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On the Cytology and Development of *Loma boopsi* n. sp. (Microspora, Glugeidae), Parasite of *Boops boops* (Pisces, Teleostei, Sparidae) from the Coasts of Senegal

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Summary: *Loma boopsi* n. sp. was found in the liver, intestine and pyloric caeca of *Boops boops* inhabiting the coasts of Senegal. Its ultrastructural characteristics were elucidated. All developmental stages had isolated nuclei and occupied the xenoma without any particular stratification. The merogonial stages were in direct contact with the host-cell cytoplasm. The merogonial plasmodium divided by plasmotomy. Sporogony occurred in sporophorous vesicles and the sporogonial plasmodium divided by plasmotomy. Two morphological types of spores were produced in the same sporophorous vesicle: ovoid spores measuring 3.70 × 2.40 µm and elongate, slightly incurvated spores which were 4.80 × 2.40 µm. They had a thick envelope (about 70 nm), a polaroplast with an anterior lamellar part and a posterior vesicular part, an isofilar polar filament displaying 12–14 (ovoid spores) or 16–18 (elongate spores) spiral turns in a single row of coils and a large posterior vacuole.

Key Words: Microsporidia; *Loma boopsi;* Ultrastructure, Fish; *Boops boops;* Senegalese coasts.

Introduction

Numerous microsporidia have been described in fish (CANNING & LOM 1986; SAKITI & BOUIX 1987; CHILMONCZYK et al. 1991; FAYE et al. 1991; FOMENA et al. 1992). They were identified using ultrastructural characteristics and the relationship between developmental stages and host-cell cytoplasm. Some microsporidia have been described in fish from Senegalese coasts (TOGUEBAYE et al. 1989; FAYE 1992; FAYE et al. 1990, 1991, 1994b). The species studied in this paper was assigned to the genus *Nosemoides* (FAYE et al. 1994a). Now, we know more about its characteristics and we place it in the genus *Loma* MORRISON and SPRAGUE, 1981.

Material and Methods

Fish were caught from the Senegalese coasts (Senegal, West Africa). A total of 132 specimens of *Boops boops* were examined. 16 were parasitized (prevalence 12.12%).

Light microscopy: Smears of fresh tissues were examined by light microscope to look for microsporidian spores. Measurements were made with an eye piece micrometer. Tissues were fixed in Carnoy's fluid and embedded in paraffin. The sections were stained with Heidenhain's azan. Semithin sections were stained with Toluidine blue.

Transmission electron microscopy: Fragments of infected tissue were fixed for 12–24 h at 4°C with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and then post-fixed for 1 h with 1% osmium tetroxide in the same buffer. After dehydration in ethanol and propylene

oxide, the fragments were embedded in Spurr's resin and sectioned with a Porter Blum MT1 microtome. Sections were stained with uranyl acetate and lead citrate. They were then observed using Jeol 100 CX II (U.C.A.D., Dakar) and Jeol 200 CX (U.M.II, Montpellier) microscopes.

Scanning electron microscopy: Spores were smeared on circular cover glasses, lightly air-dried and fixed for 12–24 h at 4°C with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). After washing in buffer and CO_2 critical point drying, the smears were covered with metallic gold and palladium and observed using. Jeol JSM 35 scanning microscope (U.C.A.D., Dakar).

Abbreviations:

ad – anchoring disc
c – cinetic center
en – endospore
er – endoplasmic reticulum
es – elongate spore
ex – exospore
ga – Golgi apparatus
ge – gut epithelium
gw – gut wall
lp – lamellar polaroplast
pf – polar filament
pm – plasma membrane
n – nucleus
os – ovoid spore
pv – posterior vacuole
s – spore
sv – sporophorous vesicle
vp – vesicular polaroplast
x – xenoma
xw – xenoma wall

Results

Location and structure of xenomas

The microsporidian formed small (1–1.5 mm in diameter), whitish xenomas (Fig. 1A) generally in the liver, and in the epithelium of the intestine (Fig. 1B) and the pyloric caeca.

The xenoma wall had an outer layer composed of conjunctive fibres (700 nm in thickness) and an inner one which is the plasma membrane of the parasitized host-cell (Fig. 1C). The different developmental stages of the microsporidian were mixed within the cyst (Fig. 1D).

Developmental cycle and ultrastructure

Merogonial stages were in direct contact with the host cell cytoplasm (Fig. 1E). They contained several isolated nuclei and were bounded by a single plasma membrane. Sometimes the stages were enveloped by a host endoplasmic reticulum. The cytoplasm contained numerous free ribosomes, some endoplasmic reticulum and vacuoles. The merogonial plasmodium divided by plasmotomy. Dividing nuclei showed cinetic centers consisting of electron dense stacked plaques in a depression of the nuclear envelope.

The initial phase of sporogony was marked by the formation of the sporophorous vesicle. The envelope of the sporophorous vesicle was formed by the generation of the blisters at the surface of the sporont (Figs. 1F, 2A and B). At the same time, the plasma membrane (the sporont wall) was thickened (Figs. 2C and D). Abundant tubular material was present in the cavity of sporophorous vesicles (Fig. 2H).

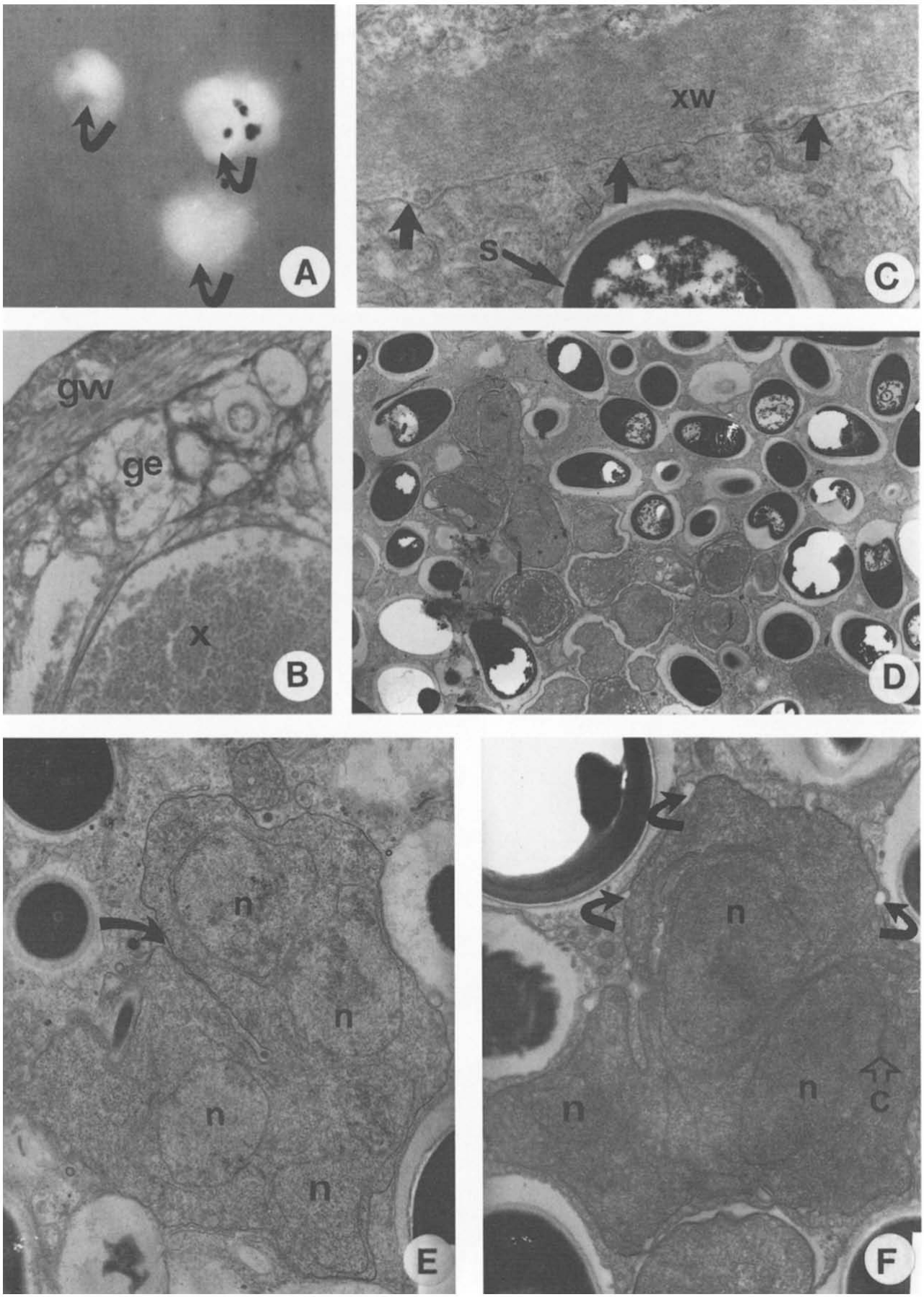
The sporonts and their successive products, sporoblasts and spores, occurred in sporophorous vesicles. The sporonts were surrounded by a thick wall (Fig. 2E). Endoplasmic reticulum was abundant in their cytoplasm sometimes forming a proeminant system of saccules around the nucleus (Figs. 2E and F). Dilated saccules formed electron lucent vacuoles (Fig. 2F). Sporonts were first uninucleate. Nuclear divisions produced sporogonial plasmodia with isolated nuclei which divided by plasmotomy (Fig. 2G). The sporogony is polysporoblastic.

Young sporoblasts were more or less spherical and surrounded by an undulated wall (Fig. 2H). The cytoplasm was highly vacuolated. Older sporoblasts were elongated and were characterized by the presence of the primordium of the polar filament (Figs. 3A and B). In transverse sections the young polar filament showed different zones: a central dense axis, a clear intermediate layer and two thin dense layers separated by a less dense zone and surrounded by an external unit membrane (Fig. 3C). The diameter of the polar filament was reduced in the last coils due to narrowing of the intermediate clear layer (Fig. 3C).

Two morphological types of mature spores were produced in the same sporophorous vesicle: ovoid spores measuring 3.70 × 2.40 (2.70–4 × 2–2.70 µm) and elongate, slightly incurvated spores measuring 4.80 × 2.40 (4.30–6.40 × 2–2.70 µm) (Figs. 3D and E). The polar filament was isofilar (about 150 nm in diameter) but the last coil was more narrow (less than 100 nm) (Fig. 4A). The filament made 12–14 (ovoid spores) or 16–18 turns

Fig. 1. Xenomas and meronts of *Loma boopsi* n. sp.

A. Three xenomas (curved arrows) on the liver (×30). **B.** Xenoma in the epithelium of the proximal gut (×120). **C.** Organization of the xenoma wall. Note the position of the host-cell plasma membrane (arrows) (×28 400). **D.** Central part of a xenoma. The different developmental stages are mixed within the xenoma (×3 900). **E.** Merogonial plasmodia dividing by plasmotomy (arrow) (×12 800). **F.** Merogonial plasmodia maturing to sporonts. The curved arrows indicate the generation of the blisters at the surface of the sporont forming the sporophorous vesicle (×16 000).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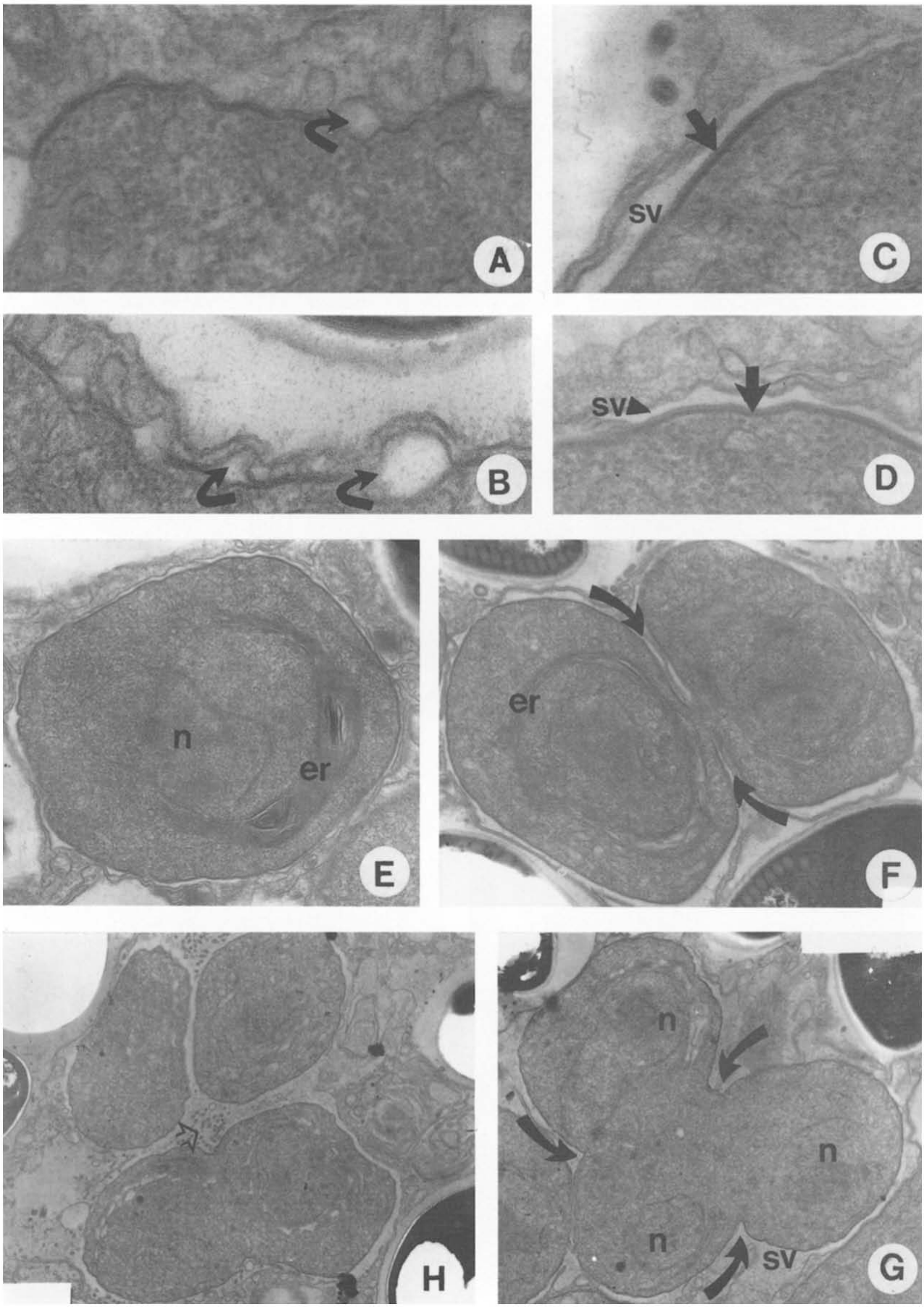
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Table 1. Some of comparative characters of *Loma* species.

Microsporidia	Hosts (site of infection)	Characters of spores (dimensions in µm)
Loma branchialis (= *Loma morhua)* MORRISON & SPRAGUE, 1981	*Melanogrammus aeglefinus, Gadus callarias, Gadus morhua* (gills, pseudobranch, digestive tract, liver, spleen, kidney)	6.3 × 3.5 (NEMECZEK 1911) ovoid to elongate-ovoid, 4.8 (4.1–6.5) × 2.3 (2–2.6) (LOM & LAIRD 1976) 5.7–6.6 × 3.5–4.2 (FANTHAM et. al. 1941) elongate-ovoid, 4.2 × 2.0 (fixed) polar filament: 16–17 or 16–19 turns (MORRISON & SPRAGUE 1981a, b)
Loma salmonae MORRISON & SPRAGUE, 1981	*Salmo gairdneri Oncorhynchus nerka, Oncorhynchus kisutch* (gills, heart, spleen, kidney, pseudobranch)	pyriform, 4.5 (4.2–5.3) × 2.2 (1.7–2.8) (fixed) (PUTZ et al. 1965) 3.7 (3.1–4.3) × 2.2 (1.6–2.4) (fixed) polar filament: 12–14 turns (MORRISON & SPRAGUE 1981c) 7.5 × 2.4 (WALES & WOLF 1955) 4.26 (3.05–5.93) × 2.07 (1.63–2.58) (fixed) polar filament: 11–12 turns (BEKHTI & BOUIX 1985) 4.4 (4–5.6) × 2.3 (2–2.4) polar filament: 14–17 turns (KENT et al. 1989)
Loma fontinalis MORRISON & SPRAGUE, 1983	*Salvelinus fontinalis* (gills)	elongate oval, 3.7 × 2.2 (fixed) polar filament: 14–15 turns (MORRISON & SPRAGUE 1983)
Loma diplodae BEKHTI & BOUIX, 1984	*Diplodus sargus* (gills)	oval, 4.17 (2.97–5.95) × 2.22 (2.12–2.97) polar filament: 17–18 turns (BEKHTI & BOUIX 1984)
Loma dimorpha LOUBÈS, MAURAND, GASC, DE BURON & BARRAL, 1984	*Gobius niger, Zosterisessor ophiocephalus* (digestive tract)	oval, 4.5 × 1.8–2 polar filament: 13–15 turns (LOUBÈS et al. 1984)
Loma trichiuri SANDEEP & KALAVATI, 1985	*Trichiurus salva* (gills)	pyriform, 3.0 (2.8–3.2) × 2.0 (1.8–2.4) (SANDEEP & KALAVATI 1985)
Loma camerounensis FOMENA, COSTE & BOUIX, 1992	*Oreochromis niloticus* (digestive tract)	oval, 3.96 ± 0.07 × 2.16 ± 0.04 polar filament: 11–12 turns (FOMENA et al. 1992)
Loma boopsi n. sp.	*Boops boops* (liver, digestive tract)	ovoid, 3.7 (2.7–4) × 2.4 (2–2.7) or elongate, 4.8 (4.3–6.4) × 2.4 (2–2.7); polar filament: 12–14 (ovoid) or 16–18 (elongate) turns

Fig. 2. Formation of the sporophorous vesicle and the sporogony of *Loma boopsi.*

A–B. Formation of the sporophorous vesicle by the generation of blisters (curved and right arrows) at the surface of the sporont (A, ×60 600; B, ×76 000). **C–D.** Details of the sporophorous vesicle and of the thickening of the sporont wall (arrows) (C, ×77 200; D, × 59 000). **E.** A young sporont in the sporophorous vesicle with one nucleus and well developed endoplasmic reticulum (×19 000): **F.** Sporont with two isolated nuclei in the sporophorous vesicle (×17 100). **G.** Plasmotomy of the sporogonial plasmodium in the sporophorous vesicle (×10 700). **H.** Isolation of sporoblasts within the sporophorous vesicle. Note the presence of tubular material in the cavity of the sporophorous vesicle (× 8500).

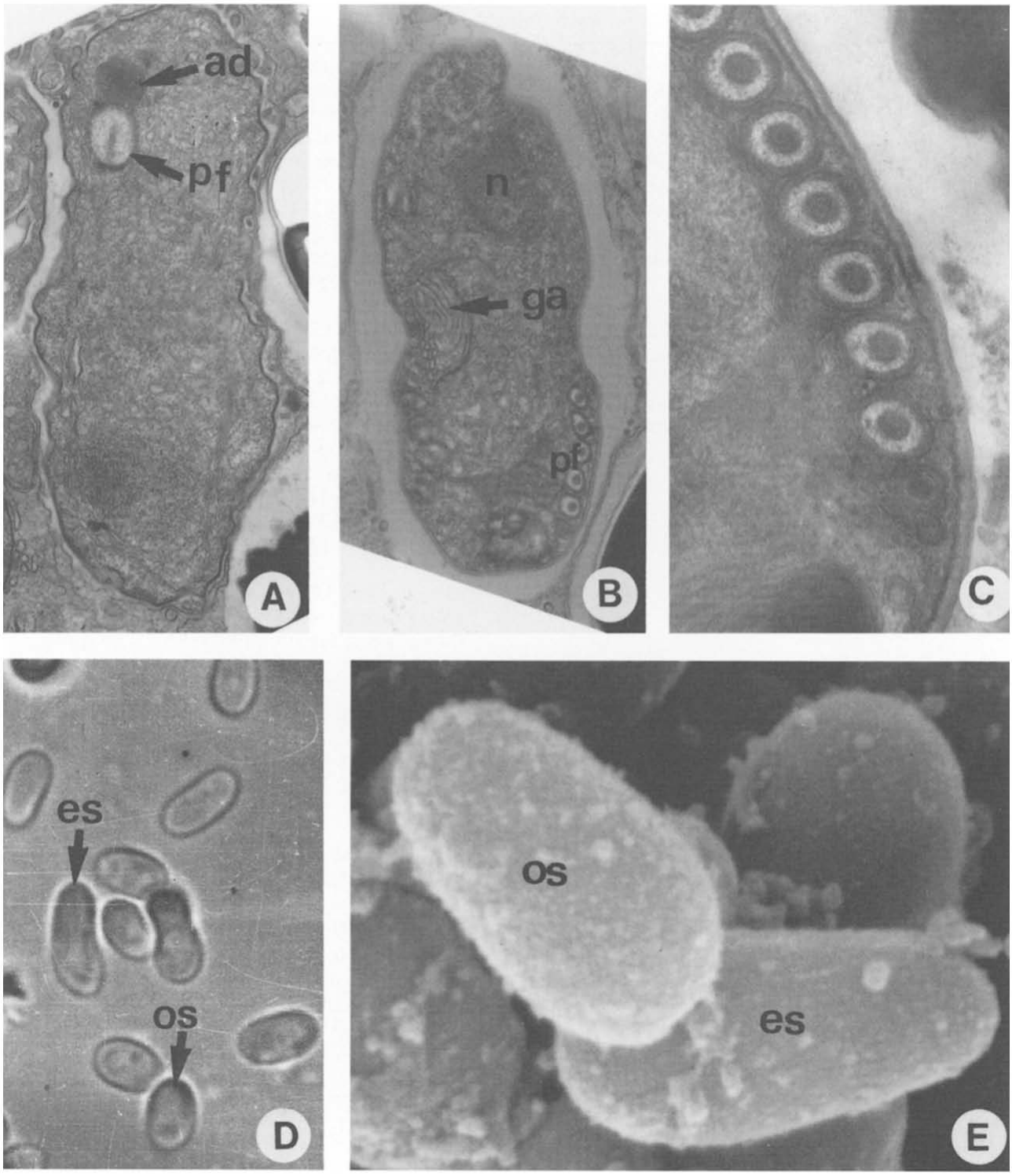

Fig. 3. Sporoblasts and spores of *Loma boopsi.*

A. Sporoblast showing early stage of polar filament formation (×19 400). **B.** Longitudinal section of a sporoblast showing the coiled part of the polar filament (×22 400). **C.** Detail of a young transversely sectioned polar filament (×64 000). **D.** Unstained smears showing the two morphological types of spores (×3 100). **E.** Scanning electron micrograph of mature spores (× 21 000).

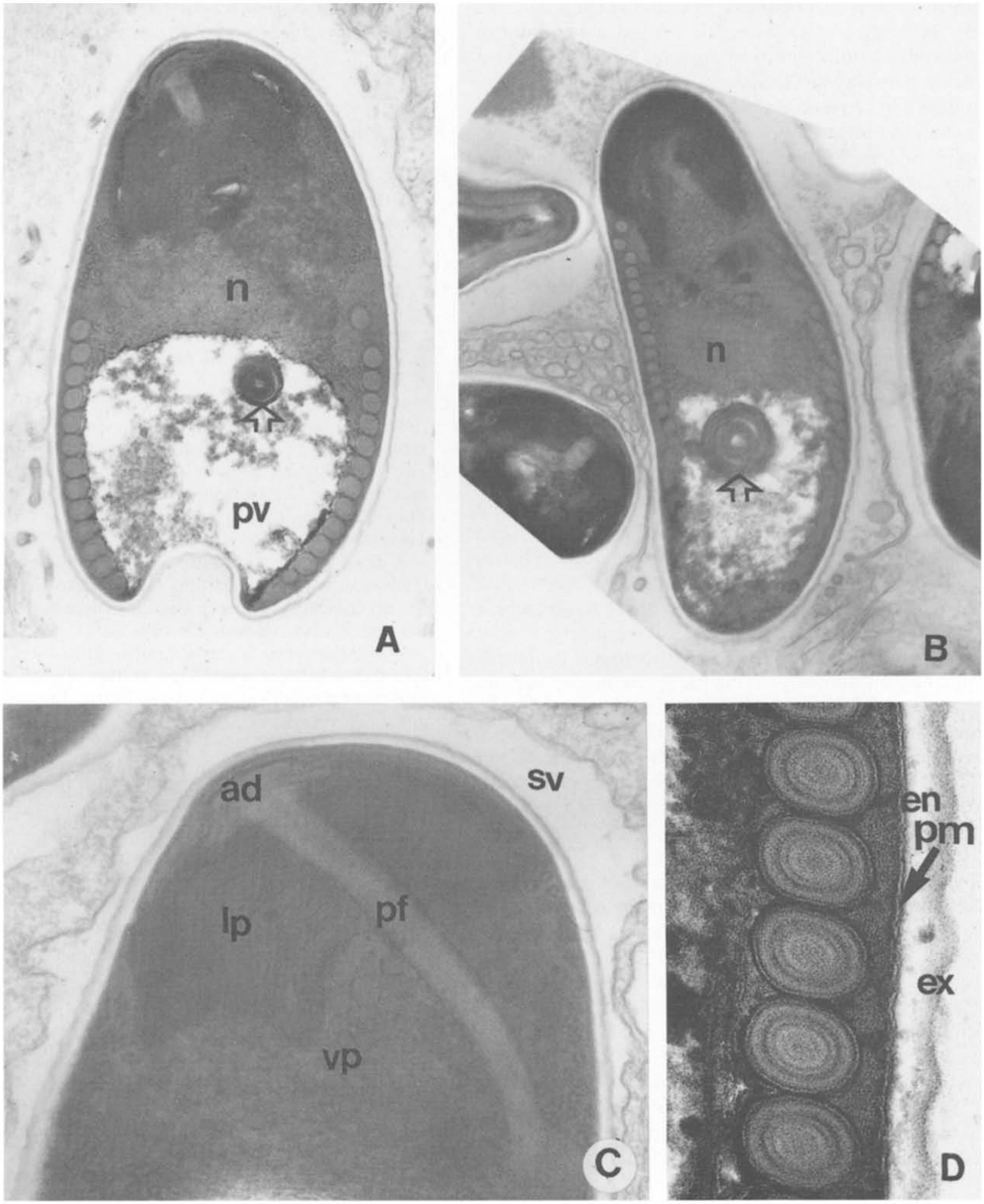

Fig. 4. Spores of *Loma boopsi.*

A–B. Longitudinal sections of the two types of mature spores. Note the presence of an electron-dense residue (arrow) in the posterior vacuole (A, ×22 000; B, ×21 000). **C.** Details of the anterior part of mature spore (×48 000). **D.** Transversely sectioned coils of the mature polar filament (×135 000).

(elongate spores) positioned in one row (Figs. 4A and B). In transverse sections, the mature polar filament showed a distinct stratification, with layers of different electron density and thickness: a central dense axis surrounded by an electron lucent layer, a thin dense layer, a thin clear layer, two peripheral thin dense layers separated by an electron lucent layer and an external unit membrane (Fig. 4D). The polaroplast had two distinct regions: a lamellar anterior part and a posterior vesicular part with polyhedral vesicles (about 100 nm in diameter) (Fig. 4C). The wall of mature spores was classical, consisting of a plasma membrane, a translucent endospore which was reduced anteriorly and a slightly corrugated and electron-dense exospore (Fig. 4D). Scanning electron microscopy revealed that the surface of the spore was rough (Fig. 3E).

Discussion

The microsporidian studied herein formed small and whitish xenomas in the liver, intestine and pyloric caeca of its host. There is no doubt that this microsporidian is a species of the genus *Loma* MORRISON and SPRAGUE, 1981. The characteristic features of this parasite, which are isolated nuclei in all stages of development, formation of blisters at the surface of the sporont at the beginning of the sporogony giving the sporophorous vesicle, the presence of tubular products in the sporophorous vesicle and the division of merogonial and sporogonial plasmodia by plasmotomy, were previously described in other *Loma* species (MORRISON & SPRAGUE 1981a, b, c, 1983; LOUBÈS et al. 1984; BEKHTI & BOUIX 1985; CANNING & LOM 1986; CANNING 1989; KENT et al. 1989; FOMENA et al. 1992; SPRAGUE et al. 1992).

To our knowledge the genus comprises seven species: *L. branchialis (= L. morhua), L. salmonae, L. fontinalis, L. diplodae, L. dimorpha, L. trichiuri* and *L. camerounensis* (MORRISON & SPRAGUE 1981a, b, c, 1983; BEKHTI & BOUIX 1985; LOUBÈS et al. 1984; SANDEEP & KALAVATI 1985; FOMENA et al. 1992). The characteristics of these species are presented in Table 1.

The species studied by us is different from these seven species. The obvious difference concerns the two types of spores produced. Only one type of spores is produced in the sporophorous vesicle by the previously described *Loma* species. The other discriminating characteristics concern the hosts, the site of infection, the characteristics of the spores (see Table 1). We can add that the polar filament of *L. diplodae* makes 17–18 turns disposed in two rows (BEKHTI & BOUIX 1985), the vesicular polaroplast of *L. diplodae* and *L. dimorpha* consists of very small vesicles (LOUBÈS et al. 1984; BEKHTI & BOUIX 1985), and the endospore of *L. camerounensis* is very thin (FOMENA et al. 1992). We conclude that the microsporidian of *Boops boops* is a new species and we propose the name *Loma boopsi,* after the generic name of its host.

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