Nosema bombi, A MICROSPORIDIAN PATHOGEN OF THE BUMBLE BEE *Bombus lucorum*

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Summary

This paper reports an examination of *N. bombi* isolated from the native bumblebee (*B. lucorum*) in China using electron microscopy to study its morphology, compare with *N. bombi* (*B. terrestris*) and *N. apis*. *N. bombi* (*B. lucorum*) is oval, showing blue refraction, its surface is smooth, no characteristic shape was found. And no difference was found between the morphology of *N. bombi* obtained from *B. lucorum* and that from *B. terrestris*, significant differences were found between the ultra-structure of *N. bombi* (*B. lucorum*) and that from *N. apis*. So the results show that *N. bombi* (*B. lucorum*) was distinct from *N. apis*.

Keywords: bumble bees, *Bombus lucorum*, *Nosema bombi*, morphology, ultra-structure.

INTRODUCTION

In recent years there has been increased interest in bumble bees as pollinators of a range of horticultural crops, especially in glasshouses (Fisher and Pomeroy 1989). Although the microsporidian pathogen of honey bees, *Nosema apis* Zander and the microsporidian pathogen of *Bombus terrestris* (L.) *Nosema bombi* have been studied in some detail (Eijnde and Vette 1993), there have been few studies of microsporidia from bumble bees in China.

Fantham and Porter (1914) were the first to describe a microsporidium from bumble bees, naming it *Nosema bombi* after the type host *Bombus agrorum* (F.). Hereafter, many countries reported that *N. bombi* was found in various *Bombus* in Europe, North America, New Zealand etc. (MacFarlane et al. 1995). In China *N. bombi* is found in *B. terrestris* imported from New Zealand, and *N. bombi* is found in the native *Bombus*, but has not been investigated, so it needs further research in China.

This paper reports an examination of *N. bombi* isolated from *B. lucorum* in China using light and electron microscopy to study its morphology, and ultra-structure. At the same time, *N. bombi* was compared with *N. apis*.

MATERIALS AND METHODS

One hundred and one infected queens of *B. lucorum* were collected in China in fields in 2003, and 96 queens of *B. terrestris* were imported from New Zealand, 200 infected workers of *Apis mellifera* were collected from a hive at the Institute of Apicultural Research CAAS in China in 2004. Collected infected insects were dissected and their guts were removed and homogenized in distilled water with homogenizer. The resultant suspension was filtered through two layers of fine nylon mesh, and the spores washed three times by repeated centrifugation (400 g, 15 min) and resuspension in distilled water. Spore suspension was used for an electron microscope study, Spores were fixed for a day with 6% (v/v)

glutaraldehyde in 0.1M sodium cacodylate buffer (SCB) (pH 7.2) at 5°C. They were washed several times with buffer, embedded in agar, which was cut into small pieces, fixed with 0.3% (w/v) osmium tetroxide in 0.1M SCB for 5 min. After several washes with 0.9% (w/v) sodium chloride solution, the spores were dehydrated in an ethanol series, embedded in Spurr (1969) resin, sectioned with glass knives, stained with uranyl acetate and lead citrate, and examined under a transmission electron microscope (TEM).

Measurements were made using an eyepiece micrometer at 1000×magnification. Spores from adult bees were measured from water mounts. Data analyses were done with SAS V6.12, values are means and S.E.

RESULTS

Mature spores were measured from water mounts of the gut tissue from infected insects (Table 1). Fresh spores of *N. apis* are significantly larger than those of *N. bombi* (*B. lucorum*), there were no significant differences between *N. bombi* (*B. lucorum*) and *N. bombi* (*B. terrestris*), their surfaces are smooth, nor any characteristic shape was found (Plate 1, Plate 2, Plate 3).

No difference were found on the ultra-structure morphology of *N. bombi* obtained from *B. lucorum* and *B. terrestris*, significant differences were found on the ultra-structure of *N. bombi* (*B. lucorum*) and *N. apis* (Plate 5, Plate 6).

Most of the spores of *N. bombi* and *N. apis* are oval in shape. Electron-microscopic studies show that their spores have a sheath of four layers. The outer one is electron-optically dense, folded in waves, and is rough in appearance. In mature spores it is more compact and smooth (Plate 5, Plate 6), whereas in young spores it is a considerably thicker and more fragile structure and almost equal in depth in all spores (Plate 7). It is thicker at the posterior pole of the spore compared with the anterior one. Electron-optically this layer is more transparent. The internal (third) layer of the sheath is much thinner, with fine structure, and electron-optically it is denser. The fourth layer of the sheath is to be observed immediately at the sporoplasm, in some cases, it is difficult to be distinguished from it and from the third. It has a very fragile and reticular structure and is electron-optically more transparent.

The sporoplasm of *N. bombi* is situated between the polaroplast and the posterior part of the spore, while the two nuclear membranes are not clearly outlined, Two

Table 1

Nosema species described from different host bees

Species	n	Spore size (μm)	Length (μm) ($\bar{X} \pm S$)	Width (μm) ($\bar{X} \pm S$)	Polar filament coils	Polar filament bevel angle $\bar{X}$
N. apis	200	5.34×2.46	5.34±0.45a	2.46±0.18a	18 ~ 44	57°
N. bombi (*B. lucorum*)	101	4.88×2.88	4.88±0.24b	2.88±0.11b	14 ~ 18	52°
N. bombi (*B. terrestris*)	96	4.88×2.88	4.88±0.04b	2.88±0.03b	14 ~ 18	52°

Note: ANOVA with length and width of spores, the different letter means significant difference, or else no difference, $P<0.05$

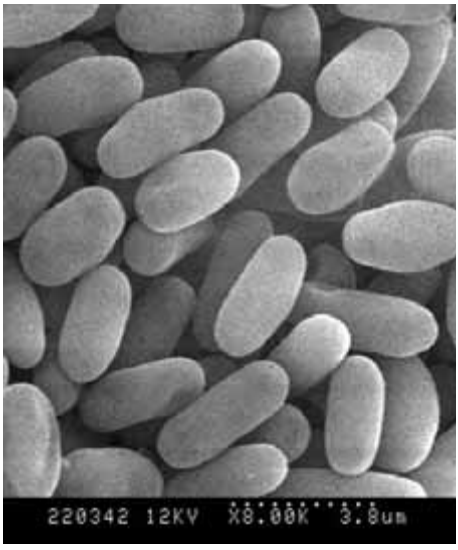

Plate 1 *N. bombi* (*B. lucorum*)

Plate 2 *N. bombi* (*B. terrestris*)

Plate 3 *N. apis*

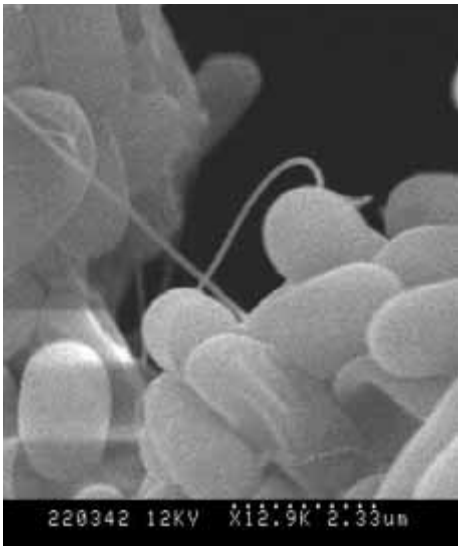

Plate 4 Polar filament

nuclei were found in the posterior half of the spore, which is similar to *N. apis*.

The polaroplast of the spore of *N. bombi* has a lamellar structure similar to its structure in *N. apis*. It occupies the anterior part of the spore and is more transparent electron-microscopically, resembling a vacuole.

The polar filament of *N. bombi* has about 14-18 coils with an angle of tilt of at least 52°. The polar filament of *N. apis* has about 18-44coils, with an angle of tilt of at least 57° (Table 1). The base of the polar fibre attached at the anterior part of the spore is the thickest. From there the filament is directed backward along the longitudinal axis of the spore where it winds into spirals. (Plate 4, Plate 5, Plate 6).

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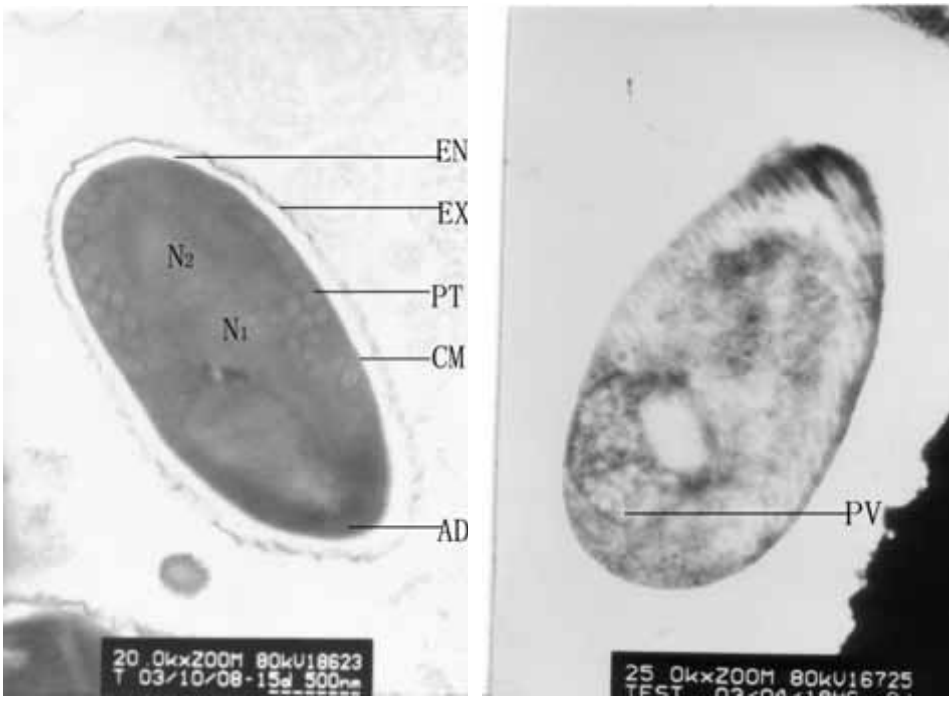

Plate 5 *N. bombi*

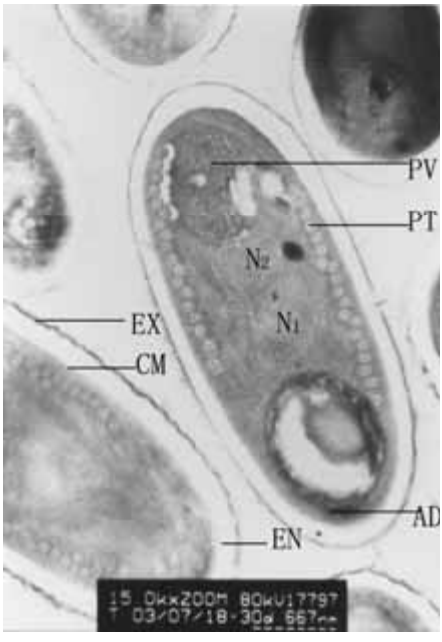

Plate 6 *N. apis*

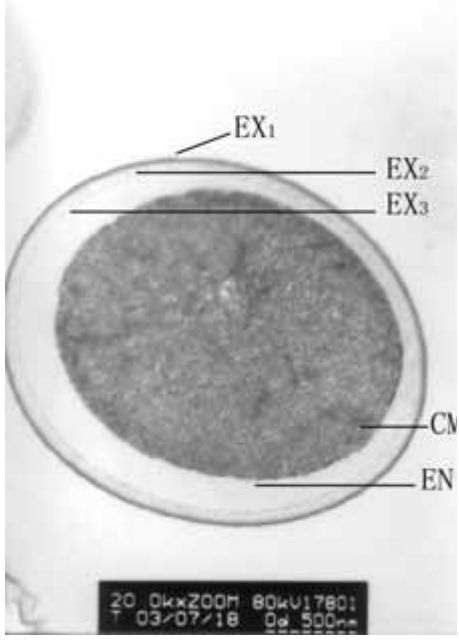

Plate 7 *Immature spore*

DISCUSSION

So far, all infections in bumble bees have been attributed to *N. bombi* Fantham and Porter (1914), although it is unclear whether all infections are in fact by the same parasite. No difference was found between the morphology of *N. bombi* obtained from *B. lucorum* and and that from *B. terrestris*, significant differences were found between the ultra-structure of *N. bombi* (*B. lucorum*) and that from *N. apis*. So the results show that *N. bombi* (*B. lucorum*) was distinct from *N. apis*. *N. bombi* obtained from *B. lucorum* and *B. terrestris* are synonymous.

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Nosema bombi MIKROSPORIDIALNY PATOGEN TRZEMIELA *Bombus lucorum*

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Streszczenie

W pracy przedstawiono wyniki przeprowadzonych przy użyciu mikroskopu elektronowego badań morfologicznych *N. bombi* wyizolowanego z rodzinnego gatunku trzmiela gajowego (*B. lucorum*) występującego w Chinach i porównania go z gatunkami *N. bombi* pochodzącego od trzmiela ziemnego (*B. terrestris*) i *N. apis* od robotnic pszczół. Spory *N. bombi* (*B. lucorum*) są kształtu owalnego, wykazują błękitną refrakcję i mają gładką powierzchnię. Nie stwierdzono charakterystycznych odrębności, jeśli chodzi o kształt. Nie stwierdzono różnic pod względem cech morfologicznych pomiędzy *N. bombi* uzyskanym z *B. lucorum* i uzyskanym z *B. terrestris*. Stwierdzono istotne różnice pod względem ultrastruktury pomiędzy *N. bombi* (*B. lucorum*) i *N. apis*. Wyniki wskazują, że *N. bombi* (*B. lucorum*) stanowi gatunek odrębny od *N. apis*.

Słowa kluczowe: trzmiel gajowy, *Bombus lucorum*, *Nosema bombi*, morfologia, ultrastruktura.