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REVIEW ARTICLE

Natural infections of *Nosema ceranae* in European honey bees

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Introduction

The classification of the large and heterogeneous group of microorganisms called protozoans has been recently updated as increased knowledge of the biology and phylogenetics is acquired. The Committee on Systematics and Evolution of the Society of Protozoologists established seven phyla in the elevated subkingdom Protozoa under the Kingdom Protista more than two decades ago (Levin, *et al.,* 1980). Later, it was suggested that the protists should be reorganized into the broader category protoctists that would also include fungi and algae besides the small eukaryotes consisting of a single or a few cells (Margulis *et al.,* 1990). However, with the rapidly increasing phylogenetic data accumulating, this higher order taxonomy is no longer accurate. Based on molecular phylogenies, microsporidia are actually included into the cluster Fungi, rank Opisthokonta which comprises the animals, the fungi and others eukaryotes (Sina *et al.,* 2005). Microsporidia are, thus, to be regarded as highly specialized parasitic fungi.

Only two microsporidian parasites are described so far from honey bees (*Nosema apis* Zander 1909 and *Nosema ceranae* Fries *et al.* 1996). *Nosema apis* was detected in the European honey bee (*Apis mellifera* L 1758) and is one of the first microsporidia to be described (Zander, 1909). Although the parasite and its life cycle have been described by many authors (see Gray *et al.*, 1969), vegetative stages are difficult to recognize and identify by light microscopy. These early descriptions have later been complemented with ultrastructural features of the parasite (Liu, 1984; Fries, 1989) and also with a molecular characterization (Gatehouse & Malone, 1998). *Nosema ceranae*, isolated from the Asian honey bee (*Apis cerana* Fabricius 1793) in China is a more recent description (Fries *et al.*, 1996). There are good reasons to assume that other microsporidia species are also present in honey bees and await full descriptions (i.e. Buys, 1977; Clark 1980).

Prior to the description of *N. ceranae,* observations of microsporidian infections in *A. cerana* had already been made (Sing, 1975). Yakobson (1992) observed microsporidia infections in both *A. cerana* and *A. mellifera* in apiaries with both honey bee species mixed and suggested that cross infection experiments using *N. apis* spores could perhaps elucidate the question of host specificity in *N. apis.* Many species of microsporidia cannot be distinguished using light microscopy and only with difficulty using electron microscopy (Larsson, 1986; Rice, 2001) and it cannot be excluded that some earlier observations of microsporidia infections in *A. cerana*, and possibly also in *A. mellifera*, may in fact have been observations of *N. ceranae.* Reports in the past of damage to A. cerana colonies attributed to *N. apis* infections (Lian, 1980) may actually be reports of *N. ceranae*, since differences between *N. ceranae* and *N. apis* may have gone unnoticed when investigated under the light microscope (Fries *et al.*, 1996).

Cross infections between the two host species have demonstrated that *N. apis* is in fact infective for *A. cerana*, but also that this parasite develops less well in the Asian host compared to the European host (Fries & Feng, 1995). It has also been stated that *N. ceranae* is infective for *A. mellifera* and multiplies more readily in *A. mellifera* than *N. apis* does in *A. cerana* (Fries, 1997), although detailed data were never published.

At the annual meeting of Society for Invertebrate Pathology in Anchorage, Alaska, 2005 it was reported that *N. ceranae* had been found in natural infections in *A. mellifera* in Taiwan (Huang *et al.*, 2005). The apiary where the infection was detected had harboured both *A. mellifera* and *A. cerana.* Thus, it was apparent that *N. ceranae* could cross the host species barrier, although no data on bee pathological repercussions due to *N. ceranae* in *Apis mellifera* were mentioned by the authors.

Almost at the same time and following progressively increased incidences of problems with nosema disease in Spain (Martin *et al.*, 2005), the laboratory of Centro Apícola Regional, involved in

diagnosis of honey bee diseases, confirmed for the first time in Europe *N. ceranae* in field samples of European honey bees (Higes *et al*., 2006). When the 16S small subunit rRNA gene from 11 samples was sequenced, it was found that the sequence obtained (GenBank Accession number DQ286728) for 10 of these samples were homologous to the original GenBank entry for *N. ceranae* (U26533). Only one out of 11 microsporidian-infected samples was found to match the GenBank entry for the *N. apis* sequence (U26534). More recently, sequences of microsporidia from samples of European honey bees from France (DQ374655) and Germany (DQ374656) have also been found to be homologous to the *N. ceranae* sequence (U26533).

The findings of *N. ceranae* in several geographically separated populations of honey bees in Europe suggest that this is not a novel introduction of a parasite into a new host species. The recognized chronic nature of infections of entomopathogenic microsporidia (Becnell & Andreadis, 1999) supports this hypothesis. Pathological data from Spanish apiaries affected by *N. ceranae* do not show a fast acting, short duration syndrome. On the contrary, only nonspecific symptoms, such as a gradual depopulation, higher autumn/winter colony death or low honey production are associated with the presence of this parasite. None of the dysentery or crawling bee behaviour usually related with *Nosema apis* infection has been reported. A similar syndrome has recently been reported from France and called "dry nosemosis" (Faucon, 2005).

Using stored samples of microsporidian-infected in honey bees, it may be possible to trace the infection back in history, but we are unlikely to unravel all relevant details. The two

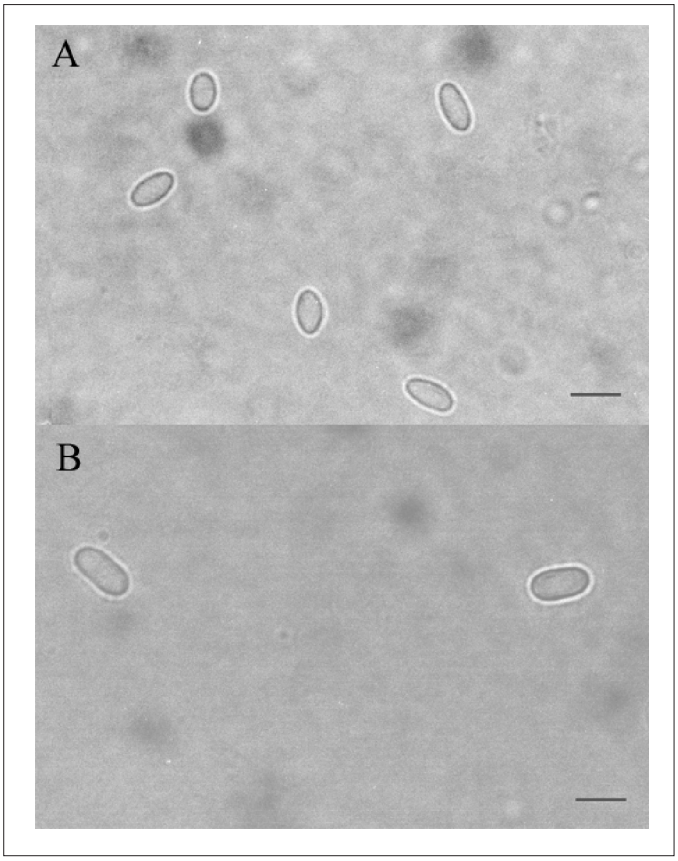

Fig. 1. Spores of *Nosema ceranae* (A) and *Nosema apis* (B) in light microscopy squash preparations. Bar = 5 μm.

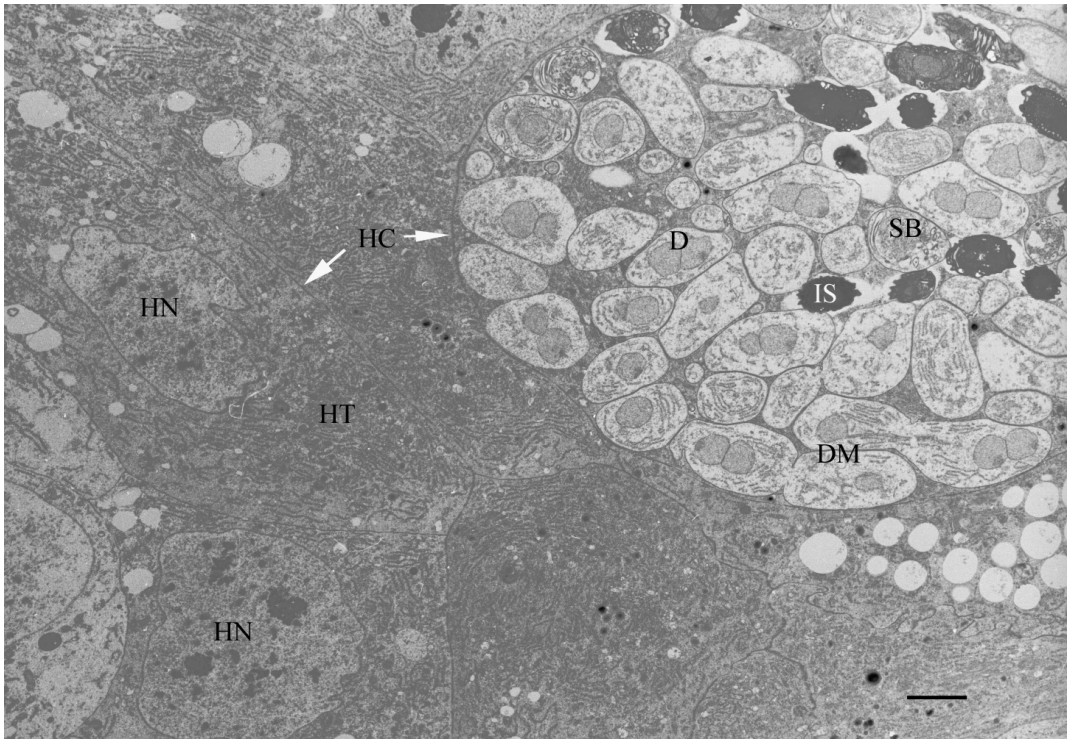

Fig. 2. Ventricular cell of *Apis cerana* infected by *Nosema ceranae*. Note infected cell isolated in surrounding healthy tissue (HT). D = diplokaryotic merozoite; DM = dividing merozoites; HC = host cell membrane (arrows); HN = host cell nucleus; IS = immature spore; SB = sporoblast with developing polar filament. Bar = 5 μm

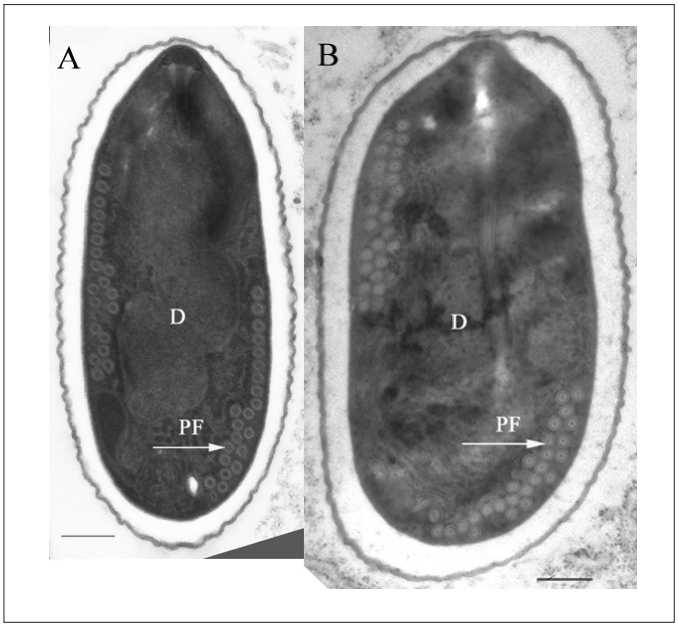

Fig. 3. Sections of spores of *Nosema ceranae* (A) and *Nosema apis* (B). D = diplokarya; PF = polar filament coils (arrows). Bars = 0.5 μm.

parasite species may in fact have existed as parallel infections for a considerable amount of time. The increased detection of *Nosema* spores in honey bees in recent years with absence of typical signs associated with *N. apis* and the recent report of *N. ceranae* from Europe, suggests that there may be a link between these phenomena. However, research is needed before it can be established if *N. ceranae* has different effects on European honey bees compared to *N. apis*.

Infections by *N. apis* can be prevented by certain beekeeping precautions and may be treated by use of the antibiotic fumagillin (Katznelson and Jamieson, 1952). However, it remains to be seen if the recommendations for nosema control, valid for *N. apis,* are also relevant for *N. ceranae*, and if the antibiotic fumagillin inhibits intracellular development of this parasite. This antibiotic is not effective in the control of *Nosema bombi,* infecting bumble bees (Whittington & Winston, 2003).

Differential diagnosis of *Nosema ceranae*

Although the differences in spore size between *N. ceranae* and *N. apis* are not immediately apparent in light microscopy, there is a consistent size difference between the spores of the two species, with *N. ceranae* being slightly smaller compared to *N. apis* (Fries *et al*., 1996). The length of unfixed spores of *N. ceranae* isolated from *A. cerana* measure 4.7±0.46 μm, range 3.3 – 5.5 μm, *n*=25.The width of the spores measure 2.7±0.25 μm, range 2.3 – 3.0 μm *n*=25 (Fries *et al*., 1996). Although there is a slight overlap, with the smallest *N. apis* spores being smaller than the largest *N. ceranae* spores, the average spore size of *N. apis* is approximately 1 μm larger in length (Figure 1). The spores of *N. ceranae* sometimes appear slightly bent which gives a less consistent appearance compared to *N. apis* (Figure 1). Another difference between the two species is how the infection is distributed in cells of newly infected ventriculi. When mature spores of *N. apis* are found in the epithelial cells of *A. mellifera*, there are always vegetative cells (merozoites) of the parasite in neighbouring cells, probably due to intracellular germination of spores with a resulting horizontal transmission between epithelial cells (Fries, 1989). In infections of *N. ceranae* in *A. cerana*, however, the infection often appears as isolated islands of single infected epithelial cells, also with mature spores developed inside the host cells (Figure 2). This has been interpreted as lack of horizontal transmission between cells, because no emptied spores are found inside the cells in *N. ceranae* infections in *A. cerana* (Fries *et al*., 1996).

When the ultrastructure of spores is examined, differences between the two species are obvious. The polar filament of *N. ceranae* is consistently shorter with 20–23 coils (Fries *et al*., 1996) compared with *N. apis,* where the number of filament coils always is larger and spores often contain around 26–32 coils (Fries, 1989) (Figure 3). Sequencing of the 16S SSU rRNA gene of microsporidian isolates clearly distinguishes the two parasite species (Fries *et al*., 1996). For screening purposes, gene sequencing is comparatively expensive, and molecular differential diagnosis of the two parasite species will not be practical until specific primers for PCR or other rapid diagnosis tools are available. Eventually, such tools will be decisive in completing a picture of the parasite's actual distribution and for determining if mixed infections of the two parasite species occur.

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