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Nosema chaetocnemae sp. n. (Microspora: Nosematidae), a Microsporidian Parasite of *Chaetocnema tibialis* (Coleoptera: Chrysomelidae)

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Summary. A microsporidian parasite infecting *Chaetocnema tibialis* is reported for the first time and described by light and electron microscopy in the present study. The infection rate in the population at Samsun (Turkey) reaches up to 42%. Spores are oval, measure 3.52 ± 0.41µm (2.85-4.27) in length and 2.09 ± 0.26 µm (1.90-2.85) in width, possess a diplokaryon and have 13 coils of the polar filament. The morphological and ultrastructural features indicate that the described microsporidian is a member of the genus *Nosema.* It is described as *Nosema chaetocnemae* sp. n. after the name of the genus of its host.

Key words: biological control, *Chaetocnema tibialis,* Microsporidia, *Nosema chaetocnemae* sp. n.

INTRODUCTION

Chaetocnema tibialis (Chrysomelidae: Coleoptera) is one of the most important pests of sugar beets in Turkey. Chemical pesticides utilized to control this pest have hazardous effects on the environment. In contrast, biological control agents have certain advantages over chemicals as control agents. They are non-polluting and thus environmentally safe and acceptable. It is believed that entomopathogenic microorganisms can decrease insect population densities and reduce the duration of outbreaks (Myers 1988). Consequently, any natural enemies of this pest are of great interest as potentially valuable biological control agents. As a group, Microsporidia are important pathogens of insects and are considered to be important regulators of the population dynamics (Linde *et al*. 2000). They form the majority of the protista pathogenic to insects and cause economically serious diseases in pest insects (Tanada and Kaya 1993).

Although chrysomelids are frequently infected by protista, no parasitic protist has been recorded up to now from *C. tibialis*. The first microsporidian described from the Chrysomelidae was *Nosema phyllotretae*, observed in *Phyllotreta atra* and *Phyllotreta undulata* (Weiser 1961). Toguebaye and Bouix (1989) presented a list of *Nosema* parasites described in the family Chrysomelidae. However, there is not a single microsporidian record from *C. tibialis*. In the present paper, we report on

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Nosema chaetocnemae sp. n., a microsporidian parasite of *C. tibialis* in Turkey.

MATERIAL AND METHODS

The adults of *Chaetocnema tibialis* were collected from March to October in 2000 in Çarşamba (Samsun) in Turkey.

Light Microscopy

Each beetle was dissected and wet smears were examined under a microscope for parasites. When infection was observed, a fragment of infected host tissue was lacerated and spread on a slide. The smear was air-dried and then fixed with methanol for 10 min. Afterwards the slides were washed with distilled water and stained for approximately 10 h in a freshly prepared 5% solution of Giemsa stain. They were then washed in running tap water, air-dried and examined under a microscope (Toguebaye *et al*. 1988). Detected spores were measured and photographed.

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 2% OsO_4 and 3% $K_4[Fe(CN_6)]$ 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, which were stained with saturated uranyl acetate and Reynolds' lead citrate (Reynolds 1963). They were examined in a Philips 208 electron microscope (TEM).

Host specificity of the parasite

We also carried out tests for host specificity of this parasite with two other chrysomelids, *Phyllotreta undulata* and *P. atra.* For this, a semi-purified spore suspension with a concentration of 2 x 10^6 spores/ml was experimentally tested. A film of the spore suspension was applied to the surface of fresh cabbage leaves and 30 beetles in each group were allowed to feed on these leaves for 25 days. Infected insects were dissected, and spores were released on the microscopic slide.

RESULTS

The infection was found in the adults of *C. tibialis* in Çarşamba (Samsun), Turkey. When the infected insects were dissected, a large quantity of spores was released on the microscopic slide. The spores of the parasite have a characteristic appearance and are easily distinguished (Figs 1-3). Fresh spores are oval and measure 3.52 ± 0.41µm (2.85-4.27) in length and 2.09 ± 0.26 µm (1.90-2.85) in width (n = 50). Microscopic examination of parasitized individuals revealed the presence of the parasite in the gut, muscles, tracheal cells and Malpighian tubules. Frequently, the tissues were completely filled with large quantities of spores. Plasmodia with 4 nuclei are formed (Fig. 4). Sporonts are diplokaryotic (Fig. 5).

Ultrastructural studies show that the polar filament of the described species has 13 coils (Fig. 6). The polar filament was measured 115 nm in diameter in the anterior 9 spirals and 75 nm in the posterior 4 spirals. The filament contains a central core surrounded by 4 concentric layers. The core is surrounded by a relatively electron-lucent layer with a substructure consisting of about 20 small granules (Fig. 7). The diplokaryotic spore has two closely approached nuclei that are slightly elongate in the direction of the major axis of the spore. They are found in the central part of the spore. The spore wall is 176.5 to 213 nm thick and made up of a clear endospore (127.5 to 158 nm) and an exospore (50 to 55 nm) (Fig. 7). Exospore is uniform. A polar sac was well developed (Fig. 8). The polaroplast seems to be relatively vesicular (Fig. 8).

***Nosema chaetocnemae* sp. n.**

Spores: oval, 3.52 x 2.09 µm (fresh spores), 13 coils, relatively vesicular polaroplast.

Location in host: gut, tracheae, muscles and Malpighian tubules.

Host: *Chaetocnema tibialis* (Coleoptera: Chrysomelidae).

Location: Çarşamba (Samsun), Turkey.

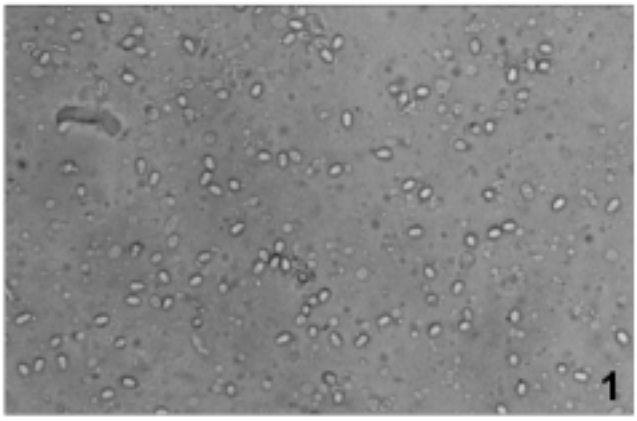
Prevalence: 112 of 431 examined beetles were infected by the parasite. Infection rate reached 42%.

Type material: the preparations for light (Preperat No: MY.P-01) and electron microscopy are stored at Department of Biology, Karadeniz Technical University, Turkey.

Etymology: the name of the species refers of its host.

We observed that last coils of the polar filament are narrow. Larsson (1986) recorded that as the most posterior coils are the most immature, an isofilar polar filament can be mistaken for anisofilar in immature spores. It has been interpreted that the polar filament of *Nosema* is isofilar (Larsson 1999, Canning and Vávra 2000). Hovewer, Toguebaye and Bouix (1986) observed anisofilar polar filament in *Nosema galerucellae* from *Galerucella luteola* (Chrysomelidae: Coleoptera).

Experimental infections showed that the parasite did not infect the other two chrysomelids, *P. undulata* and *P. atra*. An unpublished study by us confirms this result. In that study we searched for protozoan parasites of



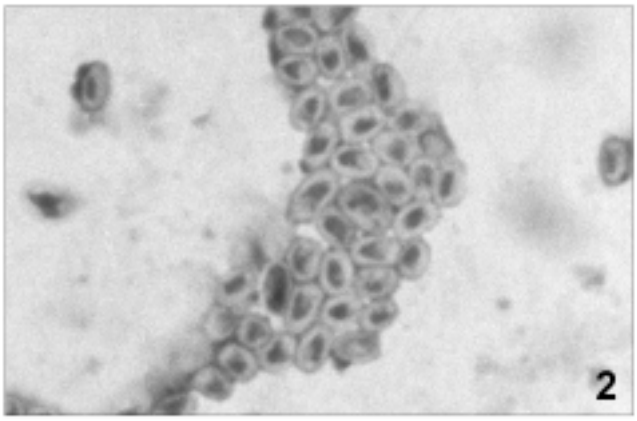
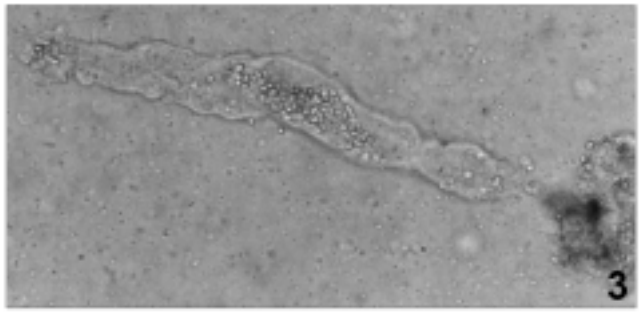


Fig. 1. Fresh spores of *N. chaetocnemae* sp. n., (x 600).
Fig. 2. *N. chaetocnemae* sp. n. spores stained with Giemsa, (x 2000).



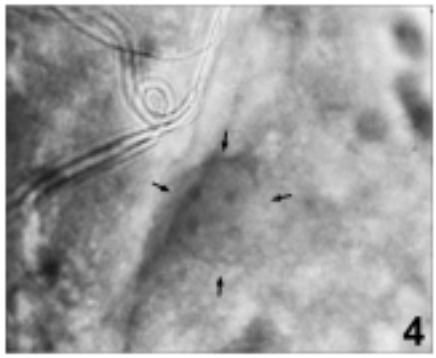


Fig. 3. *N. chaetocnemae* sp. n. spores in Malpighian tubules, (x 400).
Fig. 4. A plasmodium with 4 nuclei, (x 1500).

P. undulata and *P. atra*. We did not observe any microsporidian parasite in *P. undulata* and *P. atra* samples (more than 100 beetles of each species), although they were collected in the same plantation in which we observed the *Nosema* infection in the *C. tibialis* population.

DISCUSSION

Light and electron microscopic studies of the microsporidian parasite of *Chaetocnema tibialis* indicate that it belongs to the genus *Nosema* Naegeli, 1857. The recorded parasite has typical characters of the genus *Nosema* such as shape of the fresh (Fig. 1) and stained spores (Fig. 2), spore size, plasmodia with 4 nuclei (Fig. 4), diplokaryotic stages (Figs 5, 6), uniform exospore (Figs 6, 7) and the thickness of the spore wall (Larsson 1986, 1999). The exospore of the parasite is uniform and 50 to 55 nm. It is usually 40-60 nm in the genus *Nosema* (Larsson 1986). The polar filament was measured 115 nm in diameter in the mature coils. It is 100 nm in *N. herpobdellae* (see Spelling and Young 1983), and 117 nm in *N. tractabile* (see Larsson 1981).

Certain features observed using light and electron microscopy have led to the definition of 3 genera of microsporidians, *Nosema* Naegeli, 1857, *Unikaryon* Canning, Lai and Lie, 1974 and *Pleistophora* Gurley, 1893 in the Chrysomelidae (Toguebaye *et al*. 1988). Microsporidians of the genus *Nosema* are diplokaryotic at all developmental stages and currently recognized as being disporous, while the genera *Unikaryon* and *Pleistophora* are monokaryotic at all stages (Toguebaye *et al*. 1988).

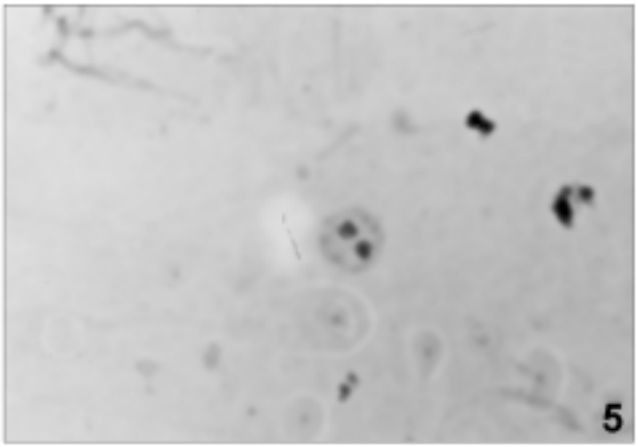
At least 200 of the 800 or so microsporidian (Microspora) species described (Canning 1990), belong to the genus *Nosema* (Sprague 1981). This seemingly disproportionate number of *Nosema* species may be due partly to incorrect identifications. Early descriptions, based mainly on spore morphology and lacking ultrastructural details, have 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). The spore is the most important life cycle stage for the identification of microsporidia above the

Table 1. *Nosema* species described in the family Chrysomelidae (Coleoptera)

Nosema species	Spore mesurations	Infected organ	Host	Locality
N. phyllotretae Weiser, 1961	4.2 x 2 to 3 µm	Adipose body	*Phyllotreta atra* *Phyllotreta undulata*	England
N. leptinotarsae Lipa, 1968	2 to 5 x 1.9 to 3.3 µm	Haemolymph	*Leptinotarsa decemlineata*	U.S.S.R.
N. gastroideae Hostounský et Weiser, 1973	3 to 4.8 x 2.5 to 3 µm	Overall infestation	*Gastrophysa polygoni* and several experimental hosts	Czechoslovakia
N. polygrammae Hostounský et Weiser, 1975	4.8 x 2.05 µm	Gut	*Polygramma undecimlineata*	Cuba
N. equestris Hostounský et Weiser, 1980	4 to 5 x 3 µm	General infestation	*Gastrophysa viridula* *Leptinotarsa decemlineata*	Czechoslovakia
N. couilloudi Toguebaye et Marchand, 1984	3.4 to 4 x 1 to 1.5 µm	Gut	*Nisotra* sp.	Senegal
N. birgii Toguebaye et Marchand, 1986	6.2 x 3.5 µm	Eggs and general	*Mesoplatys cincta* infestation, larvae and imago	Senegal
N. nisotrae Toguebaye et Marchand, 1989	5.8 x 3.1 µm	General infestation	*Nisotra* sp.	Senegal
N. galerucellae Toguebaye et Bouix, 1989	4.95 x 2.89 µm	Gut principally, adipose body, muscles, tracheae and Malpighian tubules	*Galerucella luteola*	France
N. chaetocnemae present paper	3.52 x 2.09 µm	Gut, tracheae, muscles and Malpighian tubules	*Chaetocnema tibialis*	Turkey

species level by ultrastructural studies. The spore is always present and provides abundant characters to evaluate (Larsson 1999). The ultrastructure of many *Nosema* spp. has been described (Sato *et al*. 1982, Avery and Anthony 1983, Toguebaye and Marchand 1984, Toguebaye and Bouix 1989, Hsu *et al*. 1991). These studies provide useful information for the identification of *Nosema*. The ultrastructural characteristics of spores of the genus *Nosema* was given by Sato *et al*. (1982) and Canning and Vávra (2000). Therefore, we compared the spore ultrastructure of this *Nosema* species with other *Nosema* species infecting the family Chrysomelidae (Coleoptera) in order to create new species.

In the literature there is no microsporidian record from *Chaetocnema tibialis*. The parasite presented in this paper is the first microsporidian described from *C. tibialis*. Sprague *et al*. (1992) listed the host as the first of the taxonomic characters because a host is prerequisite to the parasitism. The host affinity is generally recognized as a valid taxonomic character, at least in microsporidia infecting insects. According to Toguebaye and Bouix (1989), up to now ten species belonging to the genus *Nosema* have been described from the family Chrysomelidae; their distinctive characteristics are shown in Table 1. The spore dimension is a good feature for comparison of the ten *Nosema* species from chrysomelids. As seen in Table 1, our microsporidian differs from all *Nosema* species in spore size. On the other hand, the number of polar coils provides a further useful taxonomic criterion for differentiating species (Cheung and Wang 1995). The number of polar coils of the described parasite (13) is different from the number of coils of the 4 chrysomelid parasites, *Nosema galerucellae*



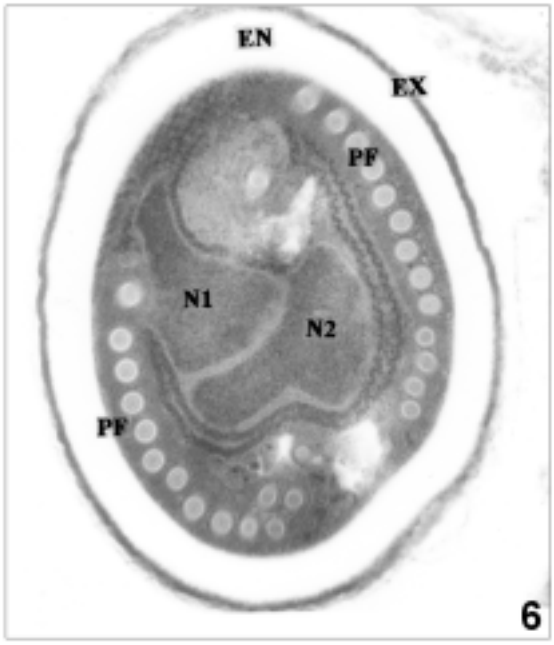
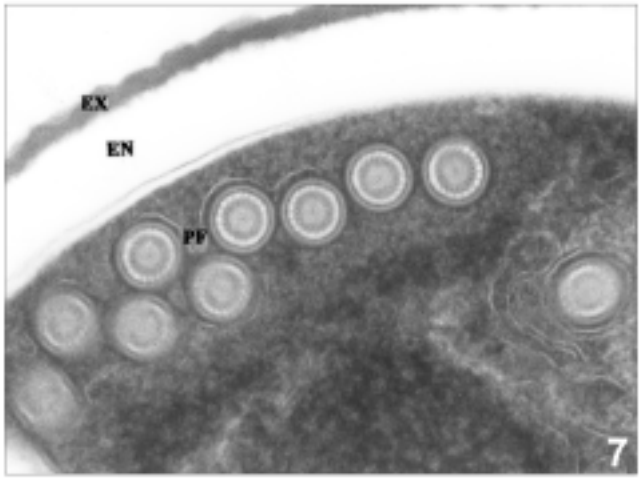


Fig. 5. Diplokaryotic sporont, (x 1000).
Fig. 6. Section of a *N. chaetocnemae* sp. n. spore showing 13 coils of the polar filament (PF) and a clearly visible diplokaryon (N1 and N2). EN - endospore, EX - exospore, (x 25,000).



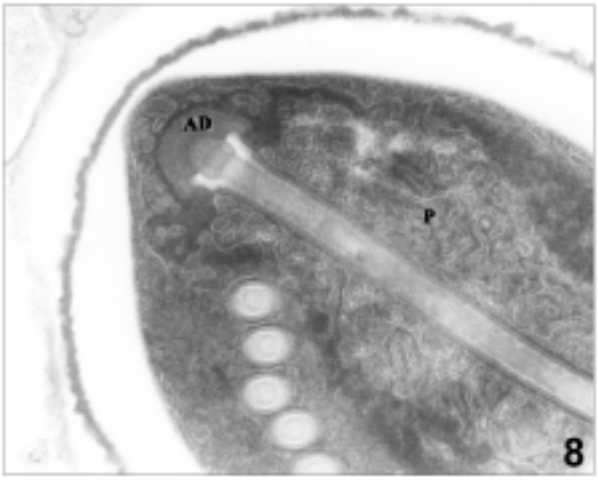


Fig. 7. Cross section of the polar filament (PF) of a *N. chaetocnemae* sp. n. spore. EN - endospore, EX - exospore, PF - polar filament, (x 80,000).
Fig. 8. Section of the anterior portion of a *N. chaetocnemae* sp. n. spore showing anchoring disc (AD) and vesicular polaroplast (P), (x 50,000).

(7-9 coils) (Toguebaye and Bouix 1989), *Nosema couilloudi* (8-10 coils) (Toguebaye and Marchand 1984), *Nosema birgii* (12-14 coils) (Toguebaye and Marchand 1986) and *Nosema nisotrae* (15-18 coils) (Toguebaye and Marchand 1989).

As seen in Table 1, in all cases, at least one character of the known species is different from this new chrysomelid parasite, either spore dimensions, number of polar filament coils, thickness of the spore wall, host and geographic location of the host, host spectrum, and infected organs. Therefore, the described characters of the *Nosema* from *C. tibialis* seem to be sufficiently distinct in order to create a new species.

Nosema chaetocnemae sp. n. infects the gut, muscles, tracheal cells and Malpighian tubules. Similar tissue infection was observed for *N. galerucellae* in *Galerucella luteola* (Toguebaye and Bouix 1989) in France. Hostounský and Weiser (1973) observed overall infestation including muscles with *Nosema gastroideae* in *Gastroidea polygoni* (Chrysomelidae: Coleoptera). They found oval or tubular masses of spores in the centre of the muscle. However, *N. couilloudi* (Toguebaye and Marchand 1984) and *N. polygrammae* (Hostounský and Weiser 1975) infect only the gut of *Nisotra* sp. and *Polygramma undecimlineata* (Chrysomelidae: Coleoptera) respectively.

When the infected insects were dissected, a large quantity of spores was released into the water on the

microscopic slide. Under natural conditions this great amount of spores is released after the death of infected beetles and this favours a quick spread of infection among the insect population. Thus, *N. chaetocnemae* sp. n. might have some importance in regulating the abundance of *C. tibialis*.

As a group, the microsporidia were thought to be the most important protozoan pathogens of insects. The systematic position of the microsporidia has been contested recently. Several molecular phylogenies suggest that microsporidia are closely related to fungi (Hirt *et al*. 1999, Keeling *et al*. 2000). However, Weiser *et al*. (2002) included this group to Protista.

In this extensive survey conducted during 2000 in Turkey, we confirmed the presence of this pathogen in *C. tibialis* populations in Çarşamba (Samsun), Turkey. The infection rate in Samsun reached 42%. Turkey represents a bridge, which joins Asia to Europe. Therefore, the results of the study are of great importance for the geographical distribution of *Nosema* species from chrysomelids (Table 1).

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