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First records of the invasive aphid species, *Aphis spiraecola*, in Kosovo, Slovakia, the Czech Republic, the United Kingdom and Denmark

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Abstract: In the last few decades, the spiraea aphid (*Aphis spiraecola* Patch) has become a widely distributed pest of apple and citrus orchards across Europe. In our study, *A. spiraecola* was observed for the first time in Kosovo, Slovakia, the Czech Republic, the United Kingdom and Denmark, in apple orchards in the growing seasons of 2016, 2018 and 2019. The presence of *A. spiraecola* was also recorded on other host plants such as the quince (*Cydonia oblonga* Miller) and Vanhoutte spiraea (*Spiraea × vanhouttei*) in Slovakia, and the quince, common pear (*Pyrus communis* Linnaeus) and firethorn (*Pyracantha coccinea* M.J. Roemer) in the United Kingdom. Based on the morphological characteristics and the sequencing of the DNA mitochondrial cytochrome c oxidase subunit 1 gene (COI fragment) barcode, our study confirms the presence of this pest in five additional regions in Europe.

Keywords: apple; pest; identification; distribution; Europe

Aphis spiraecola Patch (spiraea aphid) is a heteroeious aphid species alternating between spiraea and citrus plants as winter hosts and more than 250 taxa from several families as summer hosts, including the apple subfamily (Maloideae) (Pfeiffer et al. 1989; Satar & Uygün 2008). The economically significant damage of *A. spiraecola* to apples and other Maloideae host plants appears primarily in nurseries and in newly planted orchards as a reduction in the shoot growth, dry matter accumulation, leaf photo-

synthesis and greenness (Kaakeh et al. 1993). *Aphis spiraecola* is also a major pest of citrus (Komazaki 1990, 1994).

The origin of *A. spiraecola* is uncertain, although it most likely originated in East Asia (Blackman & Eastop 1984), and, as an invasive species, it has become an important pest of citrus and apple orchards all over the world (Blackman & Eastop 1984; Pfeiffer et al. 1989; Rakauskas et al. 2015). In Europe, *A. spiraecola* was reported in the early 1990's as a new

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widespread pest of the citrus growing areas of the Mediterranean region (Katsoyannos et al. 1997; Cambra et al. 2000; Kavallieratos et al. 2007). In the following decade, the species was recorded from apple orchards in Central Europe: in Germany in 2000 (Litterst & Thieme 2001), in Hungary in 2004 (Mezei & Kerekes 2006), and in Bulgaria and Serbia in 2007 (Andreev et al. 2007; Petrović-Obradović et al. 2009). In the recent years, *A. spiraecola* reached the Baltic region as well (Rakauskas et al. 2015). In the case of the United Kingdom, although *A. spiraecola* was recorded on an imported Yucca rooted stem consignment (Furk 1979) and on *Cotoneaster* shrubs in London (Martin 1996), there has not been any evidence of its constant presence (CABI 2020). Heie (1986) listed *A. spiraecola* as a species that is not present in Denmark, and to our knowledge, this species was not previously known to occur in Kosovo, Slovakia, and the Czech Republic (CABI 2020).

Aphis spiraecola has morphological similarities to the aphid species *Aphis pomi* DeGeer (green apple aphid), native to the Palearctic region (Baker & Turner 1916), and the two species are colonising common host plants like apples and pears (Halbert & Voegtlin 1992). The morphological discrimination of the two green aphid species is complicated, which often leads to confusion in the identification. As the life cycle of *A. spiraecola* differs from that of *A. pomi*, an accurate discrimination is required to establish effective pest control strategies (Footitt et al. 2009).

In this study, we have investigated the presence of *A. spiraecola* in five countries in Europe on apples in commercial orchards and on some other host plants using morphological and molecular identification methods.

MATERIAL AND METHODS

The green aphid (*Aphis* spp.) samples were collected in apple orchards from different locations in Kosovo, Slovakia, the Czech Republic and Denmark, across the growing seasons of 2018 and 2019, and in the United Kingdom across the growing seasons of 2016, 2018 and 2019. Samplings from other potential host plants were also carried out in Slovakia, from the Vanhoutte spirea (*Spiraea × vanhouttei*) and quince (*Cydonia oblonga* Miller), and in the United Kingdom, from the quince (*C. oblonga*), hawthorn (*Crataegus monogyna* Jacquin), medlar (*Mespilus germanica* Linnaeus), firethorn (*Pyracantha*

tha coccinea M.J. Roem.) and common pear (*Pyrus communis* Linnaeus). The aphid individuals were collected with a fine paint brush and placed in 1.5 µL Eppendorf tubes filled with 70% ethanol solution.

Identification of the samples was performed using a stereo microscope (Zeiss Stemi 200C, Carl Zeiss, Germany) based on the morphological characteristics. The number of marginal tubercles, number of caudal hairs and shape of the cauda were used as the key characteristics (Halbert & Voegtlin 1992; Rakauskas et al. 2015).

The morphological identification was supported by the sequence analysis of the DNA mitochondrial cytochrome c oxidase subunit I gene (COI fragment) barcode (Footitt et al. 2009; Rakauskas et al. 2015). The total genomic DNA was extracted from a single aphid with a DNeasy blood and tissue kit (QIAGEN, Germany) according to the manufacturer's instructions. Amplification of the COI barcode was performed in a 20 µL reaction volume containing 20–80 ng DNA, a 5× Phire Reaction Buffer, 0.4 µL of Phire Hot Start II DNA Polymerase (Thermo Scientific, Hungary), a 0.2 mM dNTP mix, 3% DMSO, 2.5 µmol of each 5' and 3' end primers [LCO 1490: 5' GGTCAACAAATCATAAAGATATTGG 3' and HCO 2198: 5' TAAACTTCAGGGTGACCAAAAAATCA 3' (Folmer et al. 1994)], and sterile distilled water. The PCR (polymerase chain reaction) was carried out in a Swift MaxPro thermocycler (ESCO Healthcare, Singapore). The cycling parameters were as follows: initial denaturation at 98 °C for 30 s, then 30 cycles of denaturation at 98 °C for 5 s, annealing at 49 °C for 5 s and extension at 72 °C for 15 s, and a final extension for 1 min at 72 °C. The PCR products were loaded on a 1% (w/v) ethidium bromide-stained agarose gel in a 1 × TBE buffer to verify the amplification. The fragment sizes were estimated by comparison with a 1 kb DNA ladder (Fermentas, Waltham, USA). The amplified fragments were purified using a CleanSweep PCR purification kit (Thermo Scientific, Hungary) for the direct sequencing. The sequencing was performed in an ABI PRISM 3100 Genetic Analyser automated sequencer (Applied Biosystems, USA). For each fragment, the nucleotide sequences were determined in both directions. The forward and reverse sequences were edited and assembled, the alignment and neighbour-joining analysis were conducted in MEGAX (Kumar et al. 2018). The DNA sequences were verified using the BLASTN algorithm from NCBI (National Center for Biotechnology Information).

RESULTS AND DISCUSSION

Based on their morphological characteristics, the *Aphis spiraecola* individuals were identified from the samples collected from apple trees in Kosovo at Llugaxhi (lat. 42.482667; long. 21.163616; 23 July, 20 August and 28 September 2018; 54, 39 and 31 individuals, respectively) and at Karavarice (lat. 42.709966; long. 21.625216; 23 July, 20 August and 28 September 2018; 65, 36 and 42 individuals, respectively). In Slovakia, *A. spiraecola* was detected on apple trees at Tvrdošovce (lat. 48.100676; long. 18.053908; 2 May 2018; 7 individuals; 19 June 2018; 42 individuals), on apples and quinces at Stúrovo (lat. 47.794591; long. 18.720009; 15 June 2018; 7 and 2 individuals, respectively) and on *Vanhoutte spirea* at Komarno (lat. 47.759638; long. 18.127365; 22 June 2018; 18 individuals) and Nitra (lat. 48.306332; long. 18.096308; 22 June 2018; 15 individuals). In the Czech Republic, *A. spiraecola* individuals were recorded on apple trees at Bílé Podolí (lat. 49.57748; long. 15.29840; 21 June 2019; 129 individuals) and at Holovousy (lat. 50.21596; long. 15.34013; 21 June 2019; 144 individuals). Out of the eight locations where apple trees were sampled in the United Kingdom, *A. spiraecola* was recorded at six locations: Ash (Canterbury) (lat. 51.302403; long. 1.291386; 13 July 2018; 8 individuals), Ditton (lat. 51.292115; long. 0.445717; 10 July 2018; 6 individuals), Lenham (lat. 51.214524; long. 0.685980; 10 July 2018; 13 individuals), Sittingbourne (lat. 51.331260; long. 0.779724; 11 July 2018; 15 individuals), West Malling (lat. 51.285483; long. 0.420669; 11 July 2018; 8 individuals), and East Malling (lat. 51.284968; long. 0.454027; 14 June, 21 July, 31 August and 28 September 2016; 23, 72, 31 and 5 individuals, respectively; 23 July 2019; 145 individuals). Of the 5 other potential host plants, *A. spiraecola* was detected on the quince (12 individuals), the common pear (5 individuals) and the firethorn (24 individuals) at East Malling (lat. 51.290962; long. 0.447164; 11 July 2018). In Denmark, four orchards on Zealand were searched at least 4 times over the growing seasons in 2018 and 2019. *Aphis spiraecola* individuals were only recorded on apple trees at The Pometum (Taastrup) (lat. 55.672327; long. 12.309266; 20 July 2019; 4 individuals).

The COI barcode of twelve *A. spiraecola* individuals were sequenced: two samples from Kosovo (Llugaxhi), one sample from Slovakia (Tvrdošovce), three samples from the Czech Republic (two from

Holovousy and one from Bílé Podolí), three samples from the United Kingdom (East Malling, West Malling and Sittingbourne), one sample from Denmark (The Pometum), and as a control, one *A. spiraecola* sample from Hungary and also one *A. pomi* sample from Hungary (Mihályi; lat. 47.523572; long. 17.098054; 29 July 2019) (Figure 1). The sequenced region covered 707 nucleotides. The forward and reverse sequences were aligned, and a consensus sequence was generated for each. All the sequences are deposited in the NCBI GenBank under the accession number MT445566-MT445577. For the sequence comparison, further *A. pomi* [KF638994 (FR), EU701478 (CA), FJ998495 (USA)] and *A. spiraecola* [FJ998605 (USA), FJ998587 (CA), FJ998581 (NZ)] COI sequences were downloaded from NCBI GenBank. For alignment purposes, these sequences were trimmed, hence, 630 bases were finally used in the alignment and cluster analysis. Comparing the *A. pomi* and *A. spiraecola* sequences, 29 SNPs (single nucleotide polymorphisms) were found differentiating the two species. Most of the *A. spiraecola* specimens constitute a single haplotype, only one specimen from Denmark differed by three SNPs from the common type along with two North American specimens [FJ998605 (USA), FJ998587 (CA)] retrieved from the GenBank.

The neighbour-joining analysis (Figure 1) revealed two independent clades with strong support (100%), one harbouring the *A. pomi* individuals, and the other the *A. spiraecola* individuals, harbouring two subclades. Footit et al. (2009), surveying *Aphis* sp. from apple trees in North America, found *A. spiraecola* to be relatively diverse, representing four COI haplotypes compared to *A. pomi*, which was found to be uniform. Rakauskas et al. (2015) studied the distribution of the two species in Europe, but they did not use the universal barcode (amplified with primers LCO 1490 and HCO 2198). They used another part of the COI region [amplified with primers *Aphis*-L-465 and *Aphis*-H-1068 designed by Turcinaviciene et al. (2006)], hence, a direct comparison of the results is not possible. However, the 222 overlapping bases are 100% identical. They also observed several haplotypes within *A. spiraecola*, but the data did not indicate any clear geographical background in the distribution of the analysed COI haplotypes.

Our results support the expectation that *A. spiraecola* is widely distributed in all apple growing regions in Europe including the Northern parts of the continent (Rakauskas et al. 2015). Although it has become

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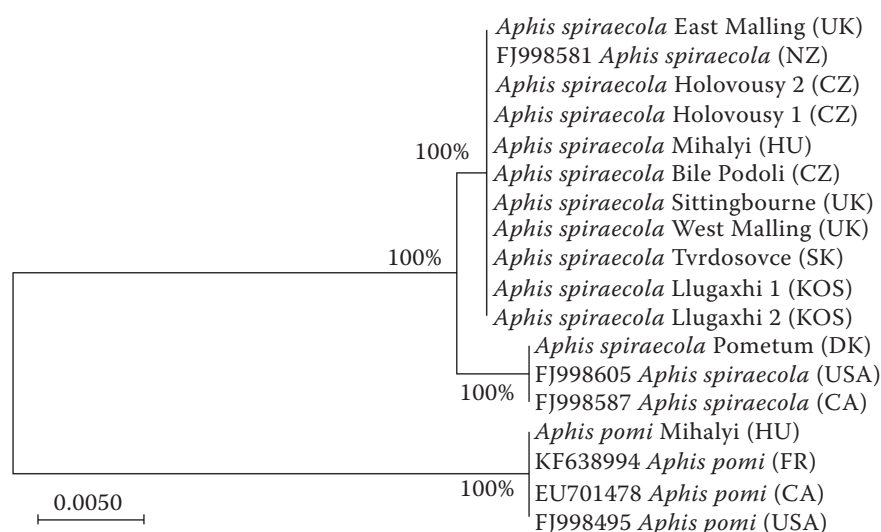


Figure 1. Neighbour-joining tree showing the phylogenetic relationships among the *A. pomi* and *A. spiraecola* individuals, based on the mitochondrial COI barcode (630 positions in the final dataset)

The analysis involved 15 nucleotide sequences (12 sequences resulting from this study and three retrieved from the NCBI GenBank database); the evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site; CA – Canada; CZ – the Czech Republic; DK – Denmark; FR – France; HU – Hungary; KOS – Kosovo; NZ – New Zealand; SK – Slovakia; UK – the United Kingdom; USA – the United States of America

a common pest of apples in Europe, the density and importance of *A. spiraecola* still lag behind those observed in North America (Brown et al. 1995; Mayer & Lunden 1996). The economic importance of this species may increase in the future due to longer and warmer growing seasons and milder winters in relation to climate change (Komazaki 1982; Qureshi 2010), switching to apples as the primary host plant, as was observed in East Asia (Komazaki et al. 1982, 1990) and in North America (Pfeiffer et al. 1989), or due to developing resistance to the insecticides commonly used (Soon Won et al. 1994; Lowery et al. 2006). Thus, the status of *A. spiraecola* as a pest of apples in Europe requires further investigations.

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