

## ***Nosemoides syacii* n. sp., a microsporidian parasite of the West African turbot *Syacium micrurum* Ranzani, 1840**

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### **Résumé**

*Nosemoides syacii* n. sp. est une nouvelle Microsporidie parasite de l'estomac, de l'intestin et du foie de *Syacium micrurum* (Poisson, Téléostéen). Elle provoque la formation de xénomes blanchâtres, allongés et ovales. Tous les stades de développement sont monocaryotiques et en contact direct avec le cytoplasme-hôte. Les plasmodes mérogoniaux et sporogoniaux se divisent par plasmotomie. La sporogonie, qui est polysporée, aboutit à la formation de spores ovoïdes, à vacuole postérieure volumineuse et mesurant  $3.8 \times 2.2 \mu\text{m}$  ( $2.9\text{--}4.9 \times 1.8\text{--}2.7 \mu\text{m}$ ). Le filament polaire est isofilaire et décrit quatre à cinq tours de spire. Le polaroplaste se compose d'une partie antérieure lamellaire et d'une partie postérieure vésiculaire.

### **Abstract**

*Nosemoides syacii* n. sp. is a new microsporidian parasite of the stomach, gut and liver of *Syacium micrurum* (Pisces: Teleoste). It forms whitish, elongate-oval xenomas. All the development stages of the microsporidia are monokaryotic and in direct contact with host cytoplasm. Merogonial and sporogonial plasmodia divide by plasmotomy. Sporogony is polysporous and results in oval spores with a conspicuous posterior vacuole which measured  $3.8 \times 2.2 \mu\text{m}$  ( $2.9\text{--}4.9 \times 1.8\text{--}2.7 \mu\text{m}$ ). The polar filament is isofilar and consists of only four to five coils. The polaroplast is made up of an anterior lamellar part and a posterior vesicular part.

### **Introduction**

Microsporidia are frequently pathogenic in fish and are therefore of a significant importance in ichthyopathology. In spite of the economic importance of several African fishes, information on their parasites are extremely fragmentary, and there are few data on Microsporidia. Paperna (1973, 1982) described a *Pleistophora*-like species

in the swim-bladder of *Haplochromis angustifrons* and *H. elegans* in Lake George, Uganda. *Glugea capverdensis* and *Pleistophora duodecimae* were two species described from the Atlantic near the region of Cape Verde (Lom *et al.*, 1980). *Nosemoides tilapiai* is a recently described species parasitising *Tilapia zillii*, *T. guineensis* and *Sarotherodon melanotheron* in Lake Nokoué and the Porto Novo Lagoon, which form the largest area of

brackish water in Benin (Sakiti & Bouix, 1987). Finally, *Loma camerounensis* was found in the cichlid *Oreochromis niloticus* from the Melen fish-rearing station in Yaoundé, Cameroon (Fomena *et al.*, 1992).

Along the coast of Senegal, several microsporidia were found in various fish during several years of surveying. *Pleistophora senegalensis* parasitising the gilt-head sea bream *Sparus aurata* was described by Faye *et al.* (1990). It was found in the intestinal wall, where it formed small xenomas in the mucosa muscularis. *Microfilum lutjani* was found on the gill filaments of *Lutjanus fulgens* and described by Faye *et al.* (1991). Spores of *M. lutjani* were characterised by the presence of a manubrium inserted on a laterally offset anchoring disc and which extended into a very short, non-coiled polar filament in the form of a hook.

During these same investigations on the microsporidia of Senegalese marine fishes, we found another new species in the gut of the teleost *Syacium micrurum* (Bothidae). The description of this parasite is presented here.

## Materials and methods

Fish samples were taken from three different fishing ports: Kayar, Dakar and Mbour along the Senegalese coast. A total of 56 specimens of *Syacium micrurum* were examined.

### Light microscopy

Smears of fresh infected tissues were examined by phase contrast microscopy for spores. Tissues were fixed in Carnoy's fluid and embedded in paraffin. The sections were stained with Heidenhain's azan stain or Masson's trichrome stain (modified by Goldner). Semi-thin sections were stained with toluidine blue.

### Transmission electron microscopy

Fragments of infected tissue were first fixed with 2.5% glutaraldehyde in sodium cacodylate buffer 0.1 M, pH 7.2 and then with 2% osmium tetroxide

in the same buffer. The fragments were embedded in Spurr's resin. Sections were stained with uranyl acetate and lead citrate. They were then observed under a Jeol 200CX (University of Montpellier II), a Siemens Elmiskop 101 and a Jeol 100CX microscopes (Cheikh Anta Diop University, Dakar).

### *Nosemoides syacii* n. sp. (Figs 1–9)

Of the 56 fishes examined, 17 were infected by microsporidia (30.4%).

#### Localisation and structure of xenomas

The most frequently infected organ is the gut, but the intestine and liver may also be attacked. Infected individuals often carry several whitish, elongate-oval xenomas of up to 1.3 mm in length.

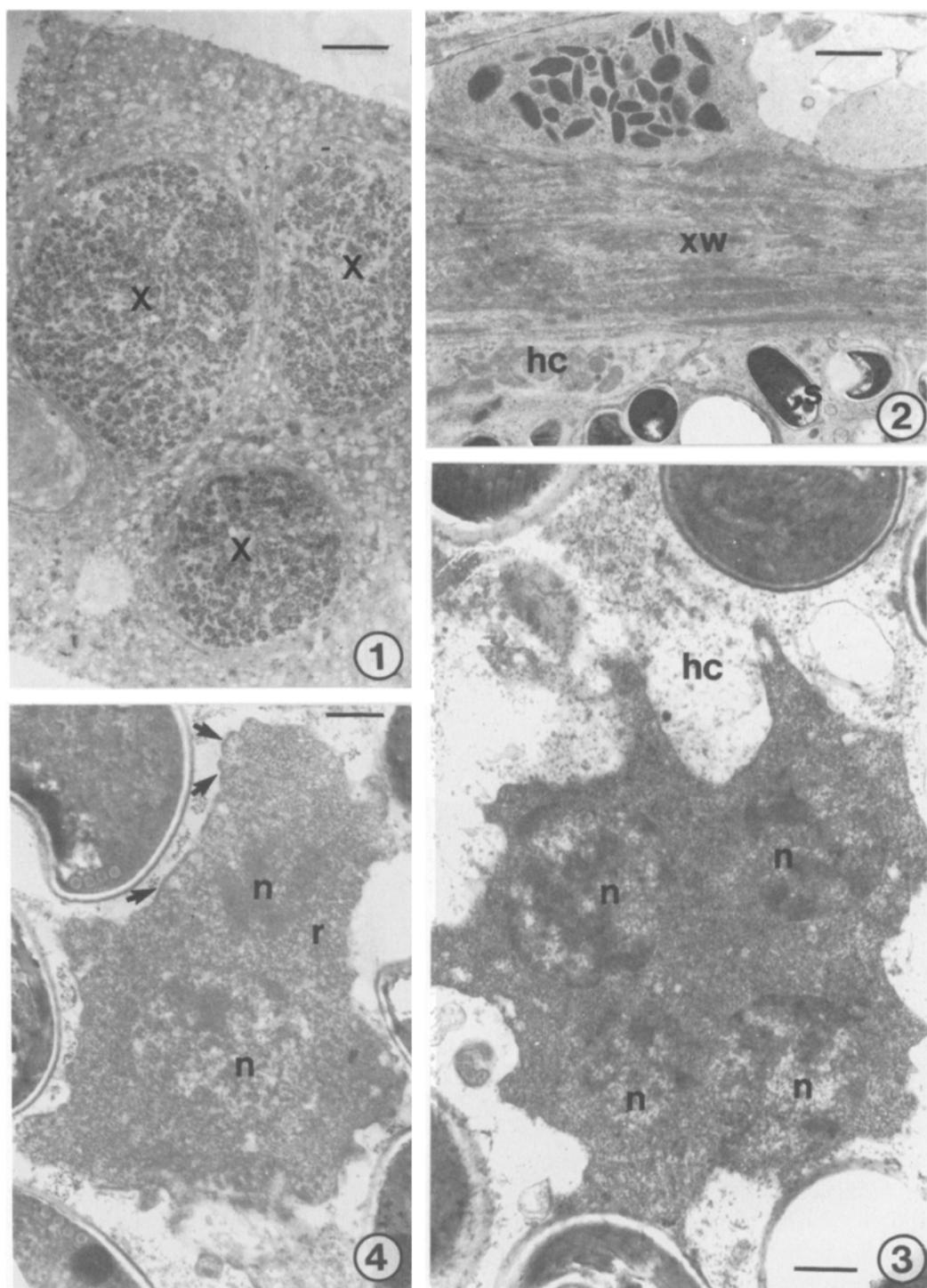
The xenomas were embedded in the stomach wall (Fig. 1). Transmission electron microscopy showed that the thick, fibrous wall of the xenomas is formed by highly developed layers of collagen fibres (Fig. 2). The xenomas contained several host nuclei which were probably derived from successive division of the original host cell nucleus, mitochondria and various development stages of the parasite without any particular arrangement.

#### Developmental cycle and ultrastructure

All of the developmental stages of the microsporidian were monokaryotic and in direct contact with the host cytoplasma (Fig. 7).

#### Meronts and merogony

The merogonic stages were represented by multi-nucleate plasmodia, with up to 6 large subspherical nuclei. The shape of the plasmodia was either lobed, almost spherical or elongate. A simple plasma membrane enclosed the plasmodium. The granular appearance of the cytoplasm was linked to the presence of abundant ribosomes (Fig. 3). Division was by plasmotomy.



Figs 1–4. *Nosemoides syacii* n. sp. 1. Semi-thin section from infected fish gut showing the xenoma. 2. Peripheral region and wall of a xenoma. The wall is formed by layers of collagen fibres. 3. Merogonial plasmodium. 4. Sporont showing patchy thickening of wall (arrows). Abbreviations: hc, host cytoplasm; n, nucleus; r, ribosomes; s, spore; X, xenomas; xw, xenoma wall. Scale-bars: 1, 250 µm; 2, 1.7 µm; 3, 0.6 µm; 4, 0.4 µm.

### *Sporonts and sporogony*

The start of sporogony was marked by a patchy thickening of the plasma membrane of the meronts, which were thus transformed into sporonts. As the sporonts developed they were covered gradually by a wall (Fig. 4). The nucleus continued to divide and the sporonts became sporogonial plasmodia whose shape was similar to that of the merogonic stages. Ribosomes were more abundant in the sporont cytoplasm, which also displayed electrolucent vacuoles and endoplasmic reticulum. Plasmotomy of the plasmodia led to the isolation of several sporoblasts.

### *Sporoblasts*

The sporoblasts were uninucleate and bounded by a thick wall. The cytoplasm displayed free ribosomes, 2–3 dense bodies and few electrolucent vacuoles (Fig. 5).

### *Spores*

The living spores were generally ovoid and contained a large, clearly defined posterior vacuole (Fig. 6). They measured 3.8 µm (2.9–4.9 µm) in length and 2.2 µm (1.8–2.7 µm) in width. The spores had a typical ultrastructure. They were uninucleate and the endospore (0.025–0.040 µm in diameter) was almost 2–3 times thicker than the exospore (<0.015 µm in diameter) (Fig. 9). Numerous ribosomes were visible in the spore cytoplasm. The polar filament originated in a sublateral anchoring disc, where it displayed a short cylindrical enlargement about 0.1 µm long and 0.16 µm wide (Figs 7,9) which continued as a straight manubrial region before becoming coiled, describing 4–5 spiral turns (Fig. 8). The average diameter of the polar filament decreased from 0.115 µm in the manubrial part to 0.08–0.09 µm in the coil. Electron microscopy of a cross-section of the polar filament beneath the plasma membrane (Fig. 8) indicated the following layers:

- (i) an outer ring (0.004 mm thick) of electron-dense material;

- (ii) an inner ring of electron-dense material (c.0.015 µm thick);
- (iii) a ring of electrolucent material;
- (iv) an electron-dense central axis, (c.0.070 µm in diameter).

The polaroplast was composed of an anterior lamellar part, with the lamellae decreasing in density posteriorly, and a posterior vesicular part (Fig. 7). One or 2 dense bodies were observed in the cytoplasm (Fig. 8).

### *Diagnosis*

*Host:* *Syacium micrurum* Ranzani, 1840 (Teleoste, Bothidae).

*Geographical distribution:* Atlantic coast, Senegal (West Africa).

*Site:* intestine, liver.

*Xenoma:* Whitish, oval or spherical, small (<1.5 mm); wall fibrous.

*Merogony:* Plasmodia with isolated nuclei and granular cytoplasm packed with ribosomes; division by plasmotomy.

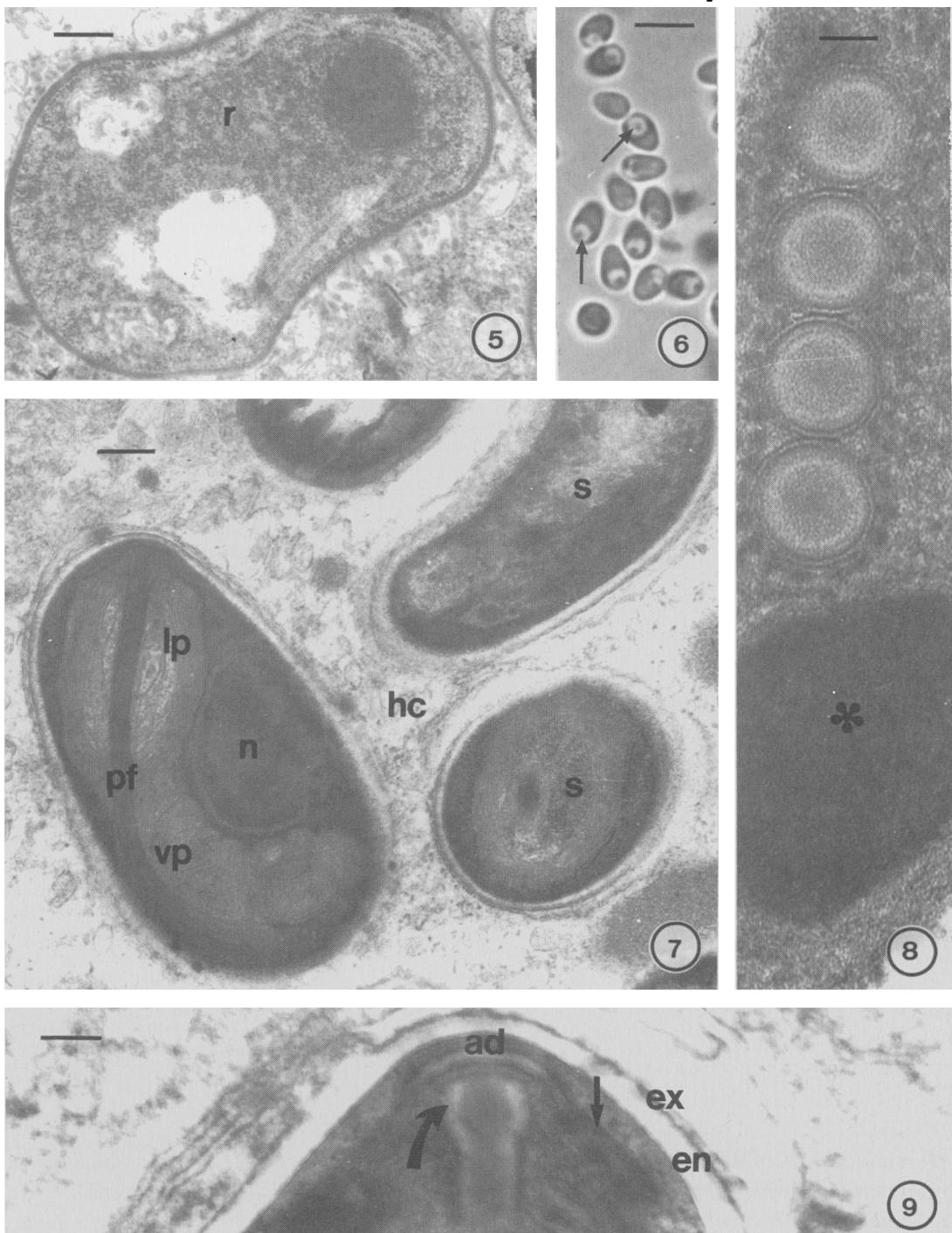
*Sporogony:* Plasmodia with isolated nuclei, cytoplasm occasionally with vacuoles, division by plasmotomy.

*Spores:* Ovoid, measuring 3.8 (2.9–4.9) × 2.2 (1.8–2.7) µm; isofilar polar filament with 4–5 spiral turns; lamellar and vesicular polaroplast; large posterior vacuole.

*Type-material* (light and electron microscope preparations): The Natural History Museum, London. Reg. No. 1994.1.14.1 (holotype), 1994.1.14.2 (paratype).

### *Discussion*

Numerous fish microsporidia belonging to 14 genera and to the collective group *Microsporidium* Balbiani, 1884 have already been described (Canning & Lom, 1986; Sakiti & Bouix, 1987; Chilmonczyk *et al.*, 1991; Faye *et al.*, 1991). The microsporidium found in the West African turbot *Syacium micrurum* is assigned to the genus *Nosemoides* Vinckier, 1975, because of the specific features of its development stages and cycle. The



Figs 5–9. *Nosemoides syacii* n. sp. 5. Sporoblast. 6. Living spores observed through photon microscope, showing development posterior vacuole (arrow). 7. Part of the cytoplasm showing some spores in direct contact with the host cytoplasm. The mature spore has a polaroplast with an anterior lamellar part and a posterior vesicular part. The vesicular polaroplast extends into the posterior part of the spore. 8. Cross-section of the polar filament coil. A reticulated body can be seen below the coil (\*). 9. Details of the anterior part of the spore. The polar filament displays a cylindrical enlargement (curved arrow). Right arrow indicates polar sac. Abbreviations: ad, anchoring disc; en, endospore; ex, exospore; hc, host cytoplasm; lp, lamellar polaroplast; n, nucleus; pf, polar filament; r, ribosomes; s, spore; vp, vesicular polaroplast. Scale-bars: 5, 0.4 µm; 6, 5 µm; 7, 0.5 µm; 8, 0.05 µm; 9, 0.1 µm.

Table 1. Characteristics of microsporidia of the genus *Nosemoides*.

Parasites species	Hosts		Infestation sites	Geographical distribution	Characters of development stages
	Group	Species			
<i>N. vivieri</i> (Vinckier, Devauchelle & Prensier, 1970) Vinckier, 1975	Gregarinida (Protozoa)	<i>Lecudina linei</i>	Cytoplasm	France	<ul style="list-style-type: none"> <li>- meront with eccentric nucleus and electron-lucent cytoplasm having endoplasmic reticulum that often has vacuoles;</li> <li>- two types of spores measuring <math>6 \times 0.7 \mu\text{m}</math> and <math>2.7 \times 1.2 \mu\text{m}</math>; polar filament with more than 9–10 spiral turns; lamellar polaroplast.</li> </ul>
<i>N. simocephali</i> Loubès & Akbarieh, 1977	Crustacea	<i>Simocephalus</i> <i>vetus</i>	Intestine	France	<ul style="list-style-type: none"> <li>- meront with dense cytoplasm packed with ribosomes;</li> <li>- sporogonial plasmodia with vesicles in cytoplasm;</li> <li>- ovoid spores measuring <math>2.5-3 \times 1.5-1.75 \mu\text{m}</math>; granular and lamellar polaroplast; polar filament with 6–7 spiral turns.</li> </ul>
<i>N. tilapia</i> Sakiti & Bouix, 1987	Fish	<i>Tilapia zillii</i> , <i>T. guineensis</i> , <i>Sarotherodon melanotheron</i>	Bronchi, intestine, liver	Benin	<ul style="list-style-type: none"> <li>- xenoma with ectoplasm;</li> <li>- meront with very dense cytoplasm displaying Golgi vesicles and vacuoles;</li> <li>- sporont with not very dense cytoplasm because of the large number of vacuoles;</li> <li>- piriform spores measuring <math>2.5-3 \times 1.5-2 \mu\text{m}</math>; polar filament with 4–5 spiral turns; lamellar and vesicular polaroplast.</li> </ul>
<i>N. syacii</i> n. sp. (current study)	Fish	<i>Syacium</i> <i>micrurum</i>	Gut, intestine, liver	Senegal	<ul style="list-style-type: none"> <li>- xenoma without ectoplasm;</li> <li>- cytoplasm with a granular appearance because of the large number of ribosomes;</li> <li>- sporont with cytoplasm that occasionally has vacuoles;</li> <li>- ovoid spores measuring <math>3.8 \times 2.2 \mu\text{m}</math> (<math>2.9-4.9 \times 1.8-2.7 \mu\text{m}</math>); large posterior vacuole; isofilar polar filament with 4–5 spiral turns; lamellar and vesicular polaroplast.</li> </ul>

genus *Nosemoides* was erected by Vinckier (1975) for a hyperparasitic species of the gregarine *Lecudina linei* and defined by the following characters: "all development stages are monokaryotic and in direct contact with host cytoplasm – polysporoblastic sporogonial plasmodia producing several sporoblasts". All these features are present in the microsporidian from *Syacium* off Senegal.

Subsequently, only one species belonging to the genus has been described in fish, i.e. *Nosemoides*

*tilapia* Sakiti & Bouix, 1987, which was found in brackish water cichlids (*Tilapia zillii*, *T. guineensis* and *Sarotherodon melanotheron*) from Benin (Sakiti & Bouix, 1987). This parasite differs from the Senegalese form in terms of the systematic position of the host fish, the site of infection and the spore shape and ultrastructure.

It is also worth mentioning that the species *Spraguea lophii* (Doflein, 1898) is dimorphic in both merogony and sporogony, with a sequence

of the "Nosema" type producing diplokaryotic spores and a sequence of the "Nosemoides" type producing monokaryotic spores (Loubes *et al.*, 1979).

Although the type-species of the genus, *Nosemoides vivieri*, is a hyperparasite and does not cause the formation of a xenoma in the host (as occurs with *N. simocephali* Loubes & Akbarieh, 1977), we still believe that the microsporidia from *Syacium micrurum* can be considered as belonging to this genus. According to several authors, the xenoma is not a useful criterion in taxonomic classification (Lom & Weiser, 1969; Lom & Laird, 1976; Berrebi & Bouix, 1978; Loubes *et al.*, 1981; Canning *et al.*, 1982; Sakiti & Bouix, 1987; Faye *et al.*, 1990). It may be that the two species from fishes with *Nosemoides*-like characters which develop in xenomas may later be shown to be unrelated to the invertebrate or hyperparasitic species which do not exhibit xenoma formation. Moreover, several genera, such as *Nosema* and *Unikaryon*, are known to be both parasites and hyperparasites (Canning & Nicholas, 1974; Sprague, 1977; Toguebaye & Marchand, 1986).

The *Nosemoides* species described in this article differs from the other three species of the genus (*N. vivieri*, *N. simocephali* and *N. tilapia*) in the structure of meronts, sporonts and spores, xenoma organisation, and the type of host (Table I). We believe, therefore, that the species associated with *Syacium micrurum* is a new species and propose to name it *Nosemoides syaci*\* n.sp. based on the generic name of its host.

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\* In his unpublished thesis, Faye (1992) referred to this species as *Nosemoides syacium* n. sp., but this was not within the rules of the ICZN.

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