Nosema jirivavrai n.sp. (Microsporea; Protozoa) from the leech *Batracobdella picta* in Ontario

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A new microsporidian species was described from the muscle and connective tissue of the glossophoniid leech *Batracobdella picta*. Subepithelial xenomas contained sporonts, sporoblasts, and spores. Merogonic stages were not observed. All sporogonic stages were diplokaryotic. Mature spores were ovoid, 3.0–3.6 × 1.8–2.2 μm. The spore wall was 190–240 nm thick, with a distinct exospore, endospore, and underlying plasmalemma. The polar filament had 12–13 coils and measured 105 nm in diameter. The angle of tilt of the anterior coils of the filament to the vertical axis of the spore was about 63°. This newly discovered parasite was named *Nosema jirivavrai*.

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Une nouvelle espèce de microsporidie a été trouvée dans les muscles et le tissu conjonctif de la sangsue glossophoniidée *Batracobdella picta*. Les xénomes sous-épithéliaux examinés contenaient des sporontes, des sporoblastes et des spores. Les stades de mérogonies n'ont pas été observés. Tous les stades de sporogonies sont diplocaryotes. Les spores à maturité sont ovoïdes et mesurent 3,0–3,6 × 1,8–2,2 μm. La paroi du spore a 190–240 nm d'épaisseur et l'exospore, l'endospore et le plasmalemme sous-jacent sont bien dessinés. Le filament polaire compte 12–13 replis en spirale et mesure 105 nm de diamètre. L'inclinaison des replis antérieurs du filalment forme un angle d'environ 63° avec l'axe vertical du spore. Le nouveau parasite porte le nom de *Nosema jirivavrai*.

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Introduction

Three species of microsporidian parasites have been described from Hirudinae. All were species of *Nosema* and were recorded from freshwater leeches from Europe.

Leeches (*Batracobdella picta* (Verrill)) with white subepithelial tumours were collected from a pond near Dundas, Ontario. Histological examination of the tumours revealed the presence of typical microsporidian spores.

In this paper, a new species of *Nosema*, representing the first microsporidian parasite recorded from leeches in North America, is described, and ultrastructural observations on the sporogonic stages of the parasite are presented.

Materials and methods

Specimens of *Batracobdella picta* were collected from a small pond adjacent to the Dundas Conservation Area near Dundas, Ontario, during April 1987. Leeches with tumours on their cuticle were cut into small cubes, fixed in 2.5% glutaraldehyde in Sorensen's phosphate buffer (pH 7.2) containing $CaCl_2$, washed in Sorensen's buffer, and postfixed in 1% OsO_4 in Sorensen's buffer containing $K_3Fe(CN)_6$. Tissue was en bloc stained using 0.5% aqueous uranyl acetate before being dehydrated through an ethanol series, infiltrated, and embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and lead citrate and observed using a Philips 201C electron microscope.

Spore dimensions were determined by measuring longitudinally sectioned mature spores in electron micrographs. The data obtained were recorded from 20 specimens.

Type specimens (0.5- to 1-μm epoxy sections stained with 0.5% toluidine blue in 1% sodium borate) containing xenomas were submitted to the National Museum of Natural Sciences, Invertebrate Collection (Parasites), Ottawa; their accession numbers are NMCP89-0011 and 0012.

Results

The white, roughly spherical tumours (0.2–1.5 mm in diameter) were irregularly distributed on both the dorsal and ventral surfaces of the leeches. Larger tumours caused localized protrusions on the cuticle.

Microscopic examination of the tumours revealed xenomas caused by a microsporidian parasite. The xenomas were usually located just beneath the epithelium (Fig. 1). The boundary layer of the xenomas was highly infolded (Fig. 2) and contained large numbers of mitochondria and abundant endoplasmic reticulum (ER) (Fig. 3).

Sporonts

The xenomas contained various stages of sporulation and mature spores (Fig. 1). The cytoplasm of the xenoma contained membrane profiles, numerous mitochondria, and granular inclusions of different sizes and densities (Figs. 4–6). The earliest stages of sporulation observed, probably sporonts, lay in direct contact with the cytoplasm of the xenoma. Young sporonts were ovoid cells bounded by two closely apposed membranes separated by a space from a third outer membrane (Fig. 4, inset). A portion of the outer two

FIGS. 1–4. Electron micrographs of sporulation stages of *Nosema jirivavrai* n.sp. Fig. 1. Body wall of *Batracobdella picta*, showing a large xenoma (arrow). Cu, cuticle. × 10 400. Fig. 2. The xenomal wall (arrow) is highly infolded. × 38 900. Fig. 3. The infoldings of the xenomal wall contain many mitochondria and endoplasmic reticulum (ER) cisternae. × 79 800. Fig. 4. Diplokaryotic sporont. The cytoplasm contains concentric ER cisternae. Dense plaques (Pl) lie in slight depressions in the membrane of each nucleus. Microtubules extend from the plaques into the nucleoplasm, where they are connected to small electron-dense bits of chromatin (Cr). A portion of the outer two membranes investing the sporont has separated from the innermost membrane, which is covered by a dense, fuzzy material (enclosed in box). × 50 300. The inset reveals more clearly the arrangement of the three membranes investing the sporonts. × 81 400.

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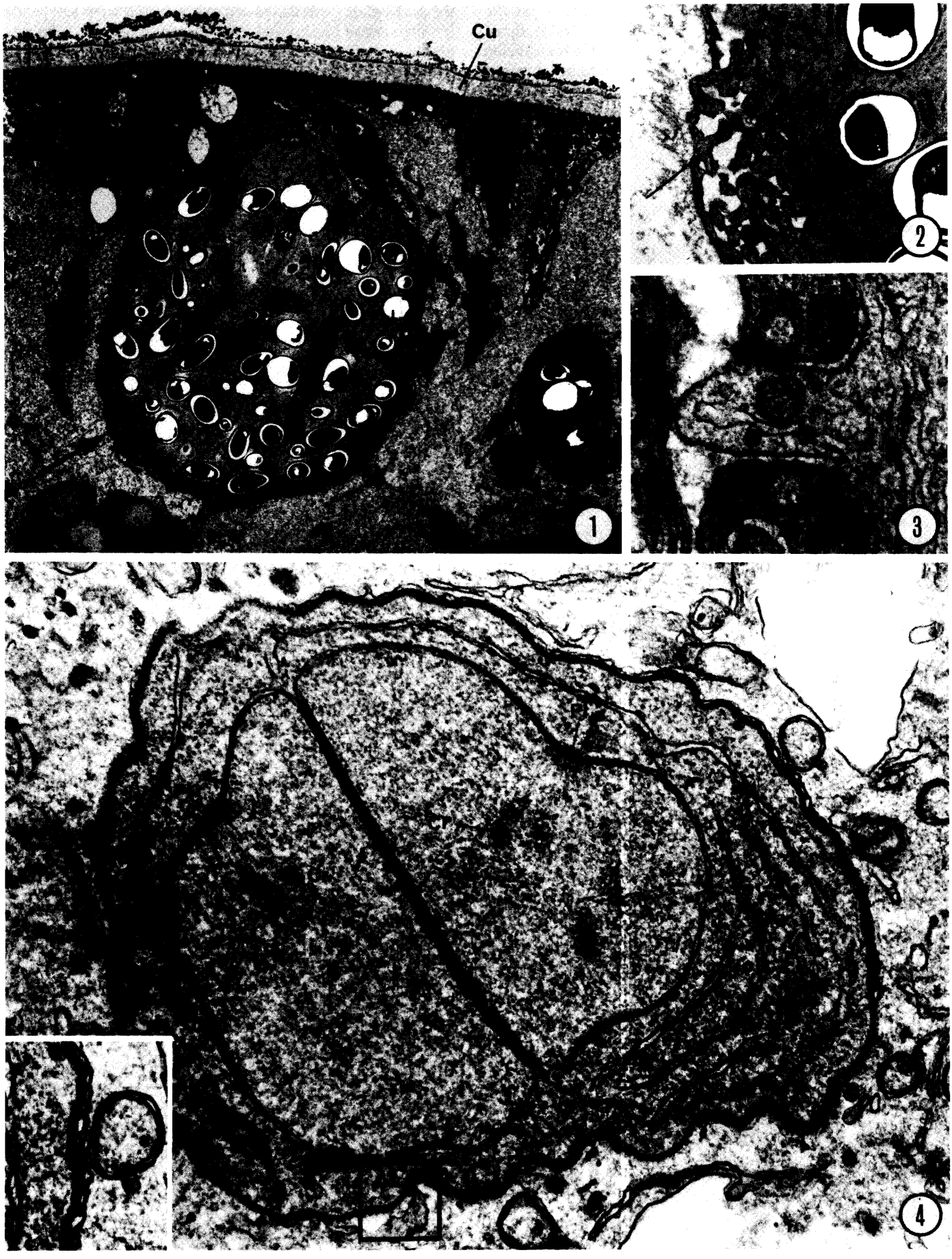


membranes surrounding a sporont was separated from the innermost membrane, which was covered by a dense, fuzzy material (Fig. 4). Each sporont contained two nuclei in the typical diplokaryon arrangement, i.e., with flattened apposed surfaces (Fig. 4). A dense plaque was located in a slight depression in the membrane of each of the two nuclei of the sporonts. In some nuclei, several microtubules radiated from the plaque into the nucleoplasm, where they were connected to small areas of condensed chromatin (Fig. 4). A similar spindle apparatus was observed in each of the two nuclei of daughter cells. Division of the sporonts was preceded by their elongation, which was followed by karyokinesis. In dividing nuclei, the structure of the spindle apparatus was modified. A dense disc was situated in a deep, densely stained invagination of the nuclear envelope (Fig. 5). A grazing surface section through the disc revealed that the surrounding sleeve of modified nuclear membrane contained material of different, periodically arranged densities (Fig. 5, inset). Spindle microtubules were seen spanning most of the length of a dividing nucleus. The condensed chromatin (probably chromosomes) persisted in the dividing nucleus (Fig. 5).

Cytokinesis occurred subsequent to nuclear division. The chromosomes were localized near the nuclear plaques at this stage of development. Binucleate daughter sporonts connected by a cytoplasmic bridge were often seen, as were localized vesicular structures at one end of the cells (Fig. 6).

Spores

Few immature spores were seen in the xenomas. Those observed were poorly preserved and provided no information on the process of spore formation. The xenomas contained many mature spores which were typical of *Nosema* species. The spores were oval and measured 3.0−3.6 × 1.8−2.2 μm (n = 20). The spore wall was composed of an outer electron-dense exospore which measured 60−80 nm and an inner electron-lucent endospore 130−160 nm thick. The endospore was markedly thinner at the anterior end of the spore (Fig. 8). The plasma membrane of the parasite lay immediately beneath the endospore (Fig. 7). An anchoring disc was located at the apex of the spore cytoplasm. The straight portion of the polar filament extended posteriorly from the disc, through the multilaminated polaroplast. Posterior to the latter structure, the filament was helically arranged, forming 12−13 coils in the peripheral cytoplasm (Fig. 8). The anterior coils were arranged at an angle of approximately 63° to the longitudinal axis of the spore. The polar filament measured about 105 nm in diameter and consisted of several layers of varying electron density (Fig. 7).

The polaroplast, which was composed of closely packed lamellae which were intimately associated with the anchoring disc and the straight portion of the polar filament, occupied about one-third of the spore cytoplasm (Fig. 7). Posterior to the polaroplast were the paired nuclei which were surrounded by three dense bands of closely-spaced ribosomes. The cytoplasm posterior to the diplokaryon appeared vacuolated (Fig. 8).

Nosema jirivavrai n.sp.

(Protozoa; Microsporea; Nosematidae)

Description

Merogonic stages

Not recognized, but cannot be excluded because only advanced xenomas were seen in the naturally infected leeches.

Sporogonic stages

Sporonts diplokaryotic; sporonts presumably divide to produce two diplokaryotic sporoblasts.

Spore

Ovoid; dimensions determined from electron micrographs, 3.0−3.6 × 1.8−2.2 μm; spore wall (exo- and endo-spore) 190−240 nm thick; region above anchoring disc considerably thinner; polar filament with 12−13 coils, diameter 105 nm; angle of tilt of coils ~63°; nuclei in diplokaryon arrangement.

HOST SPECIES: *Batracobdella picta* (Verrill) (Hirudinae: Glossiphoniidae).

TISSUES INFECTED: Xenomas, probably primarily in connective tissue and muscle cells.

TYPE LOCALITY: Pond near Dundas, Ontario, Canada (15°15′N, 80°0′W).

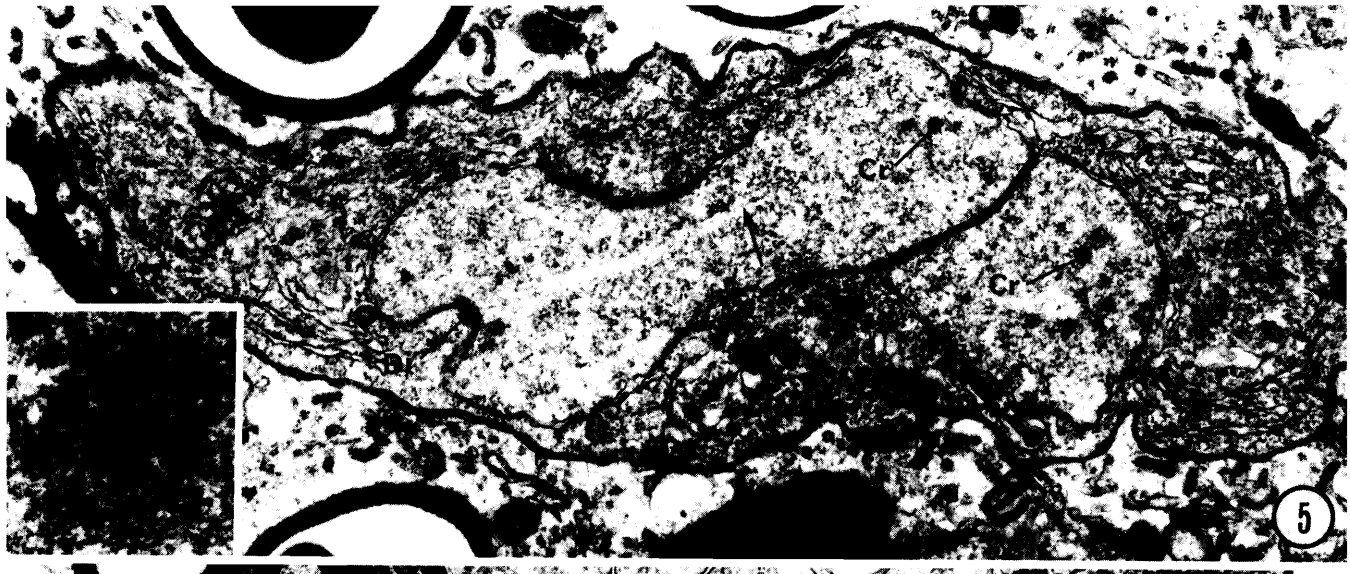
ETYMOLOGY: This species is named after Dr. Jiri Vavra, in appreciation of his extensive contribution to our knowledge of the Microspora.

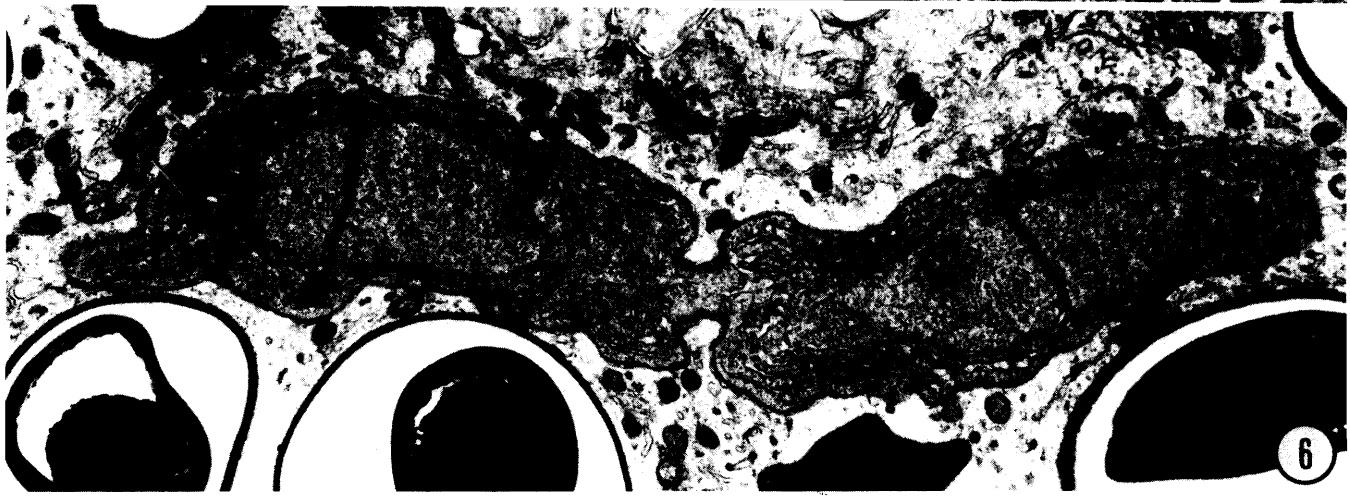
Remarks

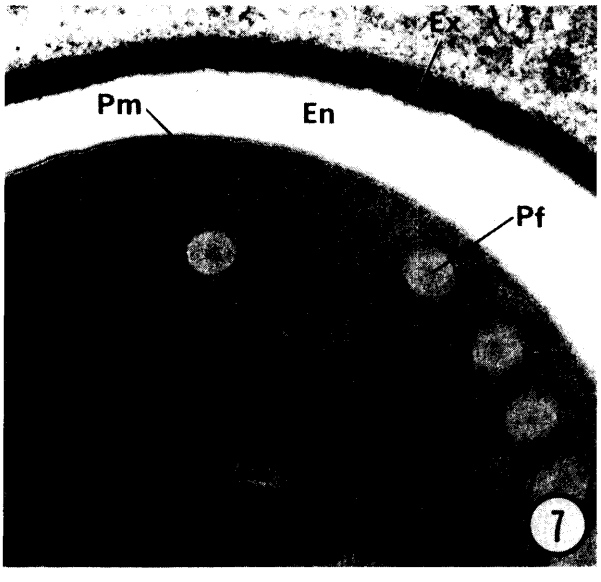
That this parasite is a species of *Nosema* is clearly evidenced by the diplokaryotic arrangement of nuclei, binary fission of the sporonts, and the absence of a pansporoblastic membrane. Differentiation of the various species of *Nosema* is difficult because the spores are similar in size and shape. Host specificity has been utilized as an important factor in differentiating *Nosema* species, but must be viewed with some caution (Larsson 1981). Other useful distinguishing features are the presence of merogony and the nature of the polar filament, i.e., its dimensions, the number and arrangement of the coils, and the angle of tilt.

A comparison of the main features of *Nosema jirivavrai* n.sp. with those of the three other species described from leeches is given in Table 1. *Nosema jirivavrai* n.sp. differs from the other species in its host and geographic location. It differs from *Nosema glossiphoniae* and *Nosema herpobdellae* in that its spores are smaller, there are fewer coils in the polar filament, and the angle of tilt is smaller. *Nosema jirivavrai* n.sp. differs less markedly from *Nosema tractabile* in that the

FIGS. 5−8. Electron micrographs of sporulation stages of *Nosema jirivavrai* n.sp. Fig. 5. Elongated sporont undergoing nuclear division. Note the dense disc (Di) located in the densely stained portion of the invaginated nuclear envelope. A spindle microtubule (arrow) extends across the dividing nucleus. Condensed chromatin (Cr) persists in the dividing nuclei. × 25 400. Inset: A grazing section through the dense disc surrounded by the modified nuclear membrane. The alternating light areas in the latter may be nuclear pores. × 61 800. Fig. 6. A dividing sporont connected by a cytoplasmic bridge. The daughter sporoblasts are diplokaryotic. Spindle plaques and condensed chromatin can be seen in the nuclei. Vesicular structures (arrow) occur at the ends of each daughter cell. × 19 800. Fig. 7. Portion of spore, illustrating dense exospore (Ex), electron-transparent endospore (En), plasma membrane (Pm), transversely sectioned filament (Pf), and lamellae of the polaroplast (*). × 85 500. Fig. 8. Mature spore. Note that the endospore is thinner at the apex adjacent to the anchoring disc (Ad). The straight portion of the polar filament (arrow) extends posteriorly from the disc through the polarplast. The polar filament is helically arranged in the peripheral cytoplasm, with about a dozen coils. Posterior to the polaroplast are paired nuclei (Nu). The nuclei are surrounded by three bands of closely spaced ribosomes (Ri). × 26 200.







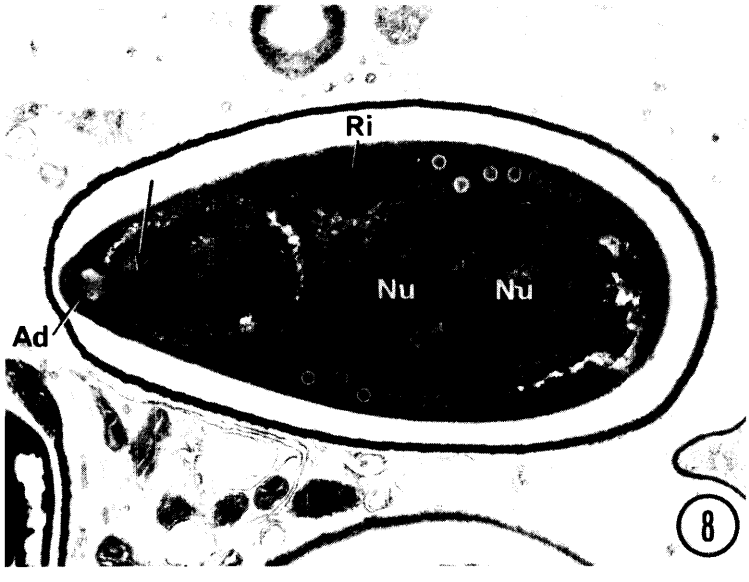


TABLE 1. A comparison of the features of *Nosema* species

	Nosema glossiphoniae	*Nosema herpobdellae*	*Nosema tractabile*	*Nosema jirivavrai* n.sp.
Host	*Glossiphonia complanata*	*Erpobdella octoculata*	*Helobdella stagnalis*	*Batracobdella picta*
Geographic location	Germany, England	Belgium, England	Sweden	Canada
Tissue localization	Muscle	Connective tissue	General	Connective tissue, muscle
Merogony	+	+	—	?
Spore dimensions	Fixed, 5.0 × 2.3 (4–5.5 × 2.0–2.5)	Fresh, 5.7 × 2.6; fixed, 4.7 × 2.3	Fresh, 3.5–4.3 × 2.1–2.5; fixed, 2.9–4.0 × 2.0	Fixed, 3.0–3.6 × 1.8–2.2
No. of coils in polar filament	17–18	14–17	13–14	12–13
Width of filament (nm)	115	100	110–120	~105
Angle of tilt of polar filament (deg.)	80	85	55	63
References	Schröeder 1914; Spelling and Young 1986	Conet 1931; Spelling and Young 1983	Larsson 1981	This paper

spore size and number of coils in the polar filament are similar. However, the angle of tilt is larger. In addition, the "cell wall" of sporonts of *N. jirivavrai* n.sp. differed strikingly from that of *N. tractabile*. In the latter species, the wall consisted of linearly arranged ridges of electron-dense material overlying the plasma membrane (Larsson 1981). Also, xenomas of *N. tractabile* were never seen in muscle tissue. Because of these and host differences, in addition to the wide geographic separation, we believe that the establishment of a new species is justified.

Discussion

Specific identification of the host cell(s) infected by *N. jirivavrai* was difficult because the wild-caught leeches examined harboured advanced infections which were characterized by the presence of large xenomas. The subepithelial localization of most of the xenomas suggested that the infected host cells were originally of muscle and (or) connective tissue origin. We were unable to determine whether the xenomas were unicellular or syncytial because host cell nuclei were not seen. Xenomas in related species may be unicellular in origin as in *N. herpobdellae* (see Spelling and Young 1983), or syncytial as in *N. tractabile* (see Larsson 1981).

The structure of the wall of the xenoma may be helpful in the differentiation of closely related microsporidian species. The highly infolded wall of xenomas of *N. jirivavrai*, which was richly endowed with mitochondria, is suggestive of high metabolic activity. Structurally similar xenomal walls were recorded from *N. herpobdellae* by Spelling and Young (1983). Unfortunately, Larsson (1981) did not describe the wall of the xenomas of *N. tractabile*. Marked structural differences have been recorded in xenomal walls of species of other microsporidian genera. In *Glugea anomala*, for example, the xenomal wall is composed of a multilaminate capsule infiltrated by layers of collagen (Canning *et al.* 1982).

In their presence and their arrangement, the three membranes investing sporonts of *N. jirivavrai* resembled those observed by Canning *et al.* (1982) around the multinucleate cylindrical stages of *Glugea anomala*. Our ultrastructural data suggest that the outer two membranes may represent a cisterna of ER, which closely invests the parasite, the plasma membrane of which is coated with a dense, fuzzy material. Canning *et al.* (1982) came to the same conclusion and suggested that the ER cisternae "provided a means by which proteins synthesized by the host cell are made available to the parasite."

The arrangement of the nuclear plaques, spindle microtubules, and chromosome-like condensations observed in dividing sporonts of *N. jirivavrai* are indicative of mitotic division and these features are similar to those described from other *Nosema* species (Larson 1981; Batson 1983; Spelling and Young 1983). Synaptonemal complexes, which have been observed in sporonts of several species of myxosporidians (Loubès 1979), were not seen in sporonts of *N. jirivavrai*.

Whether the absence of merogonic stages in xenomas of *N. jirivavrai* was due to the advanced stage of infection, or is an integral feature of the parasite's development, could not be determined in this study. Whereas one or more cycles of merogony have been recorded from several species of *Nosema*, other species have relatively simple cycles. In *N. tractabile*, for example, the sporoplasm is apparently transformed directly into a sporont. The sporont then divides to produce two sporoblasts, each of which develops into a spore. This pattern of development has also been described for *N. algerae* (by Vavra and Undeen 1970) and *N. heliothidis* (by Kramer 1959).

The description of at least three species of *Nosema* from European leeches suggests that additional microsporidians also occur in North American Hirudinae.

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