DOI: 10.2478/s11686-012-0033-6

Acta Parasitologica, 2012, 57(3), 216–220; ISSN 1230-2821

VERSITA

Menzbieria chalcographi, a new neogregarine pathogen of the great spruce bark beetle, *Dendroctonus micans* (Kugelann) (Curculionidae, Scolytinae)

Mustafa Yaman[1]* and Renate Radek[2]

[1]Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, 61080, Trabzon, Turkey;
[2]Institute of Biology/Zoology, Free University of Berlin, Working Group Protozoology, Königin-Luise-Str. 1–3, 14195 Berlin, Germany

Abstract

This study concerns a new neogregarine parasitic in the great spruce bark beetle *Dendroctonus micans* (Kugelann) (Curculionidae, Scolytinae). The rate of infection was high, reaching 27.3%. There was no difference in the rate of infection of male and female beetles. The life-cycle stages of the pathogen were described by light and electron microscopy. Each gametocyst of the neogregarine included 8–16 actinocephalid oocysts measuring 11.19 ± 0.42 × 4.99 ± 0.25 µm. The described pathogen has the typical characteristics of members of the genus *Menzbieria* within the order Neogregarinida and it was identified as *Menzbieria chalcographi*. This is the first record of an infection of *D. micans* by *M. chalcographi*. Possibly, this pathogen could be useful for the biological control of this destructive bark beetle.

Keywords

Bark beetle, biological control, *Dendroctonus micans*, *Menzbieria chalcographi*, neogregarine, pathogen

Introduction

The great spruce bark beetle *Dendroctonus micans* (Curculionidae, Scolytinae) causes serious economic losses in Europe and Asia, despite the considerable efforts invested in the mechanical and/or biological control of the beetle. One possibility to reduce or regulate the density of the bark beetle would be the detection and use of its natural pathogens (Yaman and Radek 2005, 2008a, b). The study of *D. micans* pathogens might lead to the discovery of natural factors which could suppress the populations. Several studies on the pathogens and parasites of beetles have been conducted to find an organism effective as a biological control of bark beetles. Although in recent investigations, about 20 pathogens of different bark beetles that may limit the population density of their host have been observed (Weiser *et al.* 2000, Wegensteiner 2004), studies on the pathogens of *D. micans* revealed only three pathogens (Yaman and Radek 2005; 2008a, b; Yaman 2008). In the present paper, a new pathogen of *D. micans*, the neogregarine *Menzbieria chalcographi*, is identified and characterised by light and electron microscopy.

Materials and methods

Altogether, 1860 adults of *D. micans* were collected from spruce forests on seven sampling plots in four localities in the Black Sea region of Turkey. Adults of *D. micans* were randomly collected; 252 of them were later sexed to permit comparison of the infection rate among males and females. Each beetle was dissected in insect Ringer solution, and prepared wet smears were examined for presence of the neogregarine infection under a light microscope at a magnification of ×200–1000. Positive smears were stained with Giemsa solution (Yaman and Radek 2005, Yaman *et al.* 2005). Detected fresh and stained stages were measured and photographed using an Olympus BX51 microscope equipped with a DP-25 digital camera and a DP2-BSW Soft Imaging System. Procedures for scanning (SEM) and transmission electron microscopy (TEM) followed standard protocols (Pilarska *et al.* 2002; Yaman and Radek 2005; Yaman *et al.* 2005, 2011).

*Corresponding author: muyaman@hotmail.com

Table I. *Menzbieria chalcographi* infections in *Dendroctonus micans* populations from various sampling plots

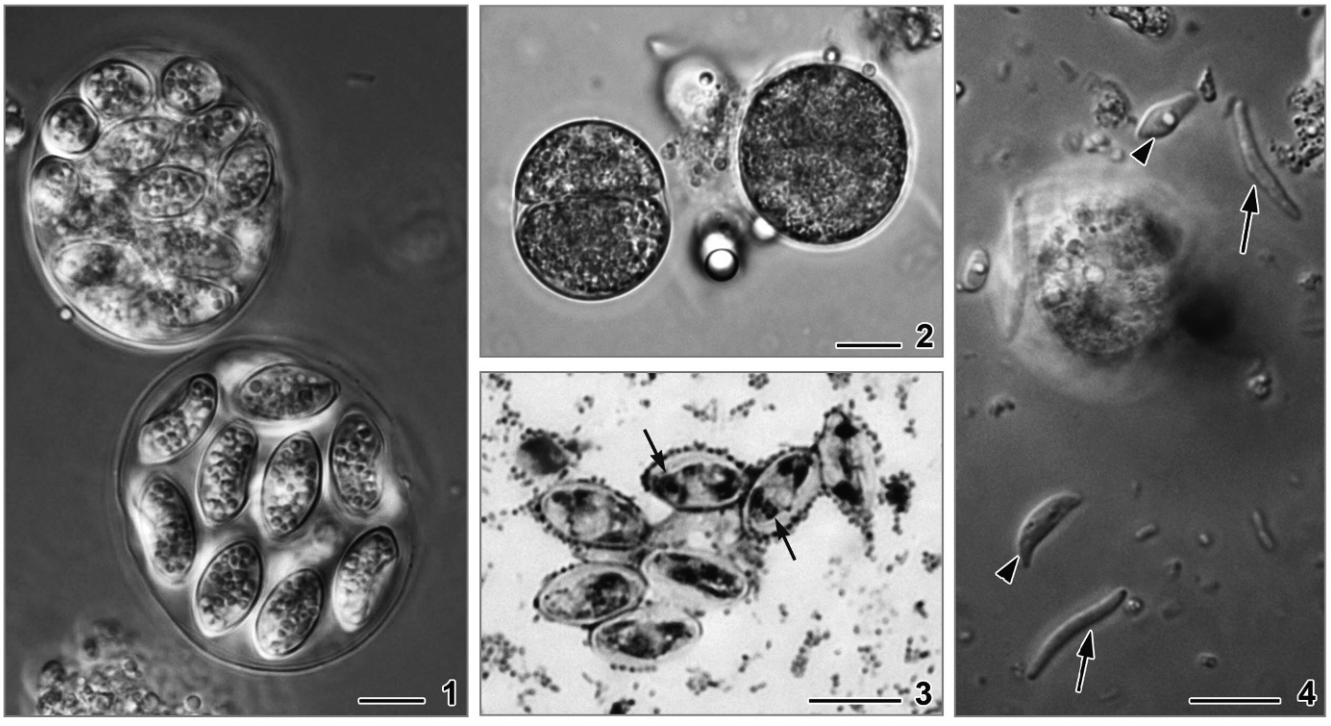
Localities		Dissected insects	Infected insects	% Infected
Artvin	Merkez	162	0	0
		106	6	5.7
	Şavşat	110	30	27.3
		48	19	12.8
	Ardanuç	103	1	0.9
Trabzon	Maçka	232	0	0
	Şalpazarı	244	0	0
Giresun	Merkez	315	0	0
Ordu	Merkez	284	1	0.4
		156	0	0
	Total	1860	57	3.1

Results and discussion

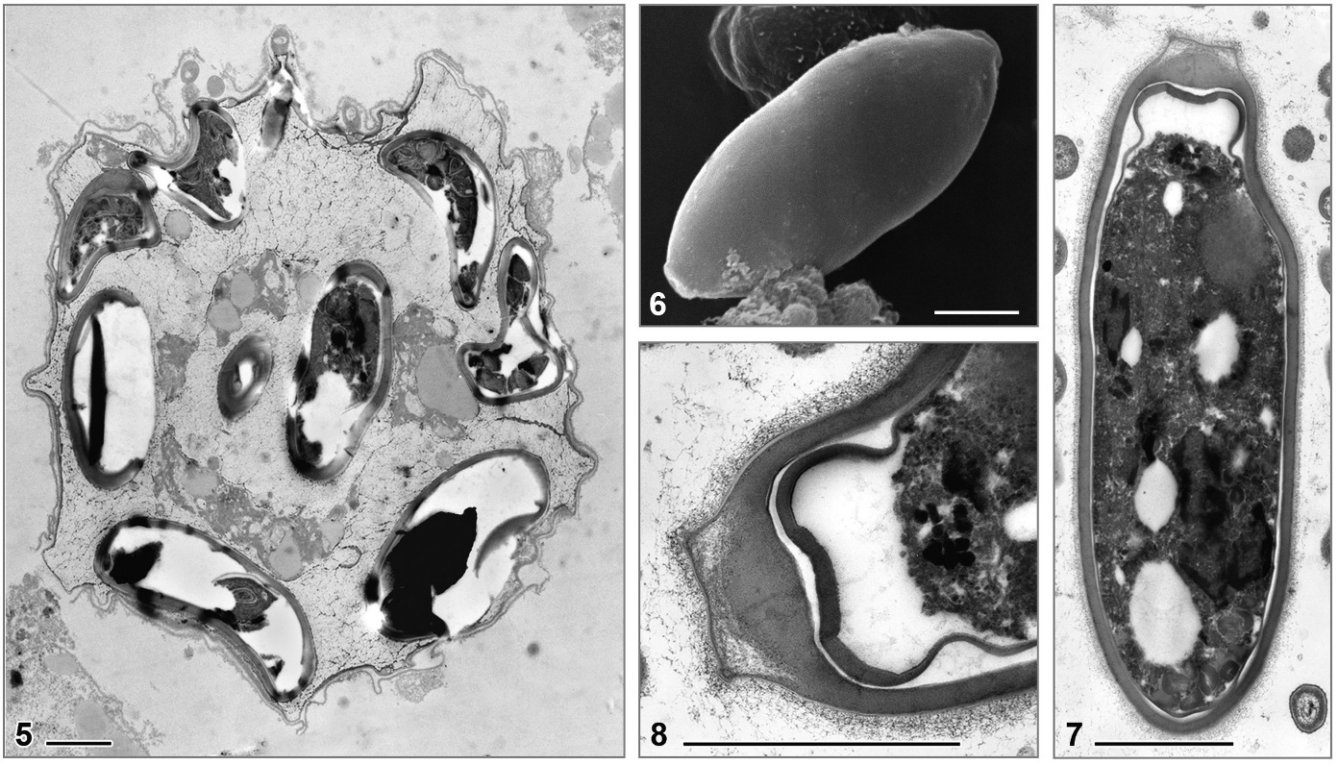
Life cycle stages and systematic affiliation

Beetles infected with neogregarine pathogens were found at two of the investigated localities (Table I). Different life stages such as gametocysts, oocysts and merozoites were observed in the fat body of the adult *D. micans* specimens (Figs 1–8). Gametocysts had a size of 20–30 µm and included 8–16 oocysts (Figs 1 and 5). The oocysts were of the actinocephalid type, meaning that they possess large polar plugs (Fig. 8). They measured 11.19 ± 0.42 × 4.99 ± 0.25 µm. Light and electron microscopic observations revealed that the neogregarine pathogen has the typical characteristics of members of the genus *Menzbieria* Bogoyavlenskaya, 1922 within the order Neogregarinida. These characteristics include merogony in body cavity, elongate first-generation merozoites and amoeboid second-generation merozoites, actinocephalid type oocysts, and more than two and maximally forty oocysts per gametocyst (Levine 1988, Undeen and Vávra 1997).

The genus *Menzbieria* has two species; the type species *M. hydrachnae* Bogoyavlenskaya, 1922 and *M. chalcographi* Weiser, 1955. *M. hydrachnae* was found in the intestine of a fresh water mite of the genus *Hydrachna* (Bogoyavlenskaya 1922). *M. chalcographi* was recorded for the first time in the bark beetle, *Pityogenes chalcographus* (Weiser 1955). Until now, *Menzbieria chalcographi* has



Figs 1–4. Light micrographs of *Menzbieria chalcographi* in *Dendroctonus micans*. **1 –** Pregametocysts and mature gametocysts including 8 to 16 oocysts; **2 –** pairing of two gamonts forming a gametocyst; **3 –** Giemsa-stained oocysts; **4** – two types of merozoites. Scale bars = 10 µm



Figs 5–8. Electron micrographs of *Menzbieria chalcographi* in *Dendroctonus micans*. **5** –Eight mature oocysts in a gametocyst. TEM. **6 –** A mature oocyst with polar plugs. SEM. **7** – Longitudinal section of an oocyst. TEM. **8** – Thick polar plug of an oocyst. TEM. Scale bars = 2 µm

been found to occur in the following bark beetles: *P. chalcographus* (Weiser 1955, Purrini 1978a, Purrini and Führer 1979, Händel *et al.* 2003, Takov *et al*. 2007), *Dryocoetes autographus* and *Hylurgops glabratus* (Purrini 1978b), *Ips typographus* (Wegensteiner and Weiser 2004, Takov *et al*. 2007, Holuša *et al*. 2009) and *Pityokteines spinidens* (Pernek *et al*. 2009). However, it has not yet been found in *D. micans*. The neogregarine pathogen from *D. micans* is very similar to *M. chalcographi* in several characteristics such as the size and shape of oocysts, the oocyst number per gametocyst, and the degree of relationship between the host species. Both isolates originate from bark beetles of the subfamily Scolytinae which live in spruce forests. Except for the small difference in size between *M. chalcographi* and our isolate, there are no morphological differences justifying the creation of a new species for the neogregarine pathogen from *D. micans.* The isolates of *M. chalcographi* from other bark beetles also showed some size differences (Purrini 1978a). We conclude that the reported pathogen is identical to *M. chalcographi* and present *D. micans* as a newly discovered host for this pathogen.

Infection rates

Infection was observed in five of ten samples; only samples from the two localities Artvin and Ordu were positive (Table I). In total, 57 of 1860 examined adults were infected with *Menzbieria chalcographi*. This corresponds to a mean infection rate of 3.1%. The prevalence of *M. chalcographi* infections in the examined beetles, however, differed considerably between localities, ranging from 0.4 to 27.3% (Table I). Such variations in infection rates were also observed for the pathogenic green alga *Helicosporidium* sp. (Yaman 2008) and the yeast *Metschnikowia typographi* (Yaman and Radek 2008b) infecting *D. micans*, as well as *M. typographi* infecting other bark beetles such as *Ips typographus* and *I. amitinus* (Weiser *et al.* 2003).

The infection rate of *D. micans* with *M. chalcographi* was sometimes quite high, reaching e.g. 27.3% in one locality (Şavşat in Artvin). This is considerably greater than the highest reported infection rates in other bark beetles, such as 0.3% in *Pityogenes chalcographus* (Händel *et al*. 2003), 8% in *Dryocoetes autographus* and 13% in *Hylurgops glabratus* (Purrini 1978b), and 0.7% in *I. typographus* (Wegensteiner and Weiser 2004).

During this study we also compared the infection levels in male and female beetles. Twenty out of the 120 investigated males and 27 of the 145 investigated females were infected by the neogregarine pathogen. This corresponds to infection levels of 18.7% in males and 18.6% in females (Table II). Thus, there were no significant differences in the infection levels, revealing that the *M. chalcographi* infection does not favor any sex in *D. micans*. In contrast, Yaman (2008) reported a higher *Helicosporidium* sp. infection rate in females than

Table II. Number and percentage of *Menzbieria chalcographi*-infected male and female *Dendroctonus micans*

Sampling plots	♂♂			♀♀		
	D	**I**	**%**	**D**	**I**	**%**
Artvin/Merkez	31	1	3.2	52	5	9.6
Artvin/Şavşat-1	26	9	34.6	41	13	31.7
Artvin/Şavşat-2	50	10	20	52	9	17.3
Total	107	20	18.7	145	27	18.6

D – no. of dissected beetles, I – no. of infected beetles.

in males of *D. micans*. Also Weiser *et al*. (2003) reported a similar result for a *M. typographi* infection among *I. typographus* and *I. amitinus* populations.

Relevance of the new pathogen

Studies on the pathogens of *D. micans* are very limited. Although the green alga *Helicosporidium* sp. (Chlorophyta) (Yaman and Radek 2005), the neogregarine *Mattesia* sp., the yeast *Metschnikowia typographi* (Yaman and Radek 2008a, b) and some bacteria (including both spore-forming and non-spore-forming species) (Yaman *et al*. 2010) have been recorded to infect *D. micans*, *Menzbieria* species had not been found before now. Thus, this is the first record of *M. chalcographi* in *D. micans.*

D. micans is currently controlled by using a predatory beetle, *Rhizophagus grandis* (Coleoptera: Rhizophagidae). This predatory beetle is reared in laboratories, using mainly *D. micans* larvae collected from infested areas as nourishment (Yaman 2008). Yaman and Radek (2007) recorded that *Helicosporidium* also infects *R. grandis*, the predatory beetle of *D. micans*, and that it may be transmitted when the beetles are fed with infected prey larvae of *D. micans*. Such an infection could reduce its efficiency of suppressing *D. micans* populations. The detection of non-infected *D. micans* populations, which are ideal nourishment for rearing *R. grandis*, is thus very important. Thus, revealing the occurrence of *Menzbieria chalcographi* in the populations of *D. micans* is also very important from this view. In a follow-up study, an infection of *R. grandis* with *M. chalcographi* will be investigated.

Acknowledgements. The study was financially supported as a research project by the Scientific and Technical Council of Turkey (105T350). The authors wish to express their thanks to Prof. Dr. Klaus Hausmann, Berlin, for making a part of this study possible.

References

Eroğlu M. 1995. Investigations on the factors affecting the population dynamic of *Dendroctonus micans* (KUG.) (Coleoptera, Scolytidae). (In Turkish) I. Ulusal Karadeniz Ormancılık Kongresi, Trabzon; Bildiriler 3. cilt, 148–159.

Fielding N.J., Evans H.F., 1997. Biological control of *Dendroctonus micans* (Scolytidae) in Great Britain. *Biocontrol News Information*, 18, 51N–60N.

Händel U., Wegensteiner R., Weiser J., Žižka Z. 2003. Occurrence of pathogens in associated living bark beetles (Col., Scolytidae) from different spruce stands in Austria. *Journal of Pest Science*, 76, 22–32.

Holuša J., Weiser J., Žižka Z. 2009. Pathogens of the spruce bark beetles *Ips typographus* and *Ips duplicatus*. *Central European Journal of Biology*, 4, 567–573. DOI: 10.2478/s11535-009-0044-y.

Levine N.D. 1988. The Protozoan Phylum Apicomplexa. CRC Press, Boca Raton, FL.

Pernek M., Matonevi D., Hranovec B., Kubini M., Wegensteiner R. 2009. Occurrence of pathogens in outbreak populations of *Pityokteines* spp. (Coleoptera, Curculionidae, Scolytinae) in silver fir forests. *Journal of Pest Science*, 82, 343–349. DOI: 10.1007/s10340-009-0259-8.

Pilarska D., Linde A., Solter L.F., Takov D., McManus M.L., Goertz D. 2002. Ultrastructure characteristic of a *Nosema* sp. (Microsporidia) from a Bulgarian population of *Euproctis chrysorrhoea* L. (Lepidoptera). *Acta Parasitologica*, 47, 1–5.

Purrini K. 1978a. Protozoen als Krankheitserreger bei einigen Borkenkäferarten (Col., Scolytidae) im Königsee-Gebiet, Oberbayern. *Anzeiger für Schädlingskunde*, 51, 171–175.

Purrini K. 1978b. *Malamoeba scolyti* sp. n. (Amoebidae, Rhizopoda, Protozoa) parasitizing the bark beetles *Dryocetes autographus* Ratz. and *Hylurgops palliatus* Gyll. (Scolytidae, Col.). *Archiv für Schädlingskunde*, 52, 167–183.

Purrini K., Führer E. 1979. Experimentelle Infektion von *Pityogenes chalcographus* L (Col., Scolytidae) durch *Malamoeba scolyti* Purrini (Amoebina, Amoebidae) und *Menzbieria chalcographi* Weiser (Neogregarina, Ophryocystidae). *Anzeiger für Schädlingskunde*, 52, 167–173.

Takov D., Doychev D., Wegensteiner R., Pilarska D. 2007. Study on the pathogens of bark beetles (Coleoptera, Scolytidae) from different coniferous stands in Bulgaria. *Acta Zoologica Bulgarica*, 59, 87–96.

Undeen A.H., Vávra J. 1997. Research methods for entomopathogenic protozoa. In: (Ed. L. Lacey) *Manual of techniques in insect pathology.* Biological Techniques Series, Academic Press, London, 117–151.

Wegensteiner R. 2004. Pathogens in bark beetles. In: (Eds. Lieutier F., Day K.R., Battisti A., Gregoire J.C. and Evans H.F.) *Bark and wood boring insects in living trees in Europe*, *a synthesis*. Kluwer, Dordrecht.

Wegensteiner R., Weiser J. 2004. Annual variation of pathogen occurrence and pathogen prevalence in *Ips typographus* (Coleoptera, Scolytidae) from the BOKU University Forest Demonstration Centre. *Journal of Pest Science*, 78, 221–228.

Weiser J. 1955. Beitrag zur Kenntnis der Parasiten des Borkenkäfers *Ips typographus* II. *Věstník Československé Společnosti Zoologické*, 19, 374–380.

Weiser J., Pultar O., Žižka Z. 2000. Biological protection of forest against bark beetle outbreaks with poxvirus and other pathogens. *IUAPPA* Section B, 168–172.

Weiser J., Wegensteiner R., Händel U., Žižka Z. 2003. Infections with the ascomycete fungus *Metschnikowia typographi* sp. nov. in the bark beetles *Ips typographus* and *Ips amitinus* (Coleoptera, Scolytidae). *Folia Microbiologica*, 48, 611–618. DOI: 10.1007/BF02993467.

Yaman M. 2008. First results on distribution and occurrence of the insect pathogenic alga *Helicosporidium* sp. (Chlorophyta: Trebouxiophyceae) in the populations of the great spruce bark beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae, Scolytinae). *North-Western Journal of Zoology*, 4, 99–107.

Yaman M., Aslan İ., Radek R. 2005. *Phyllotreta nigripens* (Coleoptera: Chrysomelidae), a new host of *Nosema phyllotretae* (Microsporida) in Turkey. *Journal of Pest Science*, 78, 239–242. DOI: 10.1007/s10340-005-0100-y.

Yaman M., Radek R. 2005. *Helicosporidium* infection of the great European spruce bark beetle, *Dendroctonus micans* (Coleoptera: Scolytidae). *European Journal of Protistology*, 41, 203–207. DOI: 10.1016/j.ejop.2005.04.002.

Yaman M., Radek R. 2007. Infection of the predator beetle *Rhizophagus grandis* Gyll. (Coleoptera, Rhizophagidae) with the insect pathogenic alga *Helicosporidium* sp. (Chlorophyta: Trebouxiophyceae). *Biological Control*, 41, 384–388. DOI: 10.1016/j.biocontrol.2007.03.001.

Yaman M., Radek R. 2008a. Pathogens and parasites of adults of the great spruce bark beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae, Scolytinae). *Journal of Pest Science*, 81, 91–97. DOI: 10.1007/s10340-007-0190-9.

Yaman M., Radek R. 2008b. Identification, distribution and occurrence of the ascomycete fungus *Metschnikowia typographi* Weiser *et al*. 2003 from the Great Spruce Bark Beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae, Scolytinae) in Turkey. *Folia Microbiologica*, 53, 427–432. DOI: 10.1007/s12223-008-0065-3.

Yaman M., Tosun O., Aslan I. 2008. On the occurrence of gregarine parasite from *Psylloides cupreus* Koch 1803 (Coleoptera: Chrysomelidae) of Turkey. *North-Western Journal of Zoology*, 4, 167–172.

Yaman M., Ertürk Ö., Aslan İ. 2010. Isolation of some pathogenic bacteria from the great spruce bark beetle, *Dendroctonus micans* and its specific predator, *Rhizophagus grandis*. *Folia Microbiologica*, 55, 35–38. DOI: 10.1007/s12223-010-0006-9.

Yaman M., Tosun O., Lipa J.J., Aslan İ. 2011a. The first records of a gregarine pathogen and a mermithid parasite from *Chrysolina fastuosa* (Scopoli 1763) (Coleoptera: Chrysomelidae). *North-Western Journal of Zoology*, 7, 105–111.

Yaman M., Özcan N., Radek R., Linde A., Lipa J.J. 2011b. Ultrastructure, characteristic features and occurrence of *Nosema leptinotarsae* Lipa, 1968, a microsporidian pathogen of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Acta Parasitologica*, 56, 1–7. DOI: 10.2478/s11686-011-0009-y.

(Accepted June 23, 2012)