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Evaluation of *Leucopis hennigrata* (Diptera: Chamaemyiidae) as a classical biological control agent of *Adelges nordmannianae* (Hemiptera: Adelgidae) in northern Europe

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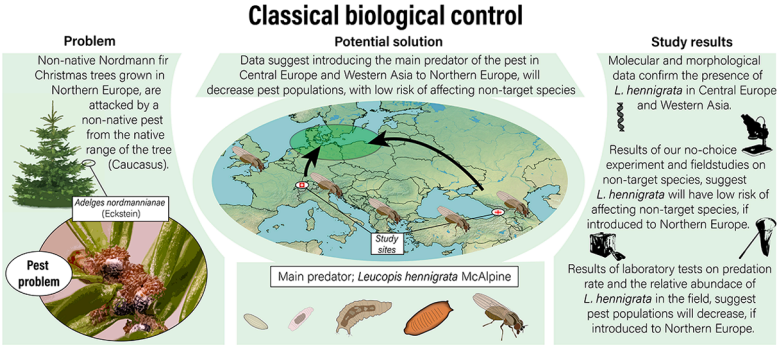
HIGHLIGHTS

- *Leucopis hennigrata* is a promising candidate for biological control of *Adelges nordmannianae* in Northern Europe.
- *L. hennigrata* is a key predator of *Ad. nordmannianae* in Western Asia and Central Europe.
- Under laboratory conditions larvae of *L. hennigrata* killed 57–117 eggs in 24 h.
- Adult *L. hennigrata* oviposited on various non-target species, but rarely completed development.
- *Leucopis hennigrata* was not found from non-target adelgids or aphids in the field.

GRAPHICAL ABSTRACT



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ABSTRACT

The silver fly *Leucopis hennigrata* McAlpine is a predator of the silver fir wooly adelgid, *Adelges nordmannianae* (Eckstein), a pest in the European production of Christmas trees. While the fly is known to be a common native predator of the adelgid in Georgia, Turkey, and central Europe, it is absent from northern Europe, where the Christmas tree production is an important industry. Therefore, *L. hennigrata* has been suggested as a classical biological control agent of *Ad. nordmannianae* in northern Europe. Here, we evaluated the fly's suitability as a classical biological control agent in terms of (1) differences between Asian and European populations, (2) its abundance relative to other predators of *Ad. nordmannianae*, (3) larval feeding efficacy, as well as (4) the physiological and (5) ecological prey preference of the fly. The results showed that despite genetic divergence between Western Asian and Central European populations, *L. hennigrata* is a dominant natural enemy of *Ad. nordmannianae* in Switzerland, the Pontic mountains in northern Turkey, and in the Greater and Lesser Caucasus mountains. Larvae of *L. hennigrata* killed on average 57–117 eggs in 24 h, depending on their size, the number of

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eggs offered to them, and possibly some environmental factors. In no-choice tests, adult flies oviposited on twigs containing different non-target adelgid, aphid, and scale insect species. Larvae of *L. hennigrata* were observed to feed on several of them. However, only the non-target species *Mindarus abietinus* Koch supported development of a single specimen to the adult stage. During field surveys in Switzerland and Georgia, *L. hennigrata* was never found on any sampled non-target species. Based on our findings, we conclude that *L. hennigrata* is a suitable classical biological control agent because it (1) is already present in countries that neighbor northern Europe, (2) is a dominant natural enemy of the pest in Switzerland, (3) is an efficient predator of the pest's eggs, and (4) seems to be specific *to Ad. nordmannianae* in its ecological range.

1. Introduction

The European production of Christmas trees is an important industry that has experienced a steep increase in production within the past several decades. The annual turnover for the industry is approximately 1.5 billion Euros (CTGCE, 2016). The main production of Christmas trees in Europe occurs in Germany (25%), Denmark (20%), Poland (11%), and Great Britain (10%), henceforth defined as northern Europe. Other countries with noteworthy production not included in this definition are France (8%), Belgium (7%), Austria (4%), and Hungary (4%) (CTGCE, 2016). Close to 80 million commercial natural Christmas trees are produced every year, of which 45 million are *Abies nordmanniana* (Spach) (Pinaceae) (CTGCE, 2016).

As in other monocultures, Christmas tree plantations harbor insect pests that cause considerable economic losses. In Europe, the most significant pest in Christmas tree plantations is the silver fir wooly adelgid *Adelges* (*Dreyfusia*) *nordmannianae* (Eckstein) (Hemiptera: Adelgidae). This species probably originates from the Pontic mountains in northern Turkey and the Greater and Lesser Caucasus mountains (Eichhorn, 1967a,b; Eichhorn, 1969a; Eichhorn, 1991; Ravn et al., 2012). Using population genetic data, Havill et al. (2020) proposed that during one of the recent mild interglacial periods, an ancestor of *Ad. nordmannianae* expanded to Europe but then was isolated in Europe away from the primary *Picea* Mill. (Pinaceae) host in the Caucasus region. These isolated populations continued to produce asexual generations on European *Abies* species, and over time they evolved into the closely related species, the balsam woolly adelgid, *Adelges (Dreyfusia) piceae* (Ratzeburg). However, during the late 1800 s *Ad. nordmannianae* was accidentally (re-) introduced to Europe on imported *Ab. nordmanniana* trees (Ravn et al., 2012; Havill et al., 2020). Both adelgids were also accidentally introduced in North America, where *Ad. piceae* in particular has caused extensive damage and death of fir trees (Montgomery & Havill, 2014; Havill et al., 2020). In a recent taxonomic revision of the species complex to which these adelgids belong, it has been shown that the two species can be separated genetically using microsatellites and also morphologically by the number of facets on the wax glands of first instars, however, hybridization between the species is known to occur (Havill et al., 2020).

Like other members of Adelgidae, *Ad. nordmannianae* has a complex life cycle alternating between sexual reproduction and gall formation on primary *Picea* hosts and asexual reproduction on secondary coniferous hosts. The primary hosts of *Ad. nordmannianae* are *Picea orientalis* L. Link (Pinaceae) (Varty, 1956; Pschorn-Walcher & Zwölfer, 1958; Eichhorn, 1967a) and *P. omorika* (Pančić) Purk. (Pinaceae) (Eichhorn, 1975). The secondary hosts of *Ad. nordmannianae* are *Ab. nordmanniana* and several other species within the genus *Abies* Mill. (Pinaceae) (Bryant, 1974; Havill et al., 2020). For a general overview of the adelgid life cycle see Havill & Foottit (2007) and for a more detailed description of *Ad. nordmannianae* biology, see Marchal (1913), Varty (1956), Eichhorn (1967a), or Eichhorn (1991).

Because the primary *Picea* hosts that are necessary for completing the sexual generation only occur in Europe as rare ornamentals, *Ad. nordmannianae* is believed to reproduce mostly asexually in this part of the world (Varty, 1956; Eichhorn, 1967a; Bryant, 1974; Havill et al., 2020). Winged individuals that are able to spread longer distances require the presence of the main *Picea* host, because only the sexuales can produce fundatrix nymphs. Therefore, it is believed that the spread of *Ad. nordmannianae* mainly occurs by the exulis first-instar crawlers, for example by wind (Clark et al., 1971; Bryant, 1974) or vectored via animals such as birds or humans (Atkins & Wood, 1968; McClure, 1990; Russo et al., 2019).

In European forests, *Ad. nordmannianae* reaches only locally high population levels, which occasionally leads to the death of young trees (Pschorn-Walcher & Zwölfer, 1958). In the Christmas tree industry, however, *Ad. nordmannianae* is regarded as the main pest (Ravn et al. 2012) because progredientes often occur in high numbers on the newly burst needles of *Ab. nordmanniana* trees, where their feeding causes the needles to curl (Pschorn-Walcher & Zwölfer, 1958), ultimately rendering the trees unsellable. In response to those damages, substantial amounts of pesticides are applied to the trees, which effectively reduces pest populations and damage (Christensen, 1994; Ravn et al., 2012). However, as concerns about negative impacts of insecticides for the environment and human health arise in the European Union, synthetic pyrethroids that have been traditionally used as insecticides in Christmas tree plantations have been banned, reduced, or highly taxed in Denmark since 2012 (Christensen, 2015). Thus, alternative sustainable methods are needed to control *Ad. nordmannianae*. Because this insect is an exotic species in Europe and may at least in some areas have arrived without its natural enemy complex, a classical biological control program, involving the introduction of a natural enemy from the area of origin, has been considered a viable option.

A literature review paired with field work in Georgia, Turkey, and Russia identified the predatory silver fly *Leucopis hennigrata* McAlpine (Diptera: Chamaemyiidae) as a suitable candidate for evaluation as a classical biological control agent in northern Europe (Ravn et al., 2012). It is a univoltine species. Eggs are laid underneath or adjacent to *Ad. nordmannianae* egg-clusters, mainly in April and May, and the larvae feed on all life stages of *Ad. nordmannianae* from April to June and then pupariate in the soil from May–June. Adults emerge in August–September and probably overwinter to mate and lay eggs in the following spring (biology reviewed by McAlpine, 1978). *Leucopis hennigrata* has been confirmed predating on *Ad. nordmannianae* in Greece (Eichhorn, 1966), Turkey (Eichhorn, 1969b; Ravn et al., 2012), Georgia, the Russian part of the Caucasus (Ravn et al., 2012), France (Wylie, 1958; Eichhorn, 1964), and central and eastern Europe (Eichhorn et al., 1962; Eichhorn, 1966; Ebejer et al., 2019), but was not found in Sweden (Pschorn-Walcher & Kraus, 1958) or Denmark (Ravn & Riis-Nielsen, 2006; Ravn et al., 2012).

Leucopis hennigrata has also been introduced to eastern and western Canada as a classical biological control agent against *Ad. piceae* in the late 1950 s and 1960 s. Clark et al. (1971) reported recoveries in the eastern introductions, but not the western, although the species was collected in western North America both before and after introductions. McAlpine (1978) had noted that the species was collected earlier in Alberta, Canada, suggesting that the species occurred there independent of the introductions. The species may have arrived with the pest around the beginning of the 20th century (Kotinsky, 1916), which is further corroborated by several specimens seen by author SG in the Canadian National Collection of Insects, Ottawa, Ontario.

The identification of the silver fly as a suitable biological control

agent in northern Europe can be summarized in the following points: (1) In the pest's area of origin, *L. hennigrata* is present in relatively high densities but is absent from northern Europe; (2) contrary to other predators, *L. hennigrata* is present especially on twigs and may therefore reduce the curling of needles, which reduces the economic value of Christmas trees; (3) the univoltinism of the species may lead to fewer problems in regard to synchronization with the target pest in the relatively cold climate of northern Europe; and (4) silver flies were suggested to be valuable biological control agents in general (Satar et al., 2015) and specifically the genus *Leucopis* has previously been used successfully as biological control agents against other adelgid species (Greathead, 1995; Zilahi-Balogh et al., 2002; Ross et al., 2012).

Here, we further investigated the potential of *L. hennigrata* as a classical biological control agent against *Ad. nordmannianae* in Christmas tree plantations of northern Europe by studying (1) differences between Asian and European populations of *L. hennigrata*, (2) the silver fly's abundance relative to other predators of *Ad. nordmannianae*, (3) the feeding efficacy of *L. hennigrata* larvae, as well as (4) the physiological and (5) ecological prey preference of the fly.

2. Material and methods

2.1. Genetic and morphological analyses

The identification of *Ad. nordmannianae* in this study relies on samples identified using genetic markers (microsatellites) and morphological characteristics from *Abies* sp. trees collected at earlier dates from our study sites in Georgia and Switzerland (see Havill et al. 2020). In Georgia, we identified 46 specimens from 17 different *Abies nordmanniana* trees and in Switzerland 31 specimens from 15 different *Abies alba* trees. These trees were more or less equally distributed among the investigated localities. Only *Ad. nordmannianae* was found in Georgia. In Europe, there is a mix of *Ad. nordmannianae*, the closely related species *Ad. piceae,* and rare hybrids between the species (Havill et al., 2020). On *Abies* hosts in Europe, twigs are the typical feeding site for *Ad. nordmannianae*, and both species can be found on the trunk. For our experiments, we focused on *Adelges* attacking twigs, which is the stage that causes the damage to Christmas trees in Denmark. In all experiments, specimens from the observed tree, or a tree in the close vicinity that also had adelgids on the twigs, was sampled and identified as *Ad. nordmannianae*. In the case of adelgid samples from tree trunks, where we did not identify the species, we refer to them as *Ad. nordmannianae/piceae*.

To determine genetic differences between *L. hennigrata* from different regions in Europe, we compared sequences from the DNA barcode region of the *cytochrome c oxidase subunit I* (*COI*) gene between specimens collected in Switzerland (n = 80) and Georgia (n = 28). Additionally, *COI* sequences from *L. hennigrata* specimens collected in Turkey (n = 7) and England (n = 1) were added to compare them with sequences identified in this study. DNA was extracted using the MagBind Blood and Tissue Kit (Omega Bio-Tek, Norcross, GA). Each specimen was pierced with a sterile insect pin, and retained as a voucher after proteinase K incubation. Larvae were slide mounted in Canada balsam. The 651 base-pair DNA barcoding portion of the *COI* gene was amplified using primers LepF1 and LepR1 (Hebert et al., 2004), using standard protocols (deWaard et al., 2008). Sequencing was performed at the Yale University DNA Analysis Facility on Science Hill on an ABI 3730 sequencer. Specimen vouchers were deposited at the Yale Peabody Museum (YPM) of Natural History and sequences were deposited in GenBank. Supplementary Table A1 shows the collection, voucher, and GenBank accession information for *L. hennigrata* specimens included in this study, as well as for the other predator and prey specimens described below.

Pairwise uncorrected p-distances among *COI* haplotypes were calculated using PAUP* v.4.0a168 and clustering among haplotypes was visualized using a neighbor joining tree with midpoint rooting. Bootstrap support was calculated with 1000 pseudo-replicates.

Table A1
Collection and voucher information for *Leucopis hennigrata* specimens included in the DNA barcode analysis. Specimen vouchers were deposited at the Peabody Museum of Natural History, Yale University, New Haven, Connecticut, USA (YPM) or the California State Collection of Arthropods, Sacramento, California, USA (CSCA).

Sample number	Collection Information	Life Stage	Specimen Voucher No.	GenBank Accession No.
18–746-01	SWITZERLAND: Jura, Delémont; Develier; 47.3604, 7.2622; coll. Mathias Just Justesen; ex. *Abies alba*; 11 May 2018	larva	YPM#ENT961299	OP950434
18–746-02	SWITZERLAND: Jura, Delémont, Develier; 47.3604, 7.2622; coll. Mathias Just Justesen; ex. *Abies alba*; 11 May 2018	larva	YPM#ENT961300	OP950435
18–746-03	SWITZERLAND: Jura, Delémont, Develier; 47.3604, 7.2622; coll. Mathias Just Justesen; ex. *Abies alba*; 11 May 2018	larva	YPM#ENT961301	OP950436
18–746-04	SWITZERLAND: Jura, Delémont, Develier; 47.3604, 7.2622; coll. Mathias Just Justesen; ex. *Abies alba*; 11 May 2018	larva	YPM#ENT961302	OP950437
18–746-05	SWITZERLAND: Jura, Delémont, Develier; 47.3604, 7.2622; coll. Mathias Just Justesen; ex. *Abies alba*; 11 May 2018	larva	YPM#ENT961303	OP950438
18–746-06	SWITZERLAND: Jura, Delémont, Develier; 47.3604, 7.2622; coll. Mathias Just Justesen; ex. *Abies alba*; 11 May 2018	larva	YPM#ENT961304	OP950439
18–746-07	SWITZERLAND: Jura, Delémont, Develier; 47.3604, 7.2622; coll. Mathias Just Justesen; ex. *Abies alba*; 11 May 2018	larva	YPM#ENT961305	OP950440
18–747-01	SWITZERLAND: Jura, Delémont, Develier; 47.3604, 7.2622; coll. Mathias Just Justesen; ex. *Abies alba*; 11 May 2018	larva	YPM#ENT961306	OP950441
18–747-02	SWITZERLAND: Jura, Delémont, Develier; 47.3604, 7.2622; coll. Mathias Just Justesen; ex. *Abies alba*; 11 May 2018	larva	YPM#ENT961307	OP950442
18–748-01	SWITZERLAND: Jura, Delémont, Seehof; 47.3026, 7.4976; coll. Mathias Just Justesen; ex. *Abies alba*; 14 May 2018	larva	N/A	OP950443
18–748-02	SWITZERLAND: Jura, Delémont, Seehof; 47.3026, 7.4976; coll. Mathias Just Justesen; ex. *Abies alba*; 14 May 2018	larva	YPM#ENT961308	OP950444
18–749-01	SWITZERLAND: Jura, Delémont, Seehof;	larva	YPM#ENT961309	OP950445

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Table A1 (*continued*)

Sample number	Collection Information	Life Stage	Specimen Voucher No.	GenBank Accession No.
	47.3371, 7.4426; coll. Mathias Just Justesen; ex. *Abies alba*; 14 May 2018			
18–750-01	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 15 May 2018	larva	YPM#ENT961310	OP950446
18–750-02	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 15 May 2018	larva	YPM#ENT961311	OP950447
18–750-03	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 15 May 2018	larva	YPM#ENT961312	OP950448
18–751-01	SWITZERLAND: Jura, Delémont, Bourrignon; 47.382, 7.2389; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	YPM#ENT961313	OP950449
18–751-02	SWITZERLAND: Jura, Delémont, Bourrignon; 47.382, 7.2389; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	YPM#ENT961314	OP950450
18–752-01	SWITZERLAND: Jura, Delémont, Bourrignon; 47.382, 7.2389; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	YPM#ENT961282	OP950451
18–752-02	SWITZERLAND: Jura, Delémont, Bourrignon; 47.382, 7.2389; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	YPM#ENT961283	OP950452
18–753-01	SWITZERLAND: Jura, Delémont, Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	YPM#ENT961284	OP950453
18–753-02	SWITZERLAND: Jura, Delémont, Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	YPM#ENT961285	OP950454
18–753-03	SWITZERLAND: Jura, Delémont, Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	N/A	OP950455
18–753-04	SWITZERLAND: Jura, Delémont, Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	YPM#ENT961286	OP950456
18–753-05	SWITZERLAND: Jura, Delémont, Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	N/A	OP950457
18–753-06	SWITZERLAND: Jura, Delémont, Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	YPM#ENT961287	OP950458
18–754-01	SWITZERLAND: Jura, Delémont, Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	YPM#ENT961288	OP950459
18–755-01	SWITZERLAND: Jura, Delémont, Bourrignon; 47.382, 7.2386; coll. Mathias Just Justesen; ex. *Abies alba*; 16 May 2018	larva	YPM#ENT961289	OP950460
18–755-02	SWITZERLAND: Jura, Delémont, Bourrignon; 47.382, 7.2386; coll. Mathias Just Justesen; ex. *Abies* alba; 16 May 2018	larva	YPM#ENT961290	OP950461
18–756-01	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961291	OP950462
18–756-02	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961292	OP950463
18–756-03	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961293	OP950464
18–756-04	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961294	OP950465
18–756-05	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961295	OP950466
18–756-06	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961296	OP950467
18–756-07	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961297	OP950468
18–756-08	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961298	OP950469

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Table A1 (*continued*)

Sample number	Collection Information	Life Stage	Specimen Voucher No.	GenBank Accession No.
18–756-09	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961272	OP950470
18–756-10	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961273	OP950471
18–756-11	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961274	OP950472
18–756-12	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961275	OP950473
18–756-13	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961276	OP950474
18–756-14	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961277	OP950475
18–756-15	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961315	OP950476
18–756-16	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961316	OP950477
18–756-17	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	N/A	OP950478
18–756-18	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961317	OP950479
18–756-19	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961318	OP950480
18–756-20	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	N/A	OP950481
18–757-01	SWITZERLAND: Jura, Delémont, Develier; 47.3603, 7.2622; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961278	OP950482
18–758-01	SWITZERLAND: Jura, Delémont; Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961279	OP950483
18–758-02	SWITZERLAND: Jura, Delémont; Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 18 May 2018	larva	YPM#ENT961280	OP950484
18–761-01	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3736, 7.3272; coll. Marc Kenis; ex. *Abies alba*; 5 May 2018	adult	CSCA#18–761-01	OP950485
18–762-01	GEORGIA: Guria, Bakhmaro; 41.85, 42.3167; coll. Mathias Just Justesen; ex. *Abies* nordmanniana; 7 June 2018	adult	CSCA#18–762-01	OP950486
18–762-02	GEORGIA: Guria, Bakhmaro; 41.85, 42.3167; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 7 June 2018	adult	CSCA#18–762-02	OP950487
18–762-03	GEORGIA: Guria, Bakhmaro; 41.85, 42.3167; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 7 June 2018	adult	CSCA#18–762-03	OP950488
18–762-04	GEORGIA: Guria, Bakhmaro; 41.85, 42.3167; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 7 June 2018	adult	CSCA#18–762-04	OP950489
19–002	UNITED KINGDOM: England, Surrey, Woking; Royal Horticultural Society Garden Wisley; 51.313784, −0.475419; coll. Fryni Drizou; ex. *Abies alba*; 10 April 2019	larva	N/A	OP950490
19–032	GEORGIA: Adjara, Tselati; 41.5004, 42.396267; coll. Marc Kenis, Hans Peter Ravn; ex. *Abies nordmanniana*; 6 June 2007	adult	CSCA#10F184	OP950491
19–033	GEORGIA: Adjara, Tselati; 41.5004, 42.3963; coll. Marc Kenis, Hans Peter Ravn; ex. *Abies nordmanniana*; 6 June 2007	adult	CSCA#10F185	N/A
19–034	GEORGIA: Adjara, Tselati; 41.5004, 42.3963; coll. Marc Kenis, Hans Peter Ravn; ex. *Abies nordmanniana*; 6 June 2007	adult	CSCA#10F186	OP950492

(*continued on next page*)

Table A1 (*continued*)

Sample number	Collection Information	Life Stage	Specimen Voucher No.	GenBank Accession No.
19–035	GEORGIA: Bolü, Sultankoy; 40.6746, 26.436; coll. Marc Kenis, Hans Peter Ravn; ex. *Abies nordmanniana*; 14 May 2007	adult	CSCA#10F190	OP950493
19–036	GEORGIA: Bolü, Atyaylasi; 40.7817, 31.4765; coll. Marc Kenis, Hans Peter Ravn; ex. *Abies nordmanniana*; 14 May 2007	adult	CSCA#10F196	OP950494
19–037	GEORGIA: Bolü, Atyaylasi; 40.7817, 31.4765; coll. Marc Kenis, Hans Peter Ravn; ex. *Abies nordmanniana*; 14 May 2007	adult	CSCA#10F197	OP950495
19–038	GEORGIA: Bolü, Atyaylasi; 40.7817, 31.4765; coll. Marc Kenis, Hans Peter Ravn; ex. *Abies nordmanniana*; 14 May 2007	adult	CSCA#10F198	OP950496
19–039	GEORGIA: Bolü, Atyaylasi; 40.7817, 31.4765; coll. Marc Kenis, Hans Peter Ravn; ex. *Abies nordmanniana*; 14 May 2007	adult	CSCA#10F203	OP950497
19–040	GEORGIA: Bolü, Atyaylasi; 40.7817, 31.4765; coll. Marc Kenis, Hans Peter Ravn; ex. *Abies nordmanniana*; 14 May 2007	adult	CSCA#10F204	OP950498
19–041	GEORGIA: Bolü, Atyaylasi; 40.7817, 31.4765; coll. Marc Kenis, Hans Peter Ravn; ex. *Abies nordmanniana*; 14 May 2007	adult	CSCA#10F205	OP950499
20–017-05	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594027	OP950500
20–017-06	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594028	OP950501
20–017-07	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594029	OP950502
20–017-08	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594030	OP950503
20–017-09	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594031	OP950504
20–017-10	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594032	OP950505
20–017-11	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594033	OP950506
20–017-12	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594034	OP950507
20–017-13	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594035	OP950508
20–017-14	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594036	OP950509
20–017-15	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594037	OP950510
20–017-16	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594038	OP950511
20–017-17	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594039	OP950512
20–017-18	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594040	OP950513
20–017-19	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	larva	YPM# ENT594041	OP950514
20–029-01	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3274; coll. Mathias Just Justesen; ex. *Abies alba*; 15 May 2019	adult	CSCA#20–029-01	OP950515
20–029-02	SWITZERLAND: Jura, Delémont, CABI Centre; 47.3733, 7.3274; coll. Mathias Just Justesen; ex. *Abies alba*; 15 May 2019	adult	CSCA#20–029-02	OP950516
20–065-01	GEORGIA: Adjara, Beshumi; 41.6332, 42.5517; coll. Mathias Just Justesen; ex. *Abies*	larva	YPM#ENT594045	OP950517

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Table A1 (*continued*)

Sample number	Collection Information	Life Stage	Specimen Voucher No.	GenBank Accession No.
	nordmanniana; 7 June 2019			
20–065-02	GEORGIA: Adjara, Beshumi; 41.6332, 42.5517; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 7 June 2019	larva	YPM#ENT594046	OP950518
20–065-03	GEORGIA: Adjara, Beshumi; 41.6332, 42.5517; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 7 June 2019	larva	YPM#ENT594047	OP950519
20–071-01	SWITZERLAND: Jura, Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 15 April 2020	larva	YPM# ENT594017	OP950520
20–071-02	SWITZERLAND: Jura, Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies* alba; 15 April 2020	larva	YPM# ENT594018	OP950521
20–072-01	SWITZERLAND: Jura, Delémont; 47.3733, 7.3276; coll. Mathias Just Justesen; ex. *Abies alba*; 27 April 2020	larva	YPM#ENT996194	OP950522
20–080-01	SWITZERLAND: Jura, Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 28 April 2020	larva	YPM# ENT594019	OP950523
20–081-01	SWITZERLAND: Jura, Bourrignon; 47.3821, 7.2388; coll. Mathias Just Justesen; ex. *Abies alba*; 15 April 2020	larva	YPM# ENT594020	OP950524
20–082-01	SWITZERLAND: Jura, St. Brais; 47.3134, 7.1277; coll. Mathias Just Justesen; ex. *Abies alba*; 25 May 2020	larva	YPM# ENT594021	OP950525
20–083-01	SWITZERLAND: Jura, St. Brais; 47.3134, 7.1277; coll. Mathias Just Justesen; ex. *Abies alba*; 29 April 2020	larva	YPM# ENT594022	OP950526
20–083-02	SWITZERLAND: Jura, St. Brais; 47.3134, 7.1277; coll. Mathias Just Justesen; ex. *Abies alba*; 29 April 2020	larva	YPM# ENT594023	OP950527
20–083-03	SWITZERLAND: Jura, St. Brais; 47.3134, 7.1277; coll. Mathias Just Justesen; ex. *Abies alba*; 29 April 2020	larva	YPM# ENT594020	OP950528
20–083-04	SWITZERLAND: Jura, St. Brais; 47.3134, 7.1277; coll. Mathias Just Justesen; ex. *Abies alba*; 29 April 2020	larva	YPM# ENT594020	OP950529
20–083-05	SWITZERLAND: Jura, St. Brais; 47.3134, 7.1277; coll. Mathias Just Justesen; ex. *Abies alba*; 29 April 2020	larva	YPM# ENT594020	OP950530
20–124-05	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–124-05	OP950531
20–126-01	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-01	OP950532
20–126-02	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-02	OP950533
20–126-03	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-03	OP950534
20–126-04	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-04	OP950535
20–126-05	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-05	OP950536
20–126-06	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-06	OP950537
20–126-07	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-07	OP950538
20–126-08	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-08	OP950539
20–126-09	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-09	OP950540
20–126-10	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-10	OP950541
20–126-11	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-11	OP950542
20–126-12	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-12	OP950543

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Table A1 (*continued*)

Sample number	Collection Information	Life Stage	Specimen Voucher No.	GenBank Accession No.
20–126-13	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-13	OP950544
20–126-14	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-14	OP950545
20–126-15	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-15	OP950546
20–126-16	SWITZERLAND: Jura, Delémont; CABI Centre; 47.3733, 7.3274; coll. Lukas Seehausen; ex. *Abies alba*; 22 May 2019	adult	CSCA#20–126-16	OP950547
20–127-01	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	adult	CSCA#20–127-01	OP950548
20–127-02	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	adult	CSCA#20–127-02	OP950549
20–127-03	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	adult	CSCA#20–127-03	OP950550
20–127-04	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	adult	CSCA#20–127-04	OP950551
20–127-05	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	adult	CSCA#20–127-05	OP950552
20–127-06	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	adult	CSCA#20–127-06	OP950553
20–127-07	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	adult	CSCA#20–127-07	OP950554
20–127-08	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	adult	CSCA#20–127-08	OP950555
20–127-09	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	adult	CSCA#20–127-09	OP950556
20–127-10	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	adult	CSCA#20–127-10	OP950557
20–127-11	GEORGIA: Guria, Bakhmaro; 41.8575, 42.3261; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 2 June 2019	adult	CSCA#20–127-11	OP950558

Leucopis hennigrata specimens from Georgia (n = 25) and Switzerland (n = 20), sequenced with this nondestructive technique, were identified morphologically by SG, who also identified the related specimens cited by Ravn et al. (2012) from Turkey (n = 13), Georgia (n = 10) and historical specimens from Germany (n = 12). Although species of *Leucopis* can be notoriously difficult to separate, with male genitalia often possessing the sole characters useful at this level, the species *L. hennigrata* is an exception as it can be reliably identified with external morphology, and confirmed using male genitalia (McAlpine, 1978; Tanasijtshuk, 1986). Keys to identify egg, larvae, and puparia are not available in the literature, so we familiarized ourselves with the life stages of the Chamaemyiidae species currently recognized from *Ad. nordmannianae/Ad. piceae*. According to Ravn et al. (2012) this included only three Chamaemyiidae species; *Cremifania nigrocellulata* Czerny, *Neoleucopis atratula* (Ratzeburg), and *Neoleucopis obscura* (Haliday). We compared the collected *L. hennigrata* eggs, larvae and puparia with available literature of the life stages of *C. nigrocellulata* (see Delucchi & Pschorn-Walcher, 1954; Brown & Clark, 1956), *N. atratula*, and *N. obscura* (see Brown & Clark, 1956). Additionally, the phenology of these species have little overlap, with *C. nigrocellulata* being active later in the year and mostly occurring on adelgids infesting the trunks (Eichhorn, 1968; Ravn et al., 2012) and *N. atratula* being active earlier and primarily on trunk infestations (Pschorn-Walcher & Zwölfer, 1956; Ravn et al., 2012). Many of the larvae collected in the field and used in the laboratory experiments were DNA barcoded and the molecular data confirmed all our identifications of *L. hennigrata*.

2.2. Abundance of L. Hennigrata

To determine the relative abundance of *L. hennigrata* compared to other insect larvae feeding on *Ad. nordmannianae* infesting twigs of *Abies alba* Mill. (Pinaceae), a total of 180 twigs (one twig = three previous-year shoots) were collected in three sites located in the Swiss Jura (Delémont: 47°22′22.51″N, 7°19′40.42″E, 547 m a.s.L.; Bourrignon: 47°22′55.7″N, 7°14′17.4″E, 851 m a.s.L.; Saint-Brais: 47°18′48.1″N, 7°07′39.5″E, 977 m a.s.L.). At four uniformly spaced dates between the beginning of April until the end of May 2020, five trees were sampled in each site by cutting three twigs per tree that were heavily infested by *Ad. nordmannianae*. Twigs were collected between breast and eye height, that is approximately between 1.3 and 2.0 m above the ground. The twigs were transported in individual plastic bags to the laboratory, where the length of the previous year's shoot was measured, dissected under a microscope, and all egg masses as well as all live predatory larvae were counted. Predatory larvae were identified to morphospecies and kept separately in alcohol. The number of *L. hennigrata* and the number of all other predators were calculated per twig. Then, absolute densities (*AD*) were calculated as:

$$AD = \frac{\sum individualsontwig}{lengthoftwig(cm)}$$

Separately for *L. hennigrata*, other predators, and *Ad. nordmannianae*.

The density of *L. hennigrata* relative to other predators (*RD*) was calculated as:

$$RD = \frac{AD_{L.hennigrata}}{AD_{otherpredators} + AD_{L.hennigrata}} \times 100$$

Additionally, the relationship of absolute *L. hennigrata* and *Ad. nordmannianae* densities was analyzed for all twigs collected during the week of highest mean densities of *L. hennigrata* (27 April to 8 May 2020).

In Georgia between 9 and 14 May 2019, a total of 1438 cm of *Ab. nordmanniana* twigs (approx. 72 twigs) infested with *Ad. nordmannianae*, were collected at the Tlughi locality (Fig. 1C) and examined under a microscope. The number of adelgid egg masses, as well as the number of predator larvae and eggs were counted. Only eggs and larvae of *L. hennigrata* were identified to species, using the morphological characteristics determined during surveys in Switzerland and during the specificity tests described below. Like for the samples from Switzerland, absolute densities of *L. hennigrata*, other predators, and *Ad. nordmannianae*, as well as the relative density of *L. hennigrata* to other predators were calculated.

Additionally, adelgid-infested twigs were examined for predators without counting *Ad. nordmannianae* egg masses and measuring twig length because this allowed for processing more samples under the given field conditions in Georgia. Twigs with the highest infestation of *Ad. nordmannianae* in any given location were collected and the number of predators were counted, again only identifying *L. hennigrata* eggs and larvae. In total, twigs were sampled with this protocol at 11 sites distributed between Bakhmaro, Guria region (n = 3), Beshumi, Adjara region (n = 5), and Tlughi, Racha region (n = 3).

2.3. Larval feeding efficacy

To investigate the efficiency of *L. hennigrata* larvae to feed on *Ad. nordmannianae* eggs, several laboratory experiments were conducted, quantifying the silver fly's kill rate (the number of predated adelgid eggs in 24 h).

In Switzerland, 20 late-instar larvae of *L. hennigrata* were collected in the Delémont valley in mid–May 2019 from *Ab. alba* branches and trunks that were heavily infested by *Ad. nordmannianae*. The silver fly larvae were then measured to the closest 0.5 mm under a microscope and placed individually in a petri dish (5 cm diameter) on a moist filter paper next to an *Ad. nordmannianae* egg mass containing a mean (±SE) of 205 ± 8 (min. = 134, max. = 253) intact eggs, which were carefully transferred from infested *Ab. alba* twigs. After 24 h, larvae were removed from the petri dish and all remains (intact eggs, damaged and empty eggs, and emerged adelgid crawlers) were counted. The number of predated eggs was calculated by subtracting the number of emerged crawlers (representing non-predated empty eggs) from the total number of damaged and empty eggs.

In 2020, the experiment was repeated in Switzerland with 10 late-instar larvae of *L. hennigrata* that were collected in the Delémont valley between 26 May and 4 June from *Ab. alba* trunks that were heavily infested with *Ad. nordmannianae/piceae*. However, this time, the petri dish (5 cm diameter) contained a higher number of intact *Ad. nordmannianae* eggs (304 ± 22; min. = 236, max. = 454) that were collected from infested branches and left with a silver fly larva for 24 h.

In Georgia, the same experiment was completed with 20 late-instar larvae of *L. hennigrata* collected in the Tlughi valley from *Ab. nordmanniana* twigs with high infestations of *Ad. nordmannianae* between 15 and 17 May 2019. Larvae were placed individually in 2.5 cm diameter arenas within 10 cm diameter petri dishes, next to an *Ad. nordmannianae* egg mass containing a mean (±SE) of 127 ± 3 (min. = 108, max. = 161) intact eggs, which were carefully transferred from an infested twig. Like in Switzerland, larvae were removed from the petri dish after 24 h. However here, the number of eaten eggs was calculated by counting eggs shaped like a half bowl as eaten and eggs with narrow and length wisely folding as natural emergence of progredientes, similar to Eichhorn (1964).

2.4. Laboratory prey preference

To determine the specificity of *L. hennigrata* adults for oviposition, a series of no-choice tests were performed in cages. For this purpose, adult *L. hennigrata* were collected at various locations in the Delémont valley in April and May 2020 from *Ab. alba* trunks that were heavily infested by *Ad. nordmannianae/piceae* and placed into 24 × 24 × 24 cm mesh cages containing water, honey water, milk, and a honey-yeast mix as food sources. All food sources were offered on soaked cotton. To determine if females generally oviposit when held in cages, two *Ab. alba* branches containing > 100.

Ad. nordmannianae egg masses were placed into each of three cages containing 15–30 adult flies. Prior to exposure to the flies, the branches were inspected under the microscope and predator eggs were removed. After three days, branches were re-inspected under a microscope to search for eggs. Any potential eggs were left on the branches and reared as described in detail below for non-target species. It was determined that females readily laid eggs on branches in the cages. Thus, no-choice tests with non-target hemipterans were conducted with species that were found in the three above-described sites in Switzerland where adult *L. hennigrata* were observed.

Hemipteran prey were collected, together with branches of their host plant, and inspected under a microscope. Existing predators and predator eggs were removed from each branch, and a sample of the prey species was taken and preserved in alcohol for DNA-based identification. DNA barcoding of the COI gene was performed as described in detail above. Supplementary Table A2 shows the collection, voucher, and GenBank accession information for these specimens. The infested branches were then divided into three bouquets, each placed into a container with water to keep them fresh and exposed for 3 days to *L. hennigrata* adults in each of the three cages described above. As the adult flies were collected from with *Ad. nordmannianae/piceae* infested *Ab. alba* trunks, it can be assumed that most of them were mated females that were drawn to the trees by the prey to oviposit. For all non-target tests, 15–30 adult flies were present in each cage. The number varied due to the fluctuating presence of flies in the field that were needed to replace dead ones in the cages. In all but one case, the cages were placed in an outdoor insectarium, a wooden cabin next to the CABI Centre in Delémont, Switzerland, with a window to allow for natural sunlight. Only exposure of *Pseudococcus comstocki* (Kuwana) (Hemiptera: Pseudococcidae) egg masses to the flies were conducted in a quarantine laboratory because this species is invasive in Switzerland and not yet present in the Swiss Canton Jura. All exposures of non-target species were conducted between 17 April and 7 May 2020, which corresponds to the time adult *L. hennigrata* and *Ad. nordmannianae* egg masses were present under natural conditions in the region. Environmental temperature in the wooden cabin was measured on an hourly basis with a HOBO® data logger.

The following non-target species were tested:

- *Adelges* (*Sacchiphantes*) *viridis* (Ratzeburg) (Hemiptera: Adelgidae) sistentes on *Larix decidua* Mill., two branches per cage, each with 14 ± 3 infested needle clusters, exposed to flies from 17 to 20 April.
- *Adelges* (*Adelges*) *laricis* Vallot (Hemiptera: Adelgidae) adult fundatrices settled at the base of galls with eggs and nymphs on *Picea abies*, 10 galls per cage on cut branches, exposed to flies from 20 to 23 April.
- *Pseudococcus comstocki* (Kuwana) (Hemiptera: Pseudococcidae) egg masses on a piece of *Malus domestica* bark, two egg masses per cage,

Table A2
Collection and voucher information for non-target species used in laboratory prey preference tests. Specimen vouchers were deposited at the Peabody Museum of Natural History, Yale University, New Haven, Connecticut, USA (YPM) or the University of Massachusetts Entomology Collection (UMEC).

Species	Collection Information	Specimen Voucher No(s).	GenBank Accession No (s).
Adelges (*Sacchiphantes*) *viridis*	SWITZERLAND: Jura, Delémont; 47.3733, 7.3251; coll. Lukas Seehausen; ex. *Larix decidua*; 17 April 2020	YPM#ENT594160, YPM#ENT5941601, YPM#ENT594163	OP950566, OP950567, OP950569
Adelges (*Adelges*) *laricis*	SWITZERLAND: Jura, Delémont; 47.3724, 7.3272; coll. Lukas Seehausen; ex. *Picea abies*; 20 April 2020	YPM#ENT594162, YPM#ENT594164, YPM#ENT594165	OP950568, OP950570, OP950571
Pineus (*Pineus*) *pini*	SWITZERLAND: Jura, Delémont; 47.3729, 7.3255; coll. Lukas Seehausen; ex. *Pinus sylvestris*; 27. April 2020	YPM#ENT594166 to YPM#ENT594172	OP950572 to OP950577
Mindarus abietinus	SWITZERLAND: Jura, Delémont; 47.3739, 7.3262; coll. Lukas Seehausen; ex. *Abies alba*; 01 May 2020	YPM#ENT594173 to YPM#ENT5941736	OP950578 to OP950581
Pineus (*Pineus*) *orientalis*	GEORGIA: Guria, Bakhmaro; 41.8503, 42.3165; coll. Mathias Just Justesen; ex. *Picea orientalis*; 24 May 2019	YPM#ENT594155 to YPM#ENT594159	OP950564, OP950565
Pineus (*Pineus*) *orientalis/pini*	GEORGIA: Racha, Tlughi; 42.4394, 43.1542; coll. Mathias Just Justesen; ex. *Abies nordmanniana*; 11 June 2019	YPM#ENT594150 to YPM#ENT594154	OP950559 to OP950563
Dynaspidiotus near *abietis*	GEORGIA: Racha, Tlughi; 42.3876, 42.9778; coll. Mathias Just Justesen; ex. *Abies nordmanniana;* 13 May 2019	UMEC#D9855A, D9856A, D9859A, D9860A	N/A
Aphis fabae	GEORGIA: Guria, Bakhmaro; 41.8534, 42.3243; coll. Mathias Just Justesen; ex. *Rumex* sp.*;* 24 May 2019	YPM#ENT594176 to ENT594178	OQ159089

exposed to flies from 23 to 26 April. Samples were verified by taxonomist but not by molecular identification.

- *Pineus orientalis/pini* (Macquart) (Hemiptera: Adelgidae) adult females, eggs, and nymphs on *Pinus sylvestris*, seven branches per cage, each with 1–7 egg masses, exposed to flies from 27 to 30 April. *Pineus orientalis* and *P. pini* are sexual and asexual sister species, respectively, that cannot be distinguished with DNA barcodes (Foottit et al., 2009) or using morphology of the exulis forms on *Pinus* (Blackman and Eastop, 2000).
- *Mindarus abietinus* Koch (Hemiptera: Aphididae) sexuparae and adults on *Abies alba*, 4–5 heavily infested twigs (containing at least three infested current-year shoots) per cage, exposed to flies from 1 to 4 May.

In the case of *Ad. viridis* on *L. decidua* one individual out of the four samples identified by DNA barcoding was found to be *Ad. laricis*, thus, both non-target species were mixed to some extent for this exposure. To determine if adults oviposit on branches without aphids or adelgids, after the exposure of non-target species also two uninfested *Ab. alba* branches were placed in each cage for three days from 4 to 7 May. After each exposure, all branches were inspected under the microscope to count *L. hennigrata* eggs. Then, the branches were placed in containers with water, which were sealed with parafilm to avoid drowning of larvae, and stored inside plastic containers that were kept in the outdoor insectarium. The plastic containers had a screened opening on the top for ventilation and a cellulose paper on the bottom to provide a hiding place for pupariating *L. hennigrata*. Upon hatching of *L. hennigrata* eggs on branches with non-target species, larvae were observed under a microscope to determine if they were feeding on any life stages of the non-target prey species. The containers with branches were also checked periodically for puparia or adults of *L. hennigrata*.

2.5. Field prey preference

To determine the prey range of *L. hennigrata* in terms of adult oviposition and larval feeding in the field, surveys of non-target adelgid and aphid species that were abundant in and around the Delémont valley, Switzerland (Fig. 1A,B), on *Larix decidua* (10 locations), *Abies alba* (one forest stand), and *Picea abies* (27 locations) were conducted between 24 April and 12 May 2020. The locations were all within 6 km of trees where *L. hennigrata* was found preying on *Ad. nordmannianae* in the same year. Three branches were collected from each of 21, 33, 97 trees of *L. decidua* infested with *Ad. viridis* sistentes, *Ab. alba* infested with *M. abietinus* sexuparae and adults, and *P. abies* infested with *Ad. laricis* females, respectively. As described above, DNA barcoding indicated that, on *L. decidua*, a mix of *Ad. viridis* and *Ad. laricis* occurred. The branches were brought to the laboratory in plastic bags and inspected under the microscope for eggs and larvae with the morphological characteristics of *L. hennigrata*. These characteristics were determined in the experiment regarding the relative abundance of *L. hennigrata* to other predators and the specificity tests with adult flies in cages.

In Georgia, non-target adelgids and aphids were surveyed in May and June 2019 in and around several locations where *Ad. nordmannianae* egg masses were found to be preyed upon by *L. hennigrata* in the same year. All samples (branches and bark pieces) were examined under a microscope and examples of the non-target species were kept in alcohol for a DNA barcoding identification as described above. Supplementary Table A2 shows the collection, voucher, and GenBank accession information for these specimens. The locations where the samples were collected in Georgia are indicated in Fig. 1C. Based on the molecular and morphological identification, the following non-target species were investigated:

- *Pineus orientalis* (Dreyfus) (Hemiptera: Adelgidae) on four bark pieces (approx. 5x5cm) from two *Picea orientalis* (n = 8) with low infestations in Bakhmaro.
- *Aphis fabae* (Scopoli) (Hemiptera: Aphididae) on *Rumex* sp. plants (n = 10) in Bakhmaro.
- *Mindarus* sp. (morphologically similar to *M. abietinus*, MJJ personal observation) on five branches of two *Ab. nordmanniana* (50 + years) trees in Bakhmaro and two *Ab. nordmanniana* (50 + years) trees Beshumi.
- *Pineus orientalis/pini* (Hemiptera: Adelgidae) on three branches of two young *Pinus sylvestris* trees (n = 6) with medium infestation levels in Tlughi. Additionally, a 15 min visual inspection of two trees was done using a 10x magnification loupe.
- *Dynaspidiotus* near *abietis* (Hemiptera: Diaspididae) from two branches of two *Ab. nordmanniana* (n = 4) trees in Tlughi. Because this species was abundant in the site, approx. 30 min were spend visually inspecting several additional branches using a 10x magnification loupe.

2.6. Statistical analysis

The influence of *Ad. nordmannianae* density on the number of *L. hennigrata* larvae on twigs as well as the effect of different non-target prey species on the number of oviposited eggs were analyzed in R using Poisson regressions (*glm* function, R Core Team, 2021). For the investigation of density dependence, the number of *L. hennigrata* larvae was

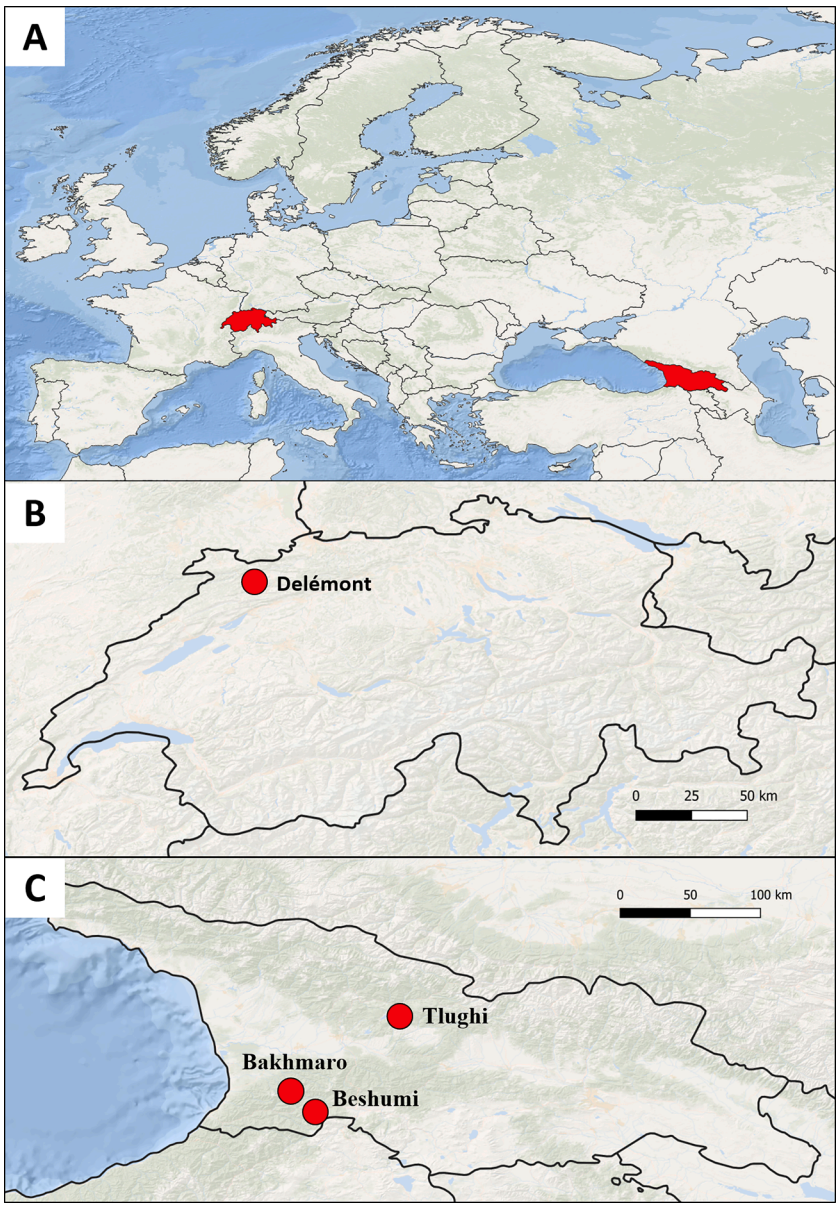

Fig. 1. Sampling locations, where the survey for *Leucopis hennigrata* eggs and larvae on non-target species were conducted at the (A) continental scale (Switzerland and Georgia are highlighted in red) and the national scale in (B) Switzerland Switzerland (Delémont valley in the Swiss canton Jura) and (C) Georgia (Bakhmaro, Beshumi, and Tlughi). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

used as dependent variable, the number of *Ad. nordmannianae* per cm twig as independent variable, and twig length in cm as offset. For the prey specificity test, the number of *L. hennigrata* eggs was a dependent variable and the non-target species on branches of their respective host tree the only independent variable. Because in both cases model residuals were significantly overdispersed, as demonstrated by comparing sum of squares of the standardized residuals to the χ^2 distribution (Gelman & Hill, 2006), a quasi-Poisson distribution was used. To compare the number of *L. hennigrata* with the total number of all other predators over the sampling period, a repeated-measures negative binomial regression was done using the *glmmTMB* function of the package with the same name (Brooks et al. 2017) in R (R Core Team, 2021). The number of predator larvae found on the twigs was the dependent variable and predator species (*L. hennigrata* or others) was the independent variable. A random effect on the sampling location accounted for the correlation between the sites that were sampled repeatedly.

3. Results

3.1. Genetic and morphological analyses

Throughout the study period, hundreds of larval and adult *L. hennigrata* have been collected. To confirm our identifications, 80 Chamaemyiidae larvae and 35 adult Chamaemyiidae flies identified as *L. hennigrata* were barcoded, and the molecular data confirmed our identifications (Table A1). In total, DNA barcode sequences were generated for 126 specimens of *L. hennigrata* (Table A1). There was a clear differentiation between *L. hennigrata* sequences from the Western Asian (Georgia and Turkey) and Central European (Switzerland and England) distribution (Fig. 2). Bootstrap support for the cluster containing the Western Asian haplotypes being separate from Central European haplotypes was 98.4%. The support value for all other clusters was<70%. Within the Asian group there was a 0.15% mean (range: 0–0.61%) pairwise divergence among the 35 *COI* sequences. Within the European group there was a mean pairwise divergence of 0.20% (range:

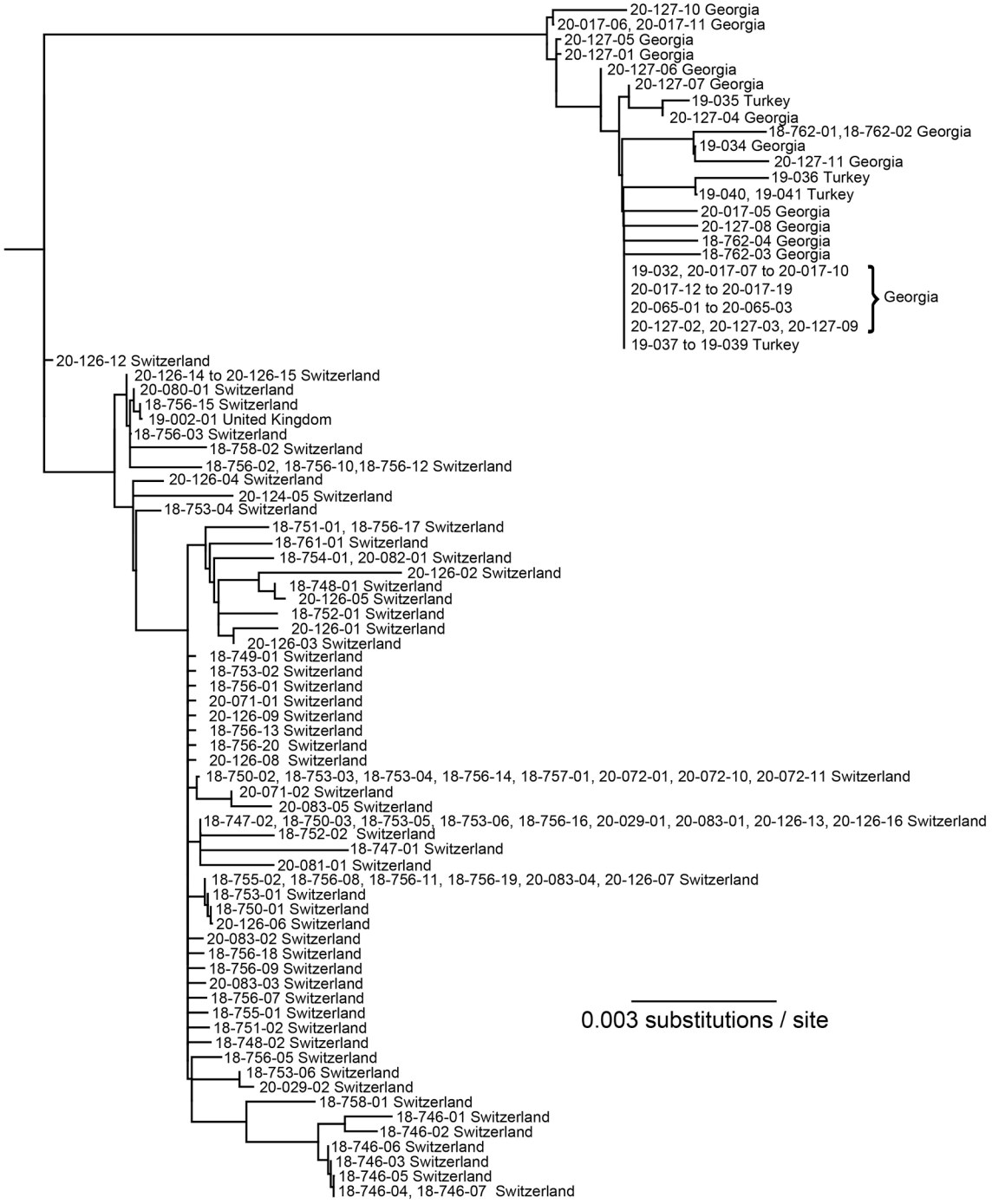

Fig. 2. Neighbor-joining tree for *COI* DNA barcoding sequences from *Leucopis hennigrata* collected in Georgia, Turkey, Switzerland and the United Kingdom. Branch tip labels show the sample number for each specimen that shared a sequence, followed by the country in which they were collected (see Table A1 for collection and voucher data).

0–1.06%) between the 81 sequences. The pairwise divergence among sequences from the different regions was 1.57% (range: 1.06–2.45%). This range of *COI* divergence could indicate longstanding isolation between the populations. The examination of *L. hennigrata* adults from Georgia and Switzerland did not find morphological differences between Western Asian and Central European specimens.

3.2. Abundance of L. Hennigrata

In Switzerland a total of 265 predatory larvae of 18 morphospecies were collected. From those, two were identified morphologically as early- (n = 57) and late-instar (n = 100) larvae of *L. hennigrata*. The molecular analysis confirmed this identification. Thus, 59% of all predatory larvae on infested twigs were found to be *L. hennigrata*. Several syrphid species were also collected and combined their numbers were noticeable (n = 79). Additionally, we collected Cecidomyiidae (n = 12) and Coccinellidae (n = 2) larvae.

The density of *L. hennigrata* compared to other predatory larvae changed over the season in April and May (Fig. 3). In mid-April, an average of one *L. hennigrata* larva per 25 cm twig was found, which was about 46% of all predatory larvae feeding on the adelgids. The highest mean densities (one individual per 10 cm twig) and relative abundance

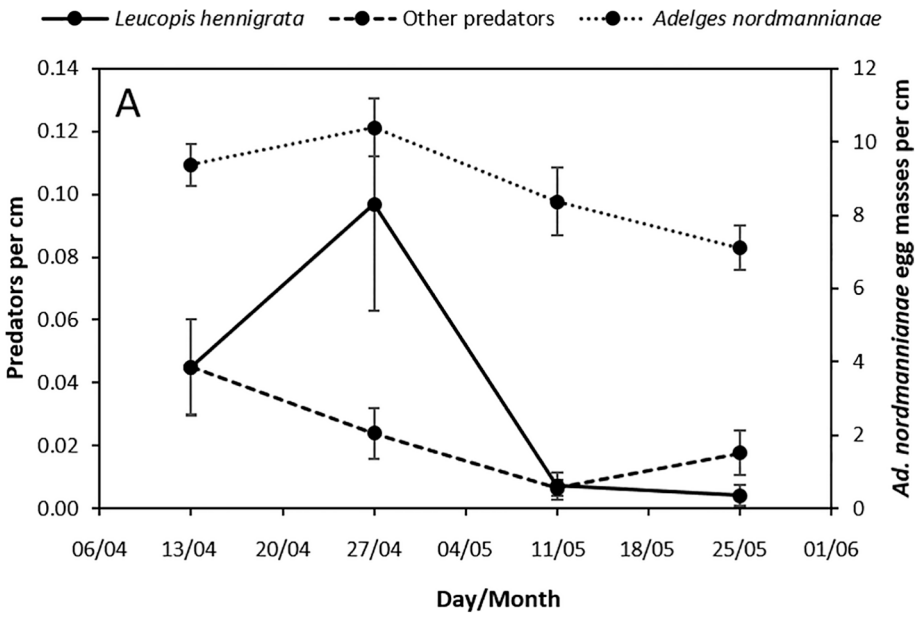

Fig. 3. Mean (±SE) density of (solid lines) *Leucopis hennigrata* and (dashed line) other species of predatory larvae on *Abies alba* twigs (previous year shoots) heavily infested by *Adelges nordmannianae* in Switzerland. The dotted line represents the pattern of *Ad. nordmannianae* egg masses density per cm twig, corresponding to the secondary axis on the right of the graph. In total 156 *L. hennigrata* specimens were collected (n = 49, n = 95, n = 7, n = 5). There was no significant difference between the number of *L. hennigrata* and the total number of other predators (repeated measures negative binomial regression).

(69%) were reached at the end of April, after which densities decreased ten-fold and also the relative abundance declined to 10% until the end of May. During the sampling dates in April, there were on average 9–10 *Ad. nordmannianae* egg masses per cm twig and in May, a slight decline to 8 and then 7 egg masses per cm twig were found (Fig. 3). There was no significant difference between the number of *L. hennigrata* and all other predators over the sampling period ($\chi^2 = 0.7304$, df = 1; P = 0.3928).

At peak densities of *L. hennigrata* (week of April 27), there was a significant positive correlation between the number of *L. hennigrata* and the number of *Ad. nordmannianae* egg masses per centimeter twig ($\chi^2 = 26.261$, df = 1, $P < 0.0001$), which can be described on the logarithmic scale with the following linear regression line: y = -4.29282 + 0.15458 × x, or the exponent of that to describe the

back-transformed data (Fig. 4). *Leucopis hennigrata* was present in low densities (one individual per 25 cm twig) starting at *Ad. nordmannianae* densities of seven egg masses per cm twig, and increased to an average of one individual per 1.8 cm twig at 24 egg masses per cm branch (Fig. 4).

In Georgia, a total of 169 predator eggs and 181 larvae were collected from branches for which also *Ad. nordmannianae* egg mass densities were determined. From these, 42 eggs and 20 larvae were identified as *L. hennigrata*, thus the silver fly accounted for 11% and 25% of all predator eggs and larvae, respectively. The majority of the remaining eggs and larvae belonged to *Laricobius caucasicus* Rost, which was very abundant in Tlughi and Bakhmaro. Additionally, a few unidentified syrphid larvae were observed with this method. Mean (±SE) numbers of *L. hennigrata*, other predators, and *Ad. nordmannianae* egg masses per cm twig were 0.04 ± 0.02 (one per 24.4 cm twig), 0.21 ± 0.08 (one per 4.8 cm twig), and 1.54 ± 0.44 (one per 0.7 cm twig), respectively. The mean density of *L. hennigrata* relative to other predators was 14.2 ± 5.5%.

On twigs from Georgia for which *Ad. nordmannianae* egg masses were not counted, 16% of all 1182 predator life stages collected were identified as *L. hennigrata*. The remaining collected predators were *L. caucasicus* (53%) and Cecidomyiidae (25%). Some syrphid larvae (5%) and Coccinellidae (0.5%) were also collected from the twigs. A high variability in the abundance of *L. hennigrata* between sites was observed. For example, at the Beshumi locality in Adjara, 162 (51%) of the 316 collected predators were *L. hennigrata*, indicating a relatively high local abundance, while in Bakhmaro and Tlughi the abundance was

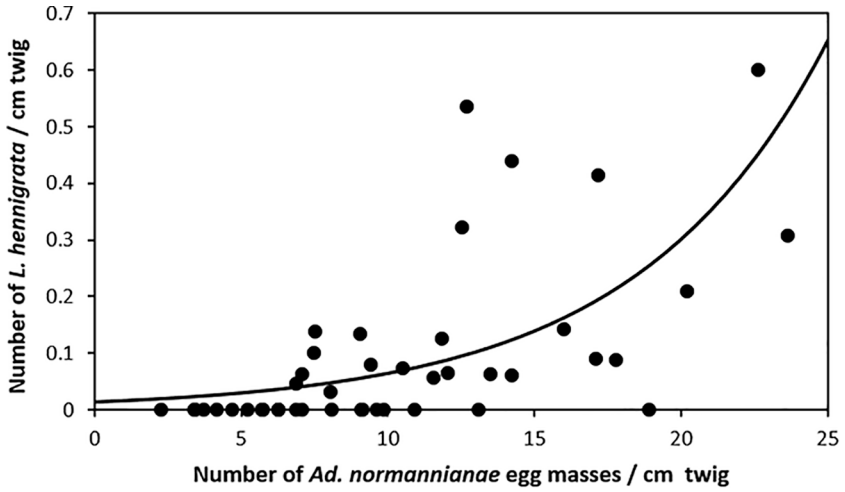

Fig. 4. Relationship in the week of April 27, 2020 between the density of *Ad. nordmannianae* egg masses and *Leucopis hennigrata* larvae (per cm) on twigs of *Abies alba* in Switzerland. The trendline is the exponential of the linear regression line on the log scale: y = -4.29282 + 0.15458 × x.

relatively low with 4% and 0.5%, respectively.

3.3. Feeding efficacy of larvae

In the 2019 experiment, four of the twenty late-instar larvae from Switzerland were excluded because the majority of *Ad. nordmannianae* eggs hatched during the experiment. The remaining 16 silver fly larvae were on average (±SE) 2.3 ± 0.2 mm in length and preyed on (killed) a mean (±SE) of 57 ± 11 eggs (min. 4, max. 135).

In the experiment that was repeated in 2020 in Switzerland, *L. hennigrata* larvae were on average (±SE) bigger, 3.1 ± 0.1 mm, and preyed on a mean (±SE) of 96 ± 20 eggs (min. = 7, max. = 183). Many of the eggs were not entirely devoured by the larvae but only pierced with their moth parts, partly emptied, and then left to dry out. This wasteful feeding markedly increased the number of killed eggs beyond those that were consumed entirely.

Six of the twenty late-instar larvae from Georgia (all above 2 mm) were excluded from the results because they pupariated during the experiment (n = 2) or escaped the paper arena (n = 4). The remaining 14 specimens preyed on a mean (±SE) of 117 ± 6 eggs (min. = 64, max. = 158).

3.4. Laboratory prey preference

During the laboratory prey range experiment in the wooden cabin in April and May 2020, average temperature was 13.8 °C (±0.2 °C SE). During the day, temperatures typically rose above 20 °C (maximum 26 °C) and during the night slightly below 10 °C (minimum 3.0 °C).

The number of eggs laid by *L. hennigrata* on *Ab. alba* branches infested with *Ad. nordmannianae* egg masses (positive control) was not determined here. But it was observed that the silver fly laid a relatively high number of eggs on infested branches and several individuals developed to the adult stage.

On average, 6, 15, 20, and 58 *L. hennigrata* eggs were oviposited on branches in the laboratory containing *Pineus orientalis/pini*, *Ad. laricis*, *Ad. viridis*, and *M. abietinus*, respectively. No fly eggs were found on or around egg masses of *P. comstocki*, but on average one *L. hennigrata* egg was laid on control branches without non-target species (Fig. 5). There was a significant difference in the number of eggs *L. hennigrata* females oviposited on branches with different non-target species ($\chi^2 = 50.91$, df = 5, $P < 0.0001$). However, only the number of eggs laid on branches with *M. abietinus* was significantly higher than the number of eggs oviposited on control branches (Fig. 5).

Leucopis hennigrata larvae were observed to readily feed on *Ad. viridis* nymphs. At the time of *L. hennigrata* egg hatch, no adults and egg masses were present yet on the needles, but formed about two weeks later on branches in the container. However, at that time, no more *L. hennigrata* larvae were observed on the branches and no pupae were found in the containers.

Upon inspection of *P. abies* branches with *Ad. laricis* fundatrices and eggs settled at the base of galls one week after exposure to *L. hennigrata* adults, all fly eggs looked collapsed and dead. Observations under the microscope revealed that some *Ad. laricis* nymphs attacked the *L. hennigrata* eggs by piercing their mouthparts into the eggs, causing them to collapse. Thus, none of the *L. hennigrata* eggs hatched. To verify nevertheless if *L. hennigrata* larvae feed on egg masses of *Ad. laricis*, some late-instar larvae were collected from *Ab. alba* trunks infested by *Ad. nordmannianae* or *Ad. piceae* and transferred into a petri dish containing *Ad. laricis* egg masses. This revealed that *L. hennigrata* larvae readily feed on *Ad. laricis* eggs in a no-choice situation.

Leucopis hennigrata larvae were found feeding on eggs of *Pineus orientalis/pini* and one observation was made suggesting that one larva also fed on an adult adelgid. Several weeks after exposure to the adult flies, a late larval instar of *L. hennigrata* was found drowned in the water for the branches. This suggests that high densities of *Pineus orientalis/pini* can support *L. hennigrata* development at least until the late larval instar.

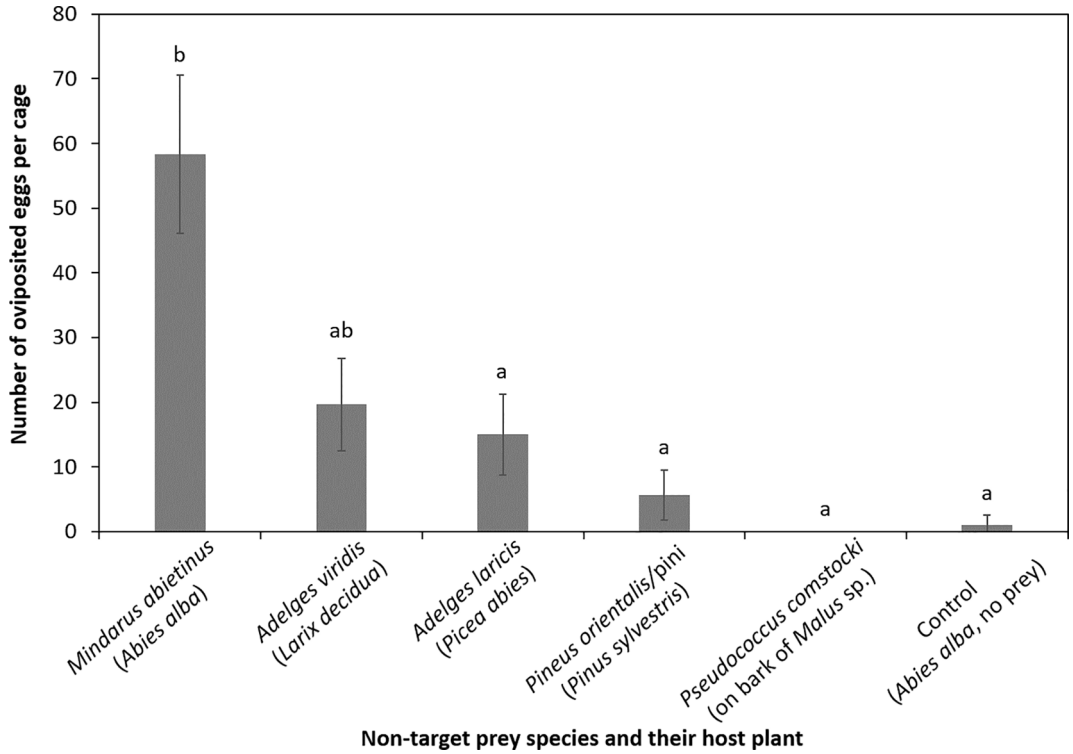

Fig. 5. Mean (±SE) number of eggs per cage (n = 3) oviposited by adult females of *Leucopis hennigrata* in cages (15–30 individuals per cage) containing different combinations of non-target prey species and their host tree species. *Pseudococcus comstocki* was exposed to flies as egg masses on a piece of bark from *Malus* sp. The control consisted of *Abies alba* branches without any prey organisms. Bars with the same lowercase letters are not significantly different at $\alpha = 0.05$ according to Tukey's HSD test.

Numerous *L. hennigrata* larvae were found to feed on *M. abietinus* nymphs. Several weeks after the exposure to adult flies, on the bottom of one container three *L. hennigrata* puparia were found, one of which developed to the adult stage.

3.5. Field prey preference

On none of the 36, 99, and 291 branches of *L. decidua* infested with *Ad. viridis*, *Ab. alba* infested with *M. abietinus*, and *P. abies* infested with *Ad. laricis*, respectively, had eggs or larvae that matched the morphological characteristics of *L. hennigrata*. However, eggs or larvae of unidentified syrphids and lacewings were relatively common. During the surveys in Georgia in 2019, no *L. hennigrata* were found on non-target species.

4. Discussion

The results of this study corroborate the potential suitability of the silver fly *L. hennigrata* as a classical biological control agent against *Ad. nordmannianae* in northern European Christmas tree plantations. This is mainly based on four criteria, which are discussed in detail below: (1) the distribution of *Leucopis hennigrata* in Europe, (2) its abundance relative to other predators, (3) its kill rate, and (4) its specificity to the target prey.

In the past, *L. hennigrata* has been studied more extensively in Europe (Pschorn-Walscher & Zwölfer, 1956; Eichhorn, 1966a; 1968; McAlpine, 1978) than in Western Asia (Eichhorn, 1969a; 2000; Ravn et al., 2012), which can be explained by the interest of Canadian researchers to find classical biological control agents in central Europe against *Ad. piceae* (Clark et al., 1971). To assure that the same predator species was studied in both Europe and Western Asia, morphological and molecular analyses became necessary and were conducted in the present study. Both DNA barcoding and morphological identification confirmed that *L. hennigrata* is present in both Europe and Western Asia. This corroborates the results of a morphological identification with specimen from Turkey and Georgia that were compared to specimens from Germany (Ravn et al., 2012). However, the *COI*-based molecular analysis found a mean 1.57% divergence between the two populations, suggesting a certain isolation of populations between two regions. It is possible that these molecular differences coincide with the relatively recent (300,000 years ago) separation of *Ad. piceae* and *Ad. nordmannianae* (Havill et al., 2020), at which point an isolated population of *L. hennigrata* may have remained in Europe feeding on *Ad. piceae*. Alternatively, *L. hennigrata* may have been reintroduced to Europe with *Ad. nordmannianae* in the late 1800 s and the differences are explained by the limited number of haplotypes that are now present in Europe and were not detected in the Western Asian population due to the limited number of samples. A more detailed molecular analysis including more samples and additional molecular loci may shed more light on the evolutionary history of *L. hennigrata* populations in Europe.

This genetic divergence raises the possibility that *L. hennigrata* from Central Europe and Western Asia might not be equally suitable and efficient as biological control agents in northern Europe. Therefore, although the silver fly species has been studied in more detail in Switzerland, we compare the results as much as possible with findings from Western Asia, specifically Georgia.

It remains to be checked whether the climate of Northern Europe is suitable for the establishment of *L. hennigrata*. The predator has been present for a long time in Central Europe and it may have failed to spread further north because of climatic factors. However, the absence of *L. hennigrata* could also be because *Abies* spp. have been planted only recently in Northern Europe where they are not native, resulting in a short period of possible dispersal to the north.

Leucopis hennigrata has been mentioned in the literature as a rather insignificant predator of *Ad. nordmannianae* attacking stems (Eichhorn, 1964), but as an important predator of *Ad. nordmannianae* attacking *Ab. alba* twigs in Switzerland and Germany (Eichhorn, 1967a,b). Also, in Turkey (Eichhorn, 1969a; Ravn et al., 2012) and Georgia (Ravn et al., 2012), *L. hennigrata* is described as the most abundant predator of *Ad. nordmannianae* infesting *Ab. nordmanniana* twigs. For Christmas tree plantations, the main problem that *Ad. nordmannianae* causes is deformations of needles. Thus, an efficient reduction of *Ad. nordmannianae* populations on twigs is a first step to limit the damage for the industry. Our results from Switzerland confirm the importance of *L. hennigrata* in terms of its absolute and relative densities to other predators on *Abies* twigs infested with *Ad. nordmannianae* (Fig. 3). Although the total number of *L. hennigrata* collected during the sampling period was higher than the total number of all other predators on the same twigs, the difference was not statistically significant because of strong variations between sites and dates. *Leucopis hennigrata* was especially abundant during peak activity of *Ad. nordmanniane* (end of April to mid-May), after which few *L. hennigrata* specimens were collected. This decline in *L. hennigrata* densities in May coincided with a decline in *Ad. nordmannianae* egg masses and a slight increase in the abundance of other predatory larvae. Given the relatively short development time of *L. hennigrata* larvae, it can be assumed that many had already pupariated in the soil by mid-May (McAlpine, 1978), explaining the low numbers of larvae recovered after this time. In Georgia, we found that the relative density of *L. hennigrata* was rather low (14%). Interestingly, the absolute density of *L. hennigrata* in Georgia corresponds to the density in the first week of observations in Switzerland (one per 25 cm twig). Because the majority of *L. hennigrata* collected in Georgia were eggs, it can be assumed that at the time the surveys were conducted, oviposition was ongoing and therefore densities are underestimated. Additionally, *Ad. nordmannianae* densities per cm twig were considerably lower in Georgia (1.5) when compared to Switzerland (mean densities between 7 and 10). Given that we found a significant positive relationship between prey and *L. hennigrata* density, at least at peak activity levels, the relatively low numbers of silver flies in Georgia may also be explained through density-dependence. This is supported by the observation in Georgia that silver fly eggs or larvae were only found on trees or branches with medium to high *Ad. nordmannianae* densities. At peak activity levels in Switzerland, *L. hennigrata* larvae were detected starting at *Ad. nordmannianae* densities of seven egg masses per cm twig, which may correspond to the amount of egg masses needed for development until the puparial stage, considering the restricted mobility of larvae. For biological control, this density-dependence suggests that *L. hennigrata* could help to decrease prey populations to a low level, after which other predators such as syrphids, cecidomyiid flies, and beetle larvae may continue preying on the adelgids (Eichhorn, 1968; Ravn & Riis-Nielsen, 2006; Ravn et al., 2012).

In Switzerland, syrphids were the second most abundant predator group (30%). In both Georgia and Switzerland, cecidomyiid larvae were quite abundant in the egg masses. Interestingly the peak activity of cecidomyiids occurred after *L. hennigrata* (Personal observation LS and MJJ), as such, this species could function as an important complement to *L. hennigrata*, in controlling *Ad. nordmannianae*. In Georgia, *L. hennigrata* was only locally abundant. In fact, the main predator at two of three localities was *L. caucasicus*. This beetle is only known from the western Caucasus and, considering its very high relative abundance in the native range of *Ad. nordmannianae*, this species also shows promise as a potential biocontrol agent in Northern Europe. The potential suitability of *L. caucasicus* as a biocontrol agent against *Ad. nordmannianae* was also noted by Ravn et al. (2012).

In our experiments examining the feeding efficacy of *L. hennigrata* larvae, we found important differences in the kill rates. In the experiment conducted in Switzerland in 2019, larvae killed a mean of 57 eggs in 24 h, whereas in 2020 a mean of 96 eggs were killed, and in Georgia, a mean of 117 eggs were killed. These variable results may be explained by the differences in size and developmental stage of the larvae used in the different experiments: the smallest larvae (mean length of 2.3 mm) were tested in the first experiment in Switzerland, and were bigger (3.1

mm) in the second one. In Georgia, larvae were not measured, but given that several larvae pupariated during the experiment, it can be assumed that most larvae were in their last instar and therefore relatively large. Additionally, the prey density and size of the experimental arena differed between the experiments, suggesting that when the size of the arena stays the same as in the experiments conducted in Switzerland (5 cm diameter), the higher number of exposed eggs in the second experiment (mean = 304) when compared to the first one (mean = 205), led to more eggs being killed. However, in a smaller arena as has been used in Georgia (2.5 cm diameter), a relatively low number of eggs (mean = 127) led to the highest kill rates. Finally, differences in temperature between the laboratories in Georgia and Switzerland could lead to difference in locomotion and energy levels and consequently to a difference in kill rate. Thus, it is difficult to know exactly how many eggs are killed under natural conditions, but the experiments are informative in that 1) the kill rate is higher with increasing prey densities, and 2) given a mean number of 160 eggs in an *Ad. nordmannianae* egg mass on twigs (Eichhorn, 1968), *L. hennigrata* might devour an entire egg mass in 1–2 days. Additionally, in terms of efficacy for biological control, it might be a useful trait of *L. hennigrata* to not always devour entire *Ad. nordmannianae* eggs but to kill additional eggs by piercing them with its mouthparts and only partially eating its contents.

Our prey specificity tests with adult *L. hennigrata* show that in cages and in the absence of *Ad. nordmannianae*, females oviposit on branches containing non-target species and larvae also feed on eggs and nymphs of several tested non-target species. We neglected to count the eggs *L. hennigrata* laid on *Ab. alba* branches infested with *Ad nordmannianae* (positive controls). Thus, no quantitative comparison between target and non-target species is possible here. Nevertheless, it is interesting to note that on all non-target species except *P. comstocki*, at least some eggs were laid. However, in most cases the number of eggs laid was relatively low and not significantly different from the number of eggs laid on *Ab. alba* branches without any adelgids (negative controls), suggesting that eggs may have been laid simply due to accumulation in the fly's oviducts.

For many of the non-target species, host tree species and adelgid/aphid densities may have been a limiting factor for oviposition and successful development. For example, *Pineus pini/orientalis* occurred only as isolated egg masses on branches of *P. sylvestris* and densities were artificially increased in our experiment by bundling several branches together. Thus, larvae that were observed feeding on egg masses may have starved after depleting one egg mass and not being mobile enough to find the next one. Supporting the unsuitability of *Pineus pini/orientalis* as a prey of *L. hennigrata*, neither eggs nor larvae of the predator were found on infested branches of *P. sylvestris* nor infested bark pieces of *Picea orientalis* under field conditions in Georgia.

Even though densities of *Ad. viridis* on *L. decidua* were relatively high on the branches exposed in cages (almost all needles were infested), the tree's morphology with the separated needle clusters may represent a problem for *L. hennigrata* larvae to find sufficient prey that develop on single needles to complete development. Additionally, once *Ad. viridis* sistentes develop to winged sexuparae, they may be too mobile to be efficiently attacked by the relatively slow *L. hennigrata* larvae. Nevertheless, *L. hennigrata* has been described to attack *Ad. viridis* on larch in Delémont, Switzerland (McAlpine, 1978; Eichhorn, 2000), although no details are published about how those attacks were determined and how frequently they occur. No eggs or larvae of *L. hennigrata* were found on the 36 *Ad. viridis* infested *L. decidua* branches we collected in and around the Delémont valley, while other predators such as Coccinellidae or Syrphidae were quite common (LS personal observation). *Larix decidua* is a species adapted to high elevations that is only planted as an ornamental tree in the hills of the Jura and therefore has a very scattered distribution in the Swiss study area.

In the case of *Ad. laricis* on *P. abies*, a possible defense mechanism against *L. hennigrata* was observed during the non-target tests: *Ad. laricis* nymphs pierced the predator's eggs with their mouth parts and killed all of the eggs before they hatched. This behavior is known from other gall-inducing social aphids, where so-called "soldiers" develop specialized mouthparts to attack and kill natural enemies that threaten the gall (Stern & Foster, 1996). On the 291 *P. abies* branches infested with *Ad. laricis*, also remains of eggs from syrphids were found that may have been killed by the soldier nymphs. However, no signs of *L. hennigrata* eggs or larvae were found on those branches.

Leucopis hennigrata only oviposited significantly more eggs on *Ab. alba* branches infested with *M. abietinus*, when compared to control branches (Fig. 5). *Mindarus abietinus* was also the only non-target species that supported a complete development of the silver fly under the experimental conditions. This development was certainly aided by the high density of sexuparae and adults that covered most of the current-year shoots on the exposed branches. Only three puparia and one adult *L. hennigrata* developed from the 58 eggs that were found on average per cage. This relative low number may be due the prey's poor quality to *L. hennigrata*, or attributed to the artificial rearing conditions on cut branches. Chamaemyiidae larvae have been found predating on *M. abietinus* in the Czech Republic, but they were not identified to species because the study focused on parasitoids (Starý, 1975). No eggs, egg remains, or preying larvae of *L. hennigrata* were found on the nearly 100 *M. abietinus* infested *Ab. alba* twigs that we collected in a region and at a time, where and when *L. hennigrata* was also found preying on *Ad. nordmannianae* in Switzerland. In Georgia, no sign of the predator was found on *Mindarus* sp. infested *Ab. nordmanniana* branches.

Besides *Ad. viridis*, *L. hennigrata* is reported preying on an unidentified aphid living in male inflorescences of *Ab. nordmanniana* in Turkey (Eichhorn, 1969a; 2000). However, also here no details are described as to how the predator was identified or how often it was found on that aphid species. The inspection of aphids and adelgids in the vicinity of *L. hennigrata* attacks on *Ad. nordmannianae* in Georgia did not identify any life stages of *L. hennigrata*. Thus, in this study, we did not identify any species other than *Ad. nordmannianae* and *Ad. piceae* that is preyed upon by *L. hennigrata* under natural conditions. Higher sample sizes and, in some cases, refined searching methods (i.e. only a hand held 10x magnification loupe was used in some cases in Georgia) may lead to higher certainty about the realized prey range of *L. hennigrata* under natural conditions.

5. Conclusions

Besides the arguments described in the introduction and by Ravn et al. (2012) relating to *L. hennigrata* being especially abundant on *Abies* twigs infested with *Ad. nordmannianae*, the univoltinism of the species leading to fewer problems in regard to synchronization, and the previous successful use of other *Leucopis* species in biocontrol, we here reveal additional points that identify *L. hennigrata* as a potentially effective classical biological control agent against *Ad. nordmannianae* in Christmas tree plantations in northern Europe: (1) We present molecular and morphological evidence that *L. hennigrata* is the same species in Western Asia and in Europe, proving that the predator already exists in neighboring countries to Northern Europe, such as Germany and the UK; (2) Our surveys on *Ab. alba* twigs show that, also in central Europe, *L. hennigrata* is a key predator of *Ad. nordmannianae* and may thus be able to significantly reduce pest populations in Northern Europe; (3) The wasteful feeding behavior of *L. hennigrata* larvae on *Ad. nordmannianae* eggs leads to a high kill rate especially for late-instar larvae at high adelgid densities; (4) Although our cage experiments show that *L. hennigrata* is physiologically able to develop on at least one non-target prey species (*M. abietinus*), no life stages of *L. hennigrata* were found on adelgids or aphids other than *Ad. nordmannianae* and *Ad. piceae* in natural settings. Thus, even though attacks on non-target species are possible, and are reported in the literature for *Ad. viridis* in Europe, the frequency of such non-target attacks seems to be very low and do not appear to threaten any species at the population level.

In case of the initiation of a classical biocontrol program with

releases of predators against *Ad. nordmannianae* as a pest in the Christmas tree production in Northern Europe, we propose *L. hennigrata* as the most suitable candidate. Considering the genetic variation in the species, possible differences in climatic adaptation and kill rates between the populations in Western Asia and Central Europe, releases of populations from both areas may increase the success of a classical biological control program.

Should *L. hennigrata* fail to establish or to control *Ad. nordmannianae* in Northern Europe, another option would be to use it as augmentative biological control agent to be released every year in Christmas tree plantations. However, for this, a cost-effective mass production system would have to be developed.

CRediT authorship contribution statement

Mathias Just Justesen: Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Project administration. **M. Lukas Seehausen:** Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Nathan P. Havill:** Formal analysis, Resources, Writing – review & editing, Visualization. **Marc Kenis:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration. **Stephen D. Gaimari:** Formal analysis, Writing – review & editing. **Izolda Matchutadze:** Investigation, Writing – review & editing. **Deanna Zembrzuski:** Formal analysis, Writing – review & editing. **Hans Peter Ravn:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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