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FELLOWSHIP FINAL REPORT

Interesting lessons we can learn using past herbarium collections for studying forest insect pest invasions

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ABSTRACT

Historical herbaria collected around the world are valuable source of data for studying past communities of folivore organisms and tracking their distributions through the time. Here we examined the world biggest herbarium collection stored in the Muséum National d'Histoire Naturelle (Paris, France) in order to explore past Tilia-feeding endophage complexes and their populations in the Holarctic and clarify the expansion history of the lime leafminer, Phyllonorycter issikii Kumata, 1963 (Lepidoptera: Gracillariidae), an invasive pest in Europe damaging limes, Tilia spp. (Malvaceae).

1- Introduction

Past herbarium collections have great value to science and serve not only important source of data for botanists. Herbarium samples collected in different parts of the world can provide unique data also for entomologists and ecologists to assess responds of herbivory arthropods to global changes, to study diversity, abundance, trophic relations and range expansions of folivore arthropods.

A study focused on herbaria sampled in the northeastern US showed herbivory increase over the past century due to warming climate, predicting further potential grow of insect damage to plants (Meineke et al., 2018). Other studies documented potential extinction or extirpation of insect taxa due to antropogenic activity. For instance, herbarium data and surveys in the field, provided evidence for putative extinction of *Phyllanthus*-feeding micromoth from the island of Mangareva (Gambier Islands, French Polynesia) (Hembry, 2013).

Larvae and pupae of endophagous insects such as leaf-miners and leaf-gallers, concealed in tissues of pressed leaves, can still be found in herbarium decades later and these archival specimens can be effectively used in taxonomic, phylogenetic, population genomic, and invasion ecology studies (Lees at al., 2011; Staats et al., 2013). Advanced molecular genetic approach, i.e. Next generation sequencing offers powerful tool to analyse DNAs degraded over the time opening great perspectives to study insect species diversity and their past distributions (Staats et al., 2013).

The aim of the study was to examine the world biggest herbarium collection in order to define past diversity of leaf-mining insects attacking limes, *Tilia* spp. (Malvaceae) and to study past

range of the lime leafminer, *Phyllonorycter issikii* (Kumata, 1963) (Lepidoptera: Gracillariidae), an invasive pest in the Palearctic.

2- Experimental details

An extensive survey of pressed *Tilia* leaves has been performed at the herbarium of the vascular plants collection (P) of the Muséum National d'Histoire Naturelle (MNHN) (Paris, France) (Figure 1). This museum stores the biggest ever herbarium collection of vascular plants accounting around 8 millions of herbarium sheets containing plant material sampled in various floristic regions around the world.

Figure 1. Herbarium depositary at the Muséum National d'Histoire Naturelle (Paris, France).

During the course of the study, about 2000 herbarium sheets containing hundreds of lime leaves sampled during the last two centuries in the Holarctic realm were carefully surveyed for presence of leaf mines.

The leaf mines found on pressed leaves were examined using binocular. Morphological character of the leaf mines such as shape of mine, presence of egg shell on the surface, mine position on the leaf, presence of frass, larva or pupa in the mine, location of the exit hole were documented. Presences of insects in the mines was ascertain by checking herbarium sheets against the light. Sampling location, date of sampling and other important information from the labels on herbarium sheets was noted. Whenever possible, abundance of mines was estimated as a number of leaves with mines per herbarium sheet and the number of mines per individual lime leaf.

Significant efforts were made to detect *Phyllonorycter*-like mines in order to find early evidences of lime leaf miner *Phyllonorycter issikii* invasion in Europe. The absence/ presence of mines, the total number of mines and leaves per herbarium sheet and the number of mines per leaf was recorded together with the locality (most often the country), the plantation type (wild, botanical gardens, parks, city plantations), and the collection date in order to track back the expansion of the pest in the Palearctic.

With the permission of the herbarium curator, the leaf mines containing larvae or pupae were opened to sample insect individuals (Figure 2).

Figure 2. Sampling archival larvae and pupae of leaf mining insects on the pressed leaves.

For opening the mines, a thin syringe and delicate entomological forceps were used.

Sampled archival larvae and pupae were placed individually into 1.5 ml tubes with hermetic lids, labeled and stored at -20C before using their tissues for following molecular genetic analysis.

Mines and their inhabitants were photographed using digital camera Sony Nex3.

3- Results and discussion

Larvae of insects from four taxonomic orders Lepidoptera, Coleoptera, Hymenoptera, and Diptera are known to mine leaves of plants (Hering, 1951). Among them, there are number of pests damaging crops, urban plantations and forests and invasive species.

Larvae of majority of species develop and pupate inside the leaf mines. Larvae of others abandon their mines and continue living freely or semi-freely (in leaf shelters). Mines of all these insects (often with inhabitants inside the mines) can be found on leaf lamina on pressed leaves.

In the Muséum National d'Histoire Naturelle, about 2000 herbarium sheets containing lime leaves have been found. They were represented by mainly by 13 *Tilia* species: *T. americana, T. amurensis, T. begoniifolia, T. cordata, T. chinensis, T. dasystyla, T. japonica, T. kiusiana, T. mandshurica, T. maximowicziana, T. platyphyllos, T. taquetii* и *T. tomentosa* collected between the end of the XVIII century and the beginning of the XXI century. Most lime specimens were collected and herbarized between 1840s – 1950s, followed by the gap in sampling that occurred in 1960s – 1980s and a slight subsequent increase in the 1990s.

Overall, 88% of all investigated herbarium sheets (precisely 1760 herbarium sheets) originated from Eurasia. Other 12% of herbarium sheets were sampled in North and South America. In Eurasia, two thirds of herbarium sheets contained lime leaves collected in European countries, Western Russia and Siberia and one third of herbarium sheets carried *Tilia* specimens gathered from East Asia (the Russian Far East, Japan, South Korea, and China). In North America, herbarium specimens were collected mainly in the US states (the majority species originated from in *Ohaio, Massachusetts, Arkansas*) and from few locations in Canada. Several herbarium sheets carried *Tilia* specimens sampled in Mexico.

About 10% of herbarium sheets from the Palearctic contained leaves with the mines left by different insects. We documented leaf mines created by least 12 insect morphospecies from four orders (Lepidoptera, Coleoptera, Hymenoptera and Diptera) (Figure 3).

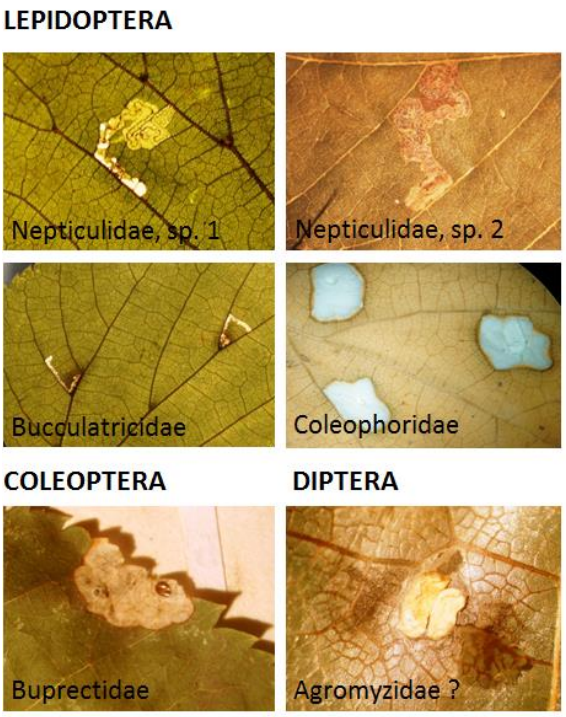

Figure 3. Archival leaf mines of insects from different orders found on the pressed leaves in the herbarium collection from the Palearctic.

The order Lepidoptera was represented by the leaf-mining insects from seven families: Gracillariidae (*Phyllonorycter*, one morpthospecies), Nepticulidae (*Stigmella*, probably three morphospecies), Incurvariidae (*Incurvaria*, one morphospecies),

Bucculatricidae (*Bucculatrix,* one morphospecies), Roeslerstammiidae (*Roeslerstammia,* one morphospecies), Tischeridae (*Tischeria,* one morphospecies), and Coleophoridae (*Coleophora,* one or more morphospecies). Coleopteran mines were assigned to *Trachys* morphospecies. Sawfly mines (Hymenoptera: Tenthredinidae) were made by the representatives of the genus *Parna*. In one case, a leaf mine containing neonate maggots of a fly, probably from the family Agromyzidae was found on *Tilia* leaves from the Palearctic. No records of leaf-mining flies attacking limes in the Palearctic are known from the literature.

Majority of leafminers's diversity on the lime leaves was documented in the Western Palearctic comparing to Eastern Palearctic that can be explained by the pronounced bias in the herbarium sampling – most samples in the herbarium originated from the west.

The mines of *Phyllonorycter*, probably all made by the lime leafminer, *Ph. issikii* Kumata, 1963 (Lepidoptera: Gracillariidae), were most abundant on the pressed leaves sampled in the Palearctic, especially in the herbarium originated from East Asia. This species is an intriguing example of fast invasion in the Palearctic. In the last few decades, this tiny moth colonized most of Europe and became a pest of European species of limes (Šefrová, 2002). Our recent phylogeographic analysis revealed unexpectedly high *Ph. issikii* genetic diversity in Europe (invasion region) vs. East Asia (putative native range), questioning the hypotheses about its expansion and the region of origin (Kirichenko et al., 2017). Herbarium surveys done in MNHN will help to check this hypothesis.

In the Nearctic, about 5% of pressed leaves of *Tilia* carried mines. The mines of a *Phyllonorycter* species, likely represented by *Ph. lucetiella* (Clemens, 1859), a North American lime leafminer were most abundant, especially in some American states.

Archival larvae and pupae sampled from herbarised leaves are being currently processed using Next generation sequencing in order to identify leafminer species communities attacking *Tilia* and to characterize genetically their past populations. This data will also be of a great value for in-deep exploration of the invasion history of the lime leafminer, *Phyllonorycter issikii* in the Palearctic.

4- Conclusion

This project highlights the importance of usage of the world biggest herbarium in studying past communities of endophagous insects, determining sources of invasive pests and tracking their expansions over the time.

5- Perspectives of future collaborations with the host laboratory

Further collaboration with the laboratory of Forest Zoology INRA will be focused on various questions linked to integrative taxonomy, phylogeography and invasive ecology of forest insect pests that will reinforce the scientific links between French and Russian academic societies in the field of forest entomology and molecular taxonomy.

6- Articles published in the framework of the fellowship

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