zool.*, **41**:308-321.
38. Weiser, J. 1985. *Phylum Microspora* Sprague, 1969. In: Lee, J. J., Hutner, S. H. & Bovee, E. C. (ed.), Illustrated guide to the Protozoa. Society of Protozoologists, Lawrence, Kansas, pp. 375-383.

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In Vitro Development of Exoerythrocytic Forms of *Plasmodium gallinaceum* Sporozoites in Avian Macrophages

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ABSTRACT. Exoerythrocytic forms of *Plasmodium gallinaceum* were cultured in vitro using salivary gland sporozoites extracted from experimentally infected *Aedes vexans* mosquitoes. The host cells were macrophage precursors from chicken bone marrow. At various times after introduction of sporozoites, the cultures were stained by Giemsa or by immunofluorescence assay (IFA) using anti-sporozoite-specific monoclonal antibodies (MAb). The time to complete parasite development in vitro was 50-70 h. By 70 h, ruptured segmenters and free merozoites were visible within the cells. Inoculation of normal chickens with infected cultures induced parasitemia after a pre-patent period of 10-11 days. In vitro young exoerythrocytic forms, late schizonts that include the matured segmenters, and free merozoites shared common antigens with the sporozoites as revealed by IFA using anti-sporozoite-specific MAbs. Our data indicate that macrophages support development of *P. gallinaceum* sporozoites and that the circumsporozoite proteins are present until the end of the primary exoerythrocytic schizogony.

Key words. Anti-sporozoite monoclonal antibodies, avian malaria, exoerythrocytic forms, *P. gallinaceum* sporozoites.

NATURAL infection of the vertebrate host with malarial parasites begins with inoculation of the sporozoite by an infected mosquito. These rapidly invade cells and undergo schizogony, resulting in the exoerythrocytic forms (EEF). In the case of mammalian malarias, the hepatocytes are host cells for EEF.

For the avian malarias, host cells are phagocytic mononuclear cells and skin macrophages at the site of the mosquito bite [3]. The mature EEF rupture the host cells releasing merozoites which invade erythrocytes. In the case of avian malaria, the EEF also will invade other cells (i.e. endothelial cells and cells of the