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RESEARCH ARTICLE

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Genetic diversity among *Neoleucopis* (Diptera: Chamaemyiidae) associated with *Marchalina hellenica* (Hemiptera: Marchalinidae) in Greece and Italy (Ischia)

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Abstract

1. *Marchalina hellenica* (Gennadius) (Hemiptera: Marchalinidae), endemic to Greece and Türkiye, infests various pine species in both countries. Apart from its native range, this species has been recorded infesting pine trees in Italy [Campania (island of Ischia) and Marche regions], Croatia, and recently in Australia. While not a severe pest in its native habitat, *M. hellenica* has been found to negatively impact pines in the invaded regions.
2. *Neoleucopis kartliana* (Tanasijtshuk) (Diptera: Chamaemyiidae), a key natural enemy of *M. hellenica* in its native range, was introduced from Greece to Ischia as a biological control agent. In the current study, *Neoleucopis* populations from 22 sites across Greece and six sites from Ischia were examined using DNA barcoding and morphological traits to determine their identities and elucidate their distributions in Greece.
3. This integrated approach allowed the separation of three genetically diverged mitochondrial DNA (mtDNA) lineages within *Neoleucopis*, leading to the conclusion via morphology that there were possibly two additional mtDNA lineages besides that of *N. kartliana*. The three *Neoleucopis* mtDNA lineages were unevenly distributed in Greece, and all individuals screened from Ischia were identified as the *N. kartliana* mtDNA lineage.
4. These findings highlighted the need for further taxonomic work and provide a deeper insight into the diversity and distribution of *Neoleucopis* mtDNA lineages in their natural range and Ischia, which are essential for optimising biological control strategies against *M. hellenica* in other regions.

KEYWORDS
biocontrol, Giant pine scale, mtCOI, occupancy, silver flies

INTRODUCTION

The family Chamaemyiidae (Diptera) comprises flies commonly referred to as silver flies. Most chamaemyiids undergo one to three generations per year (Ross et al., 2011), occurring in a diversity of habitats, such as grasslands, lowlands, or montane ecosystems (Gaimari, 2020; Tanasijtshuk, 1986). Their larvae are predatory, primarily targeting adults, nymphs and eggs of sternorrhynchous hemipterans, notably aphids, mealybugs, scales, and psyllids (Gaimari, 2020; Gaimari & Turner, 1997; McLean, 1998; Tanasijtshuk, 1986). Pupariation occurs on the twigs and branches of plants that host their prey (Gaimari et al., 2007; Grubin et al., 2011) or after falling from trees to pupariate in the soil (McAlpine & Tanasijtshuk, 1972). Collectively, chamaemyiids engage in a range of feeding behaviours, from actively preying in colonies to entirely sedentary predation on eggs enclosed within a single prey ovisac (Gaimari, 2021). Some silver fly species within specific genera can enter the dense waxy substrates created by aphids and scale insects, allowing them to feed beneath these protective coverings that are typically inaccessible to other predators (Gaimari, 2021). Some adult silver flies are also known to gently probe their prey using their fore tarsi to stimulate the release of honeydew, which they then consume (Gaimari, 2021; Gaimari & Turner, 1997; Teodorescu & Maican, 2014).

The predatory larvae of Chamaemyiidae can significantly reduce prey populations of aphids and coccids, contributing to the population regulation of these plant-sucking insects (Gaimari, 2021). Several silver fly species have been used as classical biological control agents, particularly in North America, against pests such as *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae), *Adelges piceae* (Ratzeburg) (Hemiptera: Adelgidae), and *Adelges tsugae* Annand (Hemiptera: Adelgidae) (Clark & Brown, 1962; Clausen, 1978; Gaimari & Turner, 1996; McAlpine, 1978; Noma et al., 2005; Olfert et al., 2001; Ross et al., 2011; Schooley et al., 1984). After its invasion in the 1970s in Hawaii, USA, *Pineus pini* (Macquart) (Hemiptera: Adelgidae) was effectively managed through the release of *Neoleucopis tapiae* (Blanchard), erroneously referred to as *Neoleucopis* (=*Leucopis*) *obscura* (Haliday), along with a small proportion of *N. obscura* (Culliney et al., 1988; Gaimari, 2021; Gaimari & Havill, 2021; Nakao et al., 1981). In New Zealand, the introduction of *N. tapiae* in the 1930s contributed to the effective control of *Pineus boerneri* Annand (Hemiptera: Adelgidae) on *Pinus radiata* (Gaimari & Havill, 2021; Zondag & Nutall, 1989). *Neoleucopis obscura* was also employed against *P. boerneri* in Chile, managing the pest's populations (Gaimari et al., 2024; Gaimari & Havill, 2021; Mills, 1990; Zúñiga, 1985), although this chamaemyiid species might also refer to *N. tapiae* (Gaimari, 2021; Greathead, 1995). In Australia, the release of three chamaemyiid species in the 1930s against *P. pini* had minimal impact, although one of them, *Neoleucopis atratula* (Ratzeburg), became established there (Clausen, 1978; Gaimari, 2021; Tanasijtshuk, 1996). These examples highlight the potential of Chamaemyiidae as biological control agents, though the exact impact on the target species varies.

In Europe, several *Neoleucopis* species are present. *Neoleucopis atratula* is known to prey on *Adelges* spp. (Hemiptera: Adelgidae), particularly *A. piceae*, *Adelges merkeri* (Eichhorn), *Adelges nordmannianae* (Eckstein) and *A. tsugae* (Neidermeier et al., 2020; Ravn et al., 2013), and is distributed from Britain through Central Europe, reaching the Balkan countries and Türkiye (Ebejer & Barták, 2019). *Neoleucopis obscura*, which preys on *Adelges picea* (Ratzeburg) as well as other *Adelges* spp., and less frequently *Pineus* spp., is similarly distributed from North and Central Europe to the Balkan countries and Türkiye, while *N. tapiae*, predator of *Pineus* spp., is found from Britain south to Gibraltar and east to western Russia (Ebejer & Barták, 2019; Gaimari & Havill, 2021). *Neoleucopis freyi* (McAlpine) is known only from northern Europe as a predator of *Adelges viridis* (Ratzeburg). *Neoleucopis orbiseta* (McAlpine), also known from northern Europe, has unknown biology but likely feeds on aphids (Gaimari & Havill, 2021). *Neoleucopis kartliana* is the only described *Neoleucopis* species to prey on *M. hellenica* in the Mediterranean and has been reported in Georgia, Türkiye, Greece and Italy (Gaimari et al., 2007; Gaimari & Havill, 2021; Ülgentürk et al., 2013).

The scale insect *Marchalina hellenica* (Gennadius) (Hemiptera: Marchalinidae) is indigenous to Greece and the coastal areas of Türkiye (Gounari, 2006; Gounari et al., 2023; Ülgentürk et al., 2013). Within its natural range, it serves as the primary honeydew producer that is essential for pine honey production by *Apis mellifera* L. (Hymenoptera: Apidae), contributing up to 60% of Greece's and approximately 50% of Türkiye's annual honey production (Bacandritsos et al., 2004; Gounari, 2004). In Greece, *M. hellenica* primarily feeds on *Pinus brutia* and *Pinus halepensis* while it can also feed on *Pinus pinea*, *Pinus nigra*, *Pinus maritima* and *Pinus sylvestris* (Avtzis, 1985; Kailidis, 1965), and, experimentally, *Abies cephalonica* (Bacandritsos et al., 2004). Beyond its native range, *M. hellenica* has been reported feeding on *Pinus heldreichii* var. *leucodermis*, *P. pinea* and *Pinus pinaster* on the island of Ischia (Italy) (Fimiani et al., 1994; Fimiani & Sollino, 1994), *P. halepensis* and *P. pinea* in Croatia (Masten Milek et al., 2019), and recently on *P. radiata* in Australia (Avtzis et al., 2020). Although *M. hellenica* was initially presumed to infest *Picea* sp. in Russia, Armenia and Georgia, tentative examination revealed that the scale insect in these regions was *Marchalina caucasica* Hadzibeyli (Hemiptera: Marchalinidae) (Hodgson & Gounari, 2006; Jashenko, 1999), a species that is attacked by *Neoleucopis hadzibeiliae* (Tanasijtshuk) (Tanasijtshuk, 1986). While not widely regarded as a significant pest within its natural range, control measures against *M. hellenica* are occasionally employed in urban areas to mitigate its impact on trees (Fotelli et al., 2020). Although its high population densities can lead to adverse effects such as desiccation of branches and foliage, growth reduction and crown transparency, tree mortality is believed to primarily occur in conjunction with other environmental stressors (Gallis, 2007; Mendel et al., 2016). The moderate impact of *M. hellenica* on indigenous pine trees within its native range is attributed largely to the influence of its natural enemies, particularly *N. kartliana* (Avtzis et al., 2020). The introduction of *N. kartliana* to Ischia has resulted in the successful biological control of *M. hellenica* on that island (Garonna & Viggiani, 2011). In contrast, the absence of specialized predators in the invaded areas facilitates the unimpeded population growth of *M. hellenica*, ultimately leading to adverse impacts on susceptible host trees (Fotelli et al., 2020).

The detection of *M. hellenica* in urban areas of Australia in 2014, alongside its established presence in parts of Italy [Campania (Ischia island) and recently in the Marche region] (Servizio Fitosanitario Regionale, 2021; Severini, 2024), has underscored the pest's capacity to establish itself across diverse ecosystems within its invasive range, from Mediterranean coastal areas to temperate forests (Servizio

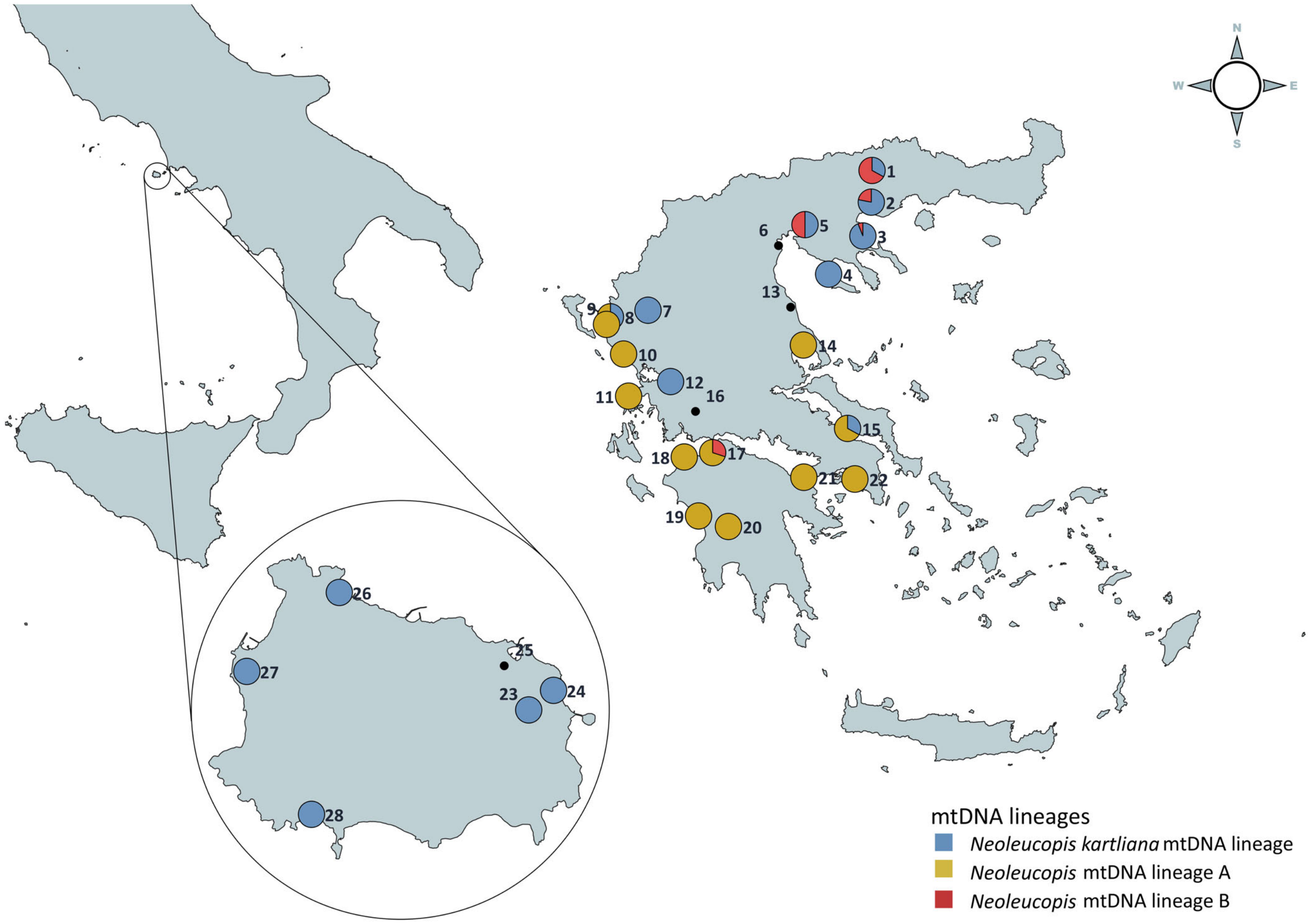

FIGURE 1 Distribution of the different *Neoleucopis* mtDNA clusters across 22 sites of Greece (1-Drama, 2-Amphipoli, 3-Stratoni, 4-Sani, 5-Thessaloniki, 6-Makriyalos, 7-Ioannina, 8-Filiates, 9-Igoumenitsa, 10-Parga, 11-Lefkada, 12-Amfilochia, 13-Larissa, 14-Volos, 15-Xirovrisi, 16-Paravola, 17-Patra, 18-Metohi, 19-Kaiafas, 20-Megalopoli, 21-Korinthos, 22-Athens) and six sites in Italy (Ischia) (23-Pineta Degli Atleti, 24-Giardino Pubblico Porto, 25-Porto d'Ischia Parking, 26-Lacco Ameno, 27-Forio, 28-Serrara Fontana). Black points indicate the sites where sequencing was unsuccessful.

Fitosanitario Regionale, 2021; Severini, 2024) and urban areas (Avtzis et al., 2020). This spread has driven further research into the biology and management of *M. hellenica* in this invaded region (Eleftheriadou et al., 2023; Jaroslow et al., 2023), the biology of *N. kartliana* in Greece (Eleftheriadou et al., 2022; Gaimari et al., 2007), and its potential as a biological control agent against *M. hellenica* (Avtzis et al., 2020; Eleftheriadou et al., 2024). Gaimari et al. (2007) and Eleftheriadou et al. (2022) offered significant insights into the biological traits of *N. kartliana* in Greece. Besides *N. kartliana*, recent investigations in northern Greece revealed an additional *Neoleucopis* lineage besides *N. kartliana*, possibly a new species referred to as "*Neoleucopis* n. sp. B" (in Eleftheriadou et al., 2024). The current study aims to (1) utilize molecular methods to differentiate the *Neoleucopis* attacking *M. hellenica* in Greece, (2) provide information about the geographic distribution of the *Neoleucopis* attacking *M. hellenica* in Greece and (3) verify which *Neoleucopis* mtDNA lineage was introduced as a classical biological control agent on the island of Ischia.

MATERIALS AND METHODS

Distribution of *Neoleucopis* in Greece

A total of 22 sites across Greece were sampled over 24 days from late May to mid-June 2022 to determine the identity and distribution of *Neoleucopis* attacking *M. hellenica* within the country. These sites included 21 locations in mainland Greece (Ioannina, Filiates, Igoumenitsa, Parga, Amphilochia, Paravola, Patra, Metohi, Kaiafas, Megalopoli, Korinthos, Attica (Paleo Faliro and Kesariani), Xirovrisi, Volos, Drama, Amphipoli, Sani, Stagira-Stratoni, Makriyalos, Larissa and Thessaloniki) and Lefkada island (Figure 1). The sampling process involved the collection of pine branches infested by *M. hellenica* using pruners. In Greece, 10 branches of *P. brutia* were collected from each site reached from ground level (approx. 3 m), and isolated in individual Nasco Whirl-Pak B01027WA sampling bags. Following collection, the samples were conveyed to the Forest Research Institute (H.A.O.

Demeter) in Thessaloniki (Greece). Counting and collection of *Neoleucopis* larvae and puparia were conducted using a Zeiss Stemi 508 stereomicroscope (Zeiss, Oberkochen, Germany). *Neoleucopis* individuals that reached the adult stage inside the sampling bags during transfer were also collected. Following inspection, the remaining branches infested with *M. hellenica* from each site were stored in ventilated cages (60 cm × 60 cm × 60 cm) within a climate chamber set to 23°C, 60% relative humidity and a 16-h light/8-h dark photoperiod to allow for the development of any individuals that may have remained undetected during examination.

Identification of the biological control agent in Ischia (Italy)

In Italy (Ischia), samples were collected from Lacco Ameno, Forio, Serrara Fontana, and three sites in Ischia Porto (Pineta degli Atleti, Giardino Pubblico and Porto d'Ischia Parking) during November 2023 and in May and June 2024 to determine the *Neoleucopis* mtDNA lineage introduced on the island. *Marchalina hellenica* ovisacs collected in May 2024 were placed in well-aerated containers (9 cm in diameter) to facilitate the potential emergence of *Neoleucopis* adults. Additionally, 10 *Neoleucopis* adult specimens collected with *M. hellenica* ovisacs in 2011 were provided by APG from voucher samples of the Department of Agricultural Sciences, University of Naples Federico II (Portici, Italy). On Ischia, sampling was conducted following the protocol used in Greece. However, due to the crown base height of the trees exceeding five meters, branches of *P. pinea* were collected by professional climbers. Samples were then transferred to the Department of Agricultural Sciences (University of Naples Federico II, Portici, Italy), where they were inspected as described above under a Leica Zoom 2000 stereomicroscope (Leica L-Z 2000, Wetzlar, Germany). *Neoleucopis* individuals from both countries were preserved in ethanol (98%) for molecular analysis.

Molecular screening and morphological identification

For samples derived from Greek sites, up to 10 individuals per site, comprising larvae, puparia and adults, underwent molecular analysis (total $n = 221$) (Eleftheriadou et al., 2025). Non-destructive DNA extraction from *Neoleucopis* specimens was conducted individually using the PureLink™ Genomic DNA Mini Kit from ThermoFisher Scientific, Life Sciences Solutions, Carlsbad, CA, USA, following the manufacturer's prescribed protocol. Samples were immersed intact in a digestion buffer plus Proteinase K and incubated overnight to extract DNA while preserving external morphology. Subsequent DNA amplification employed HCO/LCO primers (Folmer et al., 1994), targeting the standard DNA barcoding fragment within the 5' region of the mitochondrial cytochrome c oxidase subunit I (mtCOI) mitochondrial gene (658 bp), using MyTaq™ Red Mix from BioLine GmbH, Germany, in 25 μL volumes. PCR consisted of an initial denaturation step lasting 5 min at 96°C. This was followed by four cycles with 60 s at 96°C for denaturation, 47°C for annealing and 72°C for extension. The subsequent 35 cycles consisted of 60 s at 96°C for denaturation, 50°C for annealing and 72°C for extension, whereas the final extension step at 72°C lasted 5 min (Martinez-Sañudo et al., 2018). Purification of PCR products was carried out using the PureLink™ PCR Purification Kit from ThermoFisher Scientific, Life Sciences Solutions, Carlsbad, CA, USA, following the manufacturer's instructions. Sequencing was done at CEMIA SA in Larissa, Greece, utilizing an ABI 3730XL sequencer and using the aforementioned primers. The chromatograms obtained were initially visualized and manually analysed using Chromas Lite software version 2.6.6 (Technelysium Pty Ltd) and sequences were aligned with Clustal X (version 2.0.11) (Larkin et al., 2007). To avoid PCR errors, sequences containing dubious singletons were re-examined using a second PCR. After the identification of haplotypes, these were included in the construction of a Maximum Likelihood phylogenetic tree that was rooted with *Neoleucopis pinicola* (Malloch) based on its closest genetic affinity to the ingroup (NCBI Accession Number: MZ298249), using MEGA version 6 (Tamura et al., 2013) (Tamura-Nei distances, Nearest-neighbour-interchange (NNI) ML heuristic method and 1000 bootstrap replicates). For specimens collected from Ischia, 47 samples underwent molecular analysis, including larvae, puparia and adults obtained from the 2023 and 2024 samplings, alongside adults sourced from the voucher samples of 2011, following the protocol used in Greece. To assess the morphological differentiation of the mitochondrial DNA (mtDNA) clusters obtained, henceforth called mtDNA lineages, a series of specimens were sent to author SDG (15 M and 10 F of the *N. kartliana* mtDNA lineage, 36 M and 35 F of *Neoleucopis* mtDNA lineage A, and 37 M and 34 F of *Neoleucopis* mtDNA lineage B), who confirmed the identity of *N. kartliana* specimens and recognized the other two *Neoleucopis* mtDNA lineages, each as a new species (Eleftheriadou et al., 2024), which will be described in a taxonomic study by authors SDG, NE and DA. The morphological identification of the three *Neoleucopis* mtDNA lineages was based on the stereomicroscopic examination of the genitalia of adult males and females. Specifically, the male genitalia were examined for their differences in the epandrium, surstyli, pregonite, postgonite and phallus, whereas the female terminalia were assessed based on the presence or absence of thick setulae on sternites 4 and 5. For all structural characters, variation in shape, relative size, setosity and presence/absence was used to distinguish among the individuals of the *Neoleucopis* mtDNA lineages observed (Gaimari et al., 2007; Gaimari & Havill, 2021).

RESULTS

Molecular identity and distribution of *Neoleucopis* in Greece

DNA barcoding of *Neoleucopis* was conducted on 64 larvae, 48 puparia and 56 adults of mixed sex ($n = 168$ specimens in total). Sequencing attempts for *Neoleucopis* individuals from Thessaloniki, Paravola, Makriyalos and Larissa (Figure 1) were unsuccessful due to fragmented

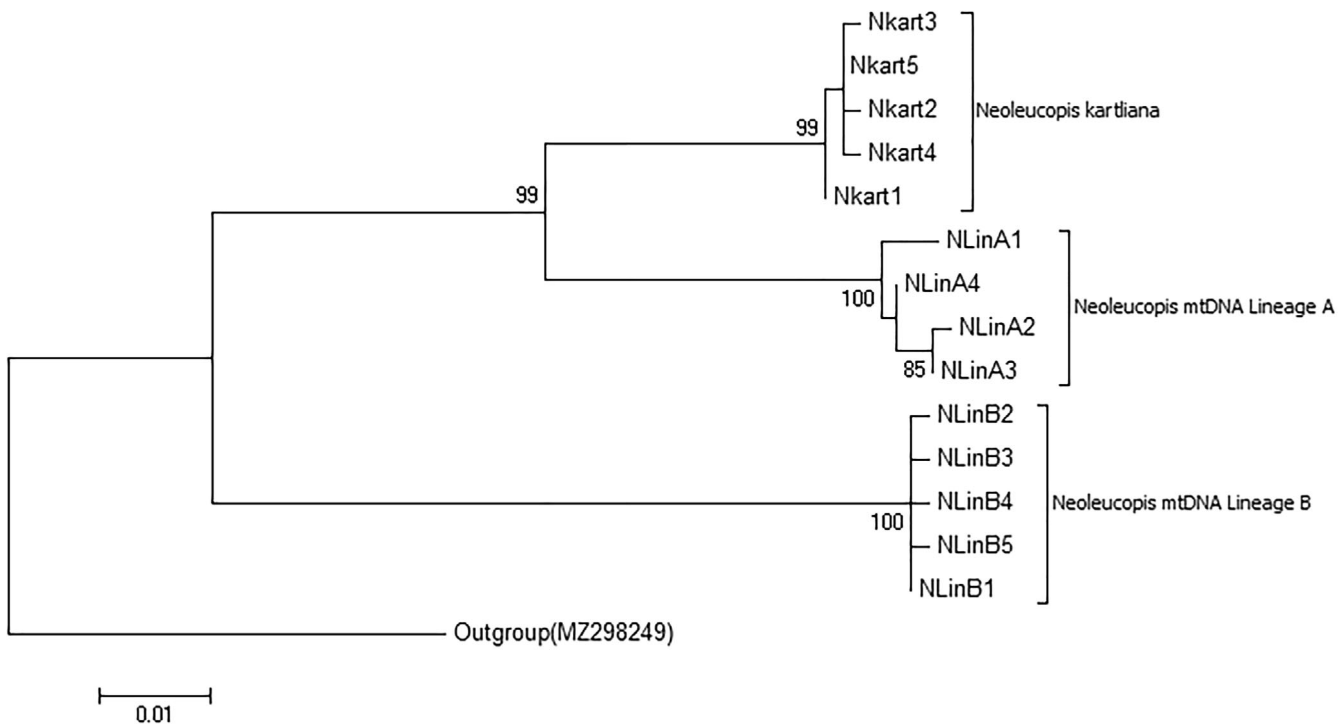

FIGURE 2 Maximum Likelihood phylogenetic tree constructed using pairwise Tamura 3 parameters and the Nearest-Neighbour-Interchange (NNI) ML heuristic method (1000 bootstrap replicates), illustrating the interspecific haplotypes among three *Neoleucopis* mtDNA clusters. Bar stands for 0.01 genetic distance, tree rooted with *N. pinicola* (NCBI Accession Number: MZ298249).

TABLE 1 Pairwise genetic distances (calculated as the number of nucleotide differences) among *Neoleucopis* mtDNA lineages (*Neoleucopis kartliana* mtDNA lineage, *Neoleucopis* mtDNA lineage A and *Neoleucopis* mtDNA lineage B).

	N. kartliana **mtDNA lineage**	***Neoleucopis*** **mtDNA lineage A**	***Neoleucopis*** **mtDNA lineage B**
N. kartliana mtDNA **lineage**	**1.600 (0.250%)**	5.556%	10.610%
Neoleucopis mtDNA **lineage A**	35.550	**3.833 (0.600%)**	10.728%
Neoleucopis mtDNA **lineage B**	67.800	68.550	**1.600 (0.250%)**

Note: Diagonal values in bold represent the average number of intraspecific mutations, with the percentage of intraspecific mutations in parentheses. Values above the diagonal values represent the average percentage of nucleotide differentiation between clusters, while values below the diagonal indicate the average number of interspecific nucleotide differences.

reads, high error rates, or possible contamination, leading to ambiguous sequences. For Thessaloniki, voucher samples of *Neoleucopis* puparia and adults originating from collections throughout 2022 were used for molecular analysis. No voucher samples were available from the remaining sites; therefore, they were not included in the analysis. The sequences from *Neoleucopis* individuals from Greece ($n = 168$) did not match any sequences deposited in the NCBI database, i.e., *N. pinicola*, *N. atratula* and *N. tapiae*, but were definitively assigned to three distinctly separated genetic clusters (Figure 2). Comparison of the sequences to specimens of *N. kartliana* identified by author SDG and those of *Neoleucopis* n. sp. B (in Eleftheriadou et al., 2024) revealed an additional, separate cluster alongside the two existing ones. Five haplotypes were identified from the 38 sequences assigned to the *N. kartliana* mtDNA lineage, four haplotypes were retrieved from the 117 sequences of the *Neoleucopis* mtDNA lineage A and five haplotypes from 13 sequences of the *Neoleucopis* mtDNA lineage B. All three clusters of haplotypes were distinctly monophyletic (Figure 2). Divergence among these three *Neoleucopis* clusters ranged from 5.556% (*N. kartliana* mtDNA lineage and *Neoleucopis* mtDNA lineage A) to 10.7% (*Neoleucopis* mtDNA lineage A and *Neoleucopis* mtDNA lineage B) (Table 1).

At the time of sampling, *Neoleucopis* mtDNA lineage A was dominant in southern and central regions of Greece, *N. kartliana* mtDNA lineage was mostly found in central and northern areas, and *Neoleucopis* mtDNA lineage B was predominantly recovered in northern Greece (Figures 1, 3). Each of the three clusters coexisted in pairs at various sites, with each possible combination present. However, all three clusters were never found to coexist at a single site.

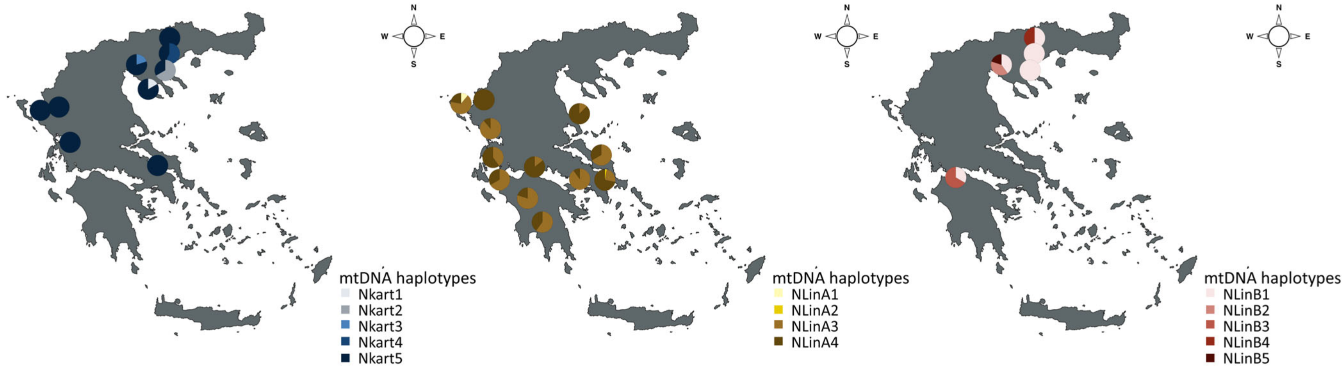

FIGURE 3 Distribution of the mtDNA clusters demonstrating the intraspecific haplotypes in *Neoleucopis kartliana* mtDNA lineage (Nkart1–5) across nine sites of Greece (Thessaloniki, Ioannina, Filiates, Amfilochia, Xirovrisi, Drama, Amphipoli, Sani and Stratoni) (left), in *Neoleucopis* mtDNA lineage A (NLinA1–4) across twelve sites of Greece (Filiates, Igoumenitsa, Parga, Lefkada, Athens, Patra, Metohi, Kaiafas, Megalopoli, Korinthos, Xirovrisi, and Volos) (center), and in *Neoleucopis* mtDNA lineage B (NLinB1–5) across five sites of Greece (Thessaloniki, Patra, Drama, Amphipoli and Stratoni) (right).

Molecular identity of *Neoleucopis* mtDNA lineages in Ischia (Italy)

Sequencing attempts for *Neoleucopis* individuals from Porto d'Ischia Parking on Ischia (Figure 1) were unsuccessful due to ambiguous sequences as mentioned above. The sequences from *Neoleucopis* individuals from Ischia from all other sites and sampling dates comprising 16 larvae, 4 puparia and 22 adults of mixed sex ($n = 42$ in total) were definitively assigned to the *N. kartliana* mtDNA lineage (Figure 2, Table 1).

Morphological differentiation

As a general finding, *Neoleucopis* mtDNA lineage A is close morphologically to *N. kartliana* mtDNA lineage, both possessing a broadly rounded epandrium, an enlarged basiphallus in the male, and thick setulae on sternites 4 and 5 in the female, but with a major difference being the lack of a pregonite in the male of *Neoleucopis* mtDNA lineage A. Both *N. kartliana* mtDNA lineage and *Neoleucopis* mtDNA lineage A are very different morphologically from *Neoleucopis* mtDNA lineage B, which is characterised by a narrow epandrium, smoothly tapering through surstylar lobes, a very thin, elongate and curved pregonite in the male, and a lack of thick setulae on sternites 4 and 5 in the female.

DISCUSSION

Based on DNA barcoding and morphological analysis, this study confirms the occurrence of three genetically distinct *Neoleucopis* mtDNA lineages in Greece that attack *M. hellenica*, including *N. kartliana*, and two additional mtDNA lineages, referred to as *Neoleucopis* mtDNA lineage A and *Neoleucopis* mtDNA lineage B. Additionally, this study confirms that the *Neoleucopis* mtDNA lineage successfully established on Ischia is the lineage assigned to *N. kartliana*. The clustering results from DNA analysis in this study aligned with the morphological differences observed in adult flies in Greece.

The morphological identification of most species of the family Chamaemyiidae relies on the examination of male genitalia, and females typically cannot be identified without close association with males (Ross et al., 2011). However, *N. kartliana* mtDNA lineage and *Neoleucopis* mtDNA lineage A do have a very distinctive female characteristic in the large patches of thick setae on sternites 4 and 5. In Greece, the occurrence of *N. kartliana* was previously verified by both DNA barcoding and morphological identification (Eleftheriadou et al., 2024; Gaimari et al., 2007). Nicolopoulos (1965) reported *N. obscura* (referred to as *Leucopis obscura* Haliday) attacking *M. hellenica* in Greece, an identification that was later questioned (Gaimari et al., 2007). Gaimari et al. (2007) suggested that this species was likely to have been *N. kartliana* or, more probably, *N. hadzibeiliae*. Nevertheless, *N. hadzibeiliae*, known to prey on *M. caucasica* in Georgia (Tanasijtshuk, 1986), was not detected preying on *M. hellenica* in Greece in the current study. It is unknown whether the species of Nicolopoulos (1965) is one or more of the *Neoleucopis* mtDNA lineages treated here, as specimens are not available for comparison.

In recent years, the identification of Chamaemyiidae adults has often consisted of destructive sampling combined with DNA barcoding (Havill et al., 2018; Neidermeier et al., 2020; Rose et al., 2020). However, the finding of reliable morphological differences among the three *Neoleucopis* mtDNA lineages will facilitate their identification in monitoring and release programmes while also being rapid and more cost-effective compared to molecular techniques. This approach, however, although effective for adults, does not resolve the challenge of distinguishing among the immature stages of the three *Neoleucopis* mtDNA lineages, for which molecular techniques remain indispensable.

The distribution of insect species is influenced by a range of biotic and abiotic factors, including climate, topography, soil composition, host plants and natural enemies (Chen, 2016; Meurisse et al., 2019;

Zhao et al., 2023; Zhu et al., 2012). The three *Neoleucopis* mtDNA lineages observed in this study exhibited distinct distributional patterns at the time of sampling: *Neoleucopis* mtDNA lineage A was found in central and southern Greece, *N. kartliana* mtDNA lineage was found in central and northern Greece, and *Neoleucopis* mtDNA lineage B predominantly in the northernmost regions of Greece. This distribution may be influenced by varying environmental conditions associated with latitude (Amundrud & Srivastava, 2020) or other factors. However, because most sampling across Greece was completed within a few weeks, the observed distribution may be due to differences in phenology across a north–south gradient and may not reflect the actual distribution of the flies. For instance, *N. kartliana* was first reported in the Attica region, southern Greece (Gaimari et al., 2007). Therefore, further collections at different times of the same year are necessary to clarify the observed distributional patterns in Greece. In addition, expanding sampling across the broader natural range of the *Neoleucopis* attacking *M. hellenica* (e.g., Türkiye) and potential introduced regions such as Croatia, where *Neoleucopis* spp. may have been introduced along with *M. hellenica*, could provide a more comprehensive understanding of their genetic structure.

Other factors may also contribute to the observed distribution patterns, such as variations in the host species. RAPD analysis conducted by Margaritopoulos et al. (2003) on *M. hellenica* across Greece revealed that southern populations differed from the northern ones. This pattern of north–south differentiation of *M. hellenica* appears to be partially congruent with the distribution pattern of the *Neoleucopis* mtDNA lineages in Greece, suggesting that this might be a factor that shaped the latter. However, to firmly draw any conclusions on this hypothesis, additional suitable molecular markers should be employed, aiming not only at the uniparental mtDNA that is proven to have low-resolution potential (Bouga et al., 2011; Eleftheriadou et al., 2023), but rather at the nuclear DNA.

The historical introduction of *M. hellenica* to different regions in Europe explains its presence on the island of Ischia, Italy, where it has been documented for three decades (Fimiani et al., 1994; Fimiani & Sollino, 1994). Beyond Ischia (Campania), *M. hellenica* has also been identified in other parts of Italy, where mandatory control measures remained in place until 2021, namely in the Marche region, with initial detections in 2018 in the municipalities of Numana, Sirolo, Ancona and Porto Recanati (Servizio Fitosanitario Regionale, 2021; Severini, 2024). The introduction of *M. hellenica* to Ischia was facilitated by Prof. P. Buchner, who introduced this species from Türkiye during his investigations into the endosymbiosis of Coccoidea in the 1960s (Tranfaglia & Tremblay, 1984). To manage the infestation of *M. hellenica* on Ischia, a small colony of morphologically identified *N. kartliana* ($n = 40$ adults of mixed sex) was introduced in 2006 from Greece as part of a biological control program (Viggiani & Mustica, 2008). Given the absence of detailed information on the precise area of Greece where these specimens were collected, it is difficult to conclude which *Neoleucopis* was introduced to Ischia. However, to date, the presence of the two new *Neoleucopis* mtDNA lineages on Ischia island has not been confirmed, and our study suggests that only the *N. kartliana* mtDNA lineage has been successfully established and is likely to be associated with the decline of the local *M. hellenica* populations as reported by Garonna and Viggiani (2011). This supports the suggestion that the *N. kartliana* mtDNA lineage is an efficient biological control agent for *M. hellenica* in its introduced range and could be a promising candidate for future classical biological control programmes, although the potential efficacy of the two new *Neoleucopis* mtDNA lineages cannot be excluded.

Understanding the conditions that favour a predatory species is critical to elucidating the natural regulation of *M. hellenica* populations in Greece and optimizing biological control strategies in invaded regions. An important aspect to investigate regarding the three *Neoleucopis* mtDNA lineages is their phenology and predator–prey synchronization to ensure effective biological control outcomes. Another key criterion for biological control agents that requires further investigation for the *Neoleucopis* spp. studied here is their prey specificity, which necessitates thorough research to estimate the consequences of their introduction in an invaded range and ensure the protection of values such as biodiversity conservation and ecosystem services (Van Lenteren et al., 2006; De Clerq et al., de Clercq et al., 2011). In addition, while samples from Türkiye were beyond the scope of this study, incorporating them in future investigations could provide valuable insights into the regional dynamics of *M. hellenica* and its predators. In conclusion, this study has highlighted the potential for these *Neoleucopis* mtDNA lineages to contribute to the biological control of *M. hellenica*. However, further research into their biology, ecology, distribution, temporal synchrony, prey specificity, potential natural enemies and their parasitism rate, and distributions through multi-year investigations at different times of the year is essential to minimize risks and maximize biological control success.

AUTHOR CONTRIBUTIONS

Nikoleta Eleftheriadou: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing – original draft; writing – review and editing. **M. Lukas Seehausen:** Data curation; methodology; validation; writing – review and editing. **Marc Kenis:** Methodology; validation; writing – review and editing. **Greg Lefoe:** Funding acquisition; validation; writing – review and editing. **Umar K. Lubanga:** Validation; writing – review and editing. **Stephen D. Gaimari:** Data curation; validation; writing – review and editing. **Nathan P. Havill:** Validation; writing – review and editing. **Antonio P. Garonna:** Resources; validation; writing – review and editing. **Nickolas G. Kavallieratos:** Supervision; validation; writing – review and editing. **Dimitrios N. Avtzis:** Conceptualization; data curation; formal analysis; methodology; project administration; resources; supervision; validation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data used in this manuscript are available from the Figshare Digital Repository at: https://doi.org/10.6084/m9.figshare.30052663.v1.

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REFERENCES

Amundrud, S.L. & Srivastava, D.S. (2020) Thermal tolerances and species interactions determine the elevational distributions of insects. *Global Ecology and Biogeography*, 29(8), 1315–1327. Available from: https://doi.org/10.1111/geb.13106

Avtzis, D.N., Lubanga, U.K., Lefoe, G.K., Kwong, R.M., Eleftheriadou, N., Andreadi, A. et al. (2020) Prospects for classical biological control of *Marchalina hellenica* in Australia. *BioControl*, 65(4), 413–423. Available from: https://doi.org/10.1007/s10526-020-10012-3

Avtzis, N. (1985) *Marchalina hellenica* (*Monophlebus hellenicus*) Gen. An important honey producing insect of Greece. *Dasiki Erevna*, 6, 51–63.

Bacandritsos, N., Saitanis, C. & Papanastasiou, I. (2004) Morphology and life cycle of *Marchalina hellenica* (Gennadius) (Hemiptera: Margarodidae) on pine (Parnis Mt.) and fir (Helmos Mt.) forests of Greece. *Annales de la Société Entomologique de France*, 40, 169–176. Available from: https://doi.org/10.1080/00379271.2004.10697413

Bouga, M., Evangelou, V., Lykoudis, D., Cakmak, I. & Hatjina, F. (2011) Genetic structure of *Marchalina hellenica* (Hemiptera: Margarodidae) populations from Turkey: preliminary mtDNA sequencing data. *Biochemical Genetics*, 49, 683–694.

Chen, Y.H. (2016) Crop domestication, global human-mediated migration, and the unresolved role of geography in pest control. *Elementa: Science of the Anthropocene*, 4, 000106.

Clark, R.C. & Brown, N.R. (1962) Studies of predators of the balsam woolly aphid, *Adelges piceae* (Ratz.) (Homoptera: Adelgidae): XI. *Cremifania nigrocellulata* Cz. (Diptera: Chamaemyiidae), an introduced predator in eastern Canada. *The Canadian Entomologist*, 94(11), 1171–1175. Available from: https://doi.org/10.4039/Ent941171-11

Clausen, C.P. (1978) Chermidae. In: Clausen, C.P. (Ed.) *Introduced parasites and predators of arthropod pests and weeds: a world review*. Agriculture Handbook 480. Washington, DC, USA: US Department of Agriculture, pp. 49–55.

Culliney, T.W., Beardsley, J.W., Jr. & Drea, J.J. (1988) Population regulation of the Eurasian pine adelgid (Homoptera: Adelgidae) in Hawaii. *Ournal of Economic Entomology*, 81(1), 142–147. Available from: https://doi.org/10.1093/jee/81.1.142

de Clercq, P., Mason, P.G. & Babendreier, D. (2011) Benefits and risks of exotic biological control agents. *BioControl*, 56, 681–698. Available from: https://doi.org/10.1007/210526-011-9372-8

Ebejer, M.J. & Barták, M. (2019) An annotated list of the Chamaemyiidae (Diptera: Acalyptrata) of Turkey with new records and additional data. *ZooKeys*, 838, 35–48.

Eleftheriadou, N., Kavallieratos, N.G., Malesios, C., Seehausen, M.L., Kenis, M., Lefoe, G. et al. (2024) Assessing the prey specificity of *Neoleucopis* spp. against *Marchalina hellenica*. *Sustainability*, 16, 2756. Available from: https://doi.org/10.3390/su16072756

Eleftheriadou, N., Lubanga, U., Lefoe, G., Seehausen, M.L., Kenis, M., Kavallieratos, N.G. et al. (2022) Phenology and potential fecundity of *Neoleucopis kartliana* in Greece. *Insects*, 13(2), 143. Available from: https://doi.org/10.3390/insects13020143

Eleftheriadou, N., Lubanga, U.K., Lefoe, G.K., Seehausen, M.L., Kenis, M., Kavallieratos, N.G. et al. (2023) Uncovering the male presence in parthenogenetic *Marchalina hellenica* (Hemiptera: Marchalinidae): insights into its mtDNA divergence and reproduction strategy. *Insects*, 14(3), 256. Available from: https://doi.org/10.3390/insects14030256

Eleftheriadou, N., Seehausen, M.L., Kenis, M., Lefoe, G., Lubanga, U.K., Gaimari, S.D. et al. (2025) Genetic diversity among Neoleucopis (Diptera: Chamaemyiidae) associated with Marchalina hellenica (Hemiptera: Marchalinidae) in Greece and Italy (Ischia). *Figshare*. Available from: https://doi.org/10.6084/m9.figshare.30052663.v1

Fimiani, P. & Sollino, G. (1994) An exotic insect dangerous to the native plants of the island of Ischia. *Informatore Agrario*, 50, 65–68.

Fimiani, P., Sollino, G. & Marotta, S. (1994) Insetti delle pinete ischitane I. La *Marchalina hellenica*. XXIV Distretto Scolastico delle isole di Ischia e Procida, Associazione per la Difesa dei Pini d'Ischia. 1–47.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

Fotelli, M.N., Lyrou, F.G., Avtzis, D.N., Maurer, D., Rennenberg, H., Spyroglou, G. et al. (2020) Effective defense of Aleppo pine against the giant scale *Marchalina hellenica* through ecophysiological and metabolic changes. *Frontiers in Plant Science*, 11, 581693. Available from: https://doi.org/10.3389/fpls.2020.581693

Gaimari, S.D. (2020) Two new genera of Nearctic Chamaemyiidae (Diptera: Lauxanioidea) associated with *Cinara* aphids (Hemiptera: Aphididae) on *Pinus*. *Zootaxa*, 4852, 61–82. Available from: https://doi.org/10.11646/zootaxa.4852.1.3

Gaimari, S.D. (2021) Chamaemyiidae. In: Kirk-Spriggs, A.H. & Sinclair, B.J. (Eds.) *Manual of Afrotropical Diptera. Volume 3. Brachycera – Cyclorrhapha, excluding Calyptratae. Suricata 8*. Pretoria, South Africa: South African National Biodiversity Institute, pp. 1791–1814.

Gaimari, S.D., González, C.R. & Elgueta, M. (2024) A catalog of the Chamaemyiidae of Chile (Diptera: Lauxanioidea). *Zootaxa*, 5399, 555–569. Available from: https://doi.org/10.11646/zootaxa.5399.5.5

Gaimari, S.D. & Havill, N.P. (2021) A new genus of Chamaemyiidae (Diptera: Lauxanioidea) predaceous on Adelgidae (Hemiptera), with a key to chamaemyiid species associated with Pinaceae-feeding Sternorrhyncha. *Zootaxa*, 5067, 1–39. Available from: https://doi.org/10.11646/zootaxa.5067.1.1

Gaimari, S.D., Milonas, P. & Souliotis, C. (2007) Notes on the taxonomy, biology and distribution of *Neoleucopis kartliana* (Tanasijtshuk) (Diptera: Chamaemyiidae). *Folia Heyrovskyana A*, 15, 7–16.

Gaimari, S.D. & Turner, W.J. (1996) Immature stages of *Leucopis ninae* Tanasijtshuk and two variant populations of *Leucopis gaimarii* Tanasijtshuk (Diptera: Chamaemyiidae) feeding on Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae). *Proceedings of the Entomological Society of Washington*, 98, 647–666.

Gaimari, S.D. & Turner, W.J. (1997) Behavioral observations on the adults and larvae of *Leucopis ninae* and *L. gaimarii* (Diptera: Chamaemyiidae), predators of Russian wheat aphid, *Diuraphis noxia* (Homoptera: Aphididae). *Journal of the Kansas Entomological Society*, 70, 153–159.

Gallis, A.T. (2007) Evaluation of the damage by insect *Marchalina hellenica* (Genn.) in eastern Attica, Greece. Conclusions for sustainable management of forest ecosystems. In: *Paper presented at: 10th international conference on environmental science and technology; 05–07 September*. Cos Island, Greece: G-NEST and University of Aegean.

Garonna, A.P. & Viggiani, G. (2011) *The establishment in Italy of Neoleucopis kartliana (Tanasjtshuk) (Diptera: Chamaemyiidae), predator of*

Marchalina hellenica (Gennadius) (Hemiptera: Margarodidae). Paper presented at: XXIII Italian National Congress of Entomology; 13–16 June. Genoa, Italy: Notiziario sulla Protezione delle Piante III Serie.

Gounari, S. (2004) Seasonal development and ovipositing behavior of *Marchalina hellenica* (Hemiptera: Margarodidae). *Entomologia Hellenica*, 15, 27–38. Available from: https://doi.org/10.12681/eh.14045

Gounari, S. (2006) Studies on the phenology of *Marchalina hellenica* (Gen.) (Hemiptera: Coccoidea: Margarodidae) in relation to honeydew flow. *Journal of Apicultural Research*, 45, 8–12. Available from: https://doi.org/10.1080/00218839.2006.11101305

Gounari, S., Zotos, C.E., Dafnis, S.D., Moschidis, G. & Papadopoulos, G.K. (2023) On the impact of critical factors to honeydew honey production: the case of *Marchalina hellenica* and pine honey. *Journal of Apicultural Research*, 62, 383–393. Available from: https://doi.org/10.1080/00218839.2021.1999684

Greathead, D.J. (1995) The *Leucopis* spp. (Diptera: Chamaemyiidae) introduced for biological control of *Pineus* sp. (Homoptera: Adelgidae) in Hawaii: implications for biological control of *Pineus*? Boerneri in Africa. *Entomologist*, 114, 83–90.

Grubin, S.M., Ross, D.W. & Wallin, K.F. (2011) Prey suitability and phenology of *Leucopis* spp. (Diptera: Chamaemyiidae) associated with hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environmental Entomology*, 40(6), 1410–1416. Available from: https://doi.org/10.1603/EN11127

Havill, N.P., Gaimari, S.D. & Caccone, A. (2018) Cryptic east-west divergence and molecular diagnostics for two species of silver flies (Diptera: Chamaemyiidae: *Leucopis*) from North America being evaluated for biological control of hemlock woolly adelgid. *Biological Control*, 121, 23–29. Available from: https://doi.org/10.1016/j.biocontrol.2018.02.004

Hodgson, C. & Gounari, S. (2006) Morphology of *Marchalina hellenica* (Gennadius) (Hemiptera: Coccoidea: Marchalinidae) from Greece, with a discussion on the identity of *M. caucasica* Hadzibeyli from the Caucasus. *Zootaxa*, 1196, 1–32. Available from: https://10.11646/zootaxa.1196.1.1

Jaroslow, D.D., Cunningham, J.P., Smith, D.I. & Steinbauer, M.J. (2023) Seasonal phenology and climate associated feeding activity of introduced *Marchalina hellenica* in Southeast Australia. *Insects*, 14, 305. Available from: https://doi.org/10.3390/insects14030305

Jashenko, R.V. (1999) Fauna, natural enemies, agricultural harm and possibility of industrial use of margarodids (Coccinea: Margarodidae) in East Europe and North Asia. *Selevinia*, 10, 43–50.

Kailidis, S.D. (1965) *Monophlebus hellenicus* (*Marchalina hellenica*) Genn. The honeydew producing insect of pine trees. *Dasika Chronika*, 81, 1–16.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H. et al. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948. Available from: https://doi.org/10.1093/bioinformatics/btm404

Margaritopoulos, J.T., Bacandritsos, N., Pekas, A.N., Stamatis, C., Mamuris, Z. & Tsitsipis, J.A. (2003) Genetic variation of *Marchalina hellenica* (Hemiptera: Margarodidae) sampled from different hosts and localities in Greece. *Bulletin of Entomological Research*, 93, 447–453.

Martinez-Sañudo, I., Mazzon, L., Simonato, M., Avtzis, D., Pujade-Villar, J. & Faccoli, M. (2018) Tracking the origin and dispersal of the Asian chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) in Europe with molecular markers. *Bulletin of Entomological Research*, 109, 300–308.

Masten Milek, T., Simala, M. & Pintar, M. (2019) First record of *Marchalina hellenica* (Gennadius) (Hemiptera: Marchalinidae) in Croatia. Paper presented at: XVth International Symposium on Scale Insect Studies; 17–20 June; Zagreb, Croatia.

McAlpine, J.F. (1978) A new dipterous predator of balsam woolly aphid from Europe and Canada (Diptera: Chamaemyiidae). *Entomologica Germanica*, 4, 349–355.

McAlpine, J.F. & Tanasijtshuk, V.N. (1972) Identity of *Leucopis argenticollis* and description of a new species (Diptera: Chamaemyiidae). *The Canadian Entomologist*, 104, 1865–1875.

McLean, I.F.G. (1998) *Leucopis psylliphaga* sp. n., a new species of silverfly (Diptera: Chamaemyiidae) from Britain. *Dipterists Digest*, 5, 49–54.

Mendel, Z., Branco, M. & Battisti, A. (2016) Invasive sap-sucker insects in the Mediterranean Basin. In: Paine, T.D. & Lieutier, F. (Eds.) *Insects and diseases of Mediterranean forest systems*. Cham: Springer, pp. 261–291. Available from: https://doi.org/10.1007/978-3-319-24744-1_10

Meurisse, N., Rassati, D., Hurley, B.P., Brockerhoff, E.G. & Haack, R.A. (2019) Common pathways by which non-native forest insects move internationally and domestically. *Journal of Pest Science*, 92, 13–27. Available from: https://doi.org/10.1007/s10340-018-0990-0

Mills, N.J. (1990) Biological control of forest aphid pests in Africa. *Bulletin of Entomological Research*, 80(1), 31–36. Available from: https://doi.org/10.1017/S0007485300045880

Nakao, H.K., Funasaki, G.Y., Higa, S.Y. & Lai, P.Y. (1981) Introductions for the biological control in Hawaii, 1977 and 1978. *Proceedings of the Hawaiian Entomological Society*, 23, 425–430.

Neidermeier, A.N., Ross, D.W., Havill, N.P. & Wallin, K.F. (2020) Temporal asynchrony of adult emergence between *Leucopis argenticollis* and *Leucopis piniperda* (Diptera: Chamaemyiidae), predators of the hemlock woolly adelgid (Hemiptera: Adelgidae), with implications for biological control. *Environmental Entomology*, 49(4), 823–828. Available from: https://doi.org/10.1093/ee/nvaa049

Nicolopoulos, C. (1965) *Morphology and biology of the species Marchalina hellenica (Gennadius) (Hemiptera: Margarodidae: Coelostomidiinae)* [PhD thesis]. Athens, Greece: Agricultural College of Athens.

Noma, T., Brewer, M.J., Pike, K.S. & Gaimari, S.D. (2005) Hymenopteran parasitoids and dipteran predators of *Diuraphis noxia* in the west-central Great Plains of North America: species records and geographic range. *BioControl*, 50, 97–111. Available from: https://doi.org/10.1007/s10526-004-4921-z

Olfert, O.O., Doane, J.F., Carl, K., Erlandson, M.A. & Goettel, M.S. (2001) *Diuraphis noxia* (Kurdjumov), Russian wheat aphid (Homoptera: Aphididae). In: Mason, P.G. & Huber, J.T. (Eds.) *Biological control programmes in Canada, 1981–2000*. UK: CABI Publishing, pp. 110–114. Available from: https://doi.org/10.1079/9780851995274.0110

Ravn, H.P., Havill, N.P., Akbulut, S., Foottit, R.G., Serin, M., Erdem, M. et al. (2013) *Dreyfusia nordmannianae* in Northern and Central Europe: potential for biological control and comments on its taxonomy. *Journal of Applied Entomology*, 137, 401–417.

Rose, A., Ross, D.W., Havill, N.P., Motley, K. & Wallin, K.F. (2020) Coexistence of three specialist predators of the hemlock woolly adelgid in the Pacific Northwest USA. *Bulletin of Entomological Research*, 110, 303–308. Available from: https://doi.org/10.1017/S0007485319000622

Ross, D.W., Gaimari, S.D., Kohler, G.R., Wallin, K.F., Grubin, S.M., Onken, B. et al. (2011) Chamaemyiid predators of the hemlock woolly adelgid from the Pacific Northwest. In: Onken, B. & Reardon, R. (Eds.) *Implementation and status of biological control of the hemlock woolly adelgid*. Fort Collins, Colorado, USA: US Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team, pp. 97–106.

Schooley, H.O., Harris, J.W.E. & Pendrel, B. (1984) *Adelges piceae* (Ratz.), balsam woolly adelgid (Homoptera: Adelgidae). In: Kelleher, J.S. & Hulme, M.A. (Eds.) *Biological control programmes in Canada, 1969–1980*. Wallingford, Oxfordshire, UK: CABI Publishing, pp. 229–234.

Servizio Fitosanitario Regionale. (2021) Regione Marche. Linee guida per contrastare la diffusione della cocciniglia greca (*Marchalina hellenica* Genn.) nel territorio della Regione Marche (Allegato n. 1). In: *Agenzia per l'Innovazione nel Settore Agroalimentare e della Pesca (AMAP)*. Marche Agricoltura Pesca: AMAP – Agenzia per l'Innovazione nel Settore Agroalimentare e della Pesca, Regione Marche.

Severini, F. (2024) Decreto del dirigente tecnico del settore "Fitosanitario e agrometeorologia, laboratori e qualità delle produzioni" – Adozione delle linee guida per contrastare la diffusione della Cocciniglia greca (*Marchalina hellenica* Genn.) nel territorio della Regione Marche. Regione Marche, Agenzia per l'Innovazione nel Settore Agroalimentare e della Pesca (AMAP). Marche Agricoltura Pesca.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725–2729. Available from: https://doi.org/10.1093/molbev/mst197

Tanasijtshuk, V.N. (1986) [Silver-flies (Chamaemyiidae)]. Fauna of the USSR, new series 134. In: *Dipterans. Zoological Institute of the Russian Academy of sciences*. Moscow, Russia: Nauka Publishers.

Tanasijtshuk, V.N. (1996) Silver-flies (Diptera: Chamaemyiidae) of Australia. *International Journal of Dipterological Research*, 7(1), 1–62.

Teodorescu, I. & Maican, S. (2014) Diversity of entomofauna (Hymenoptera: Diptera: Coleoptera) in the Comana natural park (Romania). *Romanian Journal of Biology*, 59(1), 17–34.

Tranfaglia, A. & Tremblay, E. (1984) Faunistic and systematic studies on Italian scale insects. Paper Presented at: 10th international symposium of central European entomofaunistics; 15–20 August; Budapest.

Ülgentürk, S., Szentkirályi, F., Uygun, N., Fent, M., Gaimari, S.D., Civelek, H. et al. (2013) Predators of *Marchalina hellenica* (Hemiptera: Marchalinidae) on pine forests in Turkey. *Phytoparasitica*, 41(5), 529–537. Available from: https://doi.org/10.1007/s12600-013-0313-1

Van Lenteren, J., Cock, M.J., Hoffmeister, T.S. & Sands, D.P. (2006) Host specificity in arthropod biological control, methods for testing and interpretation of the data. In: Bigler, F., Babendreier, D. & Kuhlmann, U. (Eds.) *Environmental impact of invertebrates for biological control of arthropods: methods and risk assessment*. Wallingford, Oxfordshire, UK: CABI Publishing, pp. 38–63.

Viggiani, G. & Mustica, L. (2008) Some notes on the biological control of insect pests in the island of Ischia. *Bollettino del Laboratorio di Entomologia Agraria Filippo Silvestri*, 62, 79–82.

Zhao, L., Gao, R., Liu, J., Liu, L., Li, R., Men, L. et al. (2023) Effects of environmental factors on the spatial distribution pattern and diversity of insect communities along altitude gradients in Guandi Mountain, China. *Insects*, 14, 224. Available from: https://doi.org/10.3390/insects14030224

Zhu, G., Bu, W., Gao, Y. & Liu, G. (2012) Potential geographic distribution of brown marmorated stink bug invasion (*Halyomorpha halys*). *PLoS One*, 7, e31246. Available from: https://doi.org/10.1371/journal.pone.0031246

Zondag, R. & Nutall, M.J. (1989) *Pineus laevis* Maskell, pine twig chermes or pine woolly aphid (Hemiptera: Adelgidae). In: Gameron, P., Hill, R.-L., Bain, J. & Thomas, W.P. (Eds.) *A review of invertebrate pests and weeds in New Zealand 1874 to 1987*. Wallingford, Oxfordshire, UK: Technical Communication CAB International Institute of Biological Control 10, pp. 295–297.

Zúñiga, E. (1985) Ochenta años de control biologico en Chile. Revision historica y evaluacion de los proyectos desarrollados (1903–1983). *Agricultura Téchnica*, 45, 175–183.

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