

Ultrastructure of *Unikaryon nomimoscolexi* n. sp. (Microsporida, Unikaryonidae), a parasite of *Nomimoscolex* sp. (Cestoda, Proteocephalidea) from the gut of *Clarotes laticeps* (Pisces, Teleostei, Bagridae)

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ABSTRACT: *Unikaryon nomimoscolexi* n. sp. was examined by electron microscopy from material collected in Senegal (West Africa). It parasitizes adult of *Nomimoscolex* sp. (Cestoda) from the gut of the freshwater fish *Clarotes laticeps*. The microsporidium is parasitic in parenchymal cells. All live cycle stages have isolated nuclei. Merogony and sporogony take place in direct contact with the host cell cytoplasm and ultimately give rise to unikaryotic sporoblasts and spores. The sporogony is disporoblastic. The spores are ovoid and measure, in thin sections, $3.43 \pm 0.4 \times 1.51 \pm 0.18 \mu\text{m}$. The polaroplast has an anterior lamellar part and a posterior vesicular part. The polar tube is isofilar and is arranged in 6 to 8 coils.

KEY WORDS: *Unikaryon nomimoscolexi* · Microsporida · Hyperparasite · *Nomimoscolex* sp. · Cestoda · *Clarotes laticeps* · Pisces · Freshwater · Senegal

INTRODUCTION

Nomimoscolex sp. (Cestoda, Proteocephalidae) is an intestinal parasite of *Clarotes laticeps* (Teleostei, Bagridae) from freshwater of Senegal. While studying this parasite, we found that one adult was hyperparasitized by a microsporidium. In this report, this hyperparasite is described at the electron microscope level.

MATERIAL AND METHODS

A total of 23 specimens of *Clarotes laticeps* (Rüppell, 1829), a freshwater fish, were collected from Lake Guiers in Senegal and examined. *Nomimoscolex* sp. were removed from the intestine. A microsporidium

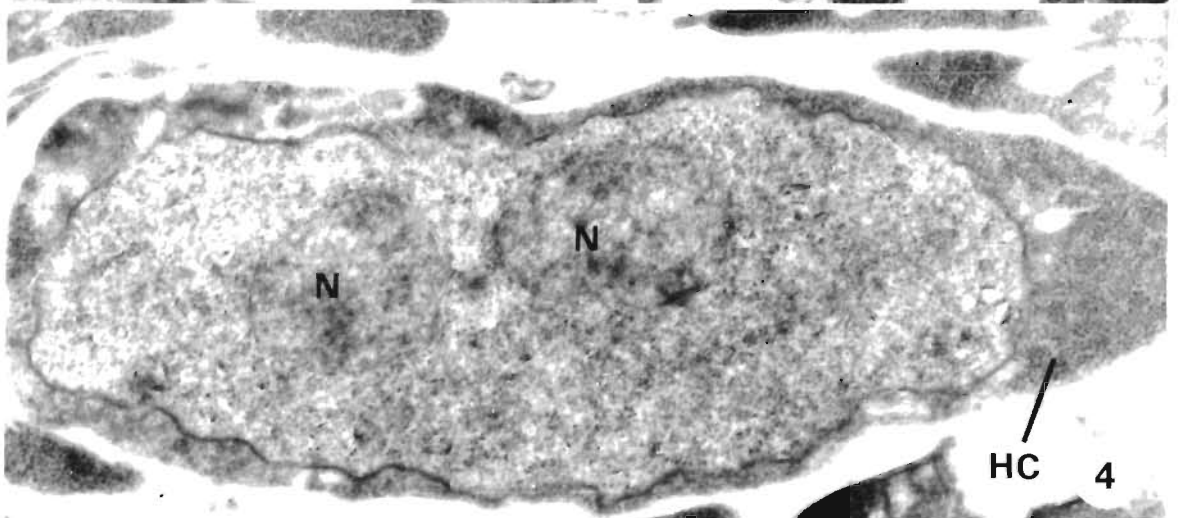
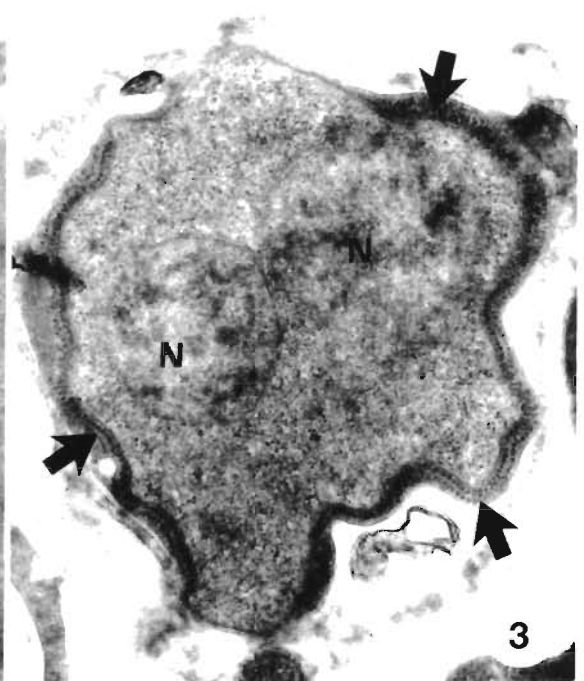
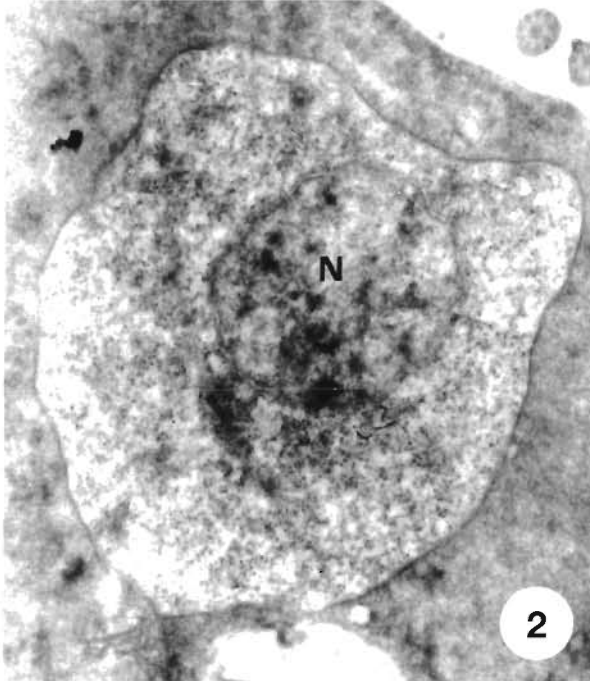
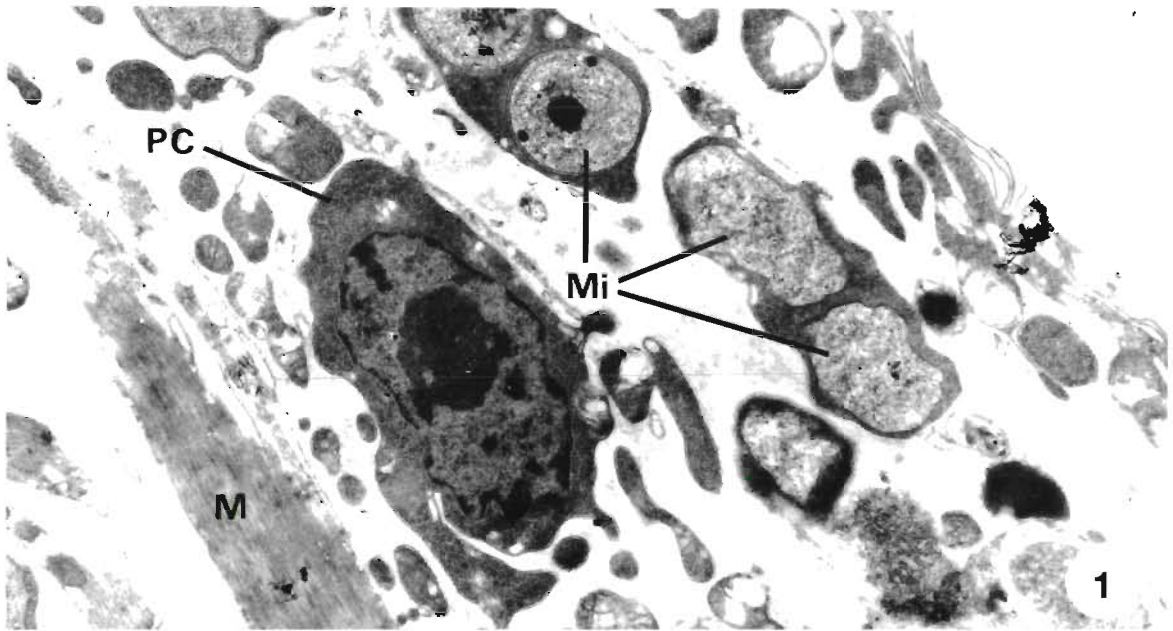
was discovered during ultrastructural study of the spermiogenesis of this cestode. This hyperparasite is described at the electron microscope level.

Small pieces of proglottids were fixed overnight in 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4°C. After washing in buffer and post-fixation in 1% (w/v) osmium tetroxide in cacodylate buffer for 1 h at 4°C, the pieces were washed, then dehydrated in ethanol, cleared in propylene oxide and embedded in spurr resin. Ultrathin sections were cut on a Reichert-Jung ultramicrotome, stained with uranyl acetate and lead citrate and examined using a JEOL 100 CX II.

RESULTS

Stages of the microsporidium were found in the parenchymal cells (Fig. 1).

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Figs. 1 to 4. Fig. 1. Ultrathin section of a parasitized zone of the host *Nomimoscolex* sp. parenchymal cells. M: muscle; Mi: microsporidium; PC: parenchymal cell ($\times 9500$). Fig. 2. Uninucleate meront of the microsporidium. N: nucleus ($\times 20\,000$). Fig. 3. Rounded binucleate meront. Note that the ribosomes of the host cell cytoplasm organise into bands (arrows) and surround the plasma membrane of the meront. N: nucleus ($\times 20\,000$). Fig. 4. Elongate binucleate meront. HC: host cell; N: nucleus ($\times 15\,000$)

Meronts are uninucleate (Fig. 2) or binucleate (Figs. 3 & 4). They are bounded by a thin surface membrane in direct contact with the host cell cytoplasm. Their cytoplasm contains numerous ribosomes and some cisternae of endoplasmic reticulum. The nuclei are small, occupying a third of the width of the cells. Uninucleate meronts are spherical cells with irregular contours. Binucleate meronts are division stages; they are rounded with irregular contours (Fig. 3) or elongate (Fig. 4).

Sporonts (Fig. 5) differ from the meronts in possessing a thick electron-dense coat around the plasma membrane and a cytoplasm with a low ribosome content. Early sporonts are uninucleate, irregular or rounded but become binucleate and elongate (Fig. 5) before division into sporoblasts. Sporonts are in direct contact with the host cell cytoplasm.

Sporoblasts (Fig. 6) are produced by sporonts in pairs and differ from earlier stages in having a thick wall (100 nm) consisting of 2 electron-dense layers separated by an electron-lucent layer. The cytoplasm contains some vacuoles. Sporoblasts become elongate, develop 6 to 8 coils of polar filament (Fig. 7) and an anchoring disc (Fig. 8). They are uninucleate and in direct contact with the host cell cytoplasm.

Mature spores are ovoid, uninucleate and in direct contact with the host cell cytoplasm (Figs. 9 & 10). A thin electron-dense exospore overlies the electron-

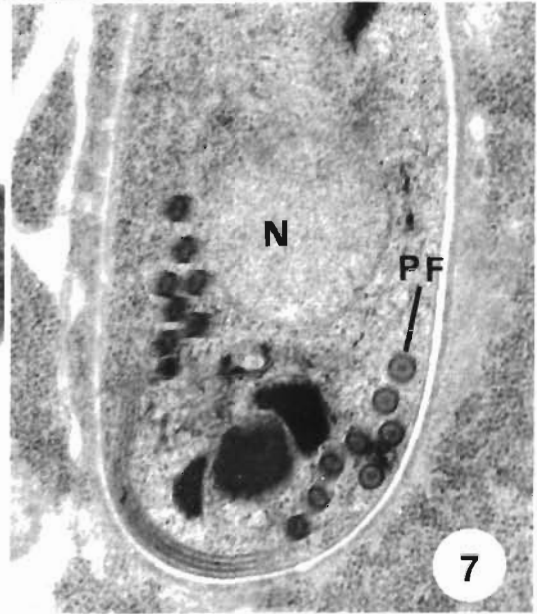
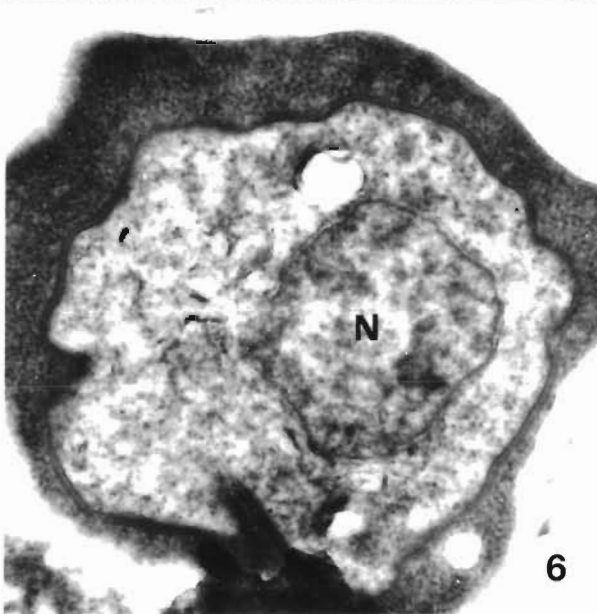
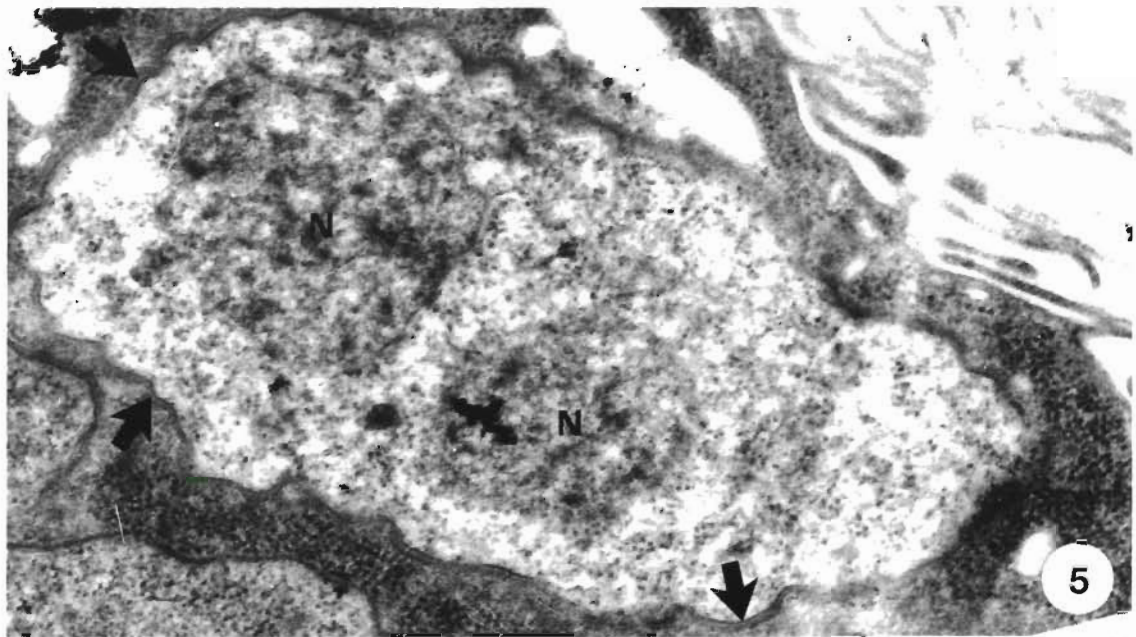
lucent endospore, which is about 150 nm in thickness. At the posterior end is a large vacuole containing electron-dense material (Fig. 9). At the anterior end is the anchoring disc which is eccentric in position (Figs. 10 & 11). The polar tube follows in an oblique course from the anchoring disc through the polaroplast (Fig. 10). It is isofilar and has 6 to 8 coils arranged in a single or 2 layers beneath the spore wall (Fig. 9). The polaroplast consists of an anterior region of closely and electron-dense packed lamellae and a posterior region consisting of numerous small vesicles (Figs. 9 to 11). In thin sections, calculated spore dimensions are $3.43 \pm 0.2 \times 1.51 \pm 0.18 \mu\text{m}$ ($N = 21$).

DISCUSSION

The microsporidium described herein belongs to the genus *Unikaryon* Canning, Lai & Lie, 1974. The essential characters of this genus are disporoblastic sporogony, nuclei unpaired in all stages and development in direct contact with the host cell cytoplasm (Sprague et al. 1992). Since 1974, 11 species have been described and placed in this genus or have been transferred into it: *U. piriformis* Canning, Lai & Lie, 1974; *U. legeri* (Dolfus, 1912) Canning & Nicholas, 1974; *U. allocreadii* Canning & Madhavi, 1977; *U. exiguum* Codreanu-

Table 1. Characteristics of *Unikaryon* spp. of platyhelminths

Species	Hosts	Development stages	Source
<i>U. piriformis</i>	<i>Echinoparyphium hystricosum</i> (Trematode from aquatic gastropod)	Spores: pyriform, $3.8 \times 2.7 \mu\text{m}$ (fresh); $3.4 \times 2.2 \mu\text{m}$ (fixed) Polar filament: length 150 μm	Canning et al. (1974)
<i>U. legeri</i>	<i>Meigymnophallus minutus</i> (Trematode from lamellibranch)	Spores: ovoid, $3.03 \pm 0.30 \times 1.76 \pm 0.02 \mu\text{m}$ (fresh); $2.9 \pm 0.13 \times 1.66 \pm 0.24 \mu\text{m}$ (fixed) Polar filament: 6–6.5 coils; length 42–49 μm Polaroplast: lamellar with 2 parts Presence of a sporophorous vesicle	Canning & Nicholas (1974); Azevedo & Canning (1987)
<i>U. allocreadii</i>	<i>Allocreadium fasciatusi</i> (Trematode from freshwater fish)	Spores: ovoid, slightly asymmetrical with one side flatter than the other, $3.5 \pm 0.3 \times 2.7 \pm 0.2 \mu\text{m}$ (fresh)	Canning & Madhavi (1977)
<i>U. slaptonleyi</i>	<i>Echinoparyphium recurratum</i> (Trematode from aquatic gastropod)	Spores pyriform, $5.01 \pm 0.2 \times 2.8 \pm 0.1 \mu\text{m}$ (fresh); $4.6 \pm 0.3 \times 2.7 \pm 0.2 \mu\text{m}$ (fixed) Polar filament: 17–21 coils; length 145 μm	Canning et al. (1983)
<i>U. nomimoscolexi</i> n. sp.	<i>Nomimoscolex</i> sp. (Cestode from freshwater fish)	Spores ovoid: $3.43 \pm 0.21 \times 1.51 \pm 0.18 \mu\text{m}$ (fixed) Polar filament : 6–8 coils Polaroplast: lamellar and vesicular	Present paper

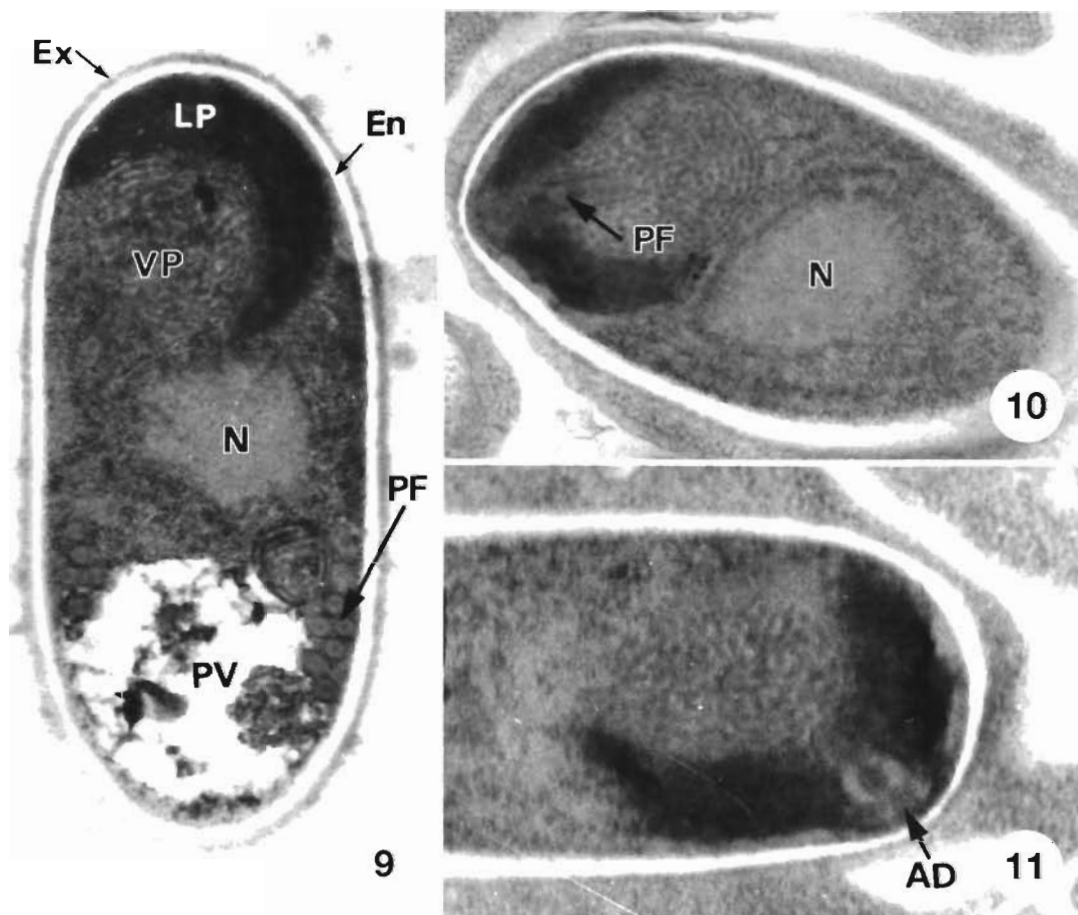


Figs. 5 to 8. Fig. 5. *Unikaryon nomimoscolexi* n. sp. Binucleate sporont with a thick wall (arrows). N: nucleus ($\times 20\,000$). Fig. 6. Sporoblast. N: nucleus ($\times 20\,000$). Fig. 7. Immature spore showing the well-formed polar tube (PF). N: nucleus ($\times 25\,000$). Fig. 8. Immature spore showing the anchoring disc (AD) of the developing polar tube. N: nucleus ($\times 25\,000$)

Balcescu, 1978; *U. minutum* Knell & Allen, 1978; *U. mytilicolae* Durfort, Vallmitjana & Vivarès, 1980; *U. slaptonleyi* Canning, Barker, Hammond & Nicholas, 1983; *U. bouixi* Toguebaye & Marchand, 1983; *U. matteii* Toguebaye & Marchand, 1984; *U. euzeti* Toguebaye & Marchand, 1988; and *U. nisotrae* Toguebaye & Marchand, 1989. *U. arachnicolum* Codreanu-Balcescu, Codreanu & Traciuc, 1981 was transferred to the genus *Oligosporidium* (see Codreanu-Balcescu et al. 1981). Of these 11 species, only *U. piriformis*, *U. legeri*, *U. allocreadii* and *U. slaptonleyi* are parasites of platyhelminths (Canning et al. 1974, 1983, Canning & Madhavi 1977, Sprague 1977, Azevedo & Canning 1987). Comparison is only valid with these 4 species (Table 1) because *U. exiguum* and *U. mytilicolae* are found in crustaceans (Codreanu-Balcescu 1978, Durfort et al.

1980) and *U. minutum*, *U. bouixi*, *U. matteii*, *U. euzeti* and *U. nisotrae* parasitize coleopteran insects (Knell & Allen 1978, Toguebaye & Marchand 1983, 1984, 1988, 1989). Table 1 clearly shows that there are numerous discriminating characters for the species described here, i.e. host, environment and structure of spores.

A few microsporidia have been reported as parasites of cestodes (Sprague 1977), but of these only *Nosema helminthorum* Moniez, 1887 can be compared with the species described in this paper. *N. helminthorum*, parasitic in the tapeworm *Moniezia expansa* (Rudolphi, 1810), has 2 cycles of development corresponding to those of the genera *Unikaryon* and *Nosema* (see Canning & Gunn 1984). The unikaryotic sequence produced meronts with large nuclei occupying at least two-thirds of the width of the cell, sporo-



Figs. 9 to 11 Mature spores of *Unikaryon nomimoscolexi* n. sp. AD: anchoring disc; En: endospore; Ex: exospore; LP: lamellar polaroplast; N: nucleus; PF: polar tube; PV: posterior vacuole; VP: vesicular polaroplast ($\times 35\,000$)

blasts having several concentric cisternae of endoplasmic reticulum around the nucleus and immature spores developing 5 to 8 coils of the polar filament (Canning & Gunn 1984). The ultrastructural features of meronts, sporoblasts and young spores and the host of the present species are distinctive, and these differences are sufficient to justify its separation from *N. helminthorum*.

The microsporidium described in this study is undoubtedly a new species for which the name *Unikaryon nomimoscolexi* n. sp. is proposed.

There is no formation of sporophorous vesicles in *Unikaryon nomimoscolexi* but *Unikaryon legeri*, which also parasitizes a platyhelminth, does form sporophorous vesicles (Azevedo & Canning 1987). It is not known whether *Unikaryon piriformis*, which is the type species of the genus, forms sporophorous vesicles. If it does, all *Unikaryon* species which develop in direct contact with the host cell cytoplasm should be transferred to a new genus; if not, a new genus should be created for *U. legeri*.

TAXONOMIC SUMMARY

Unikaryon nomimoscolexi n. sp.

Type host: *Nomimoscolexi* sp. (Cestoda, Proteocephalea) from the gut of *Clarotes laticeps* (Pisces, Teleostei, Bagridae)

Type locality: Lake Guiers (Senegal, West Africa)

Merogony: Meronts are uninucleate or binucleate. They are bounded by a thin surface membrane in direct contact with the host cell cytoplasm. Binucleate meronts divide by binary fission.

Sporogony: Sporonts are in direct contact with the host cell cytoplasm and possess a thick electron-dense coat around the plasma membrane. Early sporonts are uninucleate but become binucleate and elongate before division into 2 uninucleate sporoblasts.

Spores: Mature spores are ovoid, uninucleate and in direct contact with the host cell cytoplasm. Thin electron-dense exospore overlies the electron-lucent endospore, which is about 150 nm in thickness. At the posterior end is a large vacuole containing electron-dense material. At the anterior end is the anchoring disc which is eccentric in position. The polar tube is isofilar and has 6 to 8 coils arranged in a single or 2 layers beneath the spore wall. The polaroplast consists of an anterior region of closely and electron-dense packed lamellae and a posterior region consisting of numerous small vesicles. In thin sections, calculated spores dimensions are $3.43 \pm 0.2 \times 1.51 \pm 0.18 \mu\text{m}$.

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