

Molecular analysis of COI mtDNA in *Phytoptus* (Phytoptidae) and *Eriophyes* (Eriophyidae) species associated with galls of *Tilia* spp. (Tiliaceae): preliminary results

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Introduction

The Tiliaceae plants are important components of the urban areas, city greenery and natural habitats of Serbia. The most abundant are silver linden (*Tilia tomentosa* Moench), large-leaved linden (*Tilia platyphyllos* Scop.), and small-leaved linden (*Tilia cordata* Miller) (Josifović, 1972).

Up to now, 21 species of eriophyoid mites are known to live on linden trees (Amrine and Stasny, 1994, Boczek and Szymkowiak, 1997). Amongst them, gall-inducing species such as a complex of morphologically close *Eriophyes* and *Phytoptus* species are the most frequently recorded.

Eriophyes and *Phytoptus* mites can cause various types of injury on *Tilia* species, which differ depending of the mite species-host plant interaction.

The aim of this study was to start preliminary research of eriophyoid species associated with most frequent galls on silver linden and large-leaved linden in urban area of Belgrade using molecular marker COI mtDNA in order to obtain data which would enable clarification of the existing taxonomic issues within species complex associated with linden trees.

Materials and methods

Leaves with galls were collected for detailed analysis. Individuals of eriophyoid mites collected from galls were separated into two groups: for the morphological and molecular species identification.

Morphological study. The morphology of species was examined using a phase-contrast microscope LEICA DMLS (Hi-plan phase objectives x10, x40 and x100). Prior to light microscopy mites were examined directly under a dissection stereomicroscope MBS-9, then picked up by a fine pin, mounted on microscope slides in Hoyer's medium (Dobrivojević and Petanović, 1982), and cleared on a heating block at 70°C for 3hours.

Molecular study. Total DNA was extracted from 15-20 whole specimens, using the QIAGEN Dneasy® Blood & Tissue Kit, following the manufacturer's instructions, with modification according to Dabert et al. 2008. A fragment of 658 bp of the mitochondrial cytochrome oxidase subunit I gene (COI) was amplified using the primers LCO1490 and HCO2198 (Folmer et al., 1994). PCR amplicons were sequenced with the same primer pair as in the initial PCR procedure.



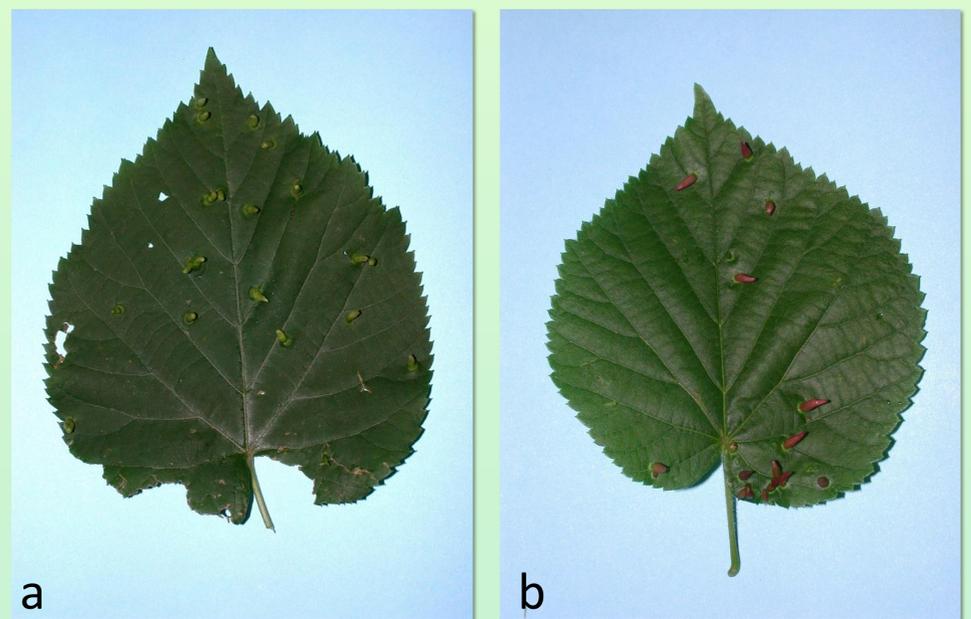
Picture 1. Green warty galls on the the upper surface and erineae on the lower surface of *T. tomentosa* leaf induced by *P. tetratrichus*.

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Results and discussion

On silver linden in the urban area of Belgrade, two types of galls caused by eriophyoid mites were found: i) green warty galls on the upper side and erineae on the underside of leaves (Picture 1) and ii) greenish-yellow nail galls on the upper surface of the leaf. (Picture 2a). On large-leaved linden red pointed nail galls on the upper surface of the leaf were monitored (Picture 2b).

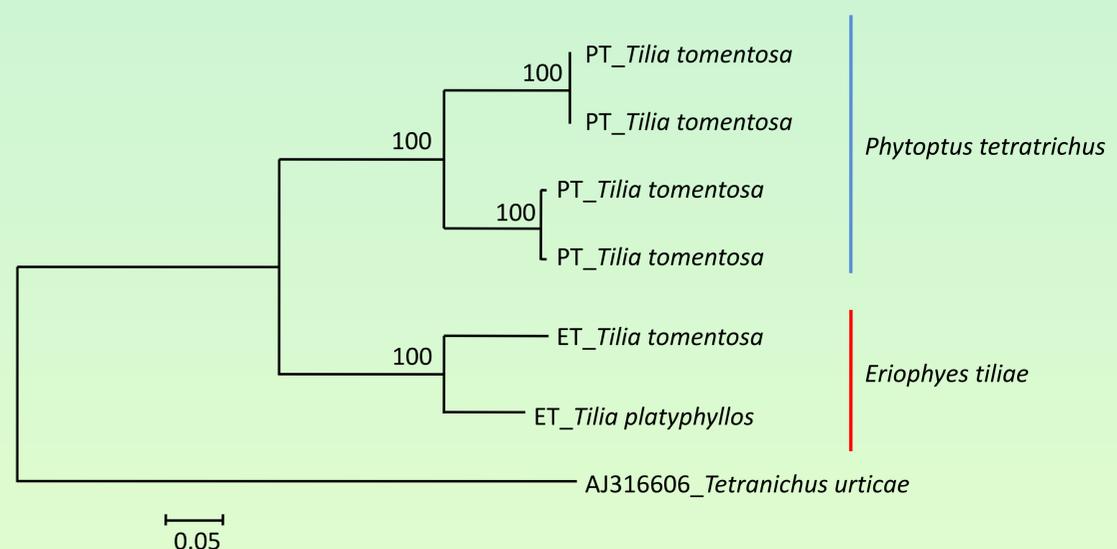


Picture 2. Galls on the upper surface of the leaf induced by *E. tiliae*: a) greenish-yellow nail galls on *T. tomentosa*; b) red pointed nail galls on *T. platyphyllos*.

Morphological study. Two species of eriophyoid mites belonging to *Phytoptus* and *Eriophyes* genera, inducing deformations on linden leaves were identified. Morphological species identification revealed that *Phytoptus tetratrichus* Nalepa 1890 is associated with warty galls on *T. tomentosa*, while *Eriophyes tiliae* (Pagenstecher, 1857) induces nail galls on both *T. tomentosa* and *T. platyphyllos*.

Molecular study. Molecular analyses were performed on four groups of *P. tetratrichus* and one group of *E. tiliae* on *T. tomentosa* as well as on one group of *E. tiliae* on *T. platyphyllos* (Picture 3). Mitochondrial COI sequence of *Tetranychus urticae* (AJ316606) (Navajas and Boursot, 2003) was used as a distant outgroup.

Four COI sequences of *P. tetratrichus* represent 2 different genotypes that vary significantly (about 12%).



Picture 3. Phylogenetic tree constructed using the Neighbor-Joining method of MEGA v5.05 for mitochondrial COI sequences of *P. tetratrichus* and *E. tiliae*.

Molecular analyses of *E. tiliae* indicate that mites associated with elongated and pointed greenish-yellow nail galls on the upperside of *T. tomentosa* leaves differ (about 9%) from mites associated with red distortions, rising up from the upper surface of the *T. platyphyllos* leaves.

To be able to clarify the relationships within *Phytoptus* and *Eriophyes* complexes of species associated with different linden species, and their evolutionary diversification, more molecular data from other *Tilia* species and diverse geographical regions is needed and research towards this direction is underway.