

***Loma camerounensis* sp. nov. (Protozoa: Microsporida) a parasite of *Oreochromis niloticus* Linnaeus, 1757 (Teleost: Cichlidae) in fish-rearing ponds in Melen, Yaoundé, Cameroon**

A. Fomena¹, F. Coste², and G. Bouix²

¹ Laboratoire de Biologie Générale, Faculté des Sciences, Université du Cameroun, Yaoundé, Cameroun

² Laboratoire de Parasitologie et Immunologie, Université Montpellier II, 34095 Montpellier Cedex 5, France

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Abstract. A new microsporidian, *Loma camerounensis*, was found in the Cichlidae *Oreochromis niloticus* from the Melen fish-rearing station in Yaoundé, Cameroon. Xenomas were located in the gut from the oesophagus to the intestine, but preferentially in the duodenum. Dimensions of the fresh spores were $3.96 \times 2.16 \mu\text{m}$. The ultrastructural study indicated the following characteristics: parasite stages arranged in a random, unstratified manner in the xenoma; merogony by multiple fission; sporogonic stages isolated within a sporophorous vesicle containing several sporoblasts and polysporoblastic sporogony. Ultimately the spores appeared to be isolated in a single vesicle, but this was a secondary phenomenon induced by the intervention of tubules that resolved in partitions of the initial sporophorous vesicle.

At the current production level of 200,000 tonnes, *Tilapia* are one of the most exploited types of fish in aquaculture, occurring in both fresh and brackish water in tropical zones. The main countries involved are Taiwan, the Phillipines, Japan and Israel, where production has increased considerably in recent years (Philippart and Mellard 1987). In Africa, aquaculture is recent and is currently practiced extensively, intensively or in combination with other types of agronomic exploitation, with tilapias being the most common fish reared in all cases (Arrignon 1989). The most abundant species is *Oreochromis niloticus*, which is encountered throughout West Africa and in the equatorial zone. This species was recently introduced into the Melen fish-rearing ponds at Yaoundé, Cameroon.

In spite of the unquestionable merits of tilapias, information on their parasites remains extremely fragmented. Numerous Myxosporidia have been found and described in the coastal lagoons in Cameroon (Fomena et al., in press) and in Benin (Sakiti et al. 1991); in the latter case, their pathogenicity was demonstrated (Sakiti et al.

1990). There are even fewer data on Microsporidia. Perna (1973, 1982) has described a *Pleistophora* sp. infecting the air bladder of the cichlids *Haplochromis angustifrons* and *H. elegans* in Lake George, Uganda. *Nosemaoides tilapia* Sakiti and Bouix, 1987 is a recently described species parasitising *T. zillii*, *T. guineensis* and *Sarotherodon melanotheron* in Lake Nokoué and the Porto Novo lagoon, which form the largest area of brackish water in Benin. The developmental stages of *N. tilapia* are never diplokaryotic and are always found in direct contact with the host cytoplasm. Xenomas primarily occur in gill tissue but can also develop in the gut and liver (Sakiti and Bouix 1987).

In *O. niloticus* from Melen (Cameroon), we found a new microsporidian whose high prevalence merits special attention. Its ultrastructural features place it in the genus *Loma* (Morisson and Sprague 1981a). We named it *L. camerounensis* on the basis of complementary data gathered on the formation and development of the sporophorous vesicle in the genus *Loma*.

Materials and methods

The fish investigated were obtained from the Melen fish-rearing station near the Obili area of Yaoundé Cameroon. A total of 275 specimens were examined over a 4-month period from December 1989 to March 1990. All regions of the gut (oesophagus, stomach, duodenum, intestine) were observed with a binocular microscope.

Light microscopy

The xenomas were removed and their contents were placed on slides in a drop of water under coverslips. The smears were then fixed and stained using the May-Grünwald-Giemsa method and examined by light microscopy.

Transmission electron microscopy

The xenomas were fixed with 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.4) for 3–4 h, washed in the buffer

and then post-fixed in 1% osmium tetroxide in the same buffer for 1 h. The samples were embedded in Spurr's resin and sectioned with a Reichert OM U2 microtome. Semithin sections were stained with toluidine blue and ultrathin sections were contrasted using Reynolds' method, and the slides were examined with a JEM 200 CX microscope (Electron Microscopy Centre, Université de Montpellier II).

Scanning electron microscopy

After identical fixation and dehydration, the samples were prepared using the CO₂ critical-point method, then coated with gold-palladium and observed using a JEOL JSM 35 microscope (Electron Microscopy Centre, Université de Montpellier II).

Results

Light microscopy observations

Location in the host. The *Loma camerounensis* xenomas were located in the gut of *Orechromis niloticus* from the oesophagus to the intestine. The duodenum was clearly the preferred site for the development of this microsporidian (100 cases of parasitism among 106 fish). We found few extra-duodenal infections (oesophagus, stomach, intestine).

Characteristics of xenomas and spores. The xenomas were embedded in the connective tissue of the intestinal submucosa (Figs. 1, 2); some were large and protruded into the intestinal cavity. The parasite could cause lysis of the connective tissue. The xenomas were whitish, almost spherical and of broadly varying dimensions (70–340 × 63–318 µm). The spores were oval (Fig. 3) and small and displayed a well-developed posterior vacuole (Fig. 5). The following measurements were obtained for fresh spores: length (L), 3.96 ± 0.07 (range, 3.20–4.50) µm; width (W), 2.16 ± 0.04 (range, 1.80–2.50) µm; L/W ratio, 1.83; and posterior vacuole, 2.12 (range, 1.8–2.5) × 1.87 (range, 1.8–2.0) µm.

Parasite prevalence. A considerable increase in prevalence was observed in February and March of 1990 (Table 1). Observations are continuing on the seasonal dynamics of the parasite.

Parasite intensity. Table 2 clearly shows the quantitative importance of parasitism by *L. camerounensis*. In addition, the mean parasite load per parasitised *O. niloticus* was 5.50 xenomas in December 1989, 23.76 in January 1990, 15.49 in February 1990 and 12.81 in March 1990.

Electron microscopy observations

Xenoma structure. The xenoma was bounded by a thin wall (0.45–0.86 µm thick) consisting of a homogeneous, continuous layer (Fig. 5). The membrane of the host cell was clear; beneath it, the peripheral cytoplasm contained numerous microvesicles. The parasite stages were dis-

Table 1. Prevalence of *Loma camerounensis*

	December 1989	January 1990	February 1990	March 1990	Totals
Fish observed (n)	38	96	88	53	275
Parasitised fish (n)	10	25	45	26	106
Prevalence (%)	26.31	26.04	51.14	49.05	38.54

Table 2. Number of xenomas per individual

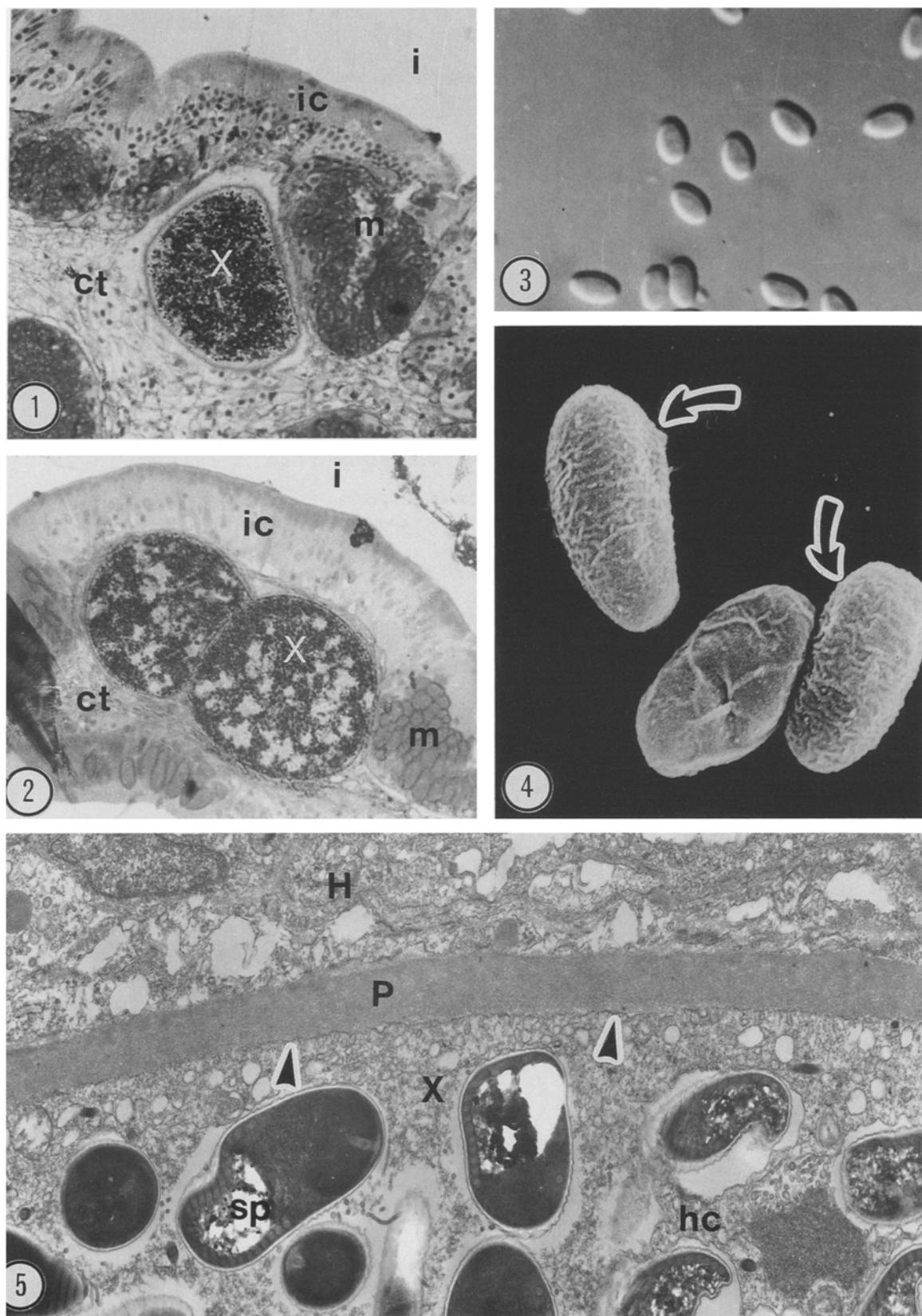
Xenomas per individual (n)	Fish (n)	Percentage
1–29	89	83.96
30–59	10	9.43
60–89	6	5.66
≥90	1	0.94

tributed in a random, unstratified manner throughout the rest of the xenoma.

Development of the parasite. The merogonic stages were in close contact with the host-cell cytoplasm. They appeared as more or less rounded, uninucleate meronts measuring 3–3.5 µm in diameter (Fig. 7) or as cylindrical vegetative plasmodia containing five or more nuclei (Fig. 6). Merogony took the form of multiple fission as in *L. salmonae* (Bekhti and Bouix 1985). The cytoplasm was not particularly dense and contained numerous ribosomes and endoplasmic-reticulum cisternae. No particular differentiation of the host cytoplasm was visible around these stages.

During differentiation of the sporophorous vesicle (Figs. 8–14), the sporogonic stages (sporonts or sporogonial plasmodia) were no longer in direct contact with the host cytoplasm from the start of sporogony and became isolated within sporophorous vesicles. The membrane of the sporophorous vesicle was formed by the generation of blisters at the surface of the sporont (Figs. 9–11). The additional membrane was created by the extrusion of blisters containing electron-dense material from the surface of the sporont (Figs. 9–14). Little by little, the sporophorous vesicle extended around the sporont or the plasmodium. Several images suggested the incorporation of amorphous, electron-dense, unstructured material in the sporophorous vesicle during this progression, but this process was difficult to distinguish precisely (Fig. 14). This material may have been the source of the large secretions observed during sporoblastogenesis.

During sporogony (Figs. 8, 9, 13–15), the sporonts (Figs. 9, 13) were rounded or oval uninucleate elements exhibiting dense cytoplasm that was rich in ribosomes, endoplasmic reticulum and vesicles. Sporogonial divisions occurred rapidly prior to the complete formation of the sporophorous vacuole. Plasmodia displaying from



Figs. 1, 2. Xenomas (X) of *Loma camerounensis* in connective tissue (ct) of the gut. i, lumen of the gut; ic, intestinal cells; m, muscularis. $\times 300$. **Fig. 3.** Fresh spores visualised by phase-contrast microscopy; $\times 2000$. **Fig. 4.** Spores seen by scanning electron microscopy;

arrows indicate the folds of the spore wall. $\times 20000$. **Fig. 5.** Xenoma wall (P), host tissues (H) and xenoma (X). hc, Host cytoplasm; sp, spore. Arrowheads indicate the position of the host-cell plasma membrane. $\times 16400$

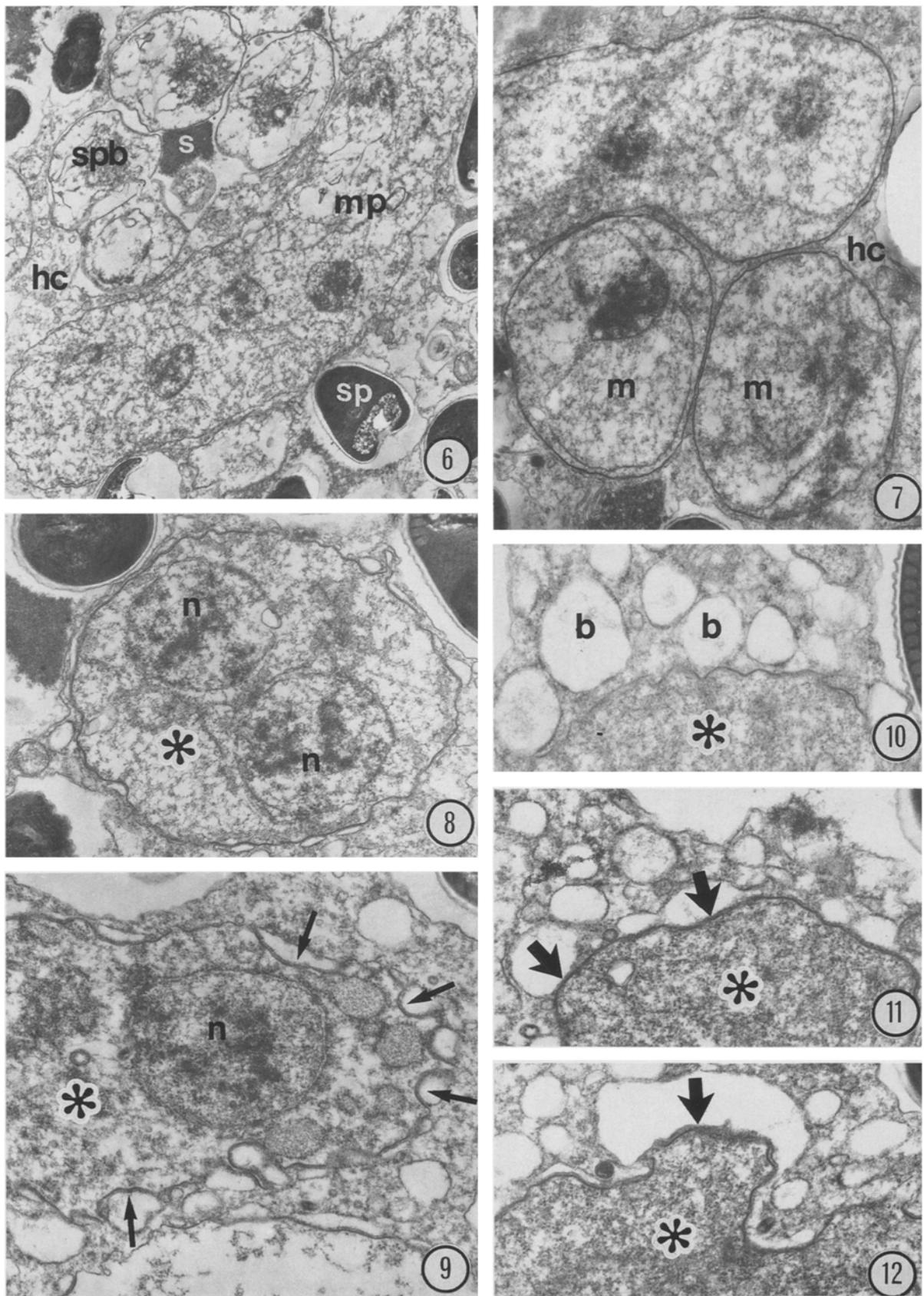


Fig. 6. Cylindrical merogonic plasmodium (*mp*) exhibiting five or more nuclei. *hc*, Host cytoplasm; *s*, secretions in the sporophorous vesicle; *sp*, spore; *spb*, sporoblast. $\times 8200$. **Fig. 7.** Spherical meronts (*m*) and host cytoplasm (*hc*). $\times 13600$. **Fig. 8.** Early sporont (*asterisk*) displaying two nuclei (*n*) before the formation of the sporophorous vesicle. $\times 16400$. **Fig. 9.** Formation of the sporophorous

vesicle around a sporont (*asterisk*) exhibiting one nucleus (*n*); arrows indicate the formation of blisters as isolated for the first time. $\times 20000$. **Figs. 10–12.** Details on the formation of the sporophorous vesicle. **Fig. 10.** Extrusion of blisters (*b*) around the sporont (*asterisk*). $\times 25000$. **Figs. 11, 12.** Progression of the phenomenon. $\times 25000$

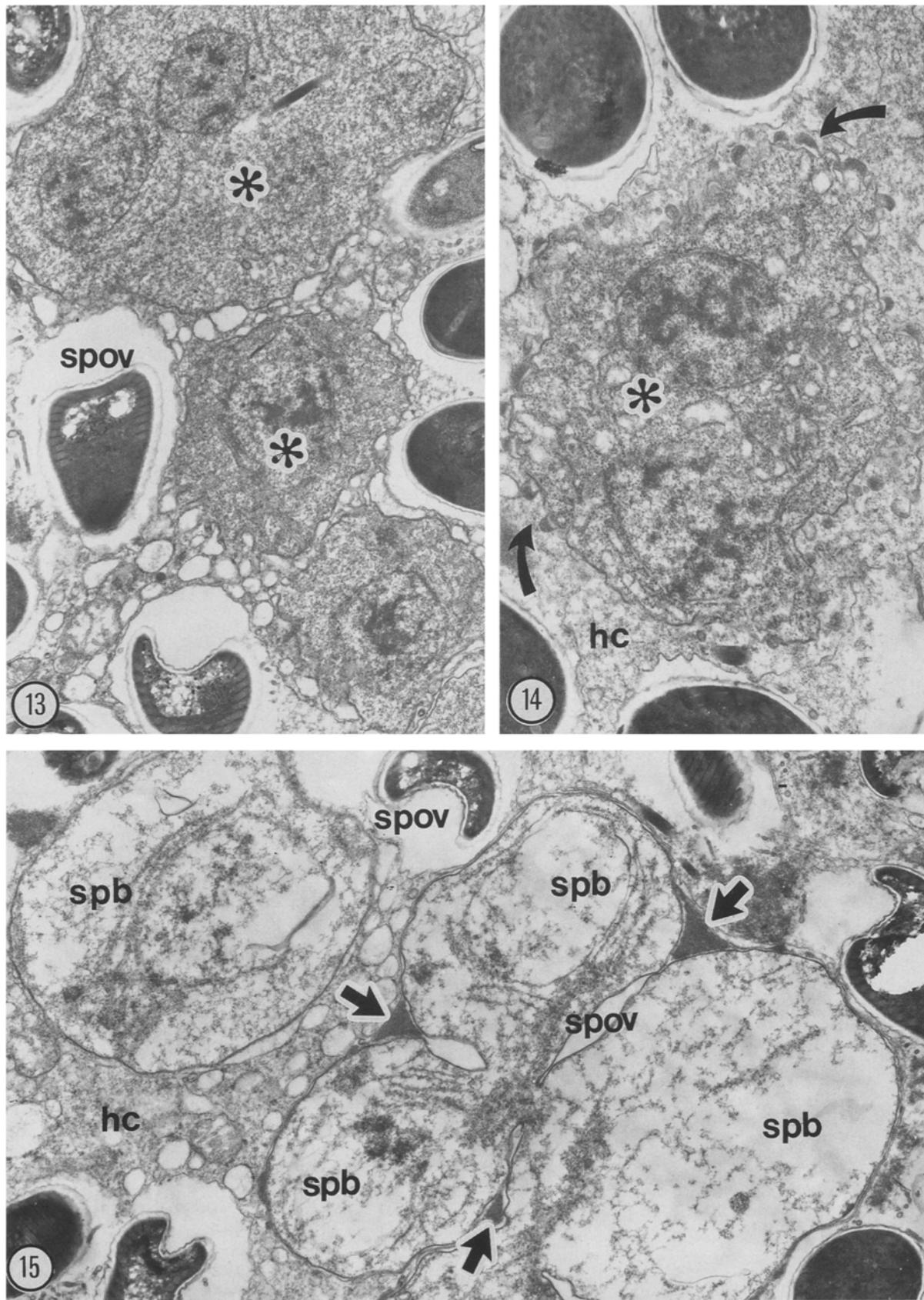
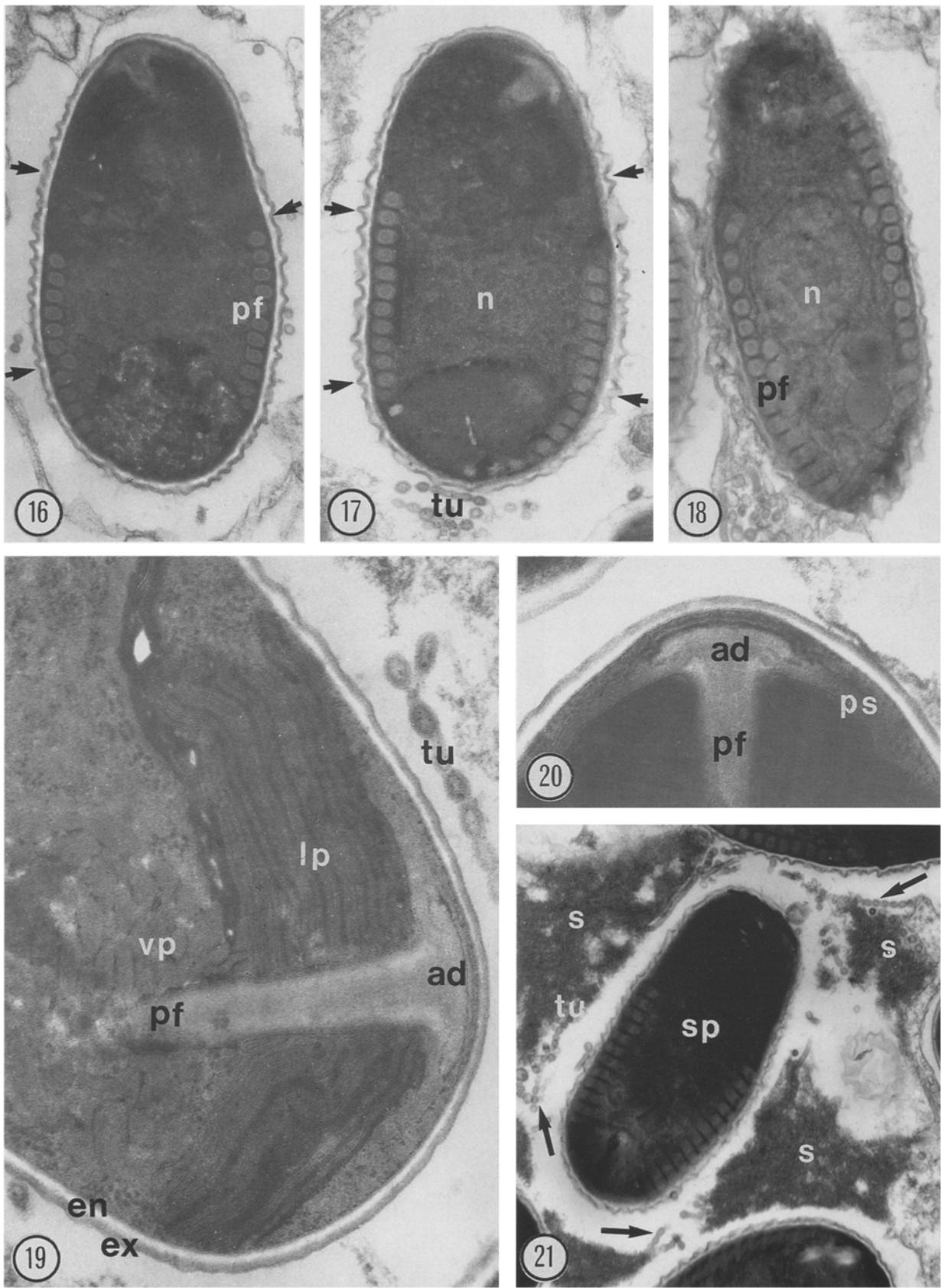


Fig. 13. Sporogonic plasmodia (asterisks) at the beginning of isolation by the extrusion of blisters. *spov*, Sporophorous vesicle. $\times 13600$. **Fig. 14.** Sporogonic plasmodium (asterisk); curved arrows indicate the hypothetic incorporation of amorphous electron-dense material in the sporophorous vesicle. $\times 20000$. **Fig. 15.** Sporogonic

rosette and the isolation of sporoblasts (*spb*) within a single sporophorous vesicle (*spov*). Secretions (arrows) occupy the spaces like wedges between the dividing sporoblasts. *hc*, Host cytoplasm. $\times 13600$



Figs. 16, 17. Two mature spores, showing a polar filament (*pf*), nucleus (*n*), ornamentation of the exospore in the form of more or less pronounced folds (*arrows*) and secretion of tubules (*tu*) in the sporophorous vesicle. $\times 28000$. **Fig. 18.** Larger abnormal spore exhibiting 17 turns of polar filament (*pf*). *n*, Nucleus. $\times 30000$. **Fig. 19.** Detail of the anterior part of a mature spore.

ad, Anchoring disc; *en*, endospore; *ex*, exospore; *lp*, lamellar polaroplast; *pf*, polar filament; *vp*, vesicular polaroplast; *tu*, tubules. $\times 80000$. **Fig. 20.** Anterior part of a spore. *ad*, Anchoring disc; *pf*, polar filament; *ps*, polar sac. $\times 60000$. **Fig. 21.** Spore (*sp*) at the beginning of its isolation in a secondary vacuole formed by tubules (*tu*, *arrows*) derived from dense secretions (*s*). $\times 20000$

two (Figs. 8, 14) to four or more nuclei (Fig. 13) were frequently encountered, indicating that sporogony is polysporoblastic. The sporogonial plasmodia formed more or less regular rosettes (Fig. 15), with four or six sporoblasts being isolated in a single sporophorous vesicle (Fig. 6).

Even before they individualised, the uninucleate sporoblasts (Figs. 6, 15) were easily recognisable by their low-density contents in which cytoplasm organelles were quite scattered. Polysporoblastic sporogony produced several sporoblasts, frequently six (Fig. 6), in the same sporophorous vesicle. The dense secretions increased in bulk and forced their way between the dividing sporoblasts like wedges (Fig. 15). During sporogenesis, tubular formations appeared to form from the dense secretions (Fig. 21). The tubules became more numerous and were distributed, often evenly, between the spores in the same sporophorous vesicle (Fig. 21). The tubules resolved in partitions that had isolated the spores. At the end of their development, the spores seemed to be isolated in a single sporophorous vesicle (Fig. 5), but was in fact a secondary phenomenon.

Mature spores were oval and uninucleate. Scanning electron microscopy demonstrated ornamentation in the form of more or less pronounced ridges (Fig. 4) that were more abundant in the posterior two-thirds of the spores and were clearly visible in the sections (Figs. 16, 17). The endospore and exospore were thinner at the anterior pole. Arising from an extremely elongated manubrial part, the isofilar polar filament (diameter, 120 nm) was coiled, exhibiting 11 or 12 turns (Fig. 16). Some slightly larger abnormal spores displayed 17 turns (Fig. 18). The polaroplast occurred in two parts: the lamellar polaroplast lying immediately below the anchoring disc and the polar sac and the vesicular polaroplast located posterior to the lamellar region (Fig. 19). The posterior vacuole consisted of granular material. The nucleus was clearly visible in the sporoplasm (Fig. 17).

Discussion

Systematic position of the Oreochromis niloticus microsporidian

The gut microsporidian of *O. niloticus* is characterised by isolated nuclei; merogonic stages that evolve in direct contact with the host-cell cytoplasm; multiple fission culminating in the formation of cylindrical, multinucleate plasmodia; sporogenous stages that are isolated in a sporophorous vesicle; and polysporoblastic sporogony. All of these features place this parasite in the genus *Loma*, created by Morrison and Sprague (1981a). In a previous report (Bekhti and Bouix 1985), the distinctive characteristics of *Loma* species have been presented in a recapitulatory table for *L. branchialis* (Morrison and Sprague 1981a), *L. salmonae* (Morrison and Sprague 1981b), *L. fontinalis* (Morrison and Sprague 1983) and *L. diplodae* (Bekhti and Bouix 1985). Another species, *L. dimorpha* (Loubès et al. 1984), has been found in Goobiidae along the Languedoc coast; its sporogony is di-

morphic, producing elongated or globular sporophorous vesicles. All of the systematic data have been covered in detail by Canning and Lom (1986).

The species described in *O. niloticus* can be distinguished from those described previously. This distinction is based on the following important characteristics:

1. The host fish is a member of a new family for the genus *Loma* (Cichlidae).
2. The geographical remoteness, since this is the first species described in sub-Saharan Africa.
3. The site in the host is strictly limited to the gut (sub-epithelial connective tissue); this is the second such case after *L. dimorpha* (Loubès et al. 1984).
4. The original ultrastructural features of the spore (particularly, the unusually thin endospore layer of the wall).

All of these points indicate that a new classification should be established for the microsporidian of *O. niloticus*. We propose the name *L. camerounensis* in reference to its country of origin, as follows:

L. camerounensis sp. nov.

Host: *O. niloticus* (Cichlidae)

Site: sub-epithelial connective tissue in the gut (mainly in the duodenum)

Xenoma: whitish, $70-340 \times 63-318 \mu\text{m}$

Characteristics:

1. The features of the genus
2. Spore shape: oval
3. Spore dimensions: $3.96 \pm 0.07 \times 2.16 \pm 0.04 \mu\text{m}$
4. Ultrastructure: polar filament exhibiting 11–12 spiral turns; a lamellar and a vesicular polaroplast; more or less distinct crest ornamentation; unusually thin endospore layer of the spore wall

Habitat: Melen fish-rearing ponds, Yaoundé, Cameroon

Type preparations (light and electron microscopy) are being kept at the General Biology Laboratory, Faculty of Sciences (Laboratoire de Biologie Générale, Faculté des Sciences), Yaoundé, Cameroon.

The sporophorous vesicle in the genus Loma

A study of *L. camerounensis* sporogony has enabled us to contribute to the knowledge about the nature of the sporophorous vesicle in the genus *Loma*. Uncertainty remains concerning the sporogonic phase: is it disporoblastic or polysporoblastic? Morrison and Sprague (1981a) have reported that the spores are isolated or grouped in pairs in *L. morhua* (= *L. branchialis*) and concluded the sporogony is disporoblastic. This finding was interpreted from the section plane, which nevertheless does not explain why most of the spores are isolated. There are two sporonts in a single sporophorous vesicle at the beginning of the sporogonic development of *L. salmonae* (Bekhti and Bouix 1985). Each of the two sporonts undergoes binary division, resulting in four sporoblasts in the same vesicle. Finally, Loubès et al. (1984) described cylindrical or morula-like plasmodia in *L. dimorpha*. The latter observations favour polysporoblastic

sporongony, and the present images of *L. camerounensis* confirmed this idea.

However, one problem remains: as several sporoblasts are enclosed in the same vesicle, how is it possible to account for the isolation of almost all of the spores at the end of sporogenesis? If the micrographs presented in previous descriptions of *L. branchialis* (Morrison and Sprague 1981a) and *L. salmonae* (Bekhti and Bouix 1985) are pertinent, our opinion is that a secondary mechanism following the formation of the sporophorous vesicle accounts for this feature. Two types of formation appear in the vesicle during the sporogonic stages: first, the dense, unstructured formations that are visible from sporoblastogenesis onwards, and subsequently, the tubular formations that sometimes form strings. Such formations have been reported in *L. branchialis* (Morrison and Sprague 1981a), *L. salmonae* (Morrison and Sprague 1981b; Bekhti and Bouix 1985), *L. dimorpha* (Loubès et al. 1984) and *L. diplodae* (Bekhti and Bouix 1985). In *L. camerounensis*, when the sporoblasts separate, the dense secretions are driven like wedges between the dividing elements. The tubules, which appear to derive from dense secretions, multiply and spread evenly between the spores (this phenomenon is observed especially at the end of sporogenesis) and then compartmentalise the initial sporophorous vesicle. The spores are finally isolated individually in secondary vesicles. Some images of *L. branchialis* (Fig. 3) presented by Morrison and Sprague (1981a) and of *L. dimorpha* (Figs. 7, 11) presented by Loubès et al. (1984) suggest that the phenomenon must be more widespread among the various species. The purpose of these intra-vacuolar formations has not been established with any certainty. Morrison and Sprague (1981a) consider that the tubules have a metabolic function, serving as a link between the developing parasite and the host-cell cytoplasm. The hypothesis presented herein does not rule out this possibility.

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