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FIRST RECORD OF *Entomophaga maimaiga* (ENTOMOPHTHORALES: ENTOMOPHTHORACEAE) IN *Lymantria dispar* POPULATIONS IN GREECE AND THE FORMER YUGOSLAVIAN REPUBLIC OF MACEDONIA

PRVI NALAZ *Entomophaga maimaiga* (ENTOMOPHTHORALES: ENTOMOPHTHORACEAE) U POPULACIJAMA *Lymantria dispar* U GRČKOJ I REPUBLICI MAKEDONIJI

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Abstract:

The entomopathogenic fungus *Entomophaga maimaiga* Humber, Shimazu & Soper (Entomophtorales: Entomophtoraceae) was found for first time in populations of gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae), in Greece and the Former Yugoslavian Republic of Macedonia (FYROM) after its introduction in Bulgaria in 1999. Monitoring studies were conducted in 2012 in oak stands in three sites in the Xanthi region in Greece, and in three sites in FYROM in the Prilep region. Gypsy moth larvae, predominately in fourth to sixth instar, were collected in May and June. During laboratory rearing, mortality of gypsy moth larvae collected in two sites in Greece ranged from 36.4–89.3%. Larval mortality of *L. dispar* in the three sites in FYROM ranged from 16.7–87.8%. Dead larvae were analysed under light microscopy for presence of *E. maimaiga* and other entomopathogens. *E. maimaiga* was recorded from one site in Greece (Kidaris vill.), and in all study sites in FYROM (Toplica, Belovodica and Krushevo vill.). Azygospores of *E. maimaiga* were found in the bodies of 78.6% of gypsy moth larvae from Kidaris, and in 8.3–16.3% of the larvae from sites in FYROM. Recent records of *E. maimaiga* in Serbia and the European part of Turkey, and present findings in Greece and FYROM, indicate that the fungus most probably has invaded gypsy moth populations in other parts of Balkan Peninsula.

KEY WORDS: gypsy moth, fungal entomopathogen, first records

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Introduction
Uvod

The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae) is one of the most important insect pests in deciduous forests of the Palaearctic zoogeographical zone and in northeastern North America where it was accidentally introduced in 1869. The preferred food plants of this polyphagous species are oaks (*Quercus* spp.). An outbreak species, gypsy moth defoliates large areas of oak forests, primarily in southeast Europe and sandy ridges in the US Appalachian Mountain chain, as well as the more recently invaded areas of North America (McManus and Csóka 2007).

Entomophaga maimaiga Humber, Shimazu and Soper (Entomophtorales: Entomophtoraceae) was described as host specific pathogen of *Lymantria dispar japonensis* Motschulsky in Japan where it causes epizootics (Shimazu and Soper 1986; Soper et al. 1988). Epizootics were also reported in South Korea, Pacific region of China, Sakhalin Islands, India, and the Primorsky region of far-eastern Russia, as well as from Poland and Yugoslavia (Hajek 1999).

E. maimaiga was first introduced into USA in 1910–1911 and 1985–1986 with inocula from Japan. Both introductions were reported as unsuccessful (Hajek et al. 2005); however, in 1989, the pathogen was reported causing epizootics in gypsy moth populations in seven northeastern US states (Andreadis and Weseloh 1990; Hajek 1999). Andreadis and Weseloh (1990) suggested that *E. maimaiga* may have survived via resting spores and spread through the North American gypsy moth population, and the ability of the pathogen to spread rapidly suggests that the fungus was probably accidentally introduced into the US prior to its detection in 1989 (Hajek 1999; Nielsen et al. 2005). *E. maimaiga* has expanded its range by natural distribution and introductions in *L. dispar* populations in North America (Solter and Hajek 2009).

In 1999–2000 *E. maimaiga* was successfully introduced in two gypsy moth populations in Bulgaria via inoculum from the US (Pilarska et al. 2000). Several years later, in forest stands situated at considerable distances from the introduction sites, the first important epizootics of the pathogen were recorded (Pilarska et al. 2006). For a period of 10–12 years, *E. maimaiga* expanded its range (naturally and by introductions) and is now found throughout Bulgaria (Georgiev et al. 2011). Proximity of sites where epizootics have occurred to neighbouring countries suggests that the pathogen probably has spread beyond the borders of Bulgaria (Georgiev et al. 2010). In 2011, *E. maimaiga* was found in the European part of Turkey (Georgiev et al. 2012a) and in Central Serbia (Tabaković-Tošić et al. 2012).

E. maimaiga is an extremely effective gypsy moth pathogen where epizootics have occurred. After the fungal epizootics in US in 1989, gypsy moth management programs were discontinued in some states. For example, in central New York State, there have been no outbreaks of gypsy moth since 1992 (Hajek 1997; Kereselidze et al. 2011). In Bulgaria, after the introduction of *E. maimaiga,* the most recent outbreak of *L. dispar* (2001–2009) included 108,000 ha, approximately 10–20% of the area typical of previous pest infestations, 492,000–1,028,000 ha (Georgiev et al. 2011).

In this study, we report on the first record of *E. maimaiga* in Greece and the Former Yugoslavian Republic of Macedonia (hereafter, FYROM). The observation of the fungus in both countries was only mentioned at the International Scientific Conference "Forests in Future-Sustainable Use, Risks and Challenges" held in Serbia in 2012 (Georgiev et al. 2012b), however no information about pathogen occurrence nor its prevalence in the gypsy moth populations studied has yet been presented.

Material and Methods
Materijali i metode

The studies were conducted during the spring of 2012. Different oak stands were visited and studied in Greece and FYROM but gypsy moth larvae were observed and collected only in three localities in the region of Xanthi (Greece) and in three sites in the region of Prilep (FYROM). Detailed site data are presented in Table 1.

The study sites in Greece consisted of mixed stands of *Quercus frainetto* Ten., *Quercus cerris* L. and *Quercus petraea* (Matt.) Liebl. In two sites in FYROM, Toplica and Belovodica vill., Macedonian endemic oak, *Quercus trojana* Webb dominated, and in the third site (Krushevo vill.), *Q. cerris* was dominant.

Collections of gypsy moth larvae were made from leaves in the lower parts of tree crowns. The age structure of gypsy moth larval population was determined by the width of epicranium. Methods for larval rearing and microscopic analyses of dead larvae were used as described in previous studies (Pilarska et al. 2006; Georgiev et al. 2011; Georgiev et al. 2012a).

Results
Rezultati

Studies in Greece – Istraživanja u Grčkoj

Sixty three and 77 gypsy moth larvae were collected on May 18 and May 31, respectively (Table 1). Gypsy moth larval stages ranged from second to fifth instar on May 18, and from second to sixth instar on May 31.

Development of gypsy moth was more advanced in Thermes, probably due to the lower altitude. In all three localities, however, the structure of larval populations suitable for study included mid- and late-instar larvae (fourth instar or older) that are susceptible to *E. maimaiga*. The relative proportion of larvae in fourth instar or older ranged from 25.0–72.7% (K/C1, T/C1), and between 85.7% and 95.9% (K/C2, T/C2).

High mortality of gypsy moth larvae was recorded during the laboratory rearing, 88.6–89.3% (K/C1, K/C2), and 36.4–51.0% (TC1, TC2) (Table). All larvae collected in Meses Thermes developed successfully to the imago stage.

Microscopic analysis of the gypsy moth larvae revealed that 78.6% (KC2) were infected with *E. maimaiga* (Table 1). In three samples (KC1, TC1, TC2) no spores of the pathogen were detected, although dead larvae showed typical symptoms of fungal infection – intact carcasses that were not degraded. Analyses did not indicate the presence of other fungal species or microsporidia.

Studies in FYROM – Istraživanja u Republici Makedoniji

A total of 142 gypsy moth caterpillars were collected and examined in all three sites in FYROM (Table 1). Gypsy moth larval development was the most advanced in Toplica and fourth to sixth instar larvae constituted 81.5% of the population, followed by the larvae from Belovodica (65.3%) and Krushevo (41.6%).

During laboratory rearing of the larvae 16.7–87.8% of all larvae died (Table 1). The carcasses showed typical symptoms of fungal infection and microscopic analysis showed that *E. maimaiga* occurred in all three localities from FYROM (Table 1).

Discussion
Rasprava

The results of this study have shown that *E. maimaiga* has expanded its range. Kidaris, Greece, where *E. maimaiga* occurred, is located in close proximity to the border with Bulgaria (7 km). The establishment of the pathogen in this site was expected because a strong epizootic occurred 30 km north in Kremen vill., Kirkovo State Forestry, Bulgaria in 2005 (Pilarska et al. 2006; Georgiev et al. 2010). The Macedonian sites Toplica, Belovodica and Krushevo are located 130–170 km from the nearest known site of *E. maimaiga* natural occurrence in Karlanovo vill. in Southwestern Bulgaria (Georgiev et al. 2011). The establishment of *E. maimaiga* at such distances, and finding the fungus in the European part of Turkey (Georgiev et al. 2012a) and Serbia where two strong outbreaks of gypsy moth (Tabaković-Tošić et al. 2012) are apparently suppressed demonstrates evidence that the *E. maimaiga* has expanded its range. It should be noted that *E. maimaiga* has been documented to spread by natural means more than 100 km in one season (Elkinton et al. 1991).

Additional evidence of *E. maimaiga* range extension is the establishment of the pathogen in 2005 in Dusheti region of Georgia (Kereselidze et al. 2011). Molecular analyses of the strain did not allow identification of the origin of *E. maimaiga* in Georgia, but it is known that airborne conidia are distributed by wind, facilitating rapid spread of the fungus at long distances (Hajek 1999; Hajek et al. 1999). The closest

Table 1. Basic data on study areas in Greece and FYROM and mortality of *L. dispar* larvae in 2012
Tablica 1. Osnovni podaci o istraživačkim lokacijama u Grčkoj i Republici Makedoniji s larvalnim mortalitetom u 2012. godini

Locality Lokalitet	Altitude, m a.s.l Nadmorska visina	Geographical Coordinates Geografske koordinate	Collection date/ Sample code Datum uzorkovanja/ šifra uzorka	No. of larvae collected Broj sakupljenih ličinki	Mortality in % Mortalitet u postotku	Mortality due to *E. maimaiga* in % Mortalitet uzrokovan *E. maimaiga* u postotku
Greece / Grčka						
Kidaris	724	41°21.753′N 025°02.237′E	18 May/KC1	44	88.6	0
			31 May/KC2	28	89.3	78.6
Thermes	446	41°20.941′N 024°58.927′E	18 May/TC1	11	36.4	0
			31 May/TC2	49	51.0	0
Meses Thermes	743	41°20.260′N 024°58.441′E	18 May	8	0	0
FYROM / Republika Makedonija						
Toplica	479	41°23.376′N 021°45.832′E	4 June	81	64.2	12.3
Belovodica	744	41°21.724′N, 021°41.794′E	4 June	49	87.8	16.3
Krushevo	892	41°22.417′N 021°16.488′E	4 June	12	16.7	8.3

known locations of *E. maimaiga* epizootics to Georgia are on the coast of the Black Sea in Bulgaria (Georgiev et al. 2011), approximately 1,400 km distance to Dusheti in northeastern Georgia.

In this study most of the dead caterpillars (KC1, TC1, TC2) with obvious signs of fungal infection revealed no spores of *E. maimaiga* or other pathogens. A similar phenomenon was observed in infection experiments with *E. maimaiga* in laboratory conditions (Pilarska et al., 2013), which suggests that, under stress, many infected larvae die before spore formation.

Recent records of *E. maimaiga* in countries neighbouring Bulgaria (Georgiev et al. 2012a; Tabaković-Tošić et al. 2012) suggest that the species has already invaded or can be expected to invade gypsy moth populations in other areas of the Balkan Peninsula (Albania, Kosovo, Montenegro, Bosnia and Herzegovina, Croatia, Slovenia) and southeastern Europe (Hungary, Romania, Moldova, etc.). Its specific characteristics, high virulence, species specificity and the ability to regulate host numbers at both high and low population densities, characterize the fungus as an environmentally safe alternative to the use of bacterial and chemical insecticides, especially in forests with recreational use and in protected areas rich in butterflies of high conservation importance (Abadjiev and Beshkov 2007). It is thus desirable to initiate programs for extending the range of *E. maimaiga*. Spread within country borders can be facilitated by release of inoculum harvested from the sites with epizootics and inoculating newly emerging gypsy moth populations.

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Zahvala

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References
Literatura

- Abadjiev, S, S. Beshkov. 2007. Prime Butterfly Areas in Bulgaria. Pensoft Pbl., Sofia-Moscow, 222 pp.
- Andreadis, T. G., R. M. Weseloh, 1990: Discovery of *Entomophaga maimaiga* in North American gypsy moth, *Lymantria dispar*, In: Proc. Natl Acad of Sci USA, 87: 2461–2465.
- Elkinton, J. S., A. E. Hajek, G. H. Boettner, E. E. Simons, 1991: Distribution and apparent spread of *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in gypsy moth (Lepidoptera: Lymantriidae) populations in North America, Environmental Entomology, 20: 1601–1605.
- Georgiev, G., D. Pilarska, P. Mirchev, B. Rossnev, P. Petkov, P. Pilarski, V. Golemansky, M. Todorov, D. Takov, Z. Hubenov, M. Georgieva, M. Matova, S. Kitanova, 2010: *Entomophaga maimaiga* – a factor for increasing stability and enhancing biodiversity in oak forests on the Balkan Peninsula, In: Lj. Rakonjac (ed.), Proc. Intl. Sci. Conf. 'Forest Ecosystems and Climate Changes', Belgrade (Serbia), vol. 1: 181–185.
- Georgiev, G., P. Mirchev, B. Rossnev, P. Petkov, M. Georgieva, M. Matova, S. Kitanova, D. Pilarska, P. Pilarski, V. Golemansky, M. Todorov, Z. Hubenov, D. Takov, 2011: Introduction of *Entomophaga maimaiga* and control of *Lymantria dispar* calamities in Bulgaria, In: S. Kitanova (ed.), Proc. of Sci. Conf. 'Sustainable Management of Oak Forests in Bulgaria', Primorsko, 72–79.
- Georgiev, G., P. Mirchev, M. Georgieva, B. Rossnev, P. Petkov, M. Matova, S. Kitanova, 2012a: First Record of Entomopathogenic Fungus *Entomophaga maimaiga* Humber, Shimazu and Soper (Entomophthorales: Entomophthoraceae) in *Lymantria dispar* (Linnaeus) (Lepidoptera: Lymantriidae) in Turkey, Acta zoologica bulgarica, 64 (2): 123–127.
- Georgiev, G., M. Tabaković-Tošić, D. Pilarska, P. Mirchev, M. Georgieva, P. Petkov, P. Pilarski, 2012b: Distribution of *Entomophaga maimaiga* Humber, Shimazu and Soper (Entomophthorales: Entomophthoraceae) on Balkan Peninsula, In: L. Rakonjac (ed.), Proc. of Int. Sci. Conf. "Forests in Future-Sustainable Use, Risks and Challenges", Belgrade,. 619–622..
- Hajek, A. E. 1997: Fungal and Viral Epizootics in Gypsy Moth (Lepidoptera: Lymantriidae) Populations in Central New York, Biological Control, 10: 58–68.
- Hajek, A. E. 1999: Pathology and epizootiology of *Entomophaga maimaiga* infections in forest Lepidoptera, Microbiology and Molecular Biology Reviews, 63: 814–835.
- Hajek, A. E., C. Olsen, J. S. Elkinton, 1999: Dynamics of Airborne Conidia of the Gypsy Moth (Lepidoptera: Lymantriidae) Fungal Pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales), Biological Control, 16: 111–117.
- Hajek, A. E., M. McManus, I. Jr. Delalibera, 2005: Catalogue of Introductions of Pathogens and Nematodes for Classical Biological Control of Insects and Mites, FHTET-2005–05. URL: http://www.fs.fed.us/foresthealth/technology/pdfs/catalogue.pdf, 59 pp.
- Kereselidze, M., D. Pilarska, A. Hajek, A. B. Jensen, A. Linde, 2011: First record of *Entomophaga maimaiga* Humber, Shimazu & Soper (Entomophthorales: Entomophthoraceae) in Georgia, Biocontrol Science and Technology, 21: 1375–1380.
- McManus, M., G. Csóka, 2007: History and Impact of Gypsy Moth in North America and Comparison to Recent Outbreaks in Europe, Acta Silvatica et Lignaria Hungarica, 3: 47–64.
- Nielsen, C., M. G. Milgroom, A. E. Hajek, 2005: Genetic diversity in the gypsy moth fungal pathogen Entomophaga maimaiga from founder populations in North America and source populations in Asia, Mycological Research, 109: 941–950.
- Pilarska, D., M. McManus, A. Hajek, F. Herard, F. Vega, P. Pilarski, G. Markova, 2000: Introduction of the entomopathogenic fungus *Entomophaga maimaiga* Hum., Shim. & Sop. (Zygomycetes: Entomophtorales) to a *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) population in Bulgaria, Journal of Pest Science, 73: 125–126.

- Pilarska, D., M. McManus, P. Pilarski, G. Georgiev, P. Mirchev, A. Linde, 2006: Monitoring the establishment and prevalence of the fungal entomopathogen *Entomophaga maimaiga* in two *Lymantria dispar* L. populations in Bulgaria, Journal of Pest Science, 79: 63–67.
- Pilarska, D., M. Todorov, P. Pilarski, V. Djorova, L. Solter, G. Georgiev, 2013: Bioassays for detection of the entomopathogenic fungus *Entomophaga maimaiga* (Entomophtorales: Entomophtoraceae) in soil from different sites in Bulgaria, Acta zoologica bulgarica (in press).
- Shimazu, M., R. S. Soper, 1986: Pathogenicity and sporulation of *Entomophaga maimaiga* Humber, Shimazu, Soper and Hajek (Entomophthorales: Entomophthoraceae) on larvae of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), Applied Entomology and Zoology, 21: 589–596.
- Solter, L. F., A. E. Hajek, 2009: Control of gypsy moth, *Lymantria dispar,* in North America since 1878, In: A. E. Hajek, M. O'Callaghan, T. Glare (eds.), Use of Microbes for Control and Eradication of Invasive Arthropods, Springer Publ. Co, 181–212.
- Soper, R. S., M. Shimazu, R. A. Humber, M. E. Ramos, A. E. Hajek, 1988: Isolation and characterization of *Entomophaga maimaiga* sp. nov., a fungal pathogen of gypsy moth, *Lymantria dispar*, from Japan, Journal of Invertebrate Pathology, 51: 229–241.
- Tabaković-Tošić, M., G. Georgiev, P. Mirchev, D. Tošić, V. Golubović-Ćurguz, 2012: *Entomophaga maimaiga* – new entomopathogenic fungus in the Republic of Serbia, African Journal of Biotechnology, 11: 8571–8577.

Sažetak

Entomopatogena gljiva *Entomophaga maimaiga* Humber, Shimazu & Soper (Entomophtorales: Entomophtoraceae) prvi je puta utvrđena u populacijama gubara, *Lymantria dispar* (L.) (Lepidoptera: Erebidae) u Grčkoj i Republici Makedoniji nakon introdukcije ovog patogena u Bugarsku 1999. godine. Tijekom 2012. godine obavljen je pregled hrastovih sastojina na tri lokacije u području Xanthi u Grčkoj i na tri lokacije u okolici Prilepa u Makedoniji. Gusjenice gubara sakupljane su većinom u četvrtom do šestom larvalnom stadiju u razdoblju svibanj–lipanj. Tijekom laboratorijskog uzgoja smrtnost gusjenica sakupljenih u Grčkoj iznosila je od 36,4 do 89,3 %. Smrtnost gusjenica s lokaliteta u Makedoniji bila je između 16,7 i 87,8 %. Uginule gusjenice analizirane su pomoću svjetlosnog mikroskopa na prisutnost *E. maimaiga* i ostalih patogena. Patogena gljiva *E. maimaiga* utvrđena je na uzorku s jednog lokaliteta u Grčkoj (Kidaris) i na uzorcima svih lokaliteta u Makedoniji (Toplica, Belovodica i Kruševo). Azigospore *E. maimaiga* utvrđene su u 78,6 % uginulih gusjenica s lokaliteta Kidaris i između 8,3 i 16,3 % uginulih gusjenica s tri lokaliteta u Makedoniji. Nedavni nalazi *E. maimaiga* u Srbiji i europskom dijelu Turske te najnoviji nalazi u Grčkoj i Republici Makedoniji ukazuju na vjerojatnost da je ovaj patogen već inficirao populacije gubara u ostalim dijelovima balkanskog poluotoka.

KLJUČNE RIJEČI: Entomopatogena gljiva, *Entomophaga maimaiga*, prvi nalaz, *Lymantria dispar*