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Distribution of *Nosema phyllotretae* (Microspora, Nosematidae) Weiser, 1961 in Populations of *Phyllotreta atra* (Coleoptera, Chrysomelidae) in Turkey

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SUMMARY: The distribution of *Nosema phyllotretae* infection of *Phyllotreta atra* populations in Turkey is reported for the first time. In total, 48 of the 1668 beetles from 24 samples collected in five localities were infected by the parasite. *Nosema* infection was found in *P. atra* adults from only one of the five localities studied. The infection average was 2.8% and it reached 42.5% in some samples. The results showed that *N. phyllotretae* infection occurs in *P. atra* populations in restricted localities within Turkey.

Key Words: Microsporidia, Coleoptera, parasite, distribution, *Nosema phyllotretae*, *Phyllotreta atra*

Nosema phyllotretae Weiser, 1961'nın Türkiye'deki *Phyllotreta atra* (Coleoptera, Chrysomelidae) Popülasyonlarındaki Dağılımı

ÖZET: Bu makalede Türkiye'deki *Phyllotreta atra* popülasyonlarında *Nosema phyllotretae* enfeksiyonunun dağılımı üzerine ilk çalışma verilmektedir. Beş lokaliteden toplanan 24 örnekten toplam 1668 böcek disekte edilmiştir; bunların 48'inde enfeksiyona rastlanmıştır. *Nosema* enfeksiyonu beş lokalitenin sadece birinden elde edilen *P. atra* erginlerinde bulunmuştur. Ortalama enfeksiyon oranı %2,8'dir ve bazı numunelerde %42,5'e kadar yükselmiştir. Sonuçlar *N. phyllotretae* enfeksiyonunun çalışılan *P. atra* popülasyonlarından sadece belli lokalitelerdekinde ortaya çıktığını göstermektedir.

Anahtar Sözcükler: Microsporidia, Coleoptera, parazit, dağılım, *Nosema phyllotretae, Phyllotreta atra*

INTRODUCTION

Phyllotreta atra (Coleoptera, Chrysomelidae) is an important pest on various Brassicaceae including cabbage, radish, turnip and rape (2). Chemical pesticides utilized to control this pest should be limited because these vegetables are commonly used for human consumption and residues are a concern. In contrast, biological control agents have several advantages over chemicals as control agents. It is believed that entomopathogenic microorganisms can decrease insect population densities and reduce the duration and severity of outbreaks (6, 7, 9).

Nosema phyllotretae was the first microsporidium described from the family Chrysomelidae (13). Martini observed microsporidian infection in *P. nemorum* L. in England (5). Weiser found this parasite in *P. undulata* beetles in Czech Republic (13). Issi & Radishcheva observed this infection in *P. atra, P. undulata* and *P. nemorum* in the European part of Russia (3). Sommer recorded a *Nosema* sp. infection in *P. undulata.* Recently (8), Yaman *et al.* characterized *N. phyllotretae* from *P. atra* in Turkey and showed that *N. phyllotretae* also infected *P. nigripens* in Turkey (18, 19). We present here the first report on the distribution and occurrence of *N. phyllotretae* in *P. atra* populations in Turkey.

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MATERIALS AND METHODS

In total, 1668 beetles were randomly collected from 24 samples in five localities from April to September, 2007 to search *N. phyllotretae* infection in *P. atra* populations (Figure 2). Collected *P. atra* adults were put into sterilized glass bottles to prevent possible contamination. Collected beetles were brought to laboratory and dissected as soon as possible. Beetle samples were collected from Trabzon and Samsun throughout the year from April to September (Table 1). For the other localities Hopa, Rize and Gümüşhane the samples were collected in summer and autumn (Table 1).

Each beetle was dissected in the insect Ringer's solution and wet smears were examined under a light microscope for the parasite at the magnification of 400X to 1000X**.** When infection was observed, the slides were air-dried and then fixed with methanol for 10 min. The slides were then washed with distilled water and stained for approximately 10 hours in the freshly prepared 5% solution of Giemsa stain. They were then

washed in running tap water, air-dried and re-examined under the microscope (10, 11).

RESULTS AND DISCUSSION

Forty eight of the totally tested 1668 beetles were infected with the microsporidium (Table 1). Microsporidian infection *in P. atra* was found only in one of five tested localities, in Gümüşhane, where 48 (30.1%) of 155 beetles were infected. In contrast, 1620 of the dissected beetles from other four localities - Samsun, Trabzon, Rize and Hopa were free from microsporidia. The infection rate in Gümüşhane averaged 26.4% and ranged from 6.0 to 42.5% in three samples (Table 1).

Table 1. *Nosema phyllotretae* infections in *Phyllotreta atra* populations in Turkey.

Sampled localities	Sampled dates	Number of examined beetles	*N. phyllotretae* infection	
			Number of infected beetles	%
Trabzon	15.04.2007	70	-	-
	26.04.2007	95	-	-
	10.05.2007	78	-	-
	20.05.2007	112	-	-
	26.05.2007	76	-	-
	17.06.2007	86	-	-
	28.06.2007	68	-	-
	01.07.2007	52	-	-
	29.07.2007	60	-	-
	16.09.2007	50	-	-
	24.09.2007	35	-	-
Samsun	08.04.2007	106	-	-
	15.05.2007	120	-	-
	24.06.2007	104	-	-
	20.07.2007	59	-	-
	20.08.2007	79	-	-
	03.09.2007	30	-	-
Hopa	11.06.2007	111	-	-
	11.09.2007	8	-	-
Rize	11.06.2007	69	-	-
	11.09.2007	45	-	-
Gümüşhane	29.05.2007	73	31	42.47
	28.06.2007	49	15	30.61
	10.09.2007	33	2	6.06
	Total	1668	48	2.8

Fresh spores were oval and measured 4.20 ± 35 (3.56-5.01) x 2.30 ± 0.17 (2.02-2.70) µm. The spores stained with Giemsa's were measured 4.03 ± 0.20 (3.75-4.40) x 2.27 ± 18 (1.92-2.60) µm (Figure 1). The major characteristics, such as spore size and morphology, diplokaryotic arrangement of nuclei in all lifecycle stages, tissue and host specificity suggest that the microsporidium found in *P. atra* population in Gümüşhane is identical to *Nosema phyllotretae* confirming its characteristic features (3, 4, 5, 13, 18, 19). The characteristic features of *N. phyllotretae* have been already studied from the same host and the same locality (18, 19). We observed the same characteristics of the pathogen during the study. Therefore we did not present this same results here.

Figure 1. Fresh spores of *N. phyllotretae*. (bar: 10µm)

The first microsporidian infection in *P. atra* was observed by Weiser (12). Spores in that study measured 4.2-6 x 2-3 µm. Weiser examined 1.600 *P. atra* and 200 *P. undulata* beetles and found that 300 (18.8 %) of 1.600 *P. atra* beetles were infected by the microsporidium, located in the fat body (12). Later this parasite was recorded in several countries from *P. undulata, P. atra, P. nemorum* and *P. nigripens* and exhibited different tissue tropism (Table 2). Martini observed microsporidian infection in Malpighian tubules, nerve ganglions and gonads of *P. nemorum* L. in England with a spore size of 3-4 x 2.5 µm (5). Weiser named this parasite *Nosema phyllotretae*, established synonymy with "*Nosema* sp. Weiser, 1954" and "*N. phyllotretae.* Weiser, 1961", and provided 4.2-6 x 2-3 µm spore size (13). Prevalence of infection was 0.3 % in Czech populations of *P. undulata* (1961). In the other study performed in Russia (3) prevalence of *N. phyllotretae* was recorded as high as 35 %; three species of *Phyllotreta (P. atra, P. undulata* and *P. nemorum)* were infected; the microsporidium was found in Malpighian tubules, nerves, gonads, tracheal matrix (*P. nemorum)* and fat body (*P. undulata)* (3). Sommer recorded *Nosema* infection in *P. undulata* and indicated 4.5 µm x 1.5 – 2.5 µm spore size (8). The author did not mention the infected tissue and the species name, because the spore measurements did not match the size of *N. phyllotretae* given by Weiser (13). Yaman *et al.* (18) recorded *N. phyllotretae* infection in a *P. atra* population from Gümüshane, Turkey, indicated 4.08 x 2.53 µm spore size and provided ultrastructural characters of this parasite. Yaman *et al.* (19) also described a *N. phyllotretae* infection in a *P. nigripens* population from Turkey for the first time, presented a new host for this parasite, spore measurements 3.97 ± 0.47 x 2.19 ± 0.36 µm

Table 2. *Nosema phyllotretae* described in *Phyllotreta* spp.

Host	Infected organ	Spore measurements	Infection rate (%)	Locality	Reference
P. atra ***P. undulata***	Fat body	4.2 x 2 to 3 µm	18.8	England	Weiser, 1953*
P. nemorum	Malpighian tubules, nerve ganglions and gonads	3-4 x 2.5 µm	-	England	Martini, 1955
P. atra ***P. undulata***	Fat body	4.2 x 2 to 3 µm	0.3	Czech Republic	Weiser, 1961
P. undulata	-	4 .5 to 1.5 x 2.5 µm	0.03	Austria, Hungary, Germany and Poland	Sommer, 1981*
P. atra ***P. undulata*** ***P. nemorum***	Malpighian tubules, nerves, gonads, tracheal matrix, fat body	-	35	Russia	Issi and Rad-ishcheva, 1979
P. undulata	Malpighian tubules, fat body, gonads	3.85 to 4.40 x 2.20 to 2.75 µm	21.62	Sweden	Lipa and Ekbom, 2003
P. atra	Malpighian tubules, fat body, gonads	4.08 x 2.53 µm	-	Turkey	Yaman et al., 2005a
P. nigripens	General infestation	3.97 x 2.19 µm	11.8	Turkey	Yaman et al., 2005b
P. atra	Malpighian tubules, fat body, gonads	4.20 x 2.30 µm	30.1	Turkey	This study

*The authors used "*Nosema* sp." for the microsporidian parasite in *Phyllotreta* spp.

and 11.8 % prevalence of infection.

N. phyllotretae infection reaches considerable rate (42.5%) in *P. atra* population in Gümüşhane, Turkey if to compare to other localities (Table 2). Although Sommer (8) mentioned that *Nosema* sp. from *P. undulata* cannot be used as a biological control agent because it is too rare and does not affect the population dynamics of the flea beetles, Martini (5), Issi & Radishcheva (3) and Lipa & Ekbom (4) emphasized that flea beetles are quite commonly infected by microsporidians. Our results from the infected locality support the latter viewpoint.

Such uneven distribution of the microsporidium found only in one of five tested localities raises questions. The only infected site, Gümüşhane is located in Central South Province of the Blacksea Region of Turkey, whereas the non-infected localities are found at Blacksea Coast (Figure 2). There are a number of mountain ranges in Anatolia (the Asian part of Turkey) which constitute effective barriers against the geographical dispersal of organisms (1). We think that the eastern Black Sea Mountains between the infected (Gümüşhane) and non-infected localities (Trabzon, Rize and Hopa) prevents diffusion of *N. phyllotretae* (Figure 2). Earlier studies (14, 15, 18, 19) on microsporidia of flea beetles support this idea. *Nosema phyllotretae* infection was observed in *P. atra* population from Gümüşhane (18) and in *P. nigripens* population from Erzurum which geographically are located similar to Gümüşhane (19), but not in Samsun and Trabzon (14, 15). Interestingly, *N. chaetocnemae* and *N. meligethi* follow analogous distribution patterns in Turkey (16, 17). *N. chaetocnemae* infects *C. tibialis* populations only in Samsun and Trabzon, but not in Gümüşhane (17). In contrast, *N. meligethi* infects *Meligethes aeneus* populations in Gümüşhane, but not in Trabzon and Samsun (16). Turkey consists of seven distinct regions. Each region of Turkey constitutes a different climatic zone and thus accommodates different groups of plants and animals (1). We think that the geographic and climatic conditions affect the occurrence and distribution of *N. phyllotretae* in Turkey.

Figure 2. Collection sites for *Phyllotreta atra*

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REFERENCES

1. **Anonymous,** 1992. Turkey's importance in the world of living things. Bersay Publications, İstanbul.

2. **Aslan İ, Özbek H,** 2000. *Phyllotreta* (Coleoptera, Chrysomelidae, Alticinae) species, their biology and damage in some vegetables in Cruciferae family. *Proceedings of The Third Vegetable Symposium*, 11-13 September 2000. Isparta. 203-207.

3. **Issi IV, Raditscheva DF,** 1979. Microsporidiosis of beetles noxious to cruciferous plants in Leningrad region. *Biul Vses Nauch-Issled Inst. Zaschity Rastenij,* 46: 19-23

4. **Lipa JJ, Ekbom B,** 2003. Parasites of *Phyllotreta undulata* L. (Coleoptera, Chrysomelidae) in Sweden. *Acta Agric Scan. Sect B Soil and Plant Sci*, 53: 90-93.

5. **Martini C,** 1955. Eine Protozoonose bei *Phyllotreta nemorum* L. (Coleoptera, Halticinae). Z. Pflanzenkh. *Pflanzenschutz,* 62: 551-552.

6. **Myers JH,** 1988. Can a general hypothesis explain population cycles of forest Lepidoptera? *Adv Ecol Res,* 18: 179-242.

7. **Pilarska D, Linde A, Goertz D, Mcmanus M, Solter L, Bochev N, Rajkova M,** 2001. First report on the distribution of microsporidian infections of browntail moth (*Euproctis chrysorrhoea* L.) population in Bulgaria. *J Pest Sci,* 74: 37-39.

8. **Sommer G,** 1981. Biology and parasites of Phyllotreta spp. (Coleoptera, Halticinae). 45 pp. Final report of work, Commonwealth Institute of Biological Control, European Station, Delemont, Switzerland.

9. **Tanada Y, Kaya HK,** 1993. Protozoan infection: Apicomplexa, Microspora, *Insect Pathology*, Academic Press, San Diego. p. 414-457

10. **Toguebaye BS, Marchand B, Bouix G,** 1988. Microsporidia of Chrysomelidae. Petitpierre E, Hsiao TH, Jolivet PH, eds. *Biology of Chrysomelidae*. Kluwer Academic Publishers. Boston. p.399-416

11. **Undeen A, Vavra J,** 1997. Research methods for entomopathogenic protozoa, Lacey L, ed. *Manual of Techniques in Insect Pathology*. Academic Press, San Diego. p. 117-151

12. **Weiser J,** 1953. K otázce biologického boje s dřepčikem na řepě. *Sborník Čsl akad zeměděl věd,* 27: 171-174

13. **Weiser J,** 1961. Die Mikrosporidien als Parasiten der Insekten. *Monogr Angew Entomol*, 17: 1-149.

14. **Yaman M,** 2002a. *Gregarina phyllotretae* Hoshide 1953, a protozoan parasite of the flea beetles, *Phyllotreta undulata* and *P. atra* (Coleoptera: Chrysomelidae) in Turkey. *Appl Entomol Zool,* 37: 649-653.

15. **Yaman M,** 2002b. *Howardula phyllotretae* (Tylenchida: Allantonematidae), a nematode parasite of *Phyllotreta undulata* and *Phyllotreta atra* (Coleoptera: Chrysomelidae) in Turkey. *J Asia-Pasific Entomol*, 5: 233-235.

16. **Yaman M,** 2007. Distribution of *Nosema meligethi* I. & R. (Microsporida) in populations of *Meligethes aeneus* (Coleoptera: Nitidulidae) in Turkey. *Entomological Research,* 37: 298-301.

17. **Yaman M,** 2008. First results on the distribution of *Nosema chaetocnemae* (Microspora) in the populations of *Chaetocnema tibialis* (Coleoptera, Chrysomelidae) in Turkey. *Türkiye Parazitol Derg,* 32: 94-98.

18. **Yaman M, Radek R, Aslan İ, Ertürk Ö,** 2005a. Characteristic features of *Nosema phyllotretae* Weiser 1961, a microsporidian parasite of *Phyllotreta atra* (Coleoptera: Chrysomelidae) in Turkey. *Zool Stud,* 44: 368-372.

19. **Yaman M, Aslan İ, Radek R,** 2005b. *Phyllotreta nigripens* (Coleoptera: Chrysomelidae), a new host of *Nosema phyllotretae* (Microsporida) in Turkey. *J Pest Sci,* 78: 239-242.