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Short communication

Ultrastructural analysis supports transferring *Nosema whitei* Weiser 1953 to the genus *Paranosema* and creation a new combination, *Paranosema whitei*

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Abstract

The current ultrastructural description of *Nosema whitei* is in agreement with the genus definition of *Paranosema* [Sokolova, Y.Y., Dolgikh, V.V., Morzhina, E.V., Nassonova, E.S., Issi, I.V., Terry, R.S., Ironside, J.E., Smith, J.E., Vossbrinck, C.R., 2003. Establishment of the new genus *Paranosema* based on the ultrastructure and molecular phylogeny of the type species *Paranosema grylli* Gen. Nov., Comb. Nov (Sokolova, Selezniov, Dolgikh, Issi 1994), from the cricket *Gryllus bimaculatus* Deg. Journal of Invertebrate Pathology 84, 159–172]. In addition to exhibiting similar spore morphology and sporogony type, *N. whitei*, like *P. grylli* and *Paranosema locustae,* possesses a distinct meront–sporont transitional stage in the life cycle; develops in the host fat body, and produces secretory material arranged in "tubular structures" during sporogony. Ultrastructural analysis supports the similarity of *N. whitei* to *P. grylli* and to *P. locustae* as predicted on the basis SSrDNA sequence data (GenBank Accession Nos. AY305323, AY305325, and AY305324). Comparative studies of these three related species provide a good example of the consistency of morphological and sequence data, and support both the placement of *N. whitei* inside the genus *Paranosema* and the validity of the new combination *Paranosema whitei* (Weiser) . Supported by Russian Foundation for Basic research: Grants 04-04-49314 and 03-04-49629.

Keywords: *Nosema whitei*; *Paranosema grylli*; *Paranosema locustae*; *Tribolium* sp.; Microsporidia; Ultrastructure; Phylogeny

1. Introduction

Comparative phylogenetic analysis of SSrDNA showed that *Nosema grylli* (GenBank Accession No. AY305324), a microsporidian parasite of the cricket *Gryllus bimaculatus*, is closely related to *Nosema locustae* (GenBank Accession No. AY305325), and to *Nosema whitei* (GenBank Accession No. AY305323) from flour beetles, *Tribolium* spp. (Sokolova et al., 2003). The sequence divergence and morphological traits clearly separate this group of parasites from the "true" *Nosema* clade with *Nosema bombycis* as a type species, which parasitizes mainly lepidopterans. The generic name of *N. grylli* and its close relative *N. locustae* therefore has been changed to *Paranosema*. *N. whitei* Weiser 1953 was left in its original status due to lack of data on fine morphology (Sokolova et al., 2003). Very few details of the *N. whitei* ultrastructure were resolved in the pioneer work

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of Milner (1972a), one of the earliest applications of transmission electron microscopy to microsporidia. The goal of this paper is to present updated ultrastructural data on *N. whitei*, and to establish its proper classification, in this case, a new combination, *Paranosema whitei* (Weiser).

2. Materials and methods

Microsporidial infection naturally occurred in a population of flour beetles, *Tribolium castaneum*, infesting private flour storage in St. Petersburg. Observation of fresh smears and Giemsa-stained preparations made from the infected insects allowed identification of the microsporidium as *Nosema whitei* based on slide collection of Irma V. Issi (All Russian Institute for Plant Protection, St. Petersburg, Russia). Dry microsporidia-killed *T. castaneum* specimens, heavily loaded with spores, were collected and refrigerated until use. Healthy cultures of *T. castaneum* were obtained from the insectarium of the Institute of Plant Protection, St. Petersburg, Russia. Insects were reared at 25 °C on a diet containing four parts of oatmeal and one part of dried yeast, with vegetable or fruit peelings to supply moisture. For experimental infection, dried infected specimens were ground to a powder in a mortar. The powder was washed several times by centrifugation, the resultant pellet consisting mostly of mature *N. whitei* spores. The final suspension containing approximately 10^6 spore/ml was mixed with the rearing medium and the beetles were exposed to this mixture. In 40 days, larvae and adults were checked for the presence of microsporidia spores. The infected individuals were dissected under the dissecting microscope, and their fat bodies were removed and processed for routine electron microscopy (Sokolova et al., 2003). Fresh or methanol fixed and stained with Trichrom or Giemsa, smears of microsporidia-infected insects were photographed and measured under Nikon Eclipse E-600 Microscope equipped with a Metaview digital camera and software (MetaView. 1998, Meta Imaging Series 4.5. Universal Imaging Corporation, West Chester, PA).

3. Results and discussion

3.1. Spore measurements

Living spores measured from 3.87 to 4.97 μm in length, and from 2.46 to 3.12 μm in width, averaging $4.64 \pm 0.098 \times 2.85 \pm 0.074$ μm ($x \pm$ SE, $n = 11$), with a width/length ratio of 0.62 ± 0.015 μm. Methanol fixed spores measured from 3.78 to 4.62 μm in length, and from 2.37 to 3.05 μm in width, averaging $4.19 \pm 0.067 \times 2.62 \pm 0.053$ μm ($n = 15$), with a width/length ratio of 0.63 ± 0.014 μm. Careful examination of the fixed and stained material revealed also the presence of slightly elongated macrospores (Fig. 1A), that averaged 5.72×3.19, ranged from 5.64 to 6.5 μm in length and 2.88 to 4.0 μm in width, and had a mean width/length ratio of 0.55 ± 0.015 ($n = 10$). Macrospores were rare; their occurrence did not exceed 1% of the total number of spores. All these spore measurements are in good accord with a previous study of this microsporidium (Milner, 1972b). Slightly larger average values for measurements of living spores were obtained by Milner, which can be explained by the fact that he did not differentiate macrospores, including them in his morphometric analyses. While being of comparable length with *Paranosema grylli* (c. 4.50 μm) and *Paranosema locustae* (c. 4.95 μm), *P. whitei* spores appeared to be wider (width/length ratio 0.62 versus 0.49 in *P. grylli* and 0.54 in *P. locustae*) and thus can be easily distinguished on smears (Fig. 1A). Interestingly, *Antonospora scoticae* (Fries et al., 1999) which is clustered as a sister group within the same clade with *P. grylli*, *P. locustae*, and *N. whitei* according to SSrDNA-based phylogenies has larger (averaging 6.8×2.7 μm) and more elongated (width/length equaling 0.40) spores, than any of these three species. These data suggest that spore size, especially the width/length index, can be a reliable visual character helpful in differentiating between related species.

3.2. Ultrastructure

Meront (Fig. 1B) ultrastructure is similar to merogonial stages of *P. grylli* and *P. locustae* (Sokolova and Lange, 2002; Sokolova et al., 2003). Round or oval cells measuring 3.8–5.0 μm in diameter were surrounded by a single plasma membrane. Nuclei were in diplokariotic arrangement. Free ribosomes were abundant. Membrane structures were associated with the expansions of perinuclear space. Conglomerates of vesicle-like profiles with coated membranes 30–60 nm in diameter were associated with smooth cisternae, similar to those described previously as meront Golgi organelles for *P. grylli* (Sokolova et al., 2001; Sokolova et al., 2003).

Meront–sporont transitional stages (Figs. 1C–E, 2A) were distinct. Cells were characterized by the appearance of electro-dense inclusions in the nucleoplasm associated with synaptonemal complexes, the markers of the meiotic prophase (Figs. 1C and 2A, inset). Cells with one, two (Figs. 1D and E) or four individual nuclei (not shown) in the view were typical for this phase of the parasite's life cycle, though resolving the nuclear condition (diplokaryotic versus haplokaryotic) is not always feasible on TEM sections. Previous light microscopy studies indicated the presence of haplokaryotic stages in all species of the genus *Paranosema* (Canning, 1953; Milner, 1972a; Sokolova et al., 2003). Haplokaryons might have been produced as a result of two sequential meiotic divisions, which may or may not have been followed by