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ORIGINAL ARTICLE

The spread of alien aphid species *Cinara curvipes* and *Cinara cedri* in Europe – the impact of climate and plant trade

Roma Durak[1]*, Karolina Piecuch[1], Beata Borowiak-Sobkowiak[2], Karina Wieczorek[3], Sławomir Bartoszewski[1], Apostolos Kapranas[4], Maciej K Konopiński[5]

[1] Faculty of Biology, Nature Protection, and Sustainable Development, University of Rzeszów, Rzeszów, Poland

[2] Departament of Entomology, Poznań University of Life Sciences, Poznań, Poland

[3] Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice, Katowice, Poland

[4] Laboratory of Applied Zoology and Parasitology, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece

[5] Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland

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*Corresponding address:
rdurak@ur.edu.pl

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Abstract

Aphids are a strict group of insects that are particularly stimulated by current climate changes. Recent modifications in their life cycles, development, migration dates and geographical ranges are attributed to changes of local climates. *Cinara curvipes*, a species trophically related mainly to *Abies* spp., has been observed in Poland since 2015, while *Cinara cedri*, feeding on *Cedrus* sp., has been observed since 2022. Their presence is always associated with mass occurrence on host plants. The aim of this study was molecular identification of the species *C. cedri* and *C. curvipes*, collected in Poland. Potential pathways of introduction and spread of these species across Europe and the world was discussed. Based on the analysis of two genetic markers (COI and EF1-α), haplotype networks illustrating the relationships between populations from different parts of Europe and the world were presented. A contrasting pattern of low intraspecific variation in *C. curvipes*, but high in *C. cedri* was demonstrated, which may be associated with the modes of reproduction, mechanisms of dispersal of these two species, as well as introgressive hybridization between *C. cedri* and aphids belonging to *Cinara (Cupressobium*). Genetic relationships between mitochondrial haplotypes have shown that these species have reached Poland from western and southern Europe. These species have spread from their natural range mainly through imported plant material. Human activity and climate warming have enabled them to successfully settle.

Keywords: aphid, haplotype networks, climate change, spread of species

Introduction

Aphids (Hemiptera, Aphidoidea) are insects known for their polymorphism, meaning that even within a single species there are significant morphological differences between morphs (including winged and wingless morphs, females and males) and successive generations. They are pests of most cultivated plants, including vegetables, ornamental plants, orchards and forests. Over 5,000 species are found worldwide (Foottit *et al.* 2008; Jayasinghe *et al.* 2022; Favret and Aphid Taxon Community 2026).

The genus *Cinara* includes some of the largest aphid species in terms of body size. They feed on the needles and branches of coniferous plants from the pine and cypress families (Eastop 1972). More than 370 species from the *Cinara* genus have been described in the literature, with over 150 originating from North America (Eastop 1972; Favret and Voegtlin 2004; Beğen *et al.* 2019; Favret and Aphid Taxon Community 2026). In Central Europe, including Poland, 26 species of this genus have been identified (Durak 2011). Most of them

feed on specific host plants, with the most common being *Pinus*, *Abies*, and *Picea* (Meseguer *et al.* 2015). Morphological differences within the *Cinara* genus are quite subtle. A characteristic feature is their yellow--brown or dark brown coloration and relatively large body size (up to 8 mm in length). However, despite the existing morphological differences, most species in the *Cinara* genus share common biological characteristics and may coexist on the same hosts (Watson *et al.* 1999; Meseguer *et al.* 2015) which makes identifying them and diagnosing host plant damage difficult.

The number of species inhabiting temperate climates is steadily increasing, as aphid biology is strongly affected by ongoing climate change. Changes have been observed in their life cycles, development, timing of migrations and their geographical distribution (Harrington *et al.* 2007). Such expansion has been observed, for instance, in the species *Cinara tujafilina* (Del Guercio) (Durak *et al.* 2008; Durak and Borowiak--Sobkowiak 2013). This anholocyclic species has not only broadened its distribution but has also evolved morphological, behavioral, and physiological adaptations to successfully overwinter in new, colder environments (Durak 2014; Durak *et al.* 2021). Many species of aphids are invasive and are continually expanding their distribution range (Singh and Singh 2016).

Cinara curvipes (Patch 1912) is a North American species colonizing fir (*Abies* sp.). It has been introduced into Europe and is currently considered an alien invasive species. According to data from the Global Biodiversity Information Facility (GBIF), this species is primarily distributed in the Northern Hemisphere (Fig. 1). Most of the populations of these aphids have been observed in their native regions in North America (GBIF) (*Cinara curvipes* 2023), while in Europe, *C. curvipes* has been recorded in many countries, including the United Kingdom, Germany, Serbia, Switzerland, the Czech Republic, Slovakia, Slovenia, Bulgaria, Hungary, Austria, Turkey, Poland and Norway (Wieczorek *et al.* 2025). *C. curvipes* is a species that usually massively infests firs, but can also occur on other plants like cedar, Douglas fir or western hemlock (Meseguer *et al.* 2015). Particular features of this species, such as the exceptionally high fecundity of viviparous females, the production of large numbers of winged morphs over many generations and the ability to achieve high reproduction also at lower ambient temperatures, enable it to spread rapidly to new areas and adapt to new environments (Wieczorek *et al.* 2025). Its presence is observed as early as in spring, which negatively impacts the host plants. Increased uptake of phloem sap by aphids can cause physiological disturbances in photosynthesis and respiration processes, resulting in reduced sugar production. These sugars are essential for the proper development of new vegetative and generative organs. The high number of aphids colonizing shoots, especially in early spring before the vegetation season begins, causes organ deformation and growth disorders in the following years. Weakened plants become more susceptible to fungal and bacterial infections, which may lead to further damage and even plant death (Alves *et al.* 2018; Zhan *et al.* 2020; Wang *et al.* 2023). Additionally, this species secretes large amounts of honeydew, which provides a substrate for sooty mold fungi. These fungi clog the plant's stomates, limiting photosynthesis, transpiration, and gas exchange (Zvereva *et al.* 2010). *Cinara cedri* (Mimeur, 1936; 2023) is a species that feeds on trees from the *Cedrus* genus. According to data from GBIF, *C. cedri* is widespread in Europe, especially in the Mediterranean basin including Cyprus, which is presumed to be their native range (Fig. 2). In Cyprus, the populations of *C. cedri* inhabiting *Cedrus libani subsp. brevifolia* are considered to be an endemic subspecies, *Cinara cedri subsp. brevifoliae*. Individual sightings have also been recorded in North America, Argentina and Asia (GBIF) (Michelena *et al.* 2005; Binazzi *et al.* 2017; Nozaki *et al.* 2022; *Cinara cedri* 2023). *Cinara cedri* mostly feed on the previous year's shoots, causing the needles to dry out and turn red. Young trees are particularly susceptible since dried needles fall off, leading to defoliation of shoot tips and canopies. The densest colonies have been observed mainly in city centers, parks, and gardens (Oğuzoğlu and Avci 2019a, b).

Fig. 1. Parthenogenetic females of *Cinara curvipes* (A), *Cinara cedri* (B)

Both *C. curvipes* and *C. cedri* are non-native and potentially invasive species in Central Europe, whose ranges have expanded significantly in recent years. *C. curvipes* has been observed in Poland since 2015 (Hałaj and Osiadacz 2015), while *C. cedri* has been observed since 2022 (Kanturski 2022). Because their presence is always associated with mass occurrence on host plants, their introduction could lead to disturbances in biodiversity. Their early spring appearance can result in several negative consequences, both for the plants themselves and for the entire ecosystem. Moreover, their expansion is favored by a warming climate, as it has been shown that climate plays as

Fig. 2A. Distribution map of *Cinara curvipes* based on data from GBIF (GBIF.org, 2025)
Source: https://www.gbif.org/species/2073287 (*Cinara curvipes* 2023)
Fig. 2B. Distribution map of *Cinara cedri* based on data from GBIF (GBIF.org, 2025)
Source: https://www.gbif.org/species/2073431 (*Cinara cedri* 2023)

much a role in the spread of *Cinara* as the relationship with host plants (Jousselin *et al.* 2013; Wieczorek *et al.* 2025). Investigating the genetic structure of populations may contribute to a better understanding of the dispersal processes of these species.

The aims of this study were (i) molecular identification of the species *C. curvipes* and *C. cedri*, collected in Poland and (ii) investigation of the pathways of introduction and the spread of these species across Europe and the world. Analyses were performed using fragments of sequences of two genes: mitochondrial cytochrome oxidase subunit I (COI) and nuclear elongation factor 1-alpha (EF1-α).

Materials and Methods

Samples

Two aphid species, *Cinara curvipes* and *C. cedri* were collected in Poznań, Poland. Species identification was conducted based on morphological characteristics using the Blackman, Eastop (Favret and Aphid Taxon Community 2026) key. *C. cedri* was collected from its host plant, *Cedrus deodara* 'Pendula', growing in a private garden. *C. curvipes*, on the other hand, was collected from *Abies concolor* in the Botanical Garden in Poznań. The aphids were preserved in 99.8% ethanol and stored at −20°C at the University of Rzeszów until analysis.

DNA Isolation and Amplification of COI and EF1-α genes

DNA was isolated from three individuals of each species using the NucleoSpin® Tissue kit, following the manufacturer's protocol. Each individual was sequenced separately for two genes. The obtained DNA extracts were stored at 4°C until amplification. Fragments of sequences of the mitochondrial cytochrome c oxidase subunit I gene (COI) and the nuclear elongation factor 1-alpha gene (EF1-α) were used for molecular characterization of the collected individuals. For the COI gene, two primer sets were used: LCO 5′-GGTCAACAAATCATAAAGATATTGG-3′) (Folmer *et al.* 1994) and a newly designed HCOminus (5′-AAATATGCTCGTGTATCAACATCTAT-3′). To increase specificity and avoid the sequencing of artifacts, a newly

designed internal primer, LCOplus primer (5′-CAATTATATAATGTAATTGTTACAATTCATGC-3′) was used along with HCO (5′-AAATATGCTCGTGTATCAACATCTAT-3′) (Folmer *et al.* 1994). Similarly, for amplification of the EF1-α gene, two primer sets were also used: EF3 (5′-GAACGTGAACGTGGTATCAC-3′) and EF6 (5′-TGACCAGGGTGGTTCAATAC-3′) (von Dohlen *et al.* 2002), along with external primers EF3b (5′-GTGAAATCGGCAGCACCCT-3′) and EF6b (5′-CACAGAGATTTCATCAAGAACATGAT-3′). PCR reactions were performed in a volume of 20 µl, containing 1 µl of template DNA, 1 µl of each primer (10 µM), 0.8 µl of dNTPs (5 mM), 2 µl of OptiTaq buffer (EURX), 0.2 µl of Taq polymerase (OptiTaq), and distilled water. The thermal profile included an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 15 seconds, primer annealing at 47–53°C for 30 seconds, and elongation at 68°C for 1 minute. Final elongation was performed at 68°C for 10 minutes. PCR products were separated on a 1.2% agarose gel in 0.5× TBE buffer. Electrophoresis was conducted at 100 V. The purified PCR products [High Pure PCR Product Purification Kit (Roche)] were sent for sequencing to Genomed (Warsaw).

Sequence analysis

The sequences were assembled into contigs and checked for potential errors using DNASTAR software (Lasergene). Neighbor-Joining trees were built, using the Kimura 2-Parameter model with 1000 bootstrap replicates separately for COI and EF1-α in MEGA 11 software (Tamura *et al.* 2021). In addition to the sequences obtained in this study (Table 1), COI and EF1-α sequences of *C. curvipes, C. cedri* and other species of the genus *Cinara* sp., from different regions of the world, were retrieved from GenBank and included in the analysis (Table 1, Table S1). Sequences from *Lachnus quercihabitans* and *Lachnus roboris* were used as outgroups in COI and EF1-α trees, respectively. Haplotype networks for *C. curvipes* and *C. cedri* were constructed (Table 1) using the Median-Joining algorithm in the PopART software (Population Analysis with Reticulate Trees) (Leigh and Bryant 2015). The number of haplotypes (H), haplotype diversity (h), nucleotide diversity (π), and mean number of nucleotide differences among the haplotypes (k) in the overall sample and both populations were calculated using DnaSP 6.12 (Rozas *et al.* 2017).

Table 1. Sequences of *Cinara cedri* and *Cinara curvipes* used for haplotype network analysis

Species	Gene	GenBank acc. no	Region
Cinara cedri	COI	KU321598.1 (Binazzi *et al.* 2017)	Cyprus
Cinara cedri	COI	KU321599.1[*]	Tuscany, Italy
Cinara cedri	COI	KJ433268.1[*]	Beijing, China
Cinara cedri	COI	KM501341.1[*]	Beijing, China
Cinara cedri	COI	KM501340.1[*]	Beijing, China
Cinara cedri	COI	KU754491.1[*]	Pula, Croatia
Cinara cedri	COI	KU754492.1[*]	Pula, Croatia
Cinara cedri	COI	KF649349.1(Jousselin *et al.* 2013)	Montferrier Lez, France
Cinara cedri	COI	KF649387.1(Jousselin *et al.* 2013)	Montferrier Lez, France
Cinara cedri	COI	KF649397.1(Jousselin *et al.* 2013)	Montferrier Lez, France
Cinara cedri	COI	KF639314.1[*]	Montferrier Lez, France
Cinara cedri	COI	KF649509.1(Jousselin *et al.* 2013)	Montferrier Lez, France
Cinara cedri	COI	KF649351.1(Jousselin *et al.* 2013)	Montferrier Lez, France
Cinara cedri	COI	KF649350.1(Jousselin *et al.* 2013)	Montferrier Lez, France
Cinara cedri	COI	KF639315.1[*]	Montferrier Lez, France
Cinara cedri	COI	KF639316.1[*]	Montferrier Lez, France
Cinara cedri	COI	LC700318.1[*]	Aichi, Japan
Cinara cedri	COI	LC700313.1[*]	Aichi, Japan
Cinara cedri	COI	LC700315.1[*]	Aichi, Japan
Cinara cedri	COI	LC700314.1[*]	Aichi, Japan
Cinara cedri	COI	LC700319.1[*]	Hyogo, Japan
Cinara cedri	COI	LC700312.1[*]	Aichi, Japan
Cinara cedri	COI	LC700316.1[*]	Aichi, Japan
Cinara cedri	COI	LC700317.1 [*]	Aichi, Japan

Table 1. Sequences of *Cinara cedri* and *Cinara curvipes* used for haplotype network analysis – continued

Species	Gene	GenBank acc. no	Region
Cinara cedri	COI	KR570197.1(Hebert *et al.*2016)	British Columbia, Canada
Cinara cedri	COI	KR573470.1(Hebert *et al.*2016)	British Columbia, Canada
Cinara cedri	COI	KR567761.1(Hebert *et al.*2016)	British Columbia, Canada
Cinara cedri	COI	LT600418.1(Manzano-Marín *et al.* 2017)	Brittany, France
Cinara cedri	COI	LT600419.1(Manzano-Marín *et al.* 2017)	Aragon, Spain
Cinara cedri	COI	PV848741	Poznań, Poland
Cinara cedri	EF1-α	KF693838.1(Jousselin *et al.* 2013)	Montferrier Lez, France
Cinara cedri	EF1-α	KF693839.1(Jousselin *et al.* 2013)	Montferrier Lez, France
Cinara cedri	EF1-α	KF693840.1(Jousselin *et al.* 2013)	Montferrier Lez, France
Cinara cedri	EF1-α	KF693980.1(Jousselin *et al.* 2013)	Montferrier Lez, France
Cinara cedri	EF1-α	KY064501.1 (Meseguer *et al.* 2017)	Montferrier Lez, France
Cinara cedri	EF1-α	FM174683.1 (Ortiz-Rivas and Martínez-Torres 2010)	Valencia, Spain
Cinara cedri	EF1-α	KM501163.1[*]	Beijing, China
Cinara cedri	EF1-α	KM501162.1[*]	Beijing, China
Cinara cedri	EF1-α	PV843800	Poznań, Poland
Cinara curvipes	COI	KR044084.1(Gwiazdowski *et al.* 2015)	Idaho, USA
Cinara curvipes	COI	KR033906.1(Gwiazdowski *et al.* 2015)	California, USA
Cinara curvipes	COI	KR032260.1(Gwiazdowski *et al.* 2015)	Washington, Canada
Cinara curvipes	COI	KR042186.1(Gwiazdowski *et al.* 2015)	New Brunswick, Canada
Cinara curvipes	COI	KR042076.1(Gwiazdowski *et al.* 2015)	New Brunswick, Canada
Cinara curvipes	COI	KR040187.1(Gwiazdowski *et al.* 2015)	New Brunswick, Canada
Cinara curvipes	COI	JF883736.1[*]	Canada
Cinara curvipes	COI	KR036122.1(Gwiazdowski *et al.* 2015)	Manitoba, Canada
Cinara curvipes	COI	KY064233.1(Meseguer *et al.* 2017)	Montferrier Lez, France
Cinara curvipes	COI	PV848739	Poznań, Poland
Cinara curvipes	EF1-α	KY064488.1(Meseguer *et al.* 2017)	Montferrier Lez, France
Cinara curvipes	EF1-α	AY472022.1(Favret and Voegtlin 2004)	Illinois, USA
Cinara curvipes	EF1-α	PV843799	Poznań, Poland

References in brackets; [*] asterisk for unpublished data, from GenBank; grey – present study

Results

Sequence analysis

The COI gene was successfully sequenced in all *C. curvipes* samples, resulting in high-quality electropherograms. Since all the sequences obtained from different individuals were identical, one consensus sequence for the species was presented. The entire fragment was 496 bp long in this species. Also, in *C. cedri* samples, a consensus sequence was obtained for all samples (437 bp). A single consensus sequence representing the EF1-α gene for *C. curvipes* was obtained, with a length of 808 bp. The EF1-α sequence obtained for *C. cedri* consisted of 518 bp. In the Polish populations, all conspecific individuals were monomorphic for the COI gene. However, when these were analyzed together with GenBank sequences, high

Table 2. Number of haplotypes and diversity indices of *Cinara curvipes* and *Cinara cedri* in a fragment of cytochrome oxidase subunit I mitochondrial gene

Parameter	*Cinara curvipes*	*Cinara cedri*
Number of sequences used	10	29
Total number of sites	387	387
Polymorphic sites (S)	2	7
Number of Haplotypes (h)	2	4
Haplotype diversity (H_d)	0.200 (SD 0.154)	0.581 (SD 0.089)
Nucleotide diversity (π)	0.00103 (SD 0.00080)	0.00650 (SD 0.00129)
Average number of nucleotide differences (k)	0.400	2.517
Total variance of k (V(k))	0.162	1.948

genetic diversity was observed in *C. cedri* haplotypes ($\pi = 0.00650$, $k = 2.517$; Table 2). In contrast, the combined *C. curvipes* dataset was notably less divergent ($\pi = 0.00103$, and $k = 0.400$).

Species identifications and relationships between species

In both *Cinara* species, COI sequences from Poland were identical with several other haplotypes from other European populations, as well as with populations from other parts of the world (Fig. 3, Table S1).

For the COI gene both *C. curvipes* and *C. cedri* formed two distinct, strongly supported, monophyletic clades, within a clade representing other species from subgenus *Cinara* (*Cinara*) (Fig. 3A), while sequences of subgenus *Cinara* (*Cupressobium*) formed a separate, monophyletic clade. Sequences from Poland grouped together with the conspecific reference sequences obtained from GenBank. Trees built using

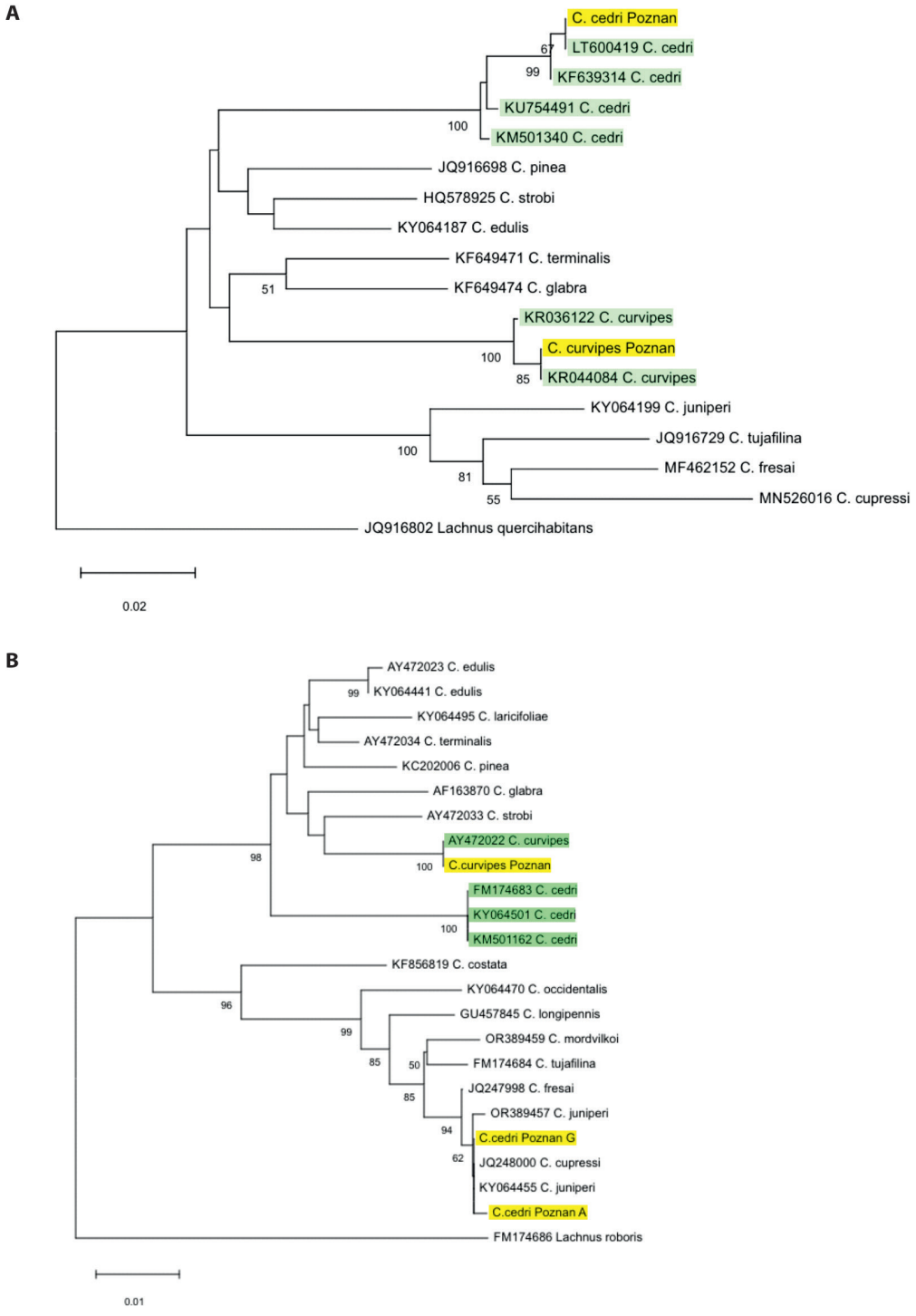

Fig. 3. Neighbor-Joining trees of *Cinara curvipes* and *Cinara cedri* for (A) mitochondrial COI gene sequences and (B) nuclear EF1-α gene sequences; bootstrap support values over 50% shown on branches

nuclear gene EF1-α grouped together the sequence of *C. curvipes* from Poland and a reference GenBank sequence of this species, while Polish sequences of *C. cedri* were grouped with sequences of *C. cupresi* and *C. juniperi* (Fig. 3B) belonging to subgenus *Cinara* (*Cupressobium*). This clade, however, also contained some sequences belonging to subgenus *Cinara* (*Cinara*) such as *C. longipennis*, *C. occidentalis* and *C. costata*.

Haplotype networks for *Cinara curvipes* and *Cinara cedri*

The haplotype networks built using two genetic markers (COI and EF1-α) illustrate the geographic patterns in the population structure of *C. curvipes* and *C. cedri*. For this analysis, all of the *C. curvipes* and *C. cedri* sequences available in the GenBank (Table 1) were used

The haplotype network of *C. curvipes* COI gene sequences (Fig. 4A) showed a low level of intraspecific variation, and two haplotypes can be distinguished: G1 and G2. The two introduced populations (Poland and France) possess a haplotype which is the most abundant in the Canadian and U.S populations (G1). The other haplotype (G2) was found only in a Canadian population from Manitoba (Fig. 4A). No variation was found in the EF1-α gene – the same haplotype was found in invasive populations from France and Poland as well as in a population from the USA. (G3) (Fig. 4B).

The haplotype network of COI sequences of *C. cedri* showed the existence of two main lineages differing by five mutations (Fig. 5A). Four haplotypes were found (H1- H4). The most abundant haplotype (H1) is present in western native populations (France and Spain) and three introduced ranges (Poland, Canada, and Japan), while haplotype H2 is present exclusively in France. The second lineage contains haplotypes from eastern native populations (Croatia, Italy, and Cyprus) but also in an introduced population in Asia (China, H3), where the derived haplotype, H4 is also present. EF1-α gene reference sequences are identical and occur both in native (France, Spain) and Asian introduced populations, while the Polish population holds solely a highly divergent haplotype H6 (Fig. 5B). The presence of the main haplotype in the populations from various countries, such as France, Spain and China, suggests that these populations share a common origin.

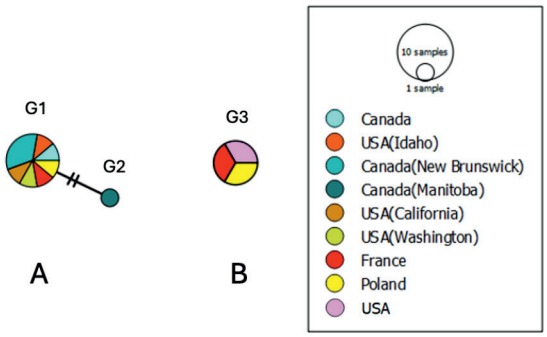

Fig. 4. Haplotype networks illustrating the genetic structure of populations of *Cinara curvipes* from different countries, based on the analysis of two genetic markers, COI (A) and EF1-α (B). The colors corresponding to specific countries/country regions are provided in the legend. Each circle represents a haplotype, and its size indicates the number of samples assigned to that haplotype. The lines between haplotypes represent mutations differentiating the individual haplotypes, with the number of dashes on the line imitating the number of mutations between them

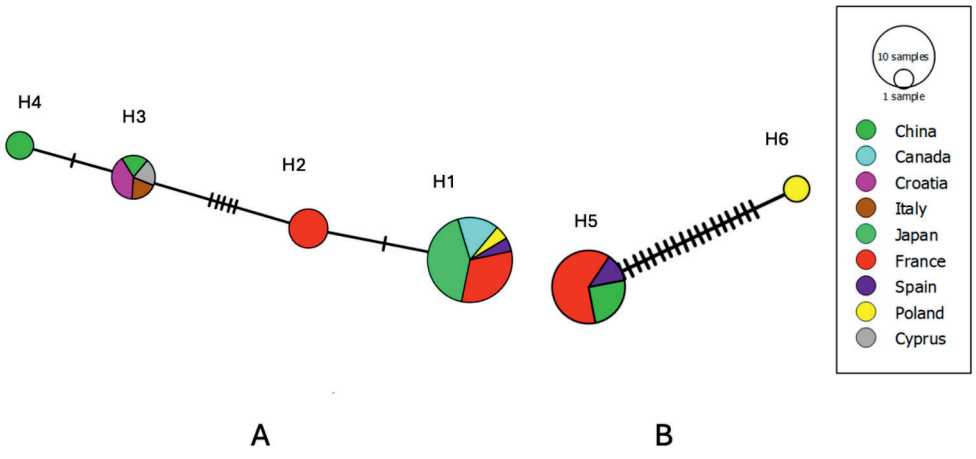

Fig. 5. Haplotype networks illustrating the genetic structure of populations of *Cinara cedri* from different countries, based on the analysis of two genetic markers, COI (A) and EF1-α (B). The colors corresponding to specific countries/country regions are provided in the legend. Each circle represents a haplotype, and its size indicates the number of samples assigned to that haplotype. The lines between haplotypes represent mutations differentiating the individual haplotypes, with the number of dashes on the line imitating the number of mutations between them

Discussion

The results from the two markers used in this study differed in power to detect the population structure of the two invasive species. The COI gene is widely used in phylogenetic studies due to both its variability, which provides a sufficient resolution at species and, in many cases, population levels, and conservativeness in certain regions, which allows designing universal primers. In insects, COI is a marker of choice in phylogenetic analyses and identification of species (Normark 2000; Hebert *et al*. 2003; Favret and Voegtlin 2004; Foottit *et al.* 2008; Chen *et al.* 2012). A significant limitation of relying solely on the COI gene is its predominantly maternal inheritance, due to its location in mitochondrial genome. It may fail to capture a complete history of taxon, especially in cases of hybridization or incomplete lineage sorting (Ballard and Whitlock 2004). The EF1-α gene, on the other hand, is located in the nuclear genome, hence, it can provide complementary information about population genetic differentiation and may better reflect the differences (Kandul *et al.* 2004; Durak *et al.* 2014).

The populations of *C. cedri* and *C. curvipes* from Poland showed genetic similarity to populations from other parts of Europe, Asia, and North America in terms of variation in the COI gene. The presented results unambiguously confirmed the presence of *C. curvipes* in Poland, placing the sequences obtained from specimens collected in Poznan in the same clades as the reference sequences of this species both in nuclear EF1-α and COI genes. On the other hand, in the individuals classified as *C. cedri* using their morphological features, while the COI gene clustered with other *C. cedri* sequences, the EF1-α sequence matched those of *C. juniperi* and *C. cupressi*. Although such incongruence between mitochondrial and nuclear sequences might suggest a hybridization event within the invasive populations, verification of this hypothesis would require analyzing additional nuclear markers and more extensive geographic sampling. The discordance between mitochondrial and nuclear genes has also been demonstrated in other aphids (Jousselin *et al.* 2024). It has been shown that phylogenetic analyses based on nuclear markers did not support the results obtained from mitochondrial analyses of many aphid subfamilies, and sometimes even failed to converge with mitochondrial phylogeny (Jousselin *et al.* 2024).

Analyses of haplotype networks revealed species specific patterns of population structure. Low genetic variability in *C. curvipes* does not allow detailed inferences about population structure of this species. Only two of the haplotypes in COI were found to have the cytochrome oxidase gene while the elongation factor gene was identical in all the individuals studied. The major haplotype, G1, is primarily associated with the Canadian and USA populations, further supporting the hypothesis of a North American source for the introduced population in Europe (Jurc *et al.* 2009). The species probably spread mainly through the migration of predominantly parthenogenetic females. Their expansion can occur through aerial plankton or with plant materials. Although the species is considered holocyclic (cyclic parthenogenesis), i.e., there is a sexual generation in its life cycle, it may also reproduce continuously parthenogenetically in areas with mild winters (Scheurer and Binazzi 2004). Previous observations of this species in Poland have not confirmed the presence of sexual generations and overwintering eggs, which could confirm the possibility that this species may also overwinter in an active form. This way of reproduction and overwintering provides *C. curvipes* with the possibility of rapid expansion. Limited genetic variation in the introduced populations might suggest parthenogenesis as the predominant mode of reproduction. Genetic bottlenecks at the front of expansion (e.g., Konopiński *et al.* 2013), along with the clonal reproduction would probably reduce variation, but limited variation in the two analyzed genes in putative source populations in North America, does not allow definite conclusions to be drawn. Populations of invasive species often show low genetic diversity between populations (Harrison and Mondor 2011). The best described species is the oleander aphid (*Aphis nerii*), which has one main haplotype that has successfully spread and dominated populations across different host plants and geographic areas (Harrison and Mondor 2011). Low genetic diversity between populations has also been observed in several major pests, including *Sitobion avenae*, *Rhopalosiphum maidis* and *Rhopalosiphum padi* (Figueroa *et al.* 2005; Guo *et al.* 2023). *R. maidis* exhibited low genetic differentiation among populations, whereas the genetic diversity of *R. padi* was overall higher than that of *R. maidis* in China as well as in Europe. The authors demonstrated that, in addition to geographical distance which can play a crucial role, the low genetic diversity between populations is also influenced by the aphid reproduction model. Obligatory parthenogenesis, which occurs mainly in *R. maidis*, reduces the chance of gene recombination, and therefore, anholocyclic populations were characterized by low genetic diversity (Guo *et al.* 2023).

C. cedri, naturally associated with the Mediterranean basin, is characterized by higher intraspecific variability. The four mitochondrial haplotypes found in this species revealed a geographical pattern. Although the two major haplogroups are separated by only two mutations, their distribution in Europe may be attributed to their independent evolutionary histories. In

the native range of the species H1/H2 haplogroup occurs in south-western Europe, while H3 was found in Italy, Croatia, and Cyprus. These two areas are known to hold independent glacial refugia for many species (e.g., Taberlet *et al.* 1998). However, to confirm this phylogeographic pattern more dense sampling and a broader range of markers would be necessary. Unfortunately, the information on EF1-α from the Balkan or Italian peninsulas leaves this part of analysis inconclusive. The sequences obtained from the Polish population of *C. cedri* suggest that the Central European population might have originated in south-western Europe. However, because the sampling of European populations is too scarce, it is not possible to disentangle the detailed expansion routes of *C. cedri*. It is uncertain if the population in western Poland emerged by gradual expansion of their range in France or if it was directly introduced by human activities. Parthenogenetic clones likely spread due to natural migration or transport via plant material (Foottit *et al.* 2008; Jurc *et al.* 2009; Nozaki *et al.* 2022). It is also possible that the populations observed in Poland resulted from expansion due to climate warming. Climate change might have allowed this thermophilic species to adapt to new areas. At the same time, the presence of suitable host plants is also important. The spread of populations is facilitated by the mobility of aphids, particularly the winged forms, which can migrate to nearby or distant locations, colonizing new areas (Dong *et al.* 1987; Xu *et al.* 2011). Previous analyses have shown close relationships between Japanese populations and those from France, Spain, and Canada, as well as genetic similarity between Chinese and Croatian populations (Ji *et al.* 2021; Nozaki *et al.* 2022). The presence of *C. cedri* in Japan was first recorded in 2021 when they were found on *Cedrus deodara* trees, which are commonly used for ornamental purposes. The appearance of this species confirms the aphid's ability to overwinter in Japan, demonstrating its adaptability and capacity to spread in the region (Nozaki *et al.* 2022).

The genetic diversity of a species can be influenced by such factors as geographical location, the host plant species and the life cycle of aphids (Caillaud and Via 2000; Vorwerk and Forneck 2006; Guo *et al.* 2023). The patterns of geographical differentiation have also been previously reported in studies in other aphid species of *Cinara*, where it was shown that geographic isolation and differences in host plants can influence genetic diversity and play a key role (Foottit *et al.* 2008; Jousselin *et al.* 2013). Human activities, such as the transport of host plants like cedars, which are increasingly planted in parks and gardens, or exotic firs and other coniferous trees planted for ornamental purposes or for Christmas tree production, may also be significant (Jurc *et al.* 2009; Nozaki *et al.* 2022). Furthermore, recent changes in the distribution of populations of both species may be promoted by climate change. Species such as *C. cedri* and *C. curvipes*, in response to rising temperatures and changing precipitation patterns, may spread in new locations outside of their natural range, appearing in areas with a moderate climate. Studies show that global warming promotes the adaptation and expansion of invasive aphid species into new areas, where environmental conditions were previously unfavorable to them (Harrington *et al.* 2007; Wieczorek *et al.* 2025). The climate changes observed in Poland, in the form of milder winters, and warmer springs and autumns, create new ecological niches suitable for *C. cedri* and *C. curvipes* aphids. A comparison of *C. cedri* and *C. curvipes* through the haplotype networks revealed significant differences in genetic structure among populations. Collectively, the results suggest that these patterns could be shaped not only by historical factors and geographic barriers, but also by ecological variables such as host plant preferences and climate change (Wang *et al.* 2017; Guo *et al.* 2023).

Conclusions

For the first time, the pathways of introduction and spread of two alien and potentially invasive aphid species, *C. curvipes* and *C. cedri* were analyzed. The genetic structure of the two species revealed lower intraspecific variation in populations of *C. curvipes*, and higher in *C. cedri*. Potential hybrid origin of the newly established population of *C. cedri* in Poland requires further investigation. It is likely that both direct human actions and climate change promote the expansion of the two species in new areas such as Central Europe.

Supplementary Materials

The following supporting information can be downloaded at Table S1: Data for aphid specimens used in this study. For each species the table includes locality, references and GenBank accession numbers for COI genetic and EF1-α markers.

Statements & Declarations

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