Ultrastructure of *Nucleospora cyclopteri*, an intranuclear microsporidian infecting the Atlantic lumpfish (*Cyclopterus lumpus* L.)

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Abstract

Nucleospora cyclopteri is an intranuclear microsporidian parasite that has recently been described infecting wild Atlantic lumpfish from Iceland. A similar report was previously made from captive lumpfish in Canada, but it is not currently known whether the same parasite is responsible for both infections. Here we present new ultrastructural data on the Icelandic parasite in order to make direct comparisons with the Canadian report. Mature spores are elongate ovoid, contain a single nucleus, an isofilar polar filament with 10-12 turns and measured 2.53 x 1.04 μm. The earliest developing stages observed were sporogonial plasmodia that contained aggregates of electron dense structures typical of microsporidia from the Enterocytozoonidae. These spore characteristics are almost identical to the Canadian form and both manuscripts report degenerate lymphocytes and comparable developing plasmodial stages. Due to these close similarities, we conclude that the same parasite, *Nucleospora cyclopteri*, is responsible for causing disease in both Canadian and Icelandic lumpfish populations.

Introduction

Nucleospora cyclopteri is an intranuclear microsporidian, from the Enterocytozoonidae, that has recently been described associated with serious kidney pathology from wild caught Icelandic lumpfish (Freeman et al., 2013). A similar microsporidian infecting captive lumpfish in Canada was reported by Mullins et al., 1994. However, it has not been possible to confirm the same parasite is responsible, as the Icelandic report focused on histopathology and DNA sequence data but did not include ultrastructural analyses. Here we provide information on the ultrastructure of Icelandic *N. cyclopteri* and compare that to the original Canadian report.

Methods

Kidneys with gross signs of infection by *N. cyclopteri*, were removed from two freshly captured spawning female fish from Húnaflói Bay in northern Iceland (66° 3'37.29"N, 20°28'18.31"W) during April 2012 and prepared for Transmission Electron Microscopy (TEM). The fish measured 40 & 44 cm in total length and were also used for the molecular characterisation of the microsporidian (data not shown; see Freeman et al., 2013 (fish 9 & 10)). Dissected kidney tissues were fixed in 2.5% glutaraldehyde for 4 hours and washed in three changes Sorenson's buffer (0.1M, pH 7.4). Samples were then post-fixed in 1% osmium tetroxide

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for one hour, before dehydration through a graded acetone series. After dehydration to 100% acetone, the samples were transferred to a 1:1 mix of low viscosity resin and acetone for 1 hr on a rotator followed by a further 2 hr in a mix of 3:1 resin: acetone. Finally, the specimens were rotated for 24 hr in 100% low viscosity resin before being embedded in Beem capsules and polymerised at 60 °C for 48 hrs. Ultrathin sections (80 nm) were mounted on 200 mesh Formvar coated copper grids and stained with uranyl acetate and lead citrate. Grids were viewed on a Tecnai Spirit G2 Biotwin TEM at 120 kV and images taken.

Results

Clusters of microsporidian spores were found in the nuclei of lymphocytes and mononuclear precursor cells. Many infected cells containing microsporidian spores were degenerate (Figure 1a); however, some remained easily recognisable clearly showing the intranuclear location of the spores (Figure 1b). Mature spores are uniformly elongate ovoid in shape with two planes of symmetry and a relatively thin spore wall (Figure 2a). An isofilar polar filament is coiled around the posterior vacuole having 10-12 turns. The single nucleus is not shrouded by the polar filament and is situated centrally in the spore. Lamellar polaroplast fills the anterior end of the spore where the polar filament attaches to the spore wall (Figure 2b). Average mature spores measurements in ultrathin sections were 2.53 x 1.04 μm (n=10). Early developmental stages were not discovered in any of the infected cells in our sections. The earliest developing stage observed was considered to be a sporogonial plasmodium, due to its multinuclear condition and the presence of a thickened plasma membrane (Figure 3a). Up to four nuclei were seen in a single plane of a plasmodium which also contained aggregates of electron dense rings. The central part of the plasmodium was filled with a ribosome-rich reticulated granular matter with electron lucent areas located adjacent to the peripheral nuclei (Figure 3b).

Discussion

We were not able to detect any merogonial stages in our samples and the earliest develop-

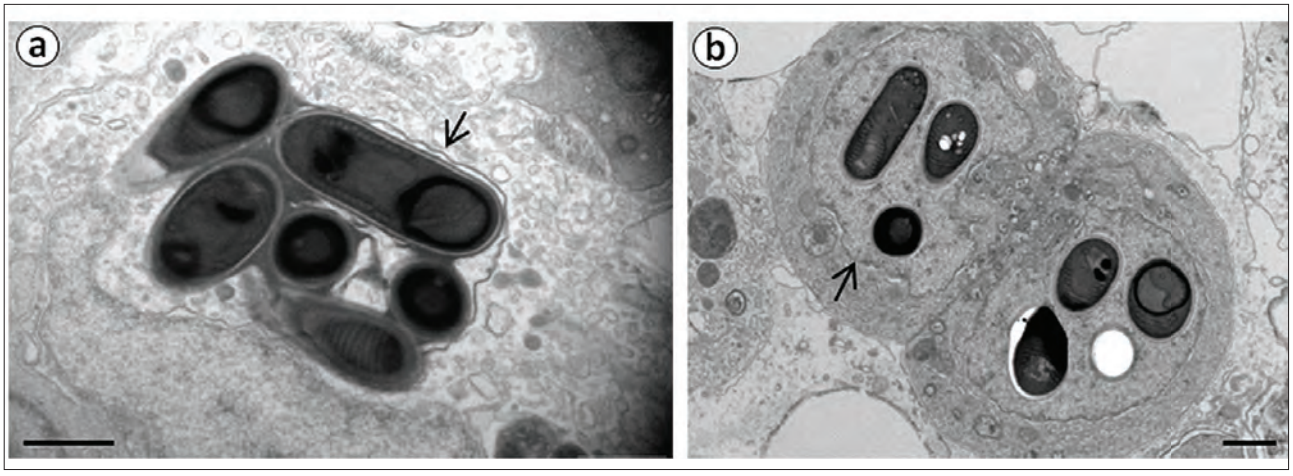

Figure 1. Mature spores of *Nucleospora cyclopteri* in the nucleus of a degenerate cell (a) and two lymphocytes (b). Black arrows indicate the nuclear membrane. Scale bars 1 μM.

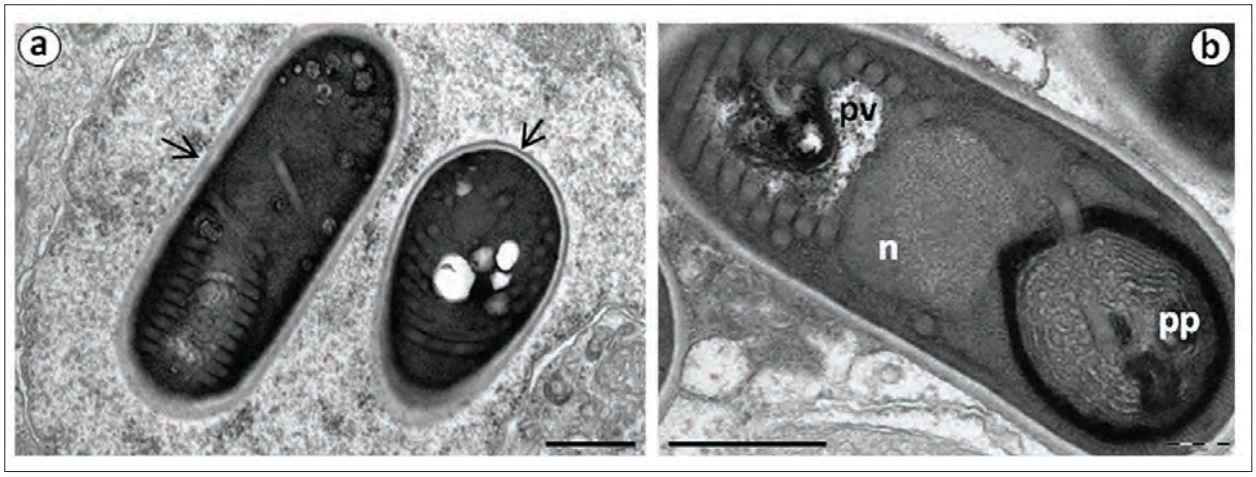

Figure 2. Mature spores of *Nucleospora cyclopteri* have a relatively thin spore wall (arrows) and contain an isofilar polar filament with 10-12 turns around the posterior vacuole (pv). Spores contain a single nucleus (n) and lamellar polaroplast (pp) at the anterior end of the spore. Scale bars 500 nm.

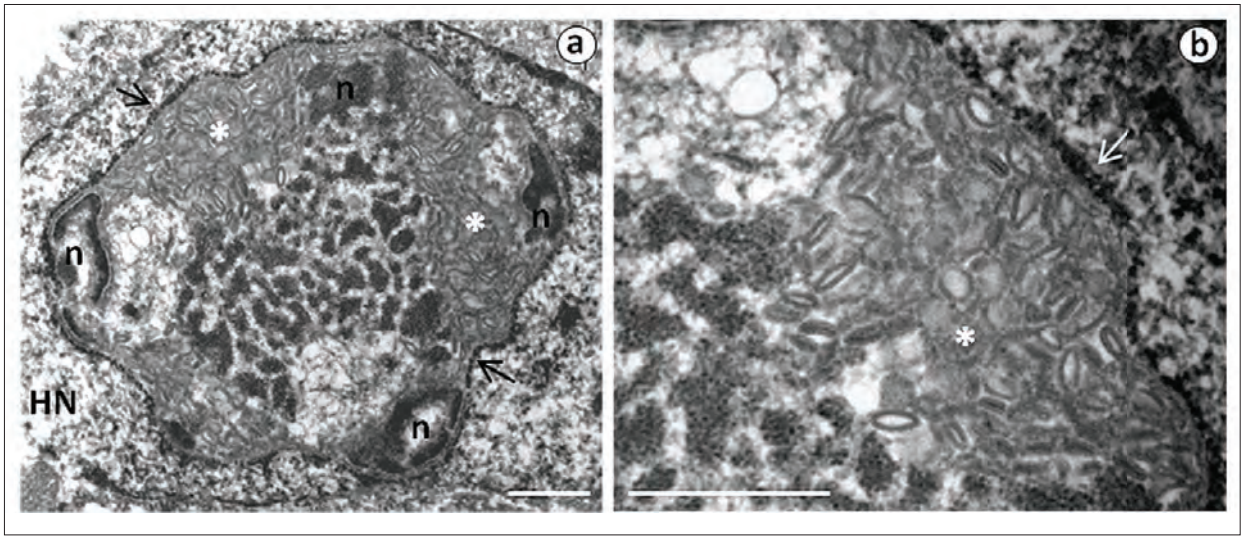

Figure 3. Multinucleate sporogonial plasmodium of *Nucleospora cyclopteri* in the host cell nucleus (HN), note the thickened plasma membrane (arrows). Numerous nuclei are visible in this plane of section of the plasmodium (n). The cytoplasm contains aggregates of electron dense discs (white asterisk), enlarged view (b). Scale bars 500 nm.

mental stage observed in this study we consider to be a sporogonial plasmodium (Figure 3). Early meronts are sometimes difficult to visualise and none were reported from Canadian lumpfish with intranuclear microsporidian infections by Mullins et al. (1994). The samples we observed were from fish with advanced clinical signs of disease, with the majority of infected lymphocytes either already degenerate or containing only mature spores. We believe that merogony must occur during the initial stages of infection and proceeds from a simple uninucleate stage to form a multinucleate meront that transforms directly into a sporogonial plasmodium without undergoing division, as shown in the congener *Nucleospora secunda* (Lom and

Dyková, 2002). The sporogonial plasmodium of *N. cyclopteri* contains electron dense discs, cylinders and tubular structures unique for the family Enterocytozoonidae (Freeman and Sommerville, 2009) which are believed to be polar tube precursors and are an ultrastructural diagnostic feature.

A typical number of mature spores of *N. cyclopteri* observed in an intact nucleus were between eight and twelve (Freeman et al., 2013). This likely represents the expected spore production from a single sporogonial plasmodium, taking into account that three or four nuclei are observed in a single plane of section (Figure 3) and spores are uninucleate (Figure 2). It may be the case that the presence of two proliferative cycles, merogony and sporogony, as described for some microsporidia, would simply result in the production of too many developing spores to be contained in a single host cell nucleus. In addition, the mechanism by which the cell nucleus becomes infected remains unknown and only one plasmodium has been observed per nucleus during our study.

The number of infected degenerate lymphocytes observed in our samples suggests that this microsporidian is pathogenic to the host fish, and its close genetic relative *Nucleospora salmonis*, has also been reported as a serious pathogen of cultured salmonids (Hedrick et al., 1991). Mullins et al. (1994) reported chronic mortalities associated with this microsporidian in captive lumpfish and we believe that *N. cyclopteri* will potentially cause problems in the future commercial production of lumpfish (Haugland et al., 2012; Freeman et al., 2013).

Mature spores, in full sagittal section, measured 2.53 x 1.04 μm with 10-12 turns of the polar filament. This is somewhat smaller than when measured fresh, 3.1 x 1.3 μm (Freeman et al., 2013), which is probably due to shrinkage during fixation or to the limitations of measuring small spores with a light microscope. The uniformly elongate ovoid shape of *N. cyclopteri* spores is not typical for the Enterocytozoonidae which usually have spores that are more subspherical/rounded or pyriform (Freeman and Sommerville, 2009), making the spore form of *N. cyclopteri* a useful diagnostic feature. Mullins et al. (1994) also measured their spores during an ultrastructural study (2.1 x 1.0 μm) typically with 11 turns of the polar filament. The ultrastructural spore dimensions and features from the Canadian and Icelandic isolates are very similar. In addition, both accounts describe degenerate infected lymphocytes and multinucleate plasmodial stages with electron dense structures in the nucleus of host fish lymphocytes. Some members of the Enterocytozoonidae are known to have a broad distribution (Freeman and Sommerville, 2011) and we consider that the same microsporidian parasite, *N. cyclopteri*, is responsible for infecting Atlantic lumpfish in Canada and Northern Europe.

Acknowledgements

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