Occurrence of *Aculus mosoniensis* (Ripka, 2014) (Acari; Prostigmata; Eriophyoidea) on tree of heaven (*Ailanthus altissima* Mill.) is expanding across Europe. First record in France confirmed by Barcoding

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Summary In May 2020, populations of Eriophyid mites were detected on tree of heaven, *Ailanthus altissima* Mill., in a recreative park in the city of Colombes, near Paris, and later in August and September in four locations, in Southern France. Morphological examination supplemented with sequencing data for the cytochrome c oxidase subunit I (*COI*) assigned all collected mites to a single species, *Aculus*

mosoniensis (Ripka) (Acari; Prostigmata; Eriophyoidea). This is the first record of this species in France and the first insights into its intraspecific variability based on a barcode dataset. In Europe, this species is considered one of the most promising biological control agents of tree of heaven. This new record provides encouraging evidence that the geographic occurrence of this species is expanding in Europe which may be indicative of its dispersal and establishment abilities, two key factors for a future biological control program.

Keywords Eriophyid mites · Invasive species · Mitochondrial DNA · Biological control · Europe

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Introduction

Ailanthus altissima (Mill.) Swingle, 1916, commonly known as tree of heaven or Faux vernis du Japon, is a fast-growing deciduous tree, native to both northeast and central China, as well as Taiwan (Hu, 1979; Kowarik & Säumel, 2007). This species was brought from China to Europe by the French missionary Pierre d'Incarville in the 1740s. Among Incarville's seed recipients, was Bernard de Jussieu, the Superintendent of the Jardin Royal des Plantes in Paris, in France. De Jussieu sowed a portion of the seeds and sent some of them to the Chelsea Physic Garden, in London

(Swingle, 1916). By the 1840s, this species, valued for its fast-growing ability and its resistance to insect infestation and damage, was being widely planted as a shade tree for parks and public promenades in Europe, particularly in France and Italy (Shah, 1997).

Widely naturalized in Europe, tree of heaven has been included on the EPPO List of Invasive Alien Plants since 2004 (EPPO, 2021), and on the list of the 100 worst alien plant species in Europe (DAISIE European Invasive Alien Species Gateway 2012). The species is regulated in several EPPO countries and many countries outside EPPO including the USA (CABI, 2021; EPPO, 2019; Motard et al., 2015; Sladonja et al., 2015). Its invasiveness is primarily attributed to five traits 1) tolerance of extreme environmental conditions although tree of heaven typically occurs in humid habitats (Albright et al., 2010); 2) production of more than 200 allelopathic substances including the phytotoxic quassinoid ailantone (Heisey, 1996; Lin et al., 1995); 3) high seed production and viability of seeds with mature females bearing highly abundant wind-dispersed samaras (Landenberger et al., 2007; Wickert et al., 2017); 4) clonal proliferation with copious sprouting after cutting (Hu, 1979); 5) limitations of herbivores insects capable of suppressing this invasive tree (Ding et al., 2006; Kiviat, 2004; Sheppard et al., 2006). In France, *A. altissima* is typically restricted to urban and suburban habitats in temperate climates but it is also frequent in rural areas of meridional and Mediterranean climates (iNaturalist, 2021; Kowarik, 1983). In southern and southwestern France, *A. altissima* is increasingly invading vineyards and is apparently difficult to control, particularly along grapevine rows where mechanical control damages grapevine roots. In addition, in organic vineyards the use of herbicides is prohibited (EPPO, 2019; 2021). In many countries, management is largely dependent on the use of systemic (and non-selective) chemical herbicides and mechanical control, and clearly lacks a long-term and sustainable control. In Europe, as the approval of chemical herbicides including glyphosate, is facing severe restrictions if not an outright ban, there is a growing need to adopt more ecologically sound and sustainable strategies such as classical biological control (Sheppard et al., 2006).

In literature surveys of natural enemies of tree of heaven in China, Ding et al. (2006) listed 46 phytophagous arthropods, 16 fungi and one potyvirus to attack tree of heaven, some apparently causing significant damages. Included in the tree of heaven feeding arthropods are four Eriophyid mites species – *Aculops ailanthi* Lin et al., 1997, *Aculops taihangensis* Hong & Xue, 2005, *Aculus altissimae* Xue & Hong, 2005, and *Aculus mosoniensis* Ripka, 2014 (Ripka & Érsek, 2014). The early synonymy between *Aculops taihangensis* and *Aculus mosoniensis* suggested by de Lillo et al. (2017) based on morphological characters was supported by a high similarity between their internal transcriber spacer 1 sequences (Cristofaro & de Lillo, 2019; Marini et al., 2021). In Europe, *Aculus mosoniensis*, first reported in Hungary (Ripka & Érsek, 2014) and recently in Italy (de Lillo et al., 2017), and the vascular wilt pathogen, *Verticillium dahlia*, isolated in Italy (Pisuttu et al., 2020), are the only two potential European biological control agents of tree of heaven. *Aculus mosoniensis* forms dense populations mainly at the under surface of the leaflets of the young compound leaves, causing leaf edges to curl upwards and turn yellowish in color. Drying of the upper parts of the stem can be observed on heavily infested plants, and young plants can desiccate and loose leaves prematurely (Cristofaro & De Lillo, 2019). In May 2020, as a part of an ongoing quest of USDA ARS to identify natural enemies of tree of heaven, weekly field observations were carried out in a recreative park in the city of Colombes, near Paris. One tree was found to display leaf rolling associated with an Eriophyid mite infestation, similar to that described by Ripka and Érsek (2014) in Hungary (Fig. 1). Leaf examination using a digital microscope (Dino-Lite Edge, Taipei, Taiwan, 100X magnification) confirmed the presence of eriophyid mites (Fig. 2). In follow up investigations, additional trees showed similar mite infestation symptoms. Since this first occurrence and later in the growing season, from August to September, four populations of eriophyid mites were found in different localities in Southern France. We here identify to species level all populations of Eriophyid mites sampled on tree of heaven in France by examining morphological characters. We also compare barcoding datasets obtained in these populations

Fig. 1 Heavily infested *A. altissima* leaves

with barcodes (mitochondrial regions on the 5' end of the cytochrome c oxidase subunit I (*COI*)) of a *A. mosoniensis* Italian population which is used in an ongoing biological control program.

Materials and methods

Mite infested leaves were collected between May and September 2020 in different locations in France (Table 1) and preserved in 70% ethanol for morphological examination at the University of Belgrade, in Serbia and in 96% ethanol for molecular characterization at EBCL in France. For morphological identification, specimens were mounted in Keifer's F medium (Amrine & Manson, 1996) and then examined using a Leica DMLS research microscope with phase-contrast. For molecular characterization of each population sampled, four to five

Fig. 2 *Aculus mosoniensis* mites on Tree of Heaven leaf

pools of 5 to 7 specimens were carefully removed off one leaf using a fine brush and examined under a stereomicroscope (Dino-Lite Edge, Taipei, Taiwan, 100X magnification). Each pool was subsequently placed into a 1.5 ml Eppendorf LoBind collecting tube. Four pools of five *A. mosoniensis* specimens collected at a site near Roma in Italy were also processed following the same protocol (Table 1). After complete evaporation of the residual ethanol using Speed-Vac, 20 μ L of 1X PCR buffer (Qiagen, Hilden, Germany), 2 μ L of Proteinase K (Qiagen, Hilden, Germany 20 mg/ml) and 20 μ L of GeneReleaser® (BioVentures, Inc., Murfreesboro, TN, USA) were added to the collecting tube which was incubated during 3 h at 56 °C using a Thermomix ®C (Eppendorf, Hamburg, Germany). Following an incubation at 95 °C for 10 min to inactivate the Proteinase K, the tube was centrifuged at 10,000 g for 5 min and the resulting supernatant was used immediately as DNA template for PCR or stored at -24 °C. PCR amplifications of the barcode region were performed in a total volume of 30 μ L containing 1 $\times$ of CoralLoad PCR Kit (Qiagen), 0.2 μ M of each degenerate primer, LCO1490puc and HCO2198puc, (Cruaud et al., 2010), 1 mM of $MgCl_2$, 0.2 mg/ml of Bovine Serum Albumin, 1 Unit of *Taq* polymerase (Qiagen) and 3.0 μ L of diluted template DNA. The thermal cycling program was as follows: 3 min at

Table 1 Collection records of *A. mosoniensis* populations used in this study

Sample ID	Locality/Country	Name of the Collector/ Agency	Collection date (dd/mm/yyyy)	GPS coordinates	Barcode Genbank Accession number
TOHA2c	Cesano near Roma/Italy	S. Barlattani; F. Marini/ BBCA	07/07/2020	42°03'50''N 12°19'44''E	MW892618
Italy1	Cesano near Roma/Italy	S. Barlattani; F. Marini/ BBCA	07/07/2020	42°03'50''N 12°19'44''E	MW892618
Italy3	Cesano near Roma/Italy	S. Barlattani; F. Marini/ BBCA	07/07/2020	42°03'50''N 12°19'44''E	MW892618
Italy5	Cesano near Roma/Italy	S. Barlattani; F. Marini/ BBCA	07/07/2020	42°03'50''N 12°19'44''E	MW892618
Colombes3	Colombes/France	Javid Kashefi/EBCL	05/26/2020	48°55'23''N 2°13'57''E	MW892615
Colombes7	Colombes/France	Javid Kashefi/EBCL	05/26/2020	48°55'23''N 2°13'57''E	MW892616
Colombes8	Colombes/France	Javid Kashefi/EBCL	05/26/2020	48°55'23''N 2°13'57''E	MW892617
Colombes9	Colombes/France	Javid Kashefi/EBCL	05/26/2020	48°55'23''N 2°13'57''E	MW892616
Colombes10	Colombes/France	Javid Kashefi/EBCL	05/26/2020	48°55'23''N 2°13'57''E	MW892615
TOHA5	Viols le Fort/France	Javid Kashefi/EBCL	08/04/2020	43°44'45.75"N 3°42'31.27"E	MW892619
Viols le Fort1	Viols le Fort/France	Javid Kashefi/EBCL	08/04/2020	43°44'45.75"N 3°42'31.27"E	MW892619
Viols le Fort3	Viols le Fort/France	Javid Kashefi/EBCL	08/04/2020	43°44'45.75"N 3°42'31.27"E	MW892619
Viols le Fort4	Viols le Fort/France	Javid Kashefi/EBCL	08/04/2020	43°44'45.75"N 3°42'31.27"E	MW892619
TOHA6	Manosque/ France	Javid Kashefi/ EBCL	09/09/2020	43°48'31.13"N 5°48'47.45"E	MW892620
Manosque1	Manosque/ France	Javid Kashefi/ EBCL	09/09/2020	43°48'31.13"N 5°48'47.45"E	MW892620
Manosque3	Manosque/ France	Javid Kashefi/ EBCL	09/09/2020	43°48'31.13"N 5°48'47.45"E	MW892620
Manosque4	Manosque/ France	Javid Kashefi/ EBCL	09/09/2020	43°48'31.13"N 5°48'47.45"E	MW892620
TOHA7	Vinon sur Verdon/France	Javid Kashefi/ EBCL	09/10/2020	43°43'50.87"N 5°48'43.96"E	MW892621
Vinon1	Vinon sur Verdon/France	Javid Kashefi/ EBCL	09/10/2020	43°43'50.87"N 5°48'43.96"E	MW892621
Vinon2	Vinon sur Verdon/France	Javid Kashefi/ EBCL	09/10/2020	43°43'50.87"N 5°48'43.96"E	MW892621
Vinon5	Vinon sur Verdon/France	Javid Kashefi/ EBCL	09/10/2020	43°43'50.87"N 5°48'43.96"E	MW892621
TOHA8	Sisteron/ France	Javid Kashefi/ EBCL	09/10/2020	44°10'15.21"N 5°56'55.18"E	MW892622
Sisteron1	Sisteron/ France	Javid Kashefi/ EBCL	09/10/2020	44°10'15.21"N 5°56'55.18"E	MW892622
Sisteron3	Sisteron/ France	Javid Kashefi/ EBCL	09/10/2020	44°10'15.21"N 5°56'55.18"E	MW892622
Sisteron4	Sisteron/ France	Javid Kashefi/ EBCL	09/10/2020	44°10'15.21"N 5°56'55.18"E	MW892622

Fig. 3 *Aculus mosiniensis*—dorsal view of prodorsal shield (phase-contrast light microscope)

94 °C, 40 cycles of 30 s at 94 °C, 30 s at 50 °C, 1 min at 72 °C and a final extension of 10 min at 72 °C. PCR products were sequenced in the forward and reverse direction using Sanger approach by Genoscreen at Lille, France. Sequences were translated into amino acids; the absence of stop codons was checked with Mega X (Kumar et al., 2018). Sequence alignment was performed using ClustalW (Higgins et al., 1994) as implemented in Mega X. Overall and pairwise distances between nucleotide sequences were calculated using Kimura's two-parameter model in Mega X.

Results and discussion

Mite specimens collected in the five locations in France were examined and determined to be *Aculus mosoniensis* based on qualitative and morphological characteristics of this species such as the design of prodorsal shield and the number of rays on tarsal empodium (Fig. 3) (de Lillo et al., 2017; Ripka & Érsek, 2014). Barcode compliant sequences of 685 bp were successfully obtained for each pool of mites sampled and none of the characteristic evidence of numts was present in the sequences obtained. All different sequences obtained in each population have been deposited in Genbank database with accession numbers

Table 2 Kimura two-parameter distances (shown as percentages with SE estimates between mitochondrial DNA *COI* sequence pairs

Level	Mean distance $\pm$ SE (%)
Within Colombes	0.89 $\pm$ 0.27
Within Italy	0
Within Southern France	0
Between Colombes and Italy	0.89 $\pm$ 0.29
Between Colombes and Southern France	0.73 $\pm$ 0.24
Between Italy and Southern France	1.38 $\pm$ 0.47

indicated in Table 1. At the time of writing, no barcode sequence data of *A. mosoniensis* or *A. taihangensis* was available in Genbank. Species identification was attempted by BOLD identification engine in Barcode of Life Data Systems (BOLD Systems v3), and all sequences including the Italian *A. mosoniensis* were assigned with similarity scores ranging from 98.6 to 99.6% to *A. taihangensis* of Chinese origin. All barcode sequences from southern France were 100% identical as well as all barcode sequences from Italy. In Colombes, we identified three haplotypes in the five pools analyzed. Genetic distances between the Italian *A. mosoniensis* and Colombes and southern France populations ranged from 0.89 to 1.388% (Table 2), which is low and compatible with the intraspecific distances reported in other eriophyid mite species such as *Aceria tosichella* Keifer (Skoracka et al., 2012a, b) and *Abacarus denticulifer* Chetverikov (Chetverikov et al., 2019).

Concluding remark

The presence of *A. mosoniensis* was confirmed in all sampled locations in France, which indicates that this mite is already established and largely distributed within France. To the best of our knowledge, this is the first report of *A. mosoniensis* in France. As this eriophyid mite is considered one of the most promising biological control agents of

Map 1 Collection map and rout in France. Yellow dots=*Aculops mosoniensis* collection sites. Greece dot=Most northern location of *Ailanthus altissima* collection point

tree of heaven in Europe, this new record provides encouraging evidence that the geographic occurrence of this species is expanding in Europe, apparently always associated with the target weed, which may be indicative of its dispersal and establishment abilities, two key factors for a future biological control program (Map 1).

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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