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Characteristic Features of *Nosema phyllotretae* Weiser 1961, a Microsporidian Parasite of *Phyllotreta atra* (Coleoptera: Chrysomelidae) in Turkey

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Mustafa Yaman, Renate Radek, İrfan Aslan, and Ömer Ertürk (2005) Characteristic features of *Nosema phyllotretae* Weiser 1961, a microsporidian parasite of *Phyllotreta atra* (Coleoptera: Chrysomelidae) in Turkey. *Zoological Studies* **44**(3): 368-372. Characteristic features of *Nosema phyllotretae* infecting *Phyllotreta atra* collected in Gümüşhane, Turkey are given in this paper. Fixed and stained spores are oval, 4.08 ± 0.56 (range, 2.90~5.20) μm in length and 2.53 ± 0.33 (range, 1.80~2.90) μm in width (n = 50) with 14 coils of the polar filament. The spore wall is 110 to 175 nm thick and made up of a clear endospore (90 to 125 nm) and a uniform exospore (50 to 64 nm). The anterior region of the polaroplast is thinly lamellar. The ultrastructural features of *Nosema phyllotretae* differ from those of other chrysomelid parasitic microsporidia.
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Key words: *Nosema phyllotretae*, Characteristic features, microsporidian parasite, *Phyllotreta atra*.

Phyllotreta atra (Coleoptera: Chrysomelidae) is one of the important pests of cabbage in the Black Sea region of Turkey. Natural enemies of this pest are of great interest. Some parasitic organisms were previously recorded from *P. atra* (Oldham 1933, Weiser 1961). Recently, a gregarine parasite, *Gregarina phyllotretae* (Yaman 2002a) and a nematode parasite, *Howardula phyllotretae* (Yaman 2002b) of *P. atra* were recorded in Turkey. It is believed that entomopathogenic microorganisms can decrease insect population densities and reduce durations of outbreaks (Myers 1988). Microsporidia are among the most important pathogens of insects with a potential to reduce pest insects (Tanada and Kaya 1993).

Of the 800 or so microsporidian species described (Canning 1990), at least 200 belong to the genus *Nosema* (Sprague 1981). This seemingly disproportionate number of *Nosema* species may be due partly to incorrect identifications. Early descriptions, mainly based on spore morphology and lacking ultrastructural details, sometimes resulted in the unnecessary creation of new species (Malone and McIvor 1995). In the most recent identification keys to microsporidian genera, it was necessary to use at least a minimum of ultrastructural characters (Larsson 1983 1988 1999). It is also necessary to know how the sporont divides and the number of sporoblasts formed. The spore is the most important life cycle stage for the identification of microsporidian species. In the present paper, the spore ultrastructure of *Nosema phyllotretae*, whose light-microscopic spore morphology was described by Weiser (1961), is presented and compared with other *Nosema* species parasitic in the family Chrysomelidae (Coleoptera). The prevalence and distribution of the parasite in the Middle and

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Eastern Black Sea Region of Turkey are given.

MATERIALS AND METHODS

Adults of *P. atra* were randomly collected and dissected from 4 different localities, Çarşamba (Samsun), Trabzon, Rize, and Gümüşhane in Turkey from Mar. to Oct. in 2000 and 2001.

Light microscopy

Each beetle was dissected, and wet smears were examined under a microscope for protozoan parasites. When a parasitic infection was observed, a fragment of the infected host tissue was dilacerated and spread on a slide. The smear was air-dried and then fixed with methanol for 10 min. After washing in distilled water, the slides were placed for approximately 10 h in a freshly prepared 5% solution of Giemsa stain. They were then washed in running water, air-dried, and examined under a microscope (Toguebaye et al. 1988). Fifty randomly selected spores were measured.

Electron microscopy

Different portions of infected beetles were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1~2 h, rinsed in cacodylate buffer, postfixed in reduced OsO_4 according to Karnovsky (1971) (a fresh 1:1 mixture of 3% $K_4(Fe(CN_6))$ and 2% OsO_4) for 1.5 h, rinsed in cacodylate buffer and dehydrated in ethanol prior to embedding in Spurr's resin (Spurr 1969). Thin sections were mounted on Pioloform-coated copper grids and stained with saturated uranyl acetate and Reynolds' lead citrate (Reynolds 1963). They were examined in a Philips 208 electron microscope.

RESULTS AND DISCUSSION

The microsporidian was found in adults of *P. atra* collected in Gümüşhane. Fixed and stained spores are oval, 4.08 ± 0.56 (range, 2.90~5.20) μm in length and 2.53 ± 0.33 (range, 1.80~2.90) μm in width (*n* = 50) (Figs. 1, 2). Sporonts are diplokaryotic (Fig. 3), and sporophorous vesicles are not formed.

Ultrastructural studies revealed that the polar filament has 13~15 (commonly 14) coils (Fig. 4). The spore has 2 closely associated nuclei (Fig. 4). The polar filament contains a central core surrounded by 4 concentric layers. The spore wall is

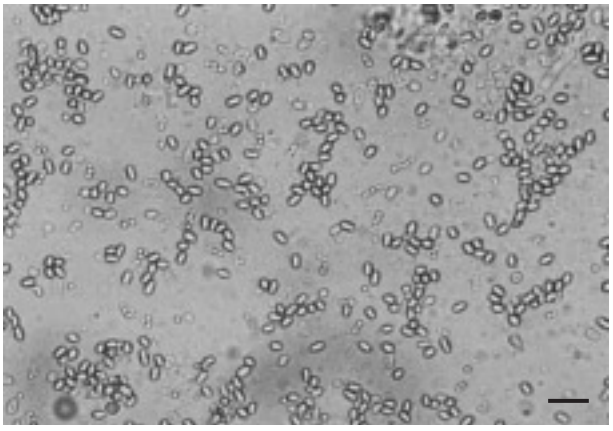

Fig. 1. Fresh spores of *Nosema phyllotretae*. (bar = 12 μm)

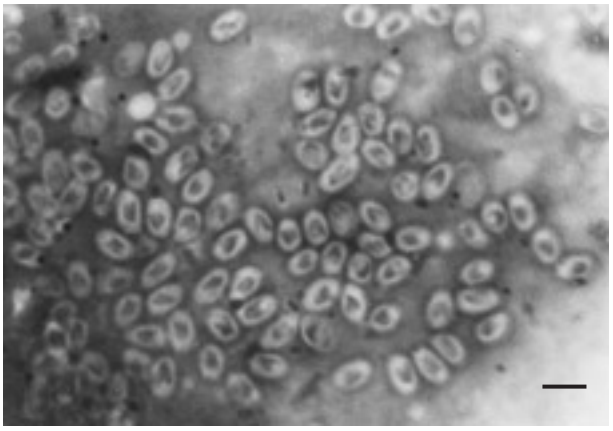

Fig. 2. Giemsa-stained spores of *Nosema phyllotretae*. (bar = 5 μm)

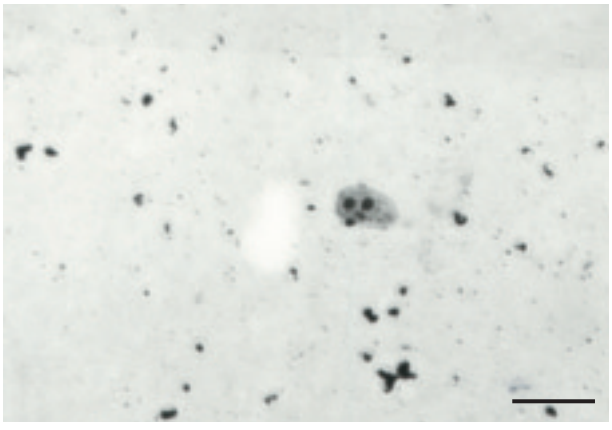

Fig. 3. Diplokaryotic sporont of *Nosema phyllotretae*. (bar = 12 μm)

110 to 175 nm thick and is made up of a clear endospore (90 to 125 nm) and a uniform exospore (50 to 64 nm) (Fig. 5). The polar filament measures 120 nm in diameter in the 10 mature spirals and then 90 nm in the last 4 immature spirals. The anterior part of the polaroplast is thinly lamellar (Fig. 6).

Light and electron microscopic characteristics of the microsporidian parasite of *P. atra* indicate that it belongs to the genus *Nosema* Naegeli, 1857. The recorded parasite has typical characters of the genus *Nosema* such as the shapes of fresh (Fig. 1) and stained spores (Fig. 2), spore size, presence of diplokaryotic stages (Figs. 3, 4), a uniform exospore (Fig. 5), and the thickness of the spore wall (Larsson 1986 1999). The exospore of the parasite is uniform and 50 to 64 nm. It is usually 40~60 nm in the genus *Nosema* (Larsson 1986).

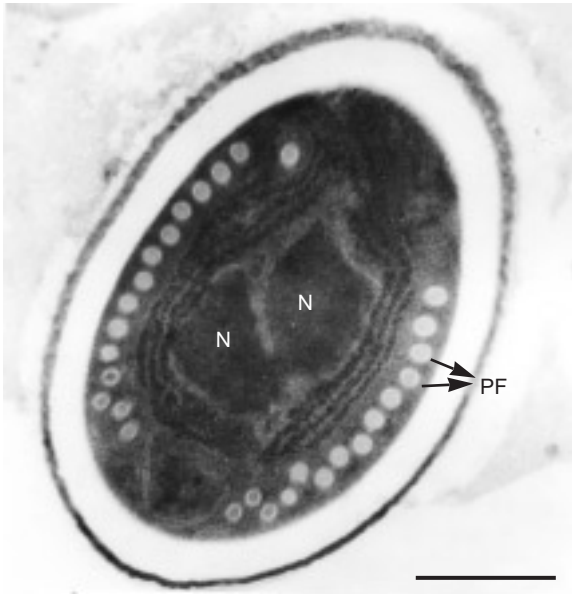

Fig. 4. Section of a *Nosema phyllotretae* spore showing the 14 coils of the polar filament (PF). N, nucleus. (bar = 1 μm)

The microscopical characters observed show that the protozoan parasite of this study is *N. phyllotretae* Weiser 1961. Fixed and stained spores of *N. phyllotretae* were 4.08 ± 0.56 μm long and 2.53 ± 0.33 μm wide. Weiser (1961) gave the dimensions as 4.2~6 x 2~3 μm. Similarly, different spore dimensions were recorded by Lipa and Hokkanen (1992) for *Nosema meligethi* originally described by Issi and Raditscheva (1979). *Nosema phyllotretae* is the first microsporidian described from the Chrysomelidae, observed in *P. atra* and *P. undulata* (Weiser 1961). Up to now, five of 11 *Nosema* species recorded from the Chrysomelidae have been studied using electron microscopy. Distinctive characteristics of these *Nosema* species described from the Chrysomelidae are shown in table 1, and as seen in that table, the ultrastructural features of *N. phyllotretae* differ from those of the other species. The number of polar filament coils provides a further useful taxonomic criterion for differentiating species (Cheung and Wang 1995). The number of polar filament coils

Table 1. Some *Nosema* species described in the family Chrysomelidae (Coleoptera) and their morphological and ultrastructural features

Nosema species	Host	Spore	Ultrastructural features			Locality
		measurements (μm)	Polaroplast	Spore wall (nm)	Polar filament	
Nosema couilloudi Toguebaye and Marchand, 1984	*Nisotra* sp.	3.4~4 x 1~1.5	Lamellar	60	8~10 coils	Senegal
Nosema birgii Toguebaye and Marchand, 1986	*Mesoplatys cincta*	6.2 x 3.5	Lamellar and vesicular	-	12~14 coils	Senegal
Nosema nisotrae Toguebaye and Marchand, 1989	*Nisotra* sp.	5.8 x 3.1	Tubular	65~155	15~18 coils	Senegal
Nosema galerucellae Toguebaye and Bouix, 1989	*Galerucella luteola*	4.95 x 2.89	Lamellar	80~100	7~9 coils	France
Nosema chaetocnemae Yaman and Radek, 2003	*Chaetocnema tibialis*	3.52 x 2.09	Relatively vesicular	176.5~213	13 coils	Turkey
Nosema phyllotretae present work	*Phyllotreta atra*[a,b]	4.2~6 x 2~3[a] 4.08 x 2.53[b]	Lamellar[b]	110~175[b]	13~15 coils[b]	Czech Republic and England[a], Turkey[b]

[a]Weiser, 1961; [b]present work.

(13~15) of *N. phyllotretae* differs from the numbers of the other five chrysomelid parasites.

It is difficult to handle microsporidian species from Coleoptera because the early descriptions were based mainly on the spore morphology as seen with light microscopy, and ultrastructural details are lacking. Therefore, the description of the spore ultrastructure of *N. phyllotretae* provided by this study will be useful for the identification and comparison of *Nosema* species from the Chrysomelidae.

In this extensive survey conducted during 2000~2001 in Turkey, we confirmed the presence of *N. phyllotretae* in *P. atra* in Gümüşhane, one of four investigated localities in Turkey during the study. The pathogen was absent from sampled populations of Samsun, Trabzon, and Rize. Yaman and Radek (2003) did not observe microsporidia in *P. atra* samples, although they were collected at the same plantation in Samsun from which a *Nosema chaetocnemae* infection of a *Chaetocnema tibialis* population was recorded.

This study confirms the data based mainly on light microscopic spore morphology given by Weiser (1961), and the light and electron micrographs provide abundant characters which can be used for evaluation (Larsson 1999). This is also the first report of *N. phyllotretae* from Turkey, and the results bring new information on the geographical distribution of *N. phyllotretae*.

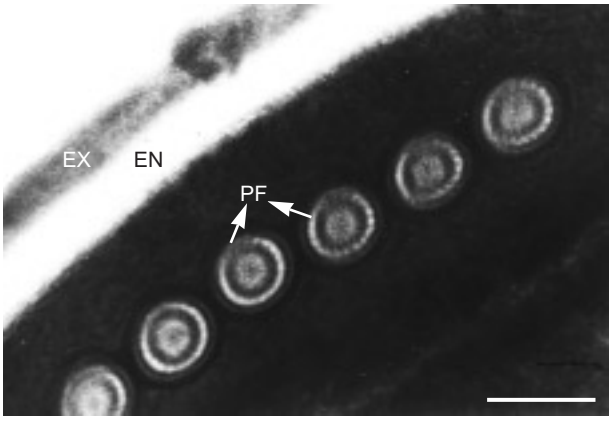

Fig. 5. Cross section of the polar filament (PF) of a *Nosema phyllotretae* spore. EN, endospore; EX, exospore. (bar = 100 nm)

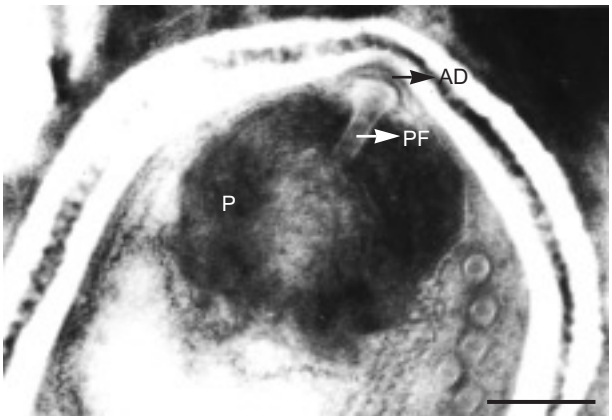

Fig. 6. Section of the anterior portion of a *Nosema phyllotretae* spore showing the anterior part of the polaroplast with thin lamellae (P), the anchoring disc (AD), and polar filament (PF). (bar = 250 nm)

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