sustainability

MDPI

Article

Assessing the Prey Specificity of *Neoleucopis* spp. against *Marchalina hellenica*

Nikoleta Eleftheriadou [1,*], Nickolas G. Kavallieratos [1], Chrisovalantis Malesios [2], M. Lukas Seehausen [3], Marc Kenis [3], Greg Lefoe [4], Umar Lubanga [4] and Dimitrios N. Avtzis [5]

1 Laboratory of Agricultural Zoology and Entomology, Faculty of Crop Science, Agricultural University of Athens, 75 Iera Odos Str., 11855 Athens, Greece; nick_kaval@aua.gr
2 Laboratory of Political Economy and European Integration, Department of Agricultural Economics and Rural Development, Agricultural University of Athens, 75 Iera Odos Str., 11855 Athens, Greece; malesios@aua.gr
3 CABI (Centre for Agriculture and Bioscience International), Rue des Grillons 1, 2800 Delémont, Switzerland; l.seehausen@cabi.org (M.L.S.); m.kenis@cabi.org (M.K.)
4 Agriculture Victoria, Department of Energy, Environment and Climate Action, AgriBio Centre, 5 Ring Road, Bundoora, VIC 3083, Australia; greg.lefoe@agriculture.vic.gov.au (G.L.); umar.lubanga@agriculture.vic.gov.au (U.L.)
5 Forest Research Institute—Hellenic Agricultural Organization Demeter, Vassilika, 57006 Thessaloniki, Greece; dimitrios.avtzis@fri.gr
* Correspondence: nikolelef@aua.gr

Abstract: *Marchalina hellenica* Gennadius (Hemiptera: Marchalinidae) is a scale insect native to Greece and Turkey and presently invasive in Australia, where it damages pine plantations. The silver fly, *Neoleucopis kartliana* Tanasijtshuk (Diptera: Chamaemyiidae), is the most abundant predator of *M. hellenica* in Greece and is presently being investigated as a potential biological control agent following the scale's introduction in Australia. This study, conducted in Northern Greece, revealed the presence of a second lineage, closely related to *N. kartliana*, referred to as *Neoleucopis* n. sp. *B*. Field surveys and laboratory experiments were conducted on *M. hellenica* and a taxonomically related scale insect, *Icerya purchasi* Maskell (Hemiptera: Monophlebidae), to test the larval growth and survival of the flies on the two prey species and assess their specificity for *M. hellenica*. The results suggest that both *Neoleucopis* spp. exhibit a high preference for *M. hellenica* when compared to *I. purchasi*. Larval growth was higher on *M. hellenica* than on *I. purchasi* but the difference was significant for *N. kartliana* only. Survival was significantly higher for both predators when provided *M. hellenica* compared to *I. purchasi*. Field surveys showed that both predators are abundant on *M. hellenica* colonies, whereas none of the two *Neoleucopis* lineages was found to have preyed on *I. purchasi*.

Keywords: silver flies; Marchalinidae; biocontrol; prey selectivity; predators

Citation: Eleftheriadou, N.; Kavallieratos, N.G.; Malesios, C.; Seehausen, M.L.; Kenis, M.; Lefoe, G.; Lubanga, U.; Avtzis, D.N. Assessing the Prey Specificity of *Neoleucopis* spp. against *Marchalina hellenica*. *Sustainability* **2024**, *16*, 2756. https://doi.org/10.3390/su16072756

Academic Editor: Antoni Sánchez

Received: 8 February 2024
Revised: 20 March 2024
Accepted: 21 March 2024
Published: 27 March 2024

1. Introduction

In the pursuit of a sustainable future, the imperative to align human activities with the preservation of ecological integrity has become increasingly prominent [1]. Sustainable development serves as a guiding principle, directing efforts toward meeting current societal needs while also safeguarding the prospects of future generations [2]. This strategic approach entails a delicate equilibrium between economic advancement, societal well-being, and environmental conservation [3]. Sustainable development acknowledges the intricate interplay between ecological health and human prosperity, advocating for a conscientious and responsible utilization of resources [4]. As global challenges, ranging from invasive species to environmental degradation, underscore the need for comprehensive solutions, the commitment to sustainable practices, including biocontrol, remains integral in ensuring the preservation of biodiversity and fostering a resilient, equitable, and enduring future [5,6].

The giant pine scale, *Marchalina hellenica* Gennadius (Hemiptera: Marchalinidae) is a univoltine sap-sucking scale insect native to the eastern Mediterranean region

(Greece and Turkey). *Marchalina hellenica* feeds on the sap of *Pinus* spp. and excretes honeydew, a sweet, glutinous, honey-like substance which is collected by bees and converted into pine honey. Pine honey production represents 60–65% of the annual honey production in Greece [7]. The importance of *M. hellenica* to apiculture, and the fact that it is rarely considered a primary factor in tree mortality [8], has led to its intentional introduction to new regions of Greece, and the island of Ischia (Italy) [9]. In these expanded ranges, *M. hellenica* has occasionally reached high population densities and has been associated with a decline in tree health and a reduction in insect biodiversity in pinewoods [8]. More recently, *M. hellenica* has invaded Croatia [10] and Australia [11]. In these countries, the impacts on tree health can be even more harmful, especially if new host associations are formed. For instance, *M. hellenica* was detected in Melbourne and Adelaide (Australia) in 2014 on a novel and highly susceptible host, *Pinus radiata* [11]. The scale's population rapidly increased and caused notable damage to *P. radiata* and other pine trees in urban and peri-urban settings [12]. Damage to *P. radiata* health is a particular concern, as it is a major component of Australia's softwood plantation estate [11,13]. The repeated invasions of *M. hellenica* underscore the urgent need for a sustainable biological control strategy. Implementing effective measures is crucial in order to mitigate its impact, preserve tree health, and maintain the equilibrium of insect biodiversity within affected ecosystems.

Research on the natural enemy complex of *M. hellenica* suggests that the silver fly *Neoleucopis kartliana* Tanasijtshuk (Diptera: Chamaemyiidae) is the most abundant predator among the scale's natural enemies in its native range (e.g., Greece and Turkey) [11,14]. Chamaemyiidae is a group of small flies that prey as larvae on soft-bodied hemipteran species, particularly aphids, mealybugs, and scales [15]. Nicolopoulos [16] reported that *Neoleucopis obscura* (Haliday) (Diptera: Chamaemyiidae) also attacked *M. hellenica* in Greece. However, it was later suggested that the *N. obscura* recorded in Greece [16] was in fact *Neoleucopis hadzibeiliae* Tanasijtshuk (Diptera: Chamaemyiidae) [17]. Based on the current knowledge, more than one *Neoleucopis* spp. prey on *M. hellenica* in Greece [11]. However, aside from *N. kartliana*, the identity of other *Neoleucopis* species in Greece remains unresolved.

Neoleucopis kartliana was purportedly introduced to the island of Ischia (Italy) for the control of *M. hellenica* [18] and has been proposed for the biological control of *M. hellenica* in Australia [11]. However, to our knowledge, no research has been conducted on the level of specificity of *N. kartliana* or any other *Neoleucopis* spp. preying on the genus *Marchalina*, which includes two known described species, *M. hellenica* and *M. caucasica* Hadzibeyli (Hemiptera: Marchalinidae) [19]. Our study was designed to assess the interaction between *Neoleucopis* spp. and an Australian scale insect species, *Icerya purchasi* Maskell (Hemiptera: Monophlebidae), closely related to *M. hellenica*, as a potential non-target species. Further research on *Neoleucopis* spp. that prey on *M. hellenica* in its native range along with prey specificity testing and risk assessment in both Greece and regions of introduction is necessary before considering *Neoleucopis* spp. for the biological control of *M. hellenica* in Australia or elsewhere.

Icerya purchasi, a native Australian scale, stands out among the Monophlebidae species prioritized for prey specificity testing [20]. *Icerya purchasi* was first recorded in Greece in 1927 and subsequently spread throughout continental Greece, where it is sympatric with *M. hellenica* [11,21]. *Icerya purchasi* belongs to the same superfamily as *M. hellenica* (Coccoidea). The two species also exhibit shared physiological characteristics, including a soft body structure, production of cottony secretions, and similarities in the morphology of their ovisac [19,22]. These attributes, and the presence of *I. purchasi* in areas of Greece where both *M. hellenica* and *Neoleucopis* naturally occur, provide an opportunity to assess its potential non-target impacts on an Australian scale present in the native range of the target pest in both laboratory and field studies. *Icerya purchasi* is notorious for being the target of the first successful classical biological control, when its predator, *Novius cardinalis* (Mulsant) (=*Rodolia cardinalis*) (Coleoptera: Coccinellidae), was introduced in California

and successfully controlled its invasive scale in citrus groves [23,24]. The ladybird was later introduced in other parts of the world, including Greece, against *I. purchasi* [25,26].

This investigation carries substantial implications for assessing the risks associated with biological control agents in the context of managing *M. hellenica* in invaded regions. It aligns with the principles of sustainable development by seeking to address the present needs without jeopardizing the ability of future generations to meet their own requirements [2]. Within the scope of our research hypotheses, we examine potential distinctions in (1) the development and survival of *Neoleucopis* spp. when exposed to either *M. hellenica* or *I. purchasi*, and (2) the occurrence of *Neoleucopis* spp. on their natural hosts within the predator's native range.

2. Materials and Methods

2.1. Prey Specificity Experiments

To study the prey specificity of *Neoleucopis* spp. larvae, the host specificity protocol of van Lenteren et al. [27] (small-arena no-choice black-box test) was followed with slight modifications so that it applied to these predatory species. The co-occurrence of the target pest (*M. hellenica*), proposed biocontrol agents (*Neoleucopis* spp.), and a priority non-target Australian scale, *I. purchasi* [20], provided an opportunity to conduct laboratory and field prey range studies in the pest's native range in Greece. *Icerya purchasi* was therefore selected for prey specificity studies in Greece. Exercising the required host plant substrate maintenance and conducting observations on live plants, as stipulated by the established protocol, was not considered essential, given that the selected developmental stage for both the target and non-target species is the egg stage, in which fitness does not depend on feeding. The larval stage of *Neoleucopis* spp. was selected, as it feeds on the eggs of suitable prey during this stage [11,17].

In May 2022 and April 2023, months selected due to the documented presence of *N. kartliana* larvae in the field, as previously reported by Eleftheriadou et al. [28], *M. hellenica*-infested *Pinus brutia* Ten. (Pinales: Pinaceae) branches were collected from the suburban forest of Thessaloniki (Greece) (40°37′58″ N, 22°58′35″ E) and *I. purchasi*-infested *Pittosporum tobira* (Thunb.) W.T. Aiton (Apiales: Pittosporaceae) branches were collected from the city of Thessaloniki, Greece (40°37′34″ N, 22°57′06″ E). The branches were subsequently transferred to the Forest Research Institute of Thessaloniki, Greece (H.A.O. Demeter). *Marchalina hellenica* and *I. purchasi* ovisacs were carefully removed from the branches using soft forceps and inspected under a stereomicroscope to remove any present predators. *Neoleucopis* spp. larvae found inside the *M. hellenica* ovisacs were counted, collected, and individually placed back onto predator-free ovisacs inside Petri dishes (5.4 cm diameter). In 2022, twenty predators were individually assigned to *M. hellenica* to serve as controls (20 replications), and an additional twenty predators were designated for *I. purchasi* (20 replications). The dishes were then placed inside a climate chamber (Termaks KB8400F, Norway) at 23 °C, 60% RH, and a 16 h light/8 h dark photoperiod [28]. The above procedure was replicated once more in 2023, with the implementation of new dishes and *Neoleucopis* spp. larvae and the use of fresh *M. hellenica* and *I. purchasi* eggs. Ovisacs were visually inspected each day to observe predation on *M. hellenica* and *I. purchasi* eggs. Before exposure to prey, the length of *Neoleucopis* spp. larvae was measured using an AxioCam 208 stereoscope camera software Zen core 3.5 (Zeiss, Oberkochen, Germany, 8.3 megapixels, 4K). This process involved gently opening the ovisacs with soft forceps and allowing the larvae to extend their bodies fully before recording the measurements. In 2022, measurements were taken again three days post installation for larvae preying on *M. hellenica* and five days post installation for those preying on *I. purchasi*, and in 2023, three days post installation for all larvae to examine whether the *Neoleucopis* spp. larvae had successfully preyed on eggs and continued their development. In addition to the size increase of larvae, the number of individuals that pupariated and the number that were emerging as adults were recorded. Following the completion of the prey specificity experiments, puparia that did not produce adult

specimens were dissected under a Zeiss Stemi 508 stereomicroscope (Zeiss, Oberkochen, Germany) using a scalpel to examine the presence of parasitoids. This examination sought to provide a comprehensive understanding of the factors influencing adult emergence, distinguishing between instances of unfavorable development and instances of parasitism, which could have affected the recorded mortality results. Subsequently, *Neoleucopis* spp. specimens that reached the adult stage were morphologically identified. For *Neoleucopis* spp. individuals that did not reach the adult stage, DNA barcoding was employed for identification, which is described in detail below (Section 2.3—Identification of the Chamaemyiid Species).

2.2. Field Surveys

To investigate whether *Neoleucopis* spp. attack the non-target species when both the target and non-target species are present in their natural habitat, branches of *P. tobira* infested with *I. purchasi* and *P. brutia* branches infested with *M. hellenica* were collected on two occasions, in May and April 2022, in Thessaloniki, Greece. The sampled *P. tobira* and *P. brutia* plants were less than 5 m apart. In addition, lightly infested *P. brutia* and *P. tobira* branches were sampled from several plants in the same area (~5 branches per plant species). The infested branches were transferred to the Forest Research Institute of Thessaloniki, Greece, and were then examined under a stereomicroscope in search of *Neoleucopis* spp. larvae. After inspection, the branches infested with *I. purchasi* were stored in small, ventilated cages (30 cm × 30 cm × 30 cm) inside a climate chamber at the aforementioned conditions to allow sufficient time for *Neoleucopis* spp. to develop to the adult stage and identify potentially undetected specimens. This was not done for branches infested with *M. hellenica*, as the presence of the fly has already been established.

2.3. Identification of the Chamaemyiid Species

Upon the conclusion of the prey specificity experiments, DNA was individually extracted from the *Neoleucopis* spp. specimens that did not reach the adult stage and remained sufficiently intact postmortem to yield viable results using PureLink™ Genomic DNA Mini Kit (ThermoFisher Scientific, Life Sciences Solutions, Carlsbad, CA, USA) following the manufacturer's protocol. DNA amplification was then performed in 25 µL volumes with HCO/LCO primers that amplify a fragment of the Cytochrome Oxidase One (COI) mitochondrial gene and with MyTaq™ Red Mix (BioLine GmbH, Luckenwalde, Germany). The thermal cycling conditions consisted of an initial denaturation step of 5 min at 96 °C, followed by 4 cycles of 60 s at 96 °C (denaturation), 60 s at 47 °C (annealing), and 60 s at 72 °C (extension). This loop was then followed by 35 additional cycles of 60 s at 96 °C, 60 s at 50 °C (annealing), and 60 s at 72 °C (extension). The final extension period was performed at 72 °C for 5 min [11]. The purification of PCR products was performed with PureLink™ PCR Purification Kit (ThermoFisher Scientific, Life Sciences Solutions, Carlsbad, CA, USA) following the manufacturer's protocol. Sequencing took place at CEMIA SA (Larissa, Greece) using an ABI 3730XL sequencer (ThermoFisher, Waltham, MA, USA). The obtained sequences were manually analyzed using Chromas Lite software version 2.01, aligned using Clustal X, and then blasted in NCBI GenBank. The morphological identification of *N. kartliana* adults and its distinction from different species was based on distinct characters of the male genitalia according to Gaimari et al. [17]. The molecular analyses revealed the presence of two *Neoleucopis* spp., *N. kartliana* and *Neoleucopis* n. sp. *B* (see Results).

2.4. Data Analysis

Analysis was conducted using R Statistical Software 4.2.2. [29]. Using the *glm* function of the stats package in R, two separate logistic regressions with binomial distribution were performed to test the influence of the two explanatory variables of prey (*M. hellenica* and *I. purchasi*) and predator (*N. kartliana* and *Neoleucopis* n. sp. *B*) on the predator's sur-

vival (live, dead) and development (increase in size or no increase). Tukey's HSD test at $p = 0.05$ was employed to compare multiple means.

Additionally, a regression-type approach was employed to explore the survival and growth dynamics of *Neoleucopis* n. sp. *B* and *N. kartliana*, examining their relationship with the potential explanatory variables of prey source (*M. hellenica* and *I. purchasi*). Differences in survival and growth between *Neoleucopis* n. sp. *B* and *N. kartliana* were assessed by incorporating the categorical variable of *Neoleucopis* lineage into the regression models. Binomial logistic regression models were utilized to link the dichotomous response variables of "survival" and "growth" to the explanatory variables of interest. Covariate selection was conducted via a backward stepwise approach to identify the best-fitting models that explain variations in survival and growth. Model selection was guided by the Akaike information criterion (AIC), with the preferred model demonstrating the lowest AIC value.

3. Results

3.1. Identification of the Chamaemyiid Species

Genetic analysis of *Neoleucopis* spp. individuals involved in the prey specificity experiments suggested the presence of two *Neoleucopis* spp., *N. kartliana* (n = 43) and possibly a different species, hereafter named *Neoleucopis* n. sp. *B* (n = 37) (intraspecific genetic distance = 5.2%). Additionally, these two *Neoleucopis* spp. display distinct morphological differences in their male terminalia, with the most notable distinctions observed in the epandrium and surstylus (Figure 1). Both DNA barcoding and morphological identification of the specimens used for the prey specificity experiments in 2022 showed that three individuals were *N. kartliana* and the remaining thirty-seven belonged to *Neoleucopis* n. sp. *B*, while in 2023, all forty individuals were *N. kartliana*.

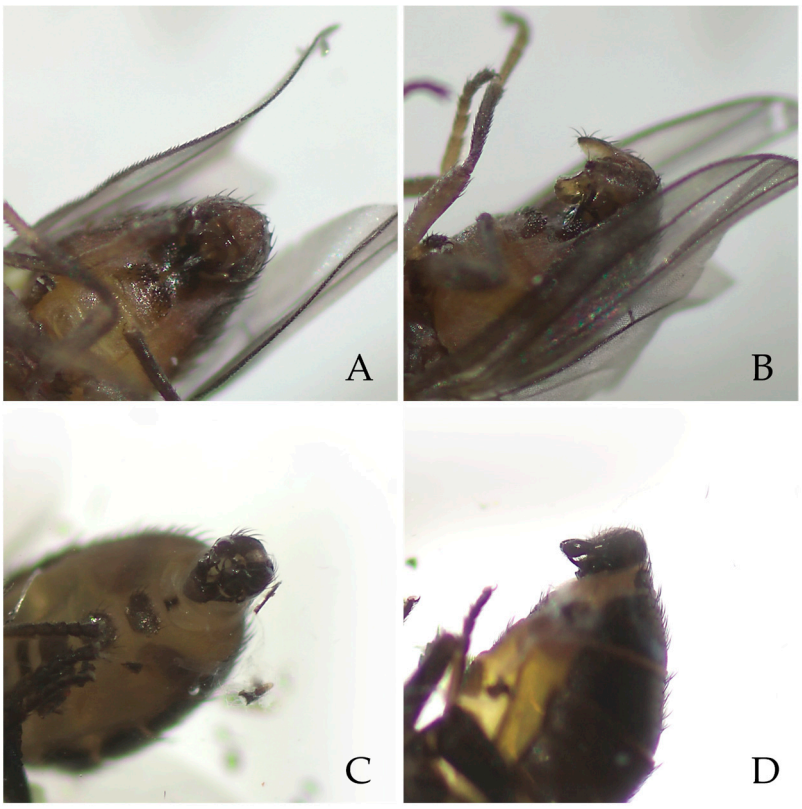

Figure 1. Anterior (left) and lateral (right) view of *N. kartliana* (**A**,**B**) and *Neoleucopis* n. sp. *B* (**C**,**D**) male terminalia.

3.2. Prey Specificity Experiments

In the controls, several *Neoleucopis* spp. larvae were observed to prey on *M. hellenica* eggs. In both 2022 and 2023, egg loss was notable in every *M. hellenica* ovisac once the inspection of the larvae was completed. During prey specificity experiments in 2022, *Neoleucopis* spp. larvae were not witnessed preying on the eggs of *I. purchasi*; however, they produced red-hued excrements, in contrast to larvae preying on *M. hellenica* eggs, which produced transparent or yellow-hued excrements. It is important to note that the quantification of egg predation or direct observation of predation was not within the scope of the present study. No parasitoid was encountered during the inspection of the *Neoleucopis* spp. puparia after the completion of the experiments.

Regarding larval growth, a significant effect was demonstrated for the prey species, as well as for the predator × prey species interaction (Table 1). While *N. kartliana* exhibited significantly lower growth on the non-target species, *I. purchasi* (17.4%), compared to the target species, *M. hellenica* (95%), the difference was not significant for *Neoleucopis* n. sp. *B* (58.8% grew on the non-target vs. 80% on the target species) (Figure 2, Table S1).

Table 1. Analysis of deviance for the results of the logistic regressions analyzing the effect of predator, prey, and their interaction on larval growth and survival to the adult stage.

Explanatory Variable	χ^2	df	p
	Larval growth		
Predator	1.966	1, 76	0.1609
Prey	24.463	1, 76	<0.0001 *
Predator × Prey	7.726	1, 76	0.0054 *
	Survival to adult		
Predator	0.407	1, 76	0.5236
Prey	64.548	1, 76	<0.0001 *
Predator × Prey	<0.001	1, 76	1.0000

Asterisks declare significant difference.

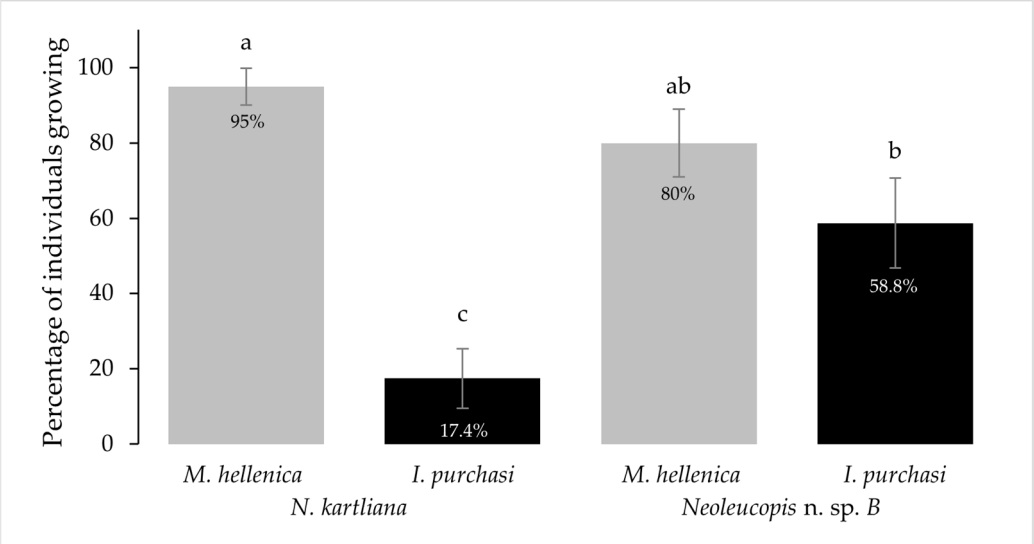

Figure 2. Bar plot of the percentage (means ± SE) of *N. kartliana* (left) and *Neoleucopis* n. sp. *B* individuals (right) growing when provided only the non-target scale insect (*I. purchasi*) (black bars) and only the target scale insect (*M. hellenica*) (grey bars) as food sources during prey specificity experiments. Error bars denoting standard error are incorporated, and significance levels are indicated. Means denoted by the same letter are not significantly different (Tukey's HSD test at $p = 0.05$).

Conversely, only the prey species demonstrated a significant effect on the survival of *Neoleucopis* spp., and no significant interaction effect was observed on survival between "predator" and "prey" (Table 1). Both predators displayed significantly higher survival

on the target species (100%) compared to the non-target (*Neoleucopis* n. sp. *B*: 17.65%, *N. kartliana*: 26.09%) (Figure 3, Table S1).

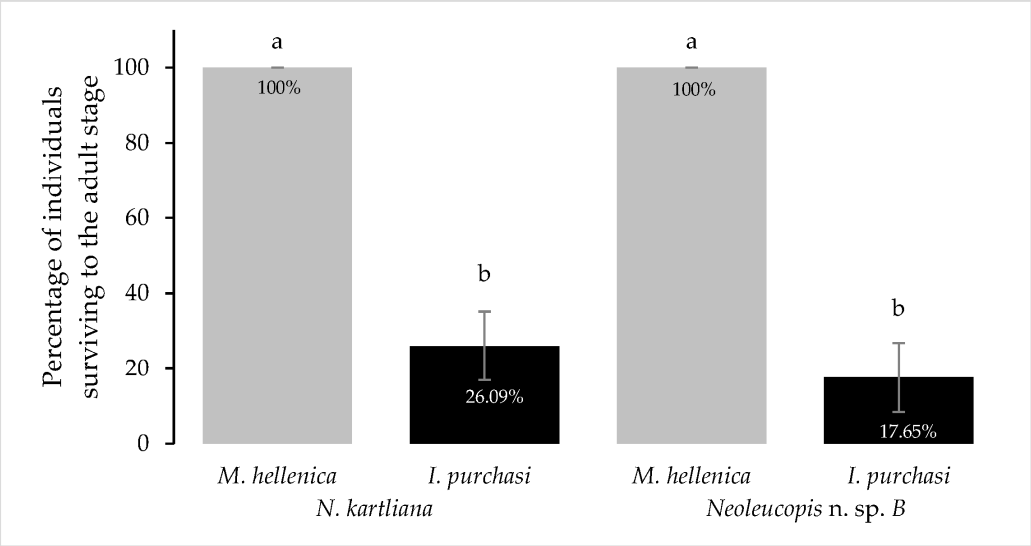

Figure 3. Bar plot of the percentage (means ± SE) of *N. kartliana* (left) and *Neoleucopis* n. sp. *B* individuals (right) that survived to the adult stage when provided only the non-target scale insect as a food source (*I. purchasi*) (black bars) and only the target scale insect (*M. hellenica*) (grey bars) during prey specificity experiments. Error bars denoting standard error are incorporated, and significance levels are indicated. Means denoted by the same letter are not significantly different (Tukey's HSD test at $p = 0.05$).

The parameter estimates of the two logistic regression models performed indicated that the food source significantly influences survival, with *M. hellenica* showing a notably positive effect compared to *I. purchasi*, enhancing the probability of survival for the *Neoleucopis* lineages tested. Specifically, the odds of survival were 6.166 times higher when provided with *M. hellenica* compared to *I. purchasi* (beta coefficient = 1.818; $p < 0.001$; odds ratio = 6.166; 95% confidence interval: 2.823–16.202) (Table 2).

Table 2. Parameter estimates of the binomial logistic regression models upon applying the backward elimination technique and retaining only the statistically significant independent variables for the responses of survival and growth of *Neoleucopis* spp.

Response	Covariate	Estimate	Significance	Odds Ratio	95% Confidence Interval of Odds Ratio
Survival	Intercept	−1.819	<0.001 *	0.162	(0.064, 0.328)
			Food source (ref. category: *I. purchasi*)		
	M. hellenica	1.818	<0.001 *	6.166	(2.823, 16.202)
Growth	Intercept	−0.752	0.003 *	0.471	(0.274, 0.760)
			Neoleucopis spp. (ref. category: *N. kartliana*)		
	Neoleucopis n. sp. *B*	0.450	0.082	1.568	(0.949, 2.635)
			Food source (ref. category: *I. purchasi*)		
	M. hellenica	0.501	0.058	1.615	(0.991, 2.825)

Asterisks declare significant differences.

In terms of larval growth, both the *Neoleucopis* lineage and the food source were found to have marginally significant effects. *Neoleucopis* n. sp. *B* exhibited a slightly higher probability of growth compared to the reference lineage, *N. kartliana*, with an odds ratio of 1.568 (beta coefficient = 0.450; $p < 0.1$; odds ratio = 1.568; 95% confidence interval: 0.949–2.635). Similarly, *M. hellenica* as a food source was associated with a greater likelihood

of growth compared to *I. purchasi*, with an odds ratio of 1.615 (beta coefficient = 0.501; $p < 0.1$; odds ratio = 1.615; 95% confidence interval: 0.949–2.635) (Table 2).

3.3. Field Surveys

No *Neoleucopis* spp. were detected during the inspection of *P. tobira* branches infested with *I. purchasi* collected from the field containing 89 ovisacs. Furthermore, *P. tobira* branches, hosting *I. purchasi* and placed in small, well-aerated containers, failed to yield any *Neoleucopis* n. sp. *B* or *N. kartliana* adults upon examination. In stark contrast, branches infested with *M. hellenica*, sourced from the same location and time bearing 24 ovisacs, revealed a notable presence of 25 *Neoleucopis* spp. larvae upon visual sample inspection (Figure S1, Table S2).

4. Discussion

Although the integration of molecular tools has greatly contributed to the initial detection of cryptic speciation that may ultimately lead to the description of new species [30], conclusions should always be drawn with cautiousness for multiple reasons [31]. The wide range of average intraspecific pairwise nucleotide differences recovered for many species does not support the occurrence of universal numerical thresholds beyond which species could be delimited solely by DNA barcoding [32,33]. Additionally, inferences based only on a single marker, most commonly a mtDNA marker, can at times be misleading [34]. In the current study, pairwise nucleotide differences between *Neoleucopis kartliana* and *Neoleucopis* n. sp. *B* exhibited an average value of 5.2%. This, coupled with the distinct morphological differences observed in the male terminalia, raises questions on their taxonomic status. Nevertheless, the distinction between the two *Neoleucopis* lineages studied here and the identification of *Neoleucopis* n. sp. *B* fall beyond the scope of this research.

The prey preference exhibited by both *Neoleucopis* spp. (*N. kartliana* and *Neoleucopis* n. sp. *B*) in our experiments is evident in their marked preference towards *M. hellenica* eggs compared to the eggs of the non-target species, *I. purchasi*, revealing a selective feeding behavior. This pronounced preference is reflected across various aspects of the parameters that were studied. Firstly, during the prey specificity experiments, the larvae of *Neoleucopis* n. sp. *B* and *N. kartliana* were observed to prey exclusively upon *M. hellenica* eggs, demonstrating a preference for this target species. In contrast, a notable absence of feeding on *I. purchasi* eggs by either predator further underscores the probability of their prey selection. In previous research, *Leucopina bellula* (Williston) (Diptera: Chamaemyiidae) demonstrated similar results when tested for its predation behavior on both target (*Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae)) and non-target insect species (*Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae), *I. purchasi*, *Icerya seychellarum* (Westwood) (Hemiptera: Monophlebidae), *Phenacoccus solenopsis* Tinsley, *Planococcus citri* Risso, and *Pseudococcus viburni* Signoret (Hemiptera: Pseudococcidae)) [35]. The results suggested that no immature specimens preyed or developed on these non-target species. However, *L. bellula* larvae successfully preyed and developed successfully on the target insect *D. opuntiae* [35].

Secondly, *N. kartliana* exhibited a significantly higher probability of growth when feeding on the target species compared to when it was supplied with the non-target species. However, a higher probability of growth on the target species was not significant for *Neoleucopis* n. sp. *B*. The larvae of the latter predator produced red-hued excrements when provided with the non-target species, in contrast to the transparent or yellow-hued excrements produced when preying on the target species. This hue was likely due to the body pigmentation of *I. purchasi*, suggesting that the larvae had preyed upon the non-target species. These red-hued excrements were not produced by *N. kartliana*, suggesting that *N. kartliana* had not fed upon *I. purchasi.* The dietary preferences of insects encompass a wide array of food sources, leading to diverse fecal compositions [36,37]. It is expected that the form, texture, and color of fecal matter would vary in response to changes in an insect's diet, with successive pellets from the same individual potentially exhibiting alterations based on recent meals [37]. The assumption that excrements display the coloration of consumed prey after feeding has also

been considered for *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) when feeding upon nymphs and adults of *Adelges tsugae* Annand (Hemiptera: Adelgidae) [38]. The fact that the probability of *Neoleucopis* n. sp. *B* growth was not significantly different between the target and non-target prey may also suggest a certain degree of feeding on the non-target species, underscoring the intricate dynamics of predator–prey interactions. Nevertheless, it has previously been noted that irrespective of whether growth manifests as continuous or discontinuous, the alignment between consumption rates and growth rates within an instar is typically not closely observed [39]. For example, Zheng et al. [40] subjected larvae of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) to varying dietary regimes involving optimal or suboptimal quantities of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs across different larval instars. The results revealed that larvae with suboptimal food supplies during the first instar exhibited significantly prolonged developmental times, reduced weight gain, and a marginally lower efficiency of food conversion to body substance compared to those with optimal diets. Conversely, suboptimally fed second instar larvae experienced slightly prolonged development but demonstrated a similar efficiency of food conversion to body substance values to optimally fed counterparts [40].

Thirdly, when feeding on *M. hellenica*, the survival rate of both *Neoleucopis* spp. reached 100%. In contrast, the survival rate was significantly lower when larvae fed on the non-target species. The emergence of some *Neoleucopis* n. sp. *B* and *N. kartliana* adults when exclusively provided with *I. purchasi* raises intriguing considerations. This phenomenon could potentially be ascribed to the larvae being initially collected from *M. hellenica* ovisacs; therefore, they might have been supplied with enough of their natural food source (*M. hellenica*) before the start of the experiments to reach the minimal viable weight for reaching the adult stage [41], suggesting a carryover effect from their natural food source. Should *I. purchasi* be deemed an unsuitable food source for *Neoleucopis* spp., it is plausible that starvation could yield comparable outcomes. In early investigations regarding the dietary requirements for reaching critical and minimal viable weight, Beadle et al. [42] documented that *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) larvae, if deprived of food prior to 70 h after egg laying (AEL), exhibited stunted growth, failed to undergo metamorphosis, and eventually perished several days into the starvation period. Conversely, larvae subjected to starvation after the 70 h AEL mark remained stunted in growth but underwent metamorphosis, resulting in the emergence of diminutive adults. The demise of larvae starved before the 70 h AEL threshold was attributed to their failure to attain minimal viable weight, indicating insufficient body fat reserves necessary for survival through the metamorphic process [42]. Park et al. [43] investigated the effect of starvation on *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae following 5- and 10-day feeding periods. They found that larvae subjected to different feeding durations exhibited distinct survival patterns during starvation. The group that was fed for 5 days and then starved showed sustained survival until approximately 20 days of starvation, followed by a rapid decline. Conversely, the group that was fed for 10 days and then starved experienced a sharp decrease in survival after 20 days of starvation, with a gradual decline thereafter over the 60-day observation period. The authors suggested that longer feeding periods may lead to larger energy reserves, extending survival duration. Nonetheless, the emergence rate for all groups exceeded 96%, indicating a successful completion of the life cycle regardless of starvation conditions [43]. This phenomenon prompts further exploration into the intricate ecological dynamics influencing the survival and developmental stages of *Neoleucopis* spp.

Numerous Chamaemyiidae species seek prey within confined spaces inaccessible to other predators, such as within densely wax-coated substrates, to find the housed aphidoid and coccoid colonies. In contrast, other chamaemyiids, such as those within *Leucopis* sensu stricto, exhibit broader feeding strategies [44]. The genera *Leucopis*, *Neoleucopis*, *Lipoleucopis*, *Cremifana*, and *Leucotaraxis* are adelgid specialists [45]. The native European *Neoleucopis atratula* (Ratzeburg) (Diptera: Chamaemyiidae) is at least genus-specific to *Adelges* spp. (Hemiptera: Adelgidae), particularly *Adelges piceae* (Ratzeburg), *A. merkeri* (Eichhorn), *A. nordmannianae* (Eckstein), and *A. tsugae* Annand [46,47]. *Neoleucopis atratula,* misidenti-

fied as *Leucopis obscura* Haliday, has already been introduced to control *A. piceae* in North America [47]. *Leucotaraxis* (=*Leucopis*) *argenticollis* (Zetterstedt) and *L. piniperda* (Malloch) are native adelgid-specific predators of *Adelges tsugae* Annand (Hemiptera: Adelgidae) in northwest USA and possible biological control agents of *A. tsugae* in the north and east USA [48]. Both *L. argenticollis* and *L. piniperda* exhibit a preference for feeding on *A. tsugae* [49]. The larvae of these flies are most abundant during the egg-laying stages of both generations of *A. tsugae* [50]. Although laboratory experiments under no-choice conditions have demonstrated that both flies can complete development on other adelgid species, their average lifespan and survival to adulthood are notably higher when reared on *A. tsugae* [50]. Similarly, in the current study, *Neoleucopis* n. sp. *B* exhibited non-significant differences in larval growth when preying on either the target or the non-target species, but survival was significantly affected, favoring *M. hellenica* as a food source. Considering the variation in the level of specificity within the Chamaemyiidae family, additional non-target species should be tested to further investigate the prey specificity of the here studied *Neoleucopis* spp. to *M. hellenica*, including through field surveys in Greece or Turkey. Of note, *M. caucasica*, the singular other species within the genus *Marchalina*, which infests *Abies nordmanniana* and *Picea orientalis* in Russia, Armenia, and Georgia [19,51], is known to be preyed on by *N. hadzibeiliae* [15,52]. Given the morphological similarities between *M. hellenica* and *M. caucasica* [19], as well as *N. kartliana* and its closely related species *N. hadzibeiliae* [17], combined with the general feeding patterns observed among chamaemyiids at the genus level, it can be assumed that *N. kartliana* is likely to prey on *M. caucasica* as well, should these two species come into contact. Further investigation of this matter is warranted. Moreover, considering the potential introduction of *Neoleucopis* spp. for the biological control of *M. hellenica* in invaded countries, it is crucial to investigate their prey specificity with multiple native species of the respective regions. This step is essential for the development of a successful biological control program tailored to the unique ecological context of each region.

So far, several chamaemyiids have been utilized as instrumental biological control agents in classical biological control programs throughout the world. Instances include the successful utilization of *N. obscura* against *Pineus boerneri* Annand (Hemiptera: Adelgidae) in Chile [53,54] and *P. pini* Goeze (Hemiptera: Adelgidae) in Hawaii [55] or *Neoleucopis tapiae* Blanchard (Diptera: Chamaemyiidae) against *P. pini* in New Zealand [45,53]. *Neoleucopis kartliana* was purportedly employed as a successful biological control agent against *M. hellenica* on the island of Ischia, where the scale became a pest after its introduction, highlighting the potential efficacy of chamaemyiids in managing invasive pests [18]. However, the absence of molecular analyses on the *Neoleucopis* species introduced in Italy underscores a critical knowledge gap. The lack of clarity regarding the precise identity of the introduced species in Italy, be it *N. kartliana*, *Neoleucopis* n. sp. *B*, or a combination of both, poses a challenge in identifying an optimal biological control agent for regions where *M. hellenica* has become invasive. Resolving this taxonomic ambiguity through comprehensive molecular analyses is indispensable for informed decision-making in devising effective and tailored biological control strategies.

The findings of this study indicate a discernible level of specificity exhibited by *Neoleucopis* n. sp. *B* and *N. kartliana* towards *Marchalina* sp. in Greece. This aligns with previous assumptions made for *N. kartliana*, recognized as a potential biological control agent against *M. hellenica* in Australia [11]. The co-occurrence of *N. kartliana* and *Neoleucopis* n. sp. *B* in northern Greece hints at a synergistic relationship, potentially enhancing the efficacy in suppressing *M. hellenica* population growth and maintaining ecological equilibrium within its natural habitat. Consequently, *Neoleucopis* n. sp. *B*, *N. kartliana*, or both, could be viable candidates for classical biological control against *M. hellenica* in Australia or other invaded regions. Such an approach holds promise for alleviating the impact of invasive species, aligning with broader goals of ecological sustainability.

The potential success of *Neoleucopis* spp. in managing *M. hellenica* underscores a crucial contribution to sustainable ecological practices. The efficient suppression of invasive species

not only safeguards the health of local ecosystems but also mitigates potential cascading effects on biodiversity. The significance of our results extends beyond immediate pest management, pointing towards a potential paradigm for sustainable ecological preservation. Future prospects involve a comprehensive exploration of the biology, ecology, and prey range of *Neoleucopis* spp., offering a foundation for the development of ecologically sound and effective strategies against invasive species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16072756/s1, Table S1: Larval growth, survival, and sex of each *Neoleucopis* lineage (*N. kartliana* and *Neoleucopis* n. sp. *B*) designated to the eggs of the target (*M. hellenica*) or the non-target (*I. purchasi*) species. Table S2: Total number of *Neoleucopis* spp. larvae encountered on branch samples infested with the non-target (*I. purchasi*) and target (*M. hellenica*) species collected on two occasions in 2022. Figure S1: *Neoleucopis* spp. larvae encountered in *Marchalina hellenica* ovisacs (A), observed to prey on the scale's eggs (B), and the absence of larvae in *Icerya purchasi* ovisacs (C).

Author Contributions: Conceptualization, N.E., N.G.K., M.L.S., M.K., G.L., U.L. and D.N.A.; methodology, N.E., M.L.S., M.K. and D.N.A.; software, N.E., C.M. and M.L.S.; validation, N.E., N.G.K., C.M., M.L.S., M.K., G.L., U.L. and D.N.A.; formal analysis, N.E., N.G.K., M.L.S., M.K., G.L., U.L. and D.N.A.; investigation, N.E., M.L.S. and D.N.A.; resources, N.E. and D.N.A.; data curation, N.E.; writing—original draft preparation, N.E., N.G.K., C.M., M.L.S., M.K., G.L., U.L. and D.N.A.; writing—review and editing, N.E., N.G.K., C.M., M.L.S., M.K., G.L., U.L. and D.N.A.; visualization, N.E. and M.L.S.; supervision, N.G.K. and D.N.A.; project administration, N.E. and D.N.A.; funding acquisition, N.E. and D.N.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Agriculture Victoria, Forest and Wood Products Australia and Australian pine plantation growers, agreement number PNC489–1819.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors extend their gratitude to Stephen D. Gaimari, Plant Pest Diagnostics Center, Department of Food and Agriculture, for his help in the morphological differentiation between the two *Neoleucopis* spp. M.L.S. and M.K. were also supported by CABI with core financial support from its member countries (see https://www.cabi.org/what-we-do/how-we-work/cabi-donors-and-partners/).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Wang, N.; Zhao, Y. Construction of an ecological security pattern in Jiangnan water network area based on an integrated approach: A case study of Gaochun, Nanjing. *Ecol. Indic.* **2024**, *158*, 111314. [CrossRef]
2. SAPEA. Science Advice for Policy by European Academies 2020. A Sustainable Food System for the European Union. SAPEA: Berlin, Germany. Available online: https://scientificadvice.eu/advice/a%E2%80%93sustainable%E2%80%93food%E2%80%93system%E2%80%93for%E2%80%93the%E2%80%93european%E2%80%93union/ (accessed on 20 January 2024).
3. Dangles, O.; Casas, J. Ecosystem services provided by insects for achieving sustainable development goals. *Ecosyst. Serv.* **2019**, *35*, 109–115. [CrossRef]
4. Xiaoping, C.; Yanqiu, W. Cultivating green growth: The interplay of communication and resource efficiency in East Asia. *Resour. Pol.* **2024**, *90*, 104776. [CrossRef]
5. Saleh, M.; Ashqar, H.I.; Alary, R.; Bouchareb, E.M.; Bouchareb, R.; Dizge, N.; Balakrishnan, D. Biodiversity for ecosystem services and sustainable development goals. In *Biodiversity and Bioeconomy. Status Quo, Challenges, and Opportunities*; Singh, K., Ribeiro, M.C., Calicioglu, Ö., Eds.; Elsevier: Amsterdam, The Netherlands, 2024; pp. 81–110. [CrossRef]
6. Mohan, C.; Robinson, J.; Vodwal, L.; Kumari, N. Sustainable development goals for addressing environmental challenges. In *Green Chemistry Approaches to Environmental Sustainability. Status, Challenges and Prospective*; Garg, V.K., Yadav, A., Mohan, C., Yadav, S., Kumari, N., Eds.; Elsevier: Amsterdam, The Netherlands, 2024; pp. 357–374. [CrossRef]
7. Gounari, S.; Zotos, C.E.; Dafnis, S.D.; Moschidis, G.; Papadopoulos, G.K. On the impact of critical factors to honeydew honey production: The case of *Marchalina hellenica* and pine honey. *J. Apic. Res.* **2021**, *62*, 383–393. [CrossRef]
8. Petrakis, P.V.; Spanos, K.; Feest, A. Insect biodiversity reduction of pinewoods in southern Greece caused by the pine scale (*Marchalina hellenica*). *For. Syst.* **2011**, *20*, 27–41. [CrossRef]
9. Mendel, Z.; Branco, M.; Battisti, A. Invasive sap–sucker insects in the Mediterranean Basin. In *Insects and Diseases of Mediterranean Forest Systems*; Payne, T.D., Lieutier, F., Eds.; Springer International Publishing: Cham, Switzerland, 2016; pp. 261–291. [CrossRef]

10. Masten Milek, T.; Simala, M.; Pintar, M. First record of *Marchalina hellenica* (Gennadius) (Hemiptera: Marchalinidae) in Croatia. In Proceedings of the XV International Symposium on Scale Insect Studies, Zagreb, Croatia, 17–20 June 2019.
11. Avtzis, D.N.; Lubanga, U.K.; Lefoe, G.K.; Kwong, R.M.; Eleftheriadou, N.; Andreadi, A.; Elms, S.; Shaw, R.; Kenis, M. Prospects for classical biological control of *Marchalina hellenica* in Australia. *BioControl* **2020**, *65*, 413–423. [CrossRef]
12. Jaroslow, D. Biology, Ecology and Impact of Giant Pine Scale, *Marchalina hellenica*, in Australia. Doctoral Dissertation, La Trobe University, Victoria, Australia, September 2023.
13. Downham, R.; Gavran, M. Australian Plantation Statistics 2017 Update. Australian Government Department of Agriculture and Water Resources, Canberra, Australia. Available online: https://data.gov.au/data/dataset/a1fcbbec-807c-438e-b8fd-db1a9a93f887 (accessed on 20 January 2024).
14. Ülgentürk, S.; Civelek, H.; Dostbil, Ö. Researches on bioecology of the giant pine scale, *Marchalina hellenica* Gennadius (Hemiptera: Marchalinidae) and relation with its predator *Neoleucopis kartliana* (Tanasijtshuk) (Diptera: Chamaemyiidae). *Mun. Ent. Zool.* **2021**, *16*, 1056–1069.
15. Gaimari, S.D. Two new genera of Nearctic Chamaemyiidae (Diptera: Lauxanioidea) associated with *Cinara aphids* (Hemiptera: Aphididae) on *Pinus*. *Zootaxa* **2020**, *4852*, 61–82. [CrossRef]
16. Nicolopoulos, C. Morphology and biology of the species *Marchalina hellenica* (Gennadius) (Hemiptera Margarodidae Coelostomidiinae). Ph.D. Thesis, Agricultural College of Athens, Athens, Greece, 1965.
17. Gaimari, S.D.; Milonas, P.; Souliotis, C. Notes of taxonomy, biology, distribution of *Neoleucopis kartliana* (Tanasijtshuk) (Diptera: Chamaemyiidae). *Folia Heyrovskyana A* **2007**, *15*, 7–16.
18. Garonna, A.P.; Viggiani, G. Sull'insediamento in Italia di *Neoleucopis kartliana* (Tanasjtshuk) (Diptera: Chamaemyiidae), predatore di *Marchalina hellenica* (Gennadius) (Hemiptera: Margarodidae) [On the establishment in Italy of *Neoleucopis kartliana* (Tanasjtshuk) (Diptera: Chamaemyiidae), predator of *Marchalina hellenica* (Gennadius) (Hemiptera: Margarodidae)]. In Proceedings of the XXIII Congresso Nazionale Italiano di Entomologia [Italian National Congress of Entomology], Genoa, Italy, 13–16 June 2011.
19. Hodgson, C.; Gounari, S. Morphology of *Marchalina hellenica* (Gennadius) (Hemiptera: Coccoidea: Marchalinidae) from Greece, with a discussion on the identity of *M. caucasica* Hadzibeyli from the Caucasus. *Zootaxa* **2006**, *1196*, 1–32. [CrossRef]
20. Mills, P.J.; Lubanga, U.K.; Lefoe, G.K. Scale insect surgery: An unusual twist to standard DNA extractions. *Austral. Entomol.* **2023**, *50*, 369–374.
21. Pellizzari, G.; Chadzidimitriou, E.; Milonas, P.; Stathas, G.J.; Kozar, F. Check list and zoogeographic analysis of the scale insect fauna (Hemiptera: Coccomorpha) of Greece. *Zootaxa* **2015**, *4012*, 57–77. [CrossRef]
22. Unruh, C.M.; Gullan, P.J. Identification guide to species in the scale insect tribe Iceryini (Coccoidea: Monophlebidae). *Zootaxa* **2008**, *1803*, 1–106. [CrossRef]
23. Bartlett, B.R. Margarodidae. In *Introduced Parasites and Predators of Arthropod Pests and Weeds: A World Review*; Clausen, C.P., Ed.; Agricultural Research Service, US Department of Agriculture: Washington, DC, USA, 1978; pp. 132–135.
24. Quezada, J.; DeBach, P. Bioecological and population studies of the cottony–cushion scale, *Icerya purchasi* Mask., and its natural enemies, *Rodolia cardinalis* Mul. and *Cryptochaetum iceryae* Will., in southern California. *Hilgardia* **1973**, *41*, 631–668. [CrossRef]
25. Katsoyannos, P. *Integrated Insect Pest Management for Citrus in Northern Mediterranean Countries*, 1st ed.; Benaki Phytopathological Institute: Athens, Greece, 1996; p. 110.
26. Stathas, G.J.; Skouras, P.J. Biological control on insect pests in citrus orchards in Greece. *IOBC–WPRS Bull.* **2013**, *95*, 1–9.
27. van Lenteren, J.; Cock, M.J.; Hoffmeister, T.S.; Sands, D.P. Host specificity in arthropod biological control, methods for testing and interpretation of the data. In *Environmental Impact of Invertebrates for Biological Control of Arthropods: Methods and Risk Assessment*; Bigler, F., Babendreier, D., Kuhlmann, U., Eds.; CABI Publishing: Wallingford, UK, 2006; pp. 38–63. [CrossRef]
28. Eleftheriadou, N.; Lubanga, U.; Lefoe, G.; Seehausen, M.L.; Kenis, M.; Kavallieratos, N.G.; Avtzis, D.N. Phenology and potential fecundity of *Neoleucopis kartliana* in Greece. *Insects* **2022**, *13*, 143. [CrossRef]
29. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing, R Core Team: Vienna, Austria, 2021.
30. Hendrich, L.; Morinière, J.; Haszprunar, G.; Hebert, P.D.; Hausmann, A.; Köhler, F.; Balke, M. A comprehensive DNA barcode database for Central European beetles with a focus on Germany: Adding more than 3500 identified species to BOLD. *Mol. Ecol. Resour.* **2015**, *15*, 795–818. [CrossRef]
31. Rubinoff, D.; Holland, B.S. Between two extremes: Mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. *Syst. Biol.* **2005**, *54*, 952–961. [CrossRef]
32. Zhang, H.; Bu, W. Exploring large-scale patterns of genetic variation in the COI gene among Insecta: Implications for DNA barcoding and threshold-based species delimitation studies. *Insects* **2022**, *13*, 425. [CrossRef]
33. Cognato, A.I.; Sun, J.H. DNA based cladograms augment the discovery of a new *Ips* species from China (Coleoptera: Curculionidae: Scolytinae). *Cladistics* **2007**, *23*, 539–551. [CrossRef]
34. Žurovcová, M.; Havelka, J.A.N.; Starý, P.E.T.R.; Věchtová, P.; Chundelova, D.; Jarošová, A.; Kučerová, L. "DNA barcoding" is of limited value for identifying adelgids (Hemiptera: Adelgidae) but supports traditional morphological taxonomy. *Eur. J. Entomol.* **2010**, *107*, 147–156. [CrossRef]
35. Mendel, Z.; Protasov, A.; Vanegas-Rico, J.M.; Lomeli-Flores, J.R.; Suma, P.; Rodríguez-Leyva, E. Classical and fortuitous biological control of the prickly pear cochineal, *Dactylopius opuntiae*, in Israel. *Biol. Control* **2020**, *142*, 104157. [CrossRef]
36. Gullan, P.J.; Cranston, P.S. *The Insects: An Outline of Entomology*, 5th ed.; Wiley Blackwell: Canberra, Australia, 2014; p. 470.

37. Weiss, M.R. Defecation behavior and ecology of insects. *Annu. Rev. Entomol.* **2006**, *51*, 635–661. [CrossRef]
38. Zilahi–Balogh, G.M.G. Biology of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) and Its Potential as a Biological Control Agent of the Hemlock Woolly Adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae) in the Eastern United States. Ph.D. Thesis, Virginia Tech, Blacksburg, VA, USA, 9 November 2001. Virginia Teck. Available online: http://hdl.handle.net/10919/29737 (accessed on 20 January 2024).
39. Farrar, R.R.; Barbour, J.D.; Kennedy, G.G. Quantifying food consumption and growth in insects. *Ann. Entomol. Soc. Am.* **1989**, *82*, 593–598. [CrossRef]
40. Zheng, Y.; Hagen, K.S.; Daane, K.M.; Mittler, T.E. Influence of larval dietary supply on the food consumption, food utilization efficiency, growth and development of the lacewing *Chrysoperla carnea*. *Entomol. Exp. Appl.* **1993**, *67*, 1–7. [CrossRef]
41. Mirth, C.K.; Riddiford, L.M. Size assessment and growth control: How adult size is determined in insects. *Bioessays* **2007**, *29*, 344–355. [CrossRef]
42. Beadle, G.W.; Tatum, E.L.; Clancy, C.W. Food level in relation to rate of development and eye pigmentation in *Drosophila melanogaster*. *Biol. Bull.* **1938**, *75*, 447–462. [CrossRef]
43. Park, K.; Lee, H.S.; Goo, T.W. Influence of starvation on the larval development of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *Int. J. Indust. Entomol.* **2018**, *37*, 100–104.
44. Gaimari, S.D. Chamaemyiidae. In *Manual of Afrotropical Diptera. Brachycera—Cyclorrhapha, Excluding Calyptratae*; Kirk–Spriggs, A.H., Sinclair, B.J., Eds.; South African National Biodiversity Institute: Pretoria, South Africa, 2021; pp. 1791–1814.
45. Motley, K. Assessing the Efficacy of Two Species of Silver Fly, *Leucopis argenticollis* and *L. piniperda*, as Biological Control Agents of Hemlock Woolly Adelgid, *Adelges tsugae*. M. Sc. Thesis, University of Vermont and State Agricultural College, Burlington, VT, USA, 8 August 2017. ProQuest. Available online: https://www.proquest.com/openview/e680f4dd0ab01befdca0ca7a76d2ab02/1?pq%E2%80%93origsite=gscholar&cbl=18750 (accessed on 20 January 2024).
46. Neidermeier, A.N.; Ross, D.W.; Havill, N.P.; Wallin, K.F. 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. *Environ. Entomol.* **2020**, *49*, 823–828. [CrossRef]
47. Ravn, H.P.; Havill, N.P.; Akbulut, S.; Foottit, R.G.; Serin, M.; Erdem, M.; Mutun, S.; Kenis, M. *Dreyfusia nordmannianae* in Northern and Central Europe: Potential for biological control and comments on its taxonomy. *J. Appl. Entomol.* **2013**, *137*, 401–417. [CrossRef]
48. Dietschler, N.J.; Bittner, T.D.; Trotter III, R.T.; Fahey, T.J.; Whitmore, M.C. Biological control of hemlock woolly adelgid: Implications of adult emergence patterns of two *Leucopis* spp. (Diptera: Chamaemyiidae) and *Laricobius nigrinus* (Coleoptera: Derodontidae) larval drop. *Environ. Entomol.* **2021**, *50*, 803–813. [CrossRef]
49. Kohler, G.R.; Stiefel, V.L.; Wallin, K.F.; Ross, D.W. Predators associated with the hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environ. Entomol.* **2008**, *37*, 494–504. [CrossRef]
50. Grubin, S.M.; Ross, D.W.; Wallin, K.F. Prey suitability and phenology of *Leucopis* spp. (Diptera: Chamaemyiidae) associated with hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environ. Entomol.* **2011**, *40*, 1410–1416. [CrossRef]
51. Jashenko, R.V. Fauna, natural enemies, agricultural harm and possibility of industrial use of margarodids (Coccinea, Margarodidae) in East Europe and North Asia. *Selevinia* **1999**, *10*, 43–50.
52. Tanasijtshuk, V.N. Мухи–серебрянки (Chamaemyiidae) [Silver–flies (Chamaemyiidae)]. In *фауна* СССР. Насекомые двукрылые *[Fauna of the USSR. New Series 134, Dipterans]*; Zoological Institute of the Russian Academy of Sciences, Nauka Publishers: Leningrad, Russia, 1986; Volume 14, pp. 1–335.
53. Gaimari, S.D.; Havill, N.P. A new genus of Chamaemyiidae (Diptera: Lauxanioidea) predaceous on Adelgidae (Hemiptera), with a key to chamaemyiid species associated with Pinaceae–feeding Sternorrhyncha. *Zootaxa* **2021**, *5067*, 1–39. [CrossRef]
54. Mills, N.J. Biological control of forest aphid pests in Africa. *Bull. Entomol. Res.* **1990**, *80*, 31–36. [CrossRef]
55. Culliney, T.W.; Beardsley, J.W., Jr.; Drea, J.J. Population regulation of the Eurasian pine adelgid (Homoptera: Adelgidae) in Hawaii. *J. Econ. Entomol.* **1988**, *81*, 142–147. [CrossRef]