Ultrastructure and development of *Nosema podocotyloidis* n. sp. (Microsporidia), a hyperparasite of *Podocotyloides magnatestis* (Trematoda), a parasite of *Parapristipoma octolineatum* (Teleostei)

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**Abstract** – *Nosema podocotyloidis* n. sp. (Microsporidia, Nosematidae) is described from *Podocotyloides magnatestis* (Trematoda: Opecoelidae), a parasite of the fish *Parapristipoma octolineatum* (Teleostei) in the Atlantic Ocean. Electron microscopy reveals that all the stages of the cycle (merogony and sporogony) are diplokaryotic and in direct contact with the cytoplasm of host cells. There is no sporophorous vesicle (pansporoblast). The earliest stages observed are meronts, which have a simple plasmic membrane. Their cytoplasm is granular, rich in ribosomes and contains some sacculi of endoplasmic reticulum. They divide by binary fission into diplokaryotic sporonts. The sporonts have a thick electron-dense wall. Their diplokaryon is slightly less electron-dense than the cytoplasm. The cytoplasm of more advanced sporonts has numerous electron-lucent vesicles. Sporonts with two diplokarya divide by binary fission into diplokaryotic sporoblasts. The older sporoblasts are irregular or elongate and the polar filament is in formation. Their cytoplasm is denser, with ribosomes and lamellae of granular endoplasmic reticulum. The sporoblasts evolve into spores. The mature spores are broadly oval and measure 3.6 (3.1–4.0) × 2.58 (1.8–3.3) µm. Their wall is 100–300 nm thick. The polar tube is isofilar with 11–16 coils, 130–155 nm in diameter and arranged in many layers in the centre of the spore. The polaroplast is divided into two regions: an outer electron-dense cup with granular content and lacking lamellae and an internal region, less electron-dense, composed of irregularly arranged sacs. The posterior vacuole, with an amorphous electron-dense content, is present. The new species is compared with other species of *Nosema* from trematodes.

**Key words:** *Nosema podocotyloidis*, Microsporidia, Hyperparasite, Digenea, *Podocotyloides magnatestis*, *Parapristipoma octolineatum*.

**Résumé** – Ultrastructure et développement de *Nosema podocotyloidis* n. sp. (Microsporidia), hyperparasite de *Podocotyloides magnatestis* (Trematoda), parasite de *Parapristipoma octolineatum* (Teleostei). *Nosema podocotyloidis* n. sp. (Microsporidia, Nosematidae) est décrit de *Podocotyloides magnatestis* (Trematode : Opecoelidae), parasite du poisson *Parapristipoma octolineatum* (Teleostei) pêché dans l’Océan Atlantique. La microscopie électronique montre que tous les stades de développement (merogonie et sporogonie) sont diplokaryotiques et en contact direct avec le cytoplasme des cellules hôtes. Il n’y a pas de pansporoblaste. Les plus jeunes stades observés sont des mérozoïtes possédant une membrane plasmique simple. Leur cytoplasme est...
Introduction

*Podocotyloides magnatestis* (Trematoda: Opecoelidae) is a parasite in the gut of the teleostean fish *Parapristipoma octolineatum* (Valenciennes, 1833) off the coast of Senegal. While studying this parasite, we found that some specimens were hyperparasitised by a microsporidia. The microsporidia are unicellular eukaryotes and intracellular parasites. Their hosts include protists, invertebrates and all paraparasitised by a microsporidia. In this paper, we describe a new species, assign it to the collective group *Microsporidium* Balbiani, 1984 and compare it with the species *Pleistophora* sp. Lie, Basch and Umathvey, 1966, *Microsporidium distomi* (Lutz and Splendore, 1908), *Microsporidium danniewskyi* (Pfeiffer, 1895) and *Microsporidium ghigii* (Guyenot and Naville, 1924).

For the genus *Pleistophora*, essential characters proposed by Canning and Nicholas [6] based on the ultrastructure of the type species *P. typicalis* from the marine fish *Myxoxcephalus scorpius* are:

- nuclei are unpaired in all stages of the development;
- merogony stages are bounded by a dense and amorphous wall which detaches from the plasma membrane at the beginning of the sporogony forming the sporophorous vesicle wall;
- division of meronts takes place by plasmotomy;
- sporogony is polysporous by successive divisions of the sporogonial plasmodium until the formation of uninucleate sporoblasts which develop into spores;
- there are numerous spores in the sporophorous vesicle.

The collective group *Microsporidium* Balbiani, 1984 has been created by Sprague [22]. It regroups the species whose generic positions are uncertain.

In this paper, we describe a new species, assign it to the genus *Nosema* Naegeli, 1857, and compare it with the species *Nosema* from trematodes previously studied using the same techniques.

Materials and methods

Specimens of *Podocotyloides magnatestis* Aleshkina and Gaevskaya, 1985 (Trematoda, Digenea) were collected live from the intestine of naturally infested *Parapristipoma octolineatum* (Pisces, Teleostei, Actinopterygii), caught off Dakar (Atlantic Ocean).

The worms were removed from their hosts, fixed in cold (4 °C) 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 7.2, rinsed in 0.1M sodium cacodylate buffer at pH 7.2, postfixed in cold (4 °C) 1% osmium tetroxyde in the same

granulaire, riche en ribosomes et contient quelques sacules de réticulum endoplasmique. Ils donnent, par division binaire, des sporontes diplocaryotiques. Les sporontes sont recouverts d’une paroi épaisse dense aux électrons. Leur diplocaryon est légèrement moins dense que le cytoplasme qui, chez les sporontes âgés, présente de nombreuses vésicules claires aux électrons. Les sporontes avec deux diplocaryons donnent, par division binaire, des sporoblastes avec un diplocaryon. Les sporoblastes âgés sont allongés ou de forme irrégulière et présentent le tube polaire en formation. Leur cytoplasme est plus dense aux électrons et riche en ribosomes libres et en réticulum endoplasmique granulaire. Les sporoblastes évoluent en spores. Les spores matures sont grossièrement ovaies et mesurent 3.6 (3.1-4.0) × 2.58 (1.8-3.3) μm. Leur paroi a une épaisseur de 100-300 nm. Le tube polaire est isofilaire, mesure 130-155 nm de diamètre et décrit 11-16 tours de spire disposés en plusieurs couches concentriques au niveau du centre de la spore. Le polaroplaste est divisé en deux régions : une externe, opaque aux électrons avec un contenu granulaire sans lamelles, et une région interne, moins opaque aux électrons, composée de sacules arrangés de façon irrégulière. La vacuole postérieure, avec un contenu amorphe opaque aux électrons est présente. La nouvelle espèce est comparée aux autres espèces de *Nosema* parasites de Trématodes.
buffer for 1 h, dehydrated in ethanol and propylene oxide, embedded in Spurr and polymerised at 60 °C for 24 h.

Ultrathin sections (60–90 nm) were cut on an ultramicrom-
tome (Power Tome PC, RMC Boeckeler). The sections were
placed on grids and stained with uranyl acetate and lead citrate.
Sections were examined on a Hitachi H-7650 transmission
electron microscope, operating at an accelerating voltage of
80 kV, in the “Service d’Étude et de Recherche en Microscopie
Électronique” of the University of Corsica (Corte, France).

Nosema podocotyloidis n. sp.

urn:lsid:zoobank.org:act:B2161001-F580-4C29-9684-CC6
E4CDB99E3

Type host: Podocotyloides magnatestis (Trematoda: Opecoelidae), a parasite of the teleost Parapristipoma octolineatum.

Type locality: Atlantic Ocean near Dakar (Senegal).

Type material: Hapantotype on grids No. 82689 deposited in the “Parasites et Ecosystèmes Méditerranéens” Laboratory, University of Corsica (France).

Site of infection: Parenchyma.

Development: All stages of merogony and sporogony are
diplokaryotic and in close contact with the host cell cytoplasm.
Sporogony is diplorsporoblastic.

Sporules: Spores, in fixed preparations, broadly oval,
3.6 (3.1–4.0) × 2.58 (1.8–3.3) μm (n = 25). Spore wall
100–300 nm thick. Polar tube isofilar, 155 nm wide, arranged
in 11–16 coils with multilayer structure. Polaroplast with an
100–300 nm thick. Polar tube isofilar, 155 nm wide, arranged

Sporo blasts and sporogenesis

The young sporoblasts were ovoid cells with one central dip-
lokaryon (Fig. 3A). They were bounded by an electron-dense coat
with a thickness of approximately 30 nm. The electron-lucent
vesicles of the cytoplasm increased in number and the endoplas-
ic reticulum became more distinct. The older sporoblasts were
irregular or elongate (Fig. 3C) and their cytoplasm was full of
ribsomes and lamellae of endoplasmic reticulum. The diplokary-
on was less electron-dense than the cytoplasm. Distinct nucleoli
were occasionally seen (Fig. 3C). The sporogenesis began with
the development of a polar tube (Fig. 3B, C). Cross-sections of
the immature polar tube appeared as symmetrical rings, each with
an electron-dense central axis surrounded by a layer of electron-
lucent material limited by a membrane (Fig. 3C). The diameter of
the immature polar tube was approximately 80–120 nm. In the
posterior end of some older sporoblasts, a prominent Golgi appa-
ratus developed near the polar tube (Fig. 3B, C). Its structure was
of traditional type. The polaroplast was the last spore structure
formed and consisted of highly electron-dense bands (Fig. 3D).
At this time, the formation of the endospore wall began.
The young spores were diplor karyotic (Fig. 3D).

Mature spores

The mature spores were broadly oval (Fig. 4A). In thin sec-
tions, the spore dimensions calculated were 3.6 (3.1–4.0) × 2.58 (1.8–3.3) μm (n = 25). The diplokaryon was found in
the posterior part of young spores (Fig. 3D) but it was not
clearly visible in mature spores.
The spore wall was about 100–300 nm thick and con-
sisted of three parts: an electron-dense exospore, a median
Figure 1. A. Ultrastructural aspects of the different developmental stages (arrows) of *Nosema podocotyloidis* n. sp. from *Podocotyloides magnatestis*. B. Ovoid meront. C. Elongate meront. D. Binary fission of meront with two diplokarya. D: diplokaryon; ER: endoplasmic reticulum; N: nucleolus; NE: nuclear envelope. Scale Bars: A, 5 μm; B, C and D, 1 μm.
electron-lucent endospore and an internal unit membrane (Fig. 4A). The endospore was reduced in thickness (100–160 nm) at the anterior end of the spore (Fig. 4B).

The polar tube was isofilar with 11–16 coils, 130–155 nm in diameter, arranged in many layers in the middle region of the spore (Fig. 4A). The fine structure of the coils showed a multilayer structure (Fig. 4C). The polar tube was attached to an anchoring disc, surrounded by the polar sac (Fig. 4B). The widest sectioned disc measured 155 nm in diameter.

The polaroplast, surrounding the anterior part of the polar tube, was divided into two regions: an anterior electron-dense cup, and a posterior region, less electron-dense, composed of irregularly arranged sacs (Fig. 4A, B, D).
In the posterior part of the spore was a posterior vacuole, more electron-dense than the cytoplasm (Fig. 4A).

Discussion

The species described here belongs to the genus *Nosema* Naegeli, 1857 as defined by Larsson [15], Sprague [22] and Canning and Vävra [9]. The following characteristics supported this generic identification:

- nuclei were paired as diplokarya at all stages of development;
- development at all stages was in direct contact with the host cell cytoplasm;
- merogony and sporogony by binary fission of diplokaryotic cells.

Eighteen species of microsporidia were described from trematodes, by Sprague [21, 22], Hussey [14], Canning [2], Canning et al. [3], Canning and Olson [8], Azevedo and Canning [1] and Levron et al. [16, 17]. Among these species, eight belong to the genus *Nosema*. They are *Nosema diplherostomi* Levron, Ternengo, Toguebaye and Marchand, 2004, *Nosema dollfusi* Sprague 1964, *Nosema eurytremae* Canning 1972, *Nosema gigantica* Canning and Madhavi 1977, *Nosema lepocreadii* Sprague 1964, *Nosema podocotyloidis* n. sp. A. Young sporoblast showing numerous electron-lucent vesicles (arrows and insert) and the endoplasmic reticulum (ER), B. A part of the sporoblast showing the polar tube (PT) and the Golgi apparatus (G). C. Elongate sporoblast. Note the presence of the Golgi apparatus (G). D. Immature spore with 12 coils of polar tube. D: diplokaryon; ER: endoplasmic reticulum; N: nucleolus; P: polaroplast; PT: polar tube; PV: posterior vacuole. Scale Bars: A, B, C and D, 1 μm.
Canning and Olson 1980, *Nosema monorchis* Levron, Temengo, Toguebaye and Marchand, 2005, *Nosema strigeoideae* Hussey, 1971 and *Nosema xiphidiocercariae* Voronin, 1974 (Table 1).

*Nosema diphterostomi* was described as a hyperparasite of adults of *Dipterostomum brusinae*, an intestinal parasite of *Diplodus annularis* [16]. The most distinctive characters of this species are the low number of coils of the polar tube (6–7 coils), the small diameter of the polar tube (100 nm) and the small size of the spores (2.1 × 1.4 μm).

*Nosema dollfusi* was described in the sporocysts of *Bucephalus cuculus*, a parasite of the oyster *Crassostrea virginica* from...
| Nosema species | Host | Hyper-host | Spore size (μm) | Number of polar tube coils | Locality | References |
|----------------|------|------------|-----------------|---------------------------|----------|------------|
| N. strigeoideae | Diplostomum flexicaudum | Stagnicola emarginata | 4.7 × 3.1 (fresh) | 11–16 | Moscow Russia | [14] |
| N. gigantica | Diplostomum flexicaudum | Stagnicola emarginata | 4.7 × 3.1 (fresh) | 11–16 | Michigan USA | [14] |
| N. podocotyloidis | Podocotyloides magnatestis | Parapristipoma octolineatum | 4.5 × 2.3 (fresh) | 11–16 | Dakar Senegal | Present study |
| N. lepocreadii | Lepocreadium manteri | Leuresthes tenuis | 4.7 (fixed) | 10 | San Diego USA | [3, 7] |
| N. monorchis | Monorchis parvus | Diplodus annularis | 4.7 × 3.1 (fixed) | 11–16 | Michigan USA | [11] |
| N. strigeoideae | Diplostomum flexicaudum | Stagnicola emarginata | 4.7 × 3.1 (fresh) | 11–16 | Moscow Russia | [14] |
| N. gigantica | Diplostomum flexicaudum | Stagnicola emarginata | 4.7 × 3.1 (fresh) | 11–16 | Michigan USA | [14] |
| N. podocotyloidis | Podocotyloides magnatestis | Parapristipoma octolineatum | 4.5 × 2.3 (fresh) | 11–16 | Dakar Senegal | Present study |
| N. lepocreadii | Lepocreadium manteri | Leuresthes tenuis | 4.7 (fixed) | 10 | San Diego USA | [3, 7] |
| N. monorchis | Monorchis parvus | Diplodus annularis | 4.7 × 3.1 (fixed) | 11–16 | Michigan USA | [11] |
| N. strigeoideae | Diplostomum flexicaudum | Stagnicola emarginata | 4.7 × 3.1 (fresh) | 11–16 | Moscow Russia | [14] |

Maryland in the USA [21]. The ultrastructure of this species is unknown. This Nosema is differentiated from N. podocotyloidis n. sp. by its host which, in the larval stage, parasitises a mollusc.

Nosema eurytremae is a microsporidian hyperparasite of larvae of the trematodes Eurytrema pancreaticum and Postar- mostomum gallinum in the land snail Bradybaena similaris. The most distinctive characters of this species are the coils of the polar tube which are arranged in a single layer close to the spore wall, the polaroplast, which consists of an anterior part composed of laminated membranes and a posterior one composed almost entirely of flattened spindle-shaped sacs, and the hosts which, in the larval stage, parasitise a land mol- lusc [11].

Nosema gigantica was found in the parenchyma of adult flukes, Allocreadium fasciatisi, living in the gut of the freshwater fish Aplochelius melastigma from India. Its spores are ellipsoid and measure 7.9 × 4.9 μm [4]. The size of the spores of this species is different from that of N. podocotyloidis n. sp.

Nosema lepocreadii is a microsporidian hyperparasite of adult flukes, Lepocreadium manteri, from the gut of the California grunion, Leuresthes tenuis. It was studied by light and electronic microscopy [7, 8]. The most distinctive ultrastructural features of this species are:

- the diplokaryotic sporoblasts are not always produced by binary fission of the sporonts but also by multiple fission of elongate sporonts with more than two diplokarya;
- the endoplasmic reticulum is abundant in all pre-spore stages;
- the polar tube is isofilar with a maximum of 10 coils arranged in a single layer;
- the subdivision of the nuclei occurs by ingestion of the inner membrane of the nuclear envelope as tongues into the nucleoplasm.

Nosema monorchis is a hyperparasite of Monorchis parvus, an intestinal parasite of Diplodus annularis. It exhibits the following distinctive characters: the great electron opacity and small size of the diplokarya; the polar tube is isofilar with 16–17 coils (90 nm diameter); the polaroplast presents an anterior part composed of laminated membranes and a posterior region with wider or irregularly arranged lamellae and a maximum wall thickness of 220 nm (exospore + endospore) [17].

Nosema strigeoideae is a hyperparasite of larval stages of Diplodistomum flexicaudum in the snail Stagnicola emarginata angulata from Michigan in the USA [14]. Its ultrastructure is unknown. It is differentiated from N. podocotyloidis n. sp. by its spores, which measure 4.7 × 3.1 μm, and by its host which, in the larval stage, parasitises a mollusc.

Nosema xiphidiocercariae is a hyperparasite of sporocysts, cercariae and metacercariae in Plagiorchidae parasites of Lymnaea pfaulna, a freshwater mollusc from Russia. The live spores measure 4.5 × 2.3 μm and the spores coloured with Giemsa measure 4.0 × 2.3 μm [22]. This Nosema is differentiated from N. podocotyloidis n. sp. by the spore size and by the fact that its host lives in a freshwater mollusc.

Therefore, we consider that N. podocotyloidis n. sp. is different from all other species of Nosema hyperparasites of trematodes.

The most prominent feature of the spores of N. podocotyloidis n. sp. is the anterior part of the polaroplast. It is an electron-dense
cup with granular contents. This type of anterior region of the polaroplast is uncommon. It has been observed only in *Nosema lepocreadii*, a hyperparasite of *Lepocreadium manteri* [8]. Usually the polaroplast has two parts, an anterior or outer region with closely-packed lamellae and a posterior or inner region with wider and less regularly arranged lamellae or vesicular units.

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