First record of Entomophaga maimaiga (Entomophthorales: Entomophthoraceae) in Georgia

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SHORT COMMUNICATION

First record of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) in Georgia

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In 2005, high levels of mortality occurred in an outbreak of the gypsy moth population in Georgia. Resting spores typical of entomophthoralean fungi were found within larval cadavers and molecular analyses confirmed that the pathogen was *Entomophaga maimaiga*. This is the first record of this entomopathogen in Georgia and in this part of Europe.

**Keywords:** *Entomophaga maimaiga*; *Lymantria dispar*; Georgia

The gypsy moth, *Lymantria dispar* (L.), is one of the most serious pest insects on deciduous and sometimes coniferous trees in Georgia. Gypsy moth has been reported from different regions in Georgia, with outbreaks occasionally causing severe defoliation. In 2004 and 2005, gypsy moth populations reached high densities over an area of 10,000 ha at 850–1000 m above sea level around Dusheti, in the Mtskheta–Mtianeti region in northeastern Georgia. Sixty percent of this region was infested and mortality of deciduous trees reached about 10% (Kereselidze, Pilarska, and Goginaschvili 2010).

*Entomophaga maimaiga* is a virulent fungal pathogen of gypsy moth larvae and was originally described from native *L. dispar* in Japan (Soper, Shimazu, Humber, Ramos, and Hajek 1988). In 1989, the pathogen was recovered in the northeastern USA where it caused epizootics in several states (Hajek et al. 1990). After 1989, this pathogen spread throughout the US area of gypsy moth invasion and today, some states no longer have gypsy moth management programs (P.C. Tobin, personal communication). *E. maimaiga* can cause high larval mortality even at low gypsy moth densities, and can help to maintain populations below damage thresholds. In addition, this pathogen is highly host specific and possesses great potential in biological control under a conservation or inoculation strategy (Hajek 2007).

In 1999, isolates of *E. maimaiga* from the USA were successfully introduced and became established in Bulgaria (Pilarska et al. 2000). Annual monitoring of the Bulgarian population has confirmed the establishment of *E. maimaiga* in Bulgaria, with epizootics occurring in 2005 (Pilarska et al. 2006). Entomophthoralean epizootics have also been recorded in a gypsy moth population in northeastern...
Poland (Glowacka-Pilot 1982). This pathogen was not identified to the species level, but the morphology of the spores was very similar to *E. maimaiga*. Undetermined entomophthoralean spores have also been reported from *L. dispar* cadavers in Yugoslavia (Injac and Vasiljevic 1978). There are, however, no reports on the occurrence of entomophthoralean pathogens in gypsy moth populations east of the Black Sea.

In this study, we report the first discovery of *E. maimaiga* in a native population of gypsy moth in Georgia. In 2005, in the Dusheti region, high natural mortality of gypsy moth larvae was observed in gypsy moth outbreak sites. Dead and living larvae were collected from the field and examined under a light microscope. Smears were made from different tissues and stained with Giemsa–Romanowski (Hazard, Ellis, and Joslyn 1981).

DNA was extracted from each of two larvae using the DNeasy Plant Kit (Qiagen, Hilden, Germany). The universal fungal primer ITS4 (White, Bruns, Lee, and Taylor 1990) and the entomophthoralean-specific primer 5.8s-5′ (Jensen and Eilenberg 2001) were used to amplify the Internal Transcribed Spacer II region (ITS II) of ribosomal DNA using a touchdown approach. An initial denaturation for 3 min at 98°C, was followed by 10 touchdown cycles (the extension temperature was reduced by 1°C per cycle) with denaturation for 10 s at 98°C, annealing for 30 s at 70–60°C, extension for 30 s at 72°C, and then 25 cycles with denaturation for 10 s at 98°C, annealing for 30 s at 60°C, extension for 30 s at 72°C and a final extension for 10 min at 72°C.

The PCR reactions were carried out in 50-μL volumes, each with 200 μM of each dNTP, 1 μM of each primer, 1 × Phusion HF Buffer (with 1.5 mM MgCl2), 0.5 unit Phusion DNA polymerase (Finnzymes; Espoo, Finland) and 2 μL template (DNA extractions). The PCR products were sent to Eurofins MWG Operon (Ebersberg, Germany) for sequencing in both directions. The sequences were aligned with publicly available ITS II *E. maimaiga* sequences as well as those from two additional *E. maimaiga* isolates sequenced in this study (see Table 1) using Bioedit v7.0.8.0. Parsimony and neighbor-joining analyses with 1000 bootstrap replications to determine the support for internal branches were performed using MEGA 4 (Tamura, Dudley, Nei, and Kumar 2007) and gaps were ignored. Maximum parsimony analysis of the ITS II sequence was used to estimate the relationship of nine *E. maimaiga* isolates originating from various parts of the world, *E. maimaiga* from the Georgian gypsy moth larvae, and two *Entomophaga aulicae* isolates that were included as an outgroup (Table 1).

Eleven out of 14 dissected larval cadavers contained resting spores similar in morphology to *E. maimaiga*. More larval cadavers were observed on trees, showing typical symptoms of *E. maimaiga* infection (Hajek and Roberts 1992), including external occurrence of conidia on some cadavers. One of the dissected larvae was found to have been killed by a nucleopolyhedrovirus (NPV), but had not been infected by *E. maimaiga*.

The molecular results confirmed that the fungal pathogen in Georgia was *E. maimaiga*. The ITS II sequences, covering 927 base pairs, only varied in a single position between the *E. maimaiga* isolates and divided the isolates into two groups. All of the US *E. maimaiga* isolates and one of the Japanese isolates grouped together, while the Georgian samples grouped together with other isolates from Japan, China,
and Russia (Figure 1). The limited variation in the ITS II sequence did not allow for precise determination of the place of origin of the Georgian *E. maimaiga*.

Although we do not know the origin of *E. maimaiga* in Georgia, the actively ejected conidia of this fungus are distributed by wind, which facilitates rapid spread of this fungus (Hajek, Olsen, and Elkinton 1999). Models by Dwyer, Elkinton, and Hajek (1998) and Weseloh (2003) suggest that dispersal of airborne *E. maimaiga* conidia occurs over both short and long distances. Longer distance dispersal probably occurs when conidia are blown above the forest canopy and travel on strong winds. Evidence of long distance dispersal of *E. maimaiga* was first found during the initial years when this fungus was detected in North America; *E. maimaiga* occurred in parts of seven northeastern states when it was first detected in 1989 and by 1992, this fungus had spread throughout the northeastern gypsy moth distribution, which included 12 states (Hajek, Elkinton, and Witcoski 1996). Although it has been difficult to estimate long distance dispersal, in North America it has been estimated that *E. maimaiga* spread >100 km per year in 1991 (Dwyer et al. 1998), which was a relatively dry spring (Hajek et al. 1996). The locations closest to Dusheti from which *E. maimaiga* has been reported are in southern Bulgaria (Pilarska et al. 2006), approximately 2000 km distant, and the prevailing wind direction is southwestern. We are not aware of other epizootics in areas between these two localities and therefore cannot determine the origin of *E. maimaiga* in Georgia. We must mention that *E. maimaiga* could potentially also have been transferred longer distances by humans, especially as resting spores in soil, e.g., in soil on soles of shoes (Hajek, Humber, and Elkinton 1995), but we are not aware of any specific human travel from locations with *E. maimaiga* in Bulgaria to the collection sites in Georgia, that would suggest this type of dispersal. The timing of the arrival of *E. maimaiga* in Georgia is unknown because resting spores can persist in forest soils for at least 10–11 years.

![Table 1. *Entomophaga maimaiga* and *E. aulicae* isolates used for the sequence analysis of the ITS II.](image)

<table>
<thead>
<tr>
<th><em>Entomophaga</em> species</th>
<th>ARSEF no.</th>
<th><em>in vivo</em> material</th>
<th>Country of origin</th>
<th>State/Prefecture/Province or nearest city</th>
<th>Year of collection</th>
<th>Genbank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. maimaiga</em></td>
<td>1400</td>
<td>Japan</td>
<td>Ishikawa</td>
<td>1984</td>
<td>DQ534745</td>
<td></td>
</tr>
<tr>
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<td>USA</td>
<td>Virginia</td>
<td>1997</td>
<td>DQ534748</td>
<td></td>
</tr>
<tr>
<td><em>E. maimaiga</em></td>
<td>6053</td>
<td>USA</td>
<td>Michigan</td>
<td>1998</td>
<td>DQ534749</td>
<td></td>
</tr>
<tr>
<td><em>E. maimaiga</em></td>
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<td>USA</td>
<td>New York</td>
<td>1996</td>
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<td></td>
</tr>
<tr>
<td><em>E. maimaiga</em></td>
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<td>USA</td>
<td>Pennsylvania</td>
<td>2003</td>
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<td></td>
</tr>
<tr>
<td><em>E. maimaiga</em></td>
<td>7127</td>
<td>Russia</td>
<td>Khabarovsk</td>
<td>1999</td>
<td>DQ534751</td>
<td></td>
</tr>
<tr>
<td><em>E. maimaiga</em></td>
<td>7139</td>
<td>China</td>
<td>Heilongjiang</td>
<td>2002</td>
<td>DQ534752</td>
<td></td>
</tr>
<tr>
<td><em>E. maimaiga</em></td>
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<td>Japan</td>
<td>Chiba</td>
<td>1998</td>
<td>DQ534750</td>
<td></td>
</tr>
<tr>
<td><em>E. maimaiga</em></td>
<td>7114</td>
<td>Japan</td>
<td>Iwate</td>
<td>2001</td>
<td>Xx4444444</td>
<td></td>
</tr>
<tr>
<td><em>E. maimaiga</em></td>
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<td>Infected cadaver</td>
<td>Georgia</td>
<td>Dusheti</td>
<td>2005</td>
<td>Xx22222222</td>
</tr>
<tr>
<td><em>E. maimaiga</em></td>
<td>7127</td>
<td>Infected cadaver</td>
<td>Georgia</td>
<td>Dusheti</td>
<td>2005</td>
<td>Xx33333333</td>
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<tr>
<td><em>E. aulicae</em></td>
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<td>USA</td>
<td>Maine</td>
<td>2003</td>
<td>DQ534753</td>
<td></td>
</tr>
<tr>
<td><em>E. aulicae</em></td>
<td>3039</td>
<td>USA</td>
<td>New York</td>
<td>1990</td>
<td>DQ534746</td>
<td></td>
</tr>
</tbody>
</table>

*Sequenced in this study.*
*ARSEF, USDA Agricultural Research Service Collection of Entomopathogenic Fungal Cultures.*
Figure 1. Relationships among isolates of *E. maimaiga* and *E. aulicae*, inferred from parsimonious analysis of the ITS II region including 594 positions. Bootstrap percentages from 1000 replicates are shown above each of the major branches. GenBank accession numbers are given before each species name (for additional information see Table 1). *Sequences produced in this study. The scale bar corresponds to 10 nucleotide changes.
thus providing a source of inoculum over time (Weseloh and Andreadis 1997; Hajek, Shimazu, and Knoblauch 2000).

This is the first record of *E. maimaiga* in Georgia as well as east of the Black Sea. Interestingly, since the first detection of *E. maimaiga* in Georgia in 2005, no further outbreaks of gypsy moth have been observed in Georgia. It can be hypothesized that in the future, outbreaks of gypsy moth may be moderated by *E. maimaiga*, at least in some areas. In central New York State, there have been no further outbreaks of gypsy moth since 1992, and this has been attributed to the continuing presence and activity of *E. maimaiga* (Hajek 1997; A.E.H, unpublished data).

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References


