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# COI GENE AS A MOLECULAR MARKER OF *Elachista* SPECIES (Lepidoptera: Elachistidae: Elachistinae) FROM DIFFERENT LITHUANIAN POPULATIONS

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*We compared COI DNA sequences of three* Elachista *species occurring in Lithuania:* Elachista maculicerusella, E. argentella*, and* E. pollinariella *(Gelechioidea: Elachistidae: Elachistinae). Also, intraspecific differences in COI DNA between moth populations were tested. A 705 bp fragment of the 3'-end of cytochrome c oxidase subunit I gene (COI) was used. This mtDNA fragment was significantly different between all studied species. Intraspecific differences were detected only for* E. maculicerusella *from different Lithuanian populations. Our results support using the COI gene for identification of* Elachista *species and as a tool for exploring intraspecific differences between populations.*

**Key words:** Elachista*, morphology, COI gene, model species, populations.*

## INTRODUCTION

*Elachista* Treitschke, 1833, is the largest genus in the family Elachistidae *sens. str.* containing about 550 named and two hundred discovered but still undescribed species worldwide (Kaila and Ståhls, 2006), Members of the genus are small moths with wingspan from 5 to 14 mm. Forewing patterns mainly consist either of a white fascia and spots on a dark background or fuscous marks on a light background; some moths are monochromatic (white, yellowish or cream). Larvae of *Elachista* are typical leaf-miners, trophically dependent on monocotyledonous grasses (Traugott-Olsen and Nielsen, 1977; Sruoga and Ivinskis, 2005).

The mitochondrial gene cytochrome oxidase I (COI) has been proposed to serve as a core of a global bioidentification system for animals (Hebert *et al*., 2003), but only one publication is known in which DNA barcodes were used to evaluate the potential of COI to differentiate closely related species of *Elachista* from Australia (Kaila and Ståhls, 2006).

The aims of this study were: (1) to select convenient methods for molecular studies of Elachistidae, (2) to test mtDNA sequences of the COI gene as a marker for analyses of intraspecific and interspecific genetic variation, and (3) to select model species for genetic polymorphism study of populations of *Elachista*.

## MATERIALS AND METHODS

We used three species of *Elachista* as model species. Samples were collected at three locations ranging from the shore of the Baltic Sea to the southeastern part of the country during the period from spring 2004 to autumn 2005 (Table 1). Distances between sampling sites ranged from 30–76 km to 330 km (Fig. 1).

*Fig. 1*. Map of collection sites in Lithuania: Vilnius, Paneriai (●); Trakai district, Aukštadvaris (■); Kaišiadorys district (○); Palanga (▲).

Table 1

COLLECTION DATA, ISOLATE NUMBER AND GENBANK ACCESSION NUMBERS OF SPECIMENS EXAMINED

| Species | Collecting data: location and date | Isolate number | Accession number GenBank |
|---|---|---|---|
| *Elachista argentella* | Vilnius, Paneriai, 28 May 2004 | 21arg | DQ666138 |
| *E. argentella* | Palanga, 21–26 August 2005 | 8arg2 | DQ666137 |
| *E pollinariella* | Trakai district, Aukštadvaris, 05 June 2004 | 21pol | DQ666140 |
| *E. pollinariella* | Palanga, 21–26 August 2005 | 5pol2 | DQ666144 |
| *E. pollinariella* | Palanga, 21–26 August 2005 | 6pol2 | DQ666139 |
| *E. maculicerusella* | Kaišiadorys district, 30 May 2004 | 15mac | DQ666143 |
| *E. maculicerusella* | Palanga, 21–26 August 2005 | 7mac2 | DQ666141 |
| *E. maculicerusella* | Palanga, 21–26 August 2005 | 8mac2 | DQ666142 |

Species were identified based on external morphology: adult specimens were examined externally using a stereo microscope (MBS-10). Then the moths were killed in 96% ethanol to avoid DNA degradation. The samples were then immediately stored at –18 °C in a refrigerator until DNA extraction. Before DNA extraction samples were washed with TBE buffer and then moths were grounded between two grainy microscope slides. The moth squash was heated at 55 °C and after centrifugation the liquid phase was used as a DNA template.

Primers used to amplify COI fragments were: LCO1490 (5'–GGT CAA CAA ATC ATA AAG ATA TTG G–3') and HCO2198 (5'–TAA ACT TCA GGG TGA CCA AAA AAT CA–3') (Folmer *et al*., 1994; Herbert *et al*., 2003). PCR reactions were performed using *Taq* DNA Polymerase (recombinant; Fermentas), under the following conditions: 36 cycles of 30 s at 94 °C, 30 s at 47°C and 72 s at 72 °C. Samples were sequenced in both directions with an ALFexpress II (Amersham Pharmacia Biotech AB) and using a Thermo Sequenase Cy 5 Dye terminator Kit (Amersham Biosciense) according to the manufacturer's protocol. The sequencing reaction consisted of 30 cycles: 30 s at 94 °C, 30 s at 55 °C and 120 s at 72 °C. Editing, contig assembly, and alignment of consensus sequences were performed with the ALFwin Sequence Analyser module version 2.11.01 (Amersham Pharmacia Biotech AB) and Bioedit version 5.0.9. The COI DNA sequences were phylogenetically analysed using MEGA version 2.1. (Kumar *et al*., 2001). Genetic distances were calculated with Kimura's two-parameter method (Kimura, 1980). A phylogenetic tree was constructed using the neighbour-joining method (Saitou and Nei, 1987).

## RESULTS

**Adult morphology.** In order to study genetic polymorphism among Lithuanian populations of *Elachista* species we selected three model species. These species were chosen because: (1) they are widespread and abundant, (2) easy detectable in the field, and (3) easy identifiable by external features. According to the latest systematic treatment of the family (Kaila, 1999), these three species belong to two different subgenera. *Elachista maculicerusella* (Bruand) is a representative of the subgenus *Elachista* whereas *E. argentella* (Clerck) and *E. pollinariella* Zeller of the subgenus *Aphelosetia*. These species are quite common throughout Lithuania (Sruoga and Ivinskis, 2005) and Europe (Kaila, 2004).

We determined several external characters of each species, which can be used for quick taxonomic identification.

*Elachista (Elachista) maculicerusella* (Bruand, 1859). The moths are rather large, with a wingspan about 10–12 mm. It is distinguished from the light-coloured species wings by a distinct, greyish brown spot located close to the dorsal margin of the wing (Fig. 2A, B). This dark spot is visible even in rather rubbed specimens (Fig. 2B). *E. maculicerusella* is one of the most common species in the Lithuanian Elachistidae fauna. The imagines are bivoltine, observed from the second half of May to early June and again from late June to late August.

*Elachista (Aphelosetia) argentella* (Clerck, 1759). The moths, with wingspan about 10–13.5 mm, are completely white (Fig. 2E). This makes them different from other Lithuanian Elachistidae species, except *Perittia farinella*, which have visible setae on antennae (Fig. 3B). Antennae of *Elachista argentella* have no visible setae (Fig. 3A). In Lithuania the species is common in grassy habitats. Adults fly during May and June.

*Elachista (Aphelosetia) pollinariella* Zeller, 1839. The moths are of moderate size, with a wingspan about 8–10 mm. The species can be identified externally by its light-coloured forewings with scattered small black-brown spots, consisting of 1–4 scales (Fig. 2C, D). *E. pollinariella* is very common and a widely distributed species in Lithuania. Adults fly from late May to late July.

**Molecular study.** We obtained 689 nucleotides from the 3' end of the COI gene. Sequences were aligned and a phylogenetic tree was constructed (Fig. 4) with the inclusion as an outgroup the GenBank mitochondrial COI sequence for *Eteobalea serratella* (AY423065). The number of parsimony informative sites was 130. The amount of the intraspecific variation of included taxa varied; no intraspecific differences were observed between *Elachista argentella* or between *E. pollinariella* individuals, but *E. maculicerusella* demonstrated intraspecific differences between geographically districted populations. The COI sequences of two *E. maculicerusella* individuals from the Palanga population were identical. The COI sequence of *E. maculicerusella*

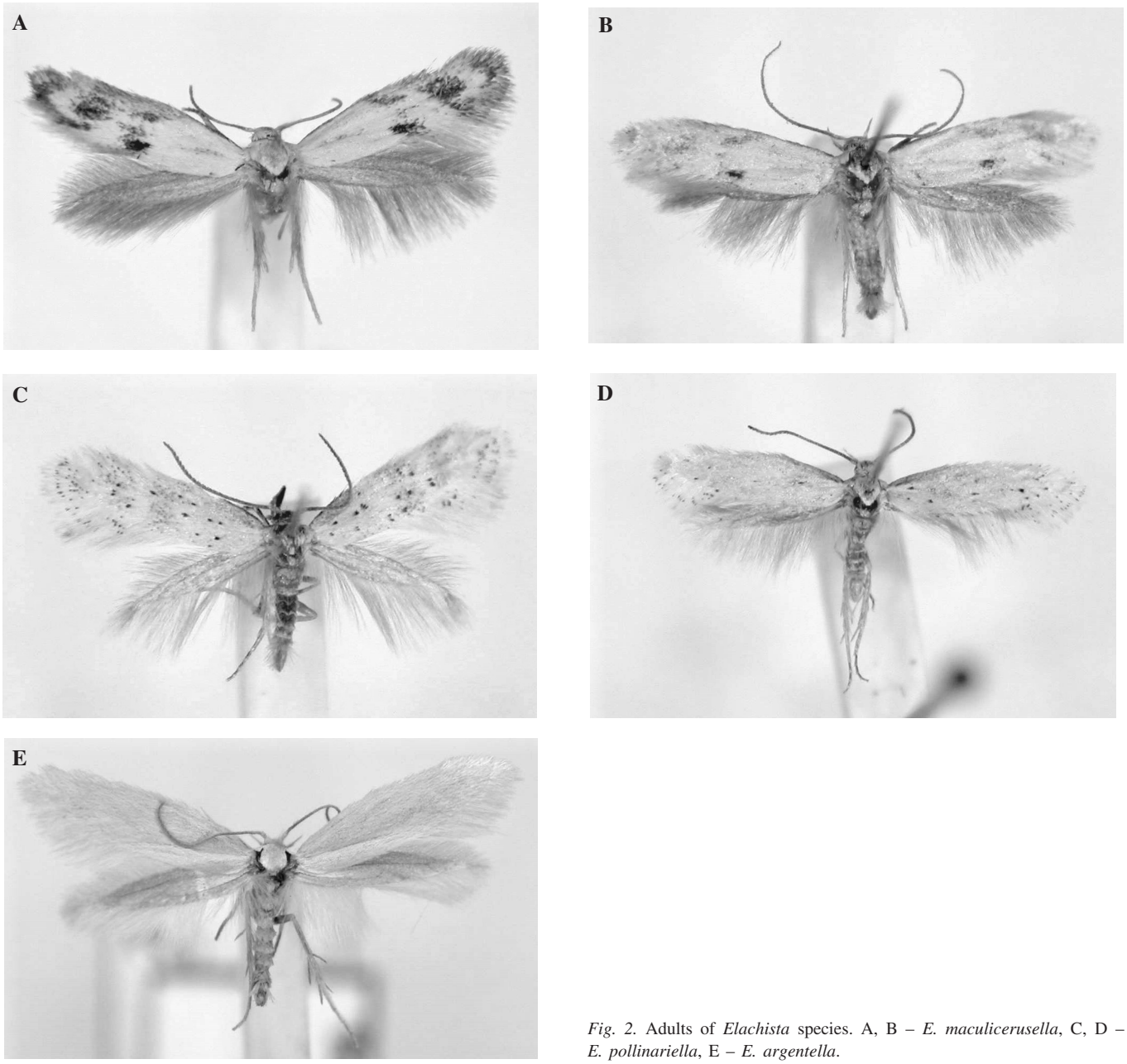


*Fig. 2.* Adults of *Elachista* species. A, B – *E. maculicerusella*, C, D – *E. pollinariella*, E – *E. argentella*.

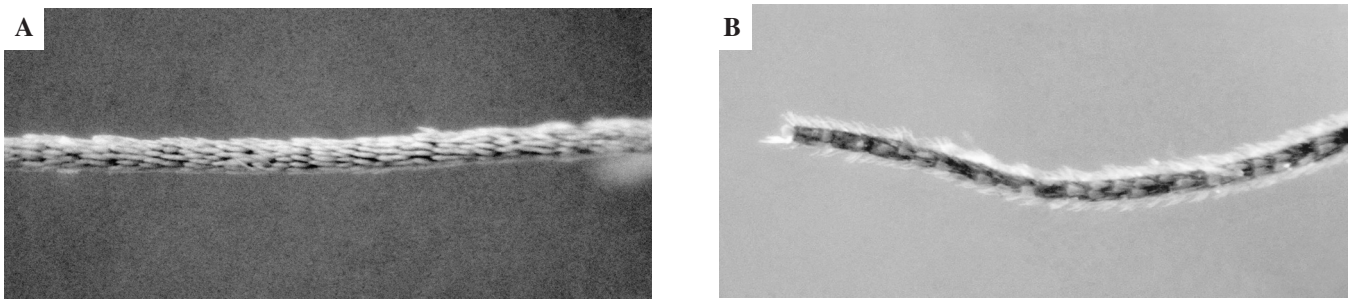


*Fig. 3.* Distinguishing features of antennae. A – not ciliated (*Elachista argentella*), B – ciliated (*Perittia farinella*).

(15mac) from the Kaišiadorys region population differed in 21 nucleotides (3.4%) from those of the Palanga population. Phylogenetic analysis of the moths based on partial mitochondrial COI gene sequences indicated that the sequences of *E. pollinariella* and *E. argentella* formed a single clade in the phylogenetic tree, different from *E. maculicerusella* clade. We found 67 nucleotides (10.5%) that differed between the sequences of *E. pollinariella* and *E. argentella*. Comparison of sequences of *E. maculicerusella* from Palanga and *E. pollinariella* showed 100 different nucleo-

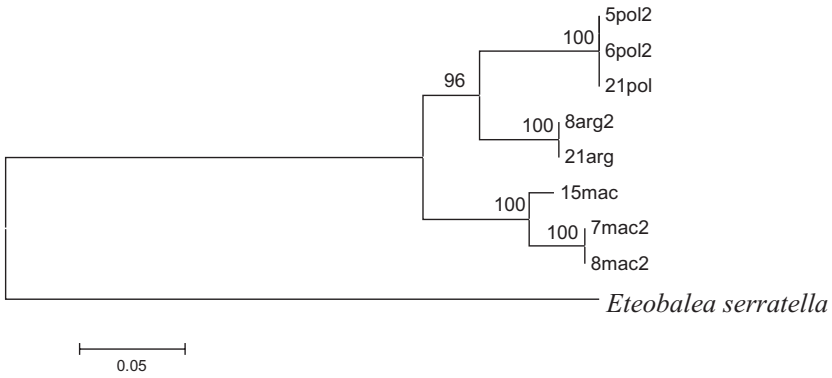


*Fig. 4.* Phylogenetic tree for specimens of *Elachista* spp. with *Eteobalea serratella* (AY423065) as an outgroup. The tree was constructed by the neighbour-joining method (bootstrap replications = 10000, complete deletion, model: nucleotide p-distance), based on analysis of 553 sites of mitochondrial COI. Bootstrap values are shown above branches.

tides (15.7%), while sequences of *E. maculicerusella* from Palanga and *E. argentella* differed in 86 nucleotides (13.5%).

## DISCUSSION

The observed huge differences between COI gene sequences in interspecific comparisons indicate that this marker can be used as a precision tool for recognising moth species. Differences between western and southeastern Lithuanian populations could be a result of the different phylogeographic history of *E. maculicerusella* populations. Homogeneity in west and southeastern populations of *E. pollinariella* and *E. argentella* suggest that each of these species colonized Lithuania from a single source.

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COI GĒNS KĀ MOLEKULĀRAIS MARĶIERIS *Elachista* SUGĀM (Lepidoptera: Elachistidae: Elachistinae) NO DAŽĀDĀM LIETUVAS POPULĀCIJĀM

Tika salīdzinātas COI gēna mtDNS sekvences trim Lietuvā sastopamām *Elachista* sugām: *Elachista maculicerusella*, *E. argentella* and *E. pollinariella* (Gelechioidea: Elachistidae: Elachistinae). Atrastas būtiskas pētīto sekvenču atšķirības starp sugām. Savukārt sugu robežās atšķirības atrastas tikai starp *E. maculicerusella* dažādām Lietuvas populācijām. COI gēnu variabilitāti var izmantot kā *Elachista* sugu noteikšanai, tā arī šo sugu populāciju daudzveidības pētījumos.