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ORIGINAL CONTRIBUTION

Dreyfusia nordmannianae in Northern and Central Europe: potential for biological control and comments on its taxonomy

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Abstract

The silver fir woolly adelgid, *Dreyfusia nordmannianae*, is the most severe pest occurring on *Abies nordmanniana* in Central and Northern Europe. The adelgid is particularly damaging to trees in Christmas tree plantations. *Dreyfusia nordmannianae* is native to the Caucasus region and alien to Europe, where its natural enemy complex is less diverse compared to its area of origin. Mitochondrial and nuclear DNA sequence data from the samples of *D. nordmannianae* collected in its native range and Europe and from the samples of *Dreyfusia piceae* and *Dreyfusia prelli* collected in Europe and North America were examined for phylogenetic structure. There was no evidence of differentiation, suggesting that these *Dreyfusia* species have recently diverged or require taxonomic revision. All existing published and unpublished reports on natural enemies of *D. nordmannianae* in its place of origin were reviewed, with the purpose of selecting agents for classical biological control in Europe. The literature review suggested that the most promising agent was the Chamaemyiidae fly, *Leucopis hennigrata*. A new survey in *D. nordmannianae*'s area of origin – Turkey, Georgia and Russia – showed that *L. hennigrata* was present in all localities. It was particularly abundant in Turkey, where its impact on populations of *D. nordmannianae* appears to be high. Its use as a biological control agent is discussed, as well as other biological control strategies.

Introduction

The silver fir woolly adelgid, *Dreyfusia nordmannianae* Eckst., was introduced to Europe from the Caucasus area in late 1800s on imported Nordmann fir, *Abies nordmanniana* (Stev.) Spach. The adelgid moved to European silver fir, *Abies alba* Mill., and soon adapted to this new host and spread throughout the geographic distribution of the tree species (Eichhorn 1968, 1991). In its region of origin, *D. nordmannianae* has a 2-year life cycle with Oriental spruce, *Picea orientalis* L. Link., as its primary host where there is a sexual generation and gall formation. *Abies nordmanniana* is the secondary host that supports only parthenogenetic generations (Eichhorn 1991). In Europe, however, *P. orientalis* is a rare ornamental tree and *D. nordmannianae* develops continuously on *Abies* spp. The adelgid exists in two forms, one that attacks twigs (the most damaging) and another that attacks stems (Eichhorn 1967a). It overwinters as first and/or second instars (hiemosistens) close to the base of the needles of young twigs. During March and April, they develop into mature females. Each female then lays 110–500 eggs in wax-covered clusters during April and May. Eggs hatch around the time when buds are expanding and the newly hatched crawlers settle on the stems and needles of the new growth. They soon develop into winged and wingless adult females. The former fly away to the primary host, while the latter stay and produce another generation on *A. nordmanniana*. The importance of particular natural enemies as potential biological controls for the suppression of *D. nordmannianae* will depend on how well synchronized they are to the life cycle of the adelgid.

Dreyfusia nordmannianae is part of a group of morphologically similar species (*D. nordmannianae*, *Dreyfusia piceae* Ratzeburg, *Dreyfusia merkeri* Eichhorn, *Dreyfusia prelli* Grossmann, *Dreyfusia nebrodensis* Binazzi and Covazzi, and *Dreyfusia schneideri* Börner) found in Europe and western Asia. Four additional *Dreyfusia* species are endemic to southern and eastern Asia, *Dreyfusia todomatsui* Inouye, *Dreyfusia joshii* Schneider-Orelli and Schneider, *Dreyfusia knucheli* Schneider-Orelli and Schneider and *Dreyfusia pindrowi* Yaseen and Ghani. The morphological differences that distinguish the *Dreyfusia* species found in Europe (e.g. the shape and distribution of dorsal sclerites and wax pores) are quite subtle and difficult to interpret (Mantovani et al. 2001; Havill and Foottit 2007). In fact, even the morphological differences used to distinguish the eight adelgid genera described by Börner and Heinze (1957) are considered by some authors to be too subtle and therefore inappropriate for this level of classification. Annand (1928), Blackman and Eastop (1994) and Havill and Foottit (2007) prefer to recognize two genera, *Adelges* and *Pineus*, because this reflects adelgid phylogeny more accurately.

Preliminary molecular studies by Mantovani et al. (2001) suggested some differentiations among species in the European *Dreyfusia* complex but the results were inconclusive, in part due to limited geographic sampling. Additional studies found that sequences from the mitochondrial cytochrome c oxidase I (COI) 'DNA barcoding' region, which is commonly used as an identification tool for many insect groups, were not able to distinguish *D. nordmannianae* from *D. piceae* and *D. prelli* (Foottit et al. 2009; Zurovcova et al. 2010). Havill et al. (2007) also found no differentiation between *D. nordmannianae* and *D. piceae* using a combination of mitochondrial and nuclear DNA sequences. The fact that some species in this complex are holocyclic and some are anholocyclic suggests that there have been important historical and climatic influences on host relationships (Steffan 1968); however, the extent to which morphological variability is genetically fixed vs. influenced by host plant physiology or other environmental effects has not been determined.

In Europe, *A. nordmanniana* is extensively grown as Christmas trees. The production of Christmas trees in Europe is estimated to be about 35 million trees per year and expected to increase to about 60 million trees per year in the next 5 years (K. Østergaard, Danish Christmas Trees, pers. comm. 2012). The production area presently covers about 60 000 ha (K. Østergaard, Danish Christmas Trees, personal communication 2012). For Denmark alone, the number of exported Christmas trees is about 10 million *Abies* each year with a value of up to 200 million € (Mainz 2012). *Dreyfusia nordmannianae* is the most significant pest problem and the target of most insecticidal treatments in Christmas tree plantations in Northern Europe. In Denmark, it is estimated that about two-thirds of the area grown with Christmas trees is sprayed for adelgid control every year (Christensen 1995). Because the use of chemical insecticides is increasingly banned or limited because of its negative impact on the environment and human health, the demand for organically grown Christmas trees is increasing and sustainable methods to replace insecticides for adelgid control are desperately needed. Because *D. nordmannianae* is an alien species that most likely arrived without a large part of its natural enemy complex, a classical biological control programme involving the introduction of natural enemies from the region of origin should be considered (Van Driesche and Bellows 1996).

Adelgidae are known for being among the few insect groups without parasitoids. Pathogens have been mentioned only occasionally as natural enemies of Adelgidae (e.g. Schimitschek 1952; Gouli et al. 1997). In contrast, adelgids are known to be preyed upon by a rich community of invertebrate predators. In their reviews on the predators of the balsam woolly adelgid, *D. piceae,* a European species accidentally introduced in North America, Pschorn-Walcher and Zwölfer (1956) reported over 30 species of predators occurring in Europe. More recently, surveys for predators of the hemlock woolly adelgid, *Adelges tsugae* Annand, in North America and Asia also revealed high numbers of species (Yu et al. 2000; Cheah et al. 2004).

Predators of *D. nordmannianae* have been less studied than those of the two previously mentioned adelgids. Data before 1960 are listed in the study by Herting (1972). Of particular interest were the surveys by Varty (1956), Wylie (1958), Pschorn-Walcher and Zwölfer (1956) and Pschorn-Walcher and Kraus (1958) in Scotland, France, Switzerland and Sweden, respectively, but it is the work by Otto Eichhorn and his colleagues which is by far the most complete. During the 1960s, they studied five fir adelgids occurring in Europe, *D. nordmannianae*, *D. piceae*, *D. prelli*, *D. merkeri* and an undescribed *Dreyfusia* sp. These studies included taxonomy, identification, mortality factors, population dynamics and control (e.g. Eichhorn 1964, 1967a,b, 1968, 1969a,b, 1991; Eichhorn et al. 1968). They investigated the predator complex of these adelgids and their importance for adelgid population dynamics (Eichhorn et al. 1962, 1968; Eichhorn 1964, 1966, 1968, 2000). The study on the predators of *D. nordmannianae* in Turkey (Eichhorn 1969a; revisited in Eichhorn 2000) is of particular importance because it provides the only data on natural enemies of the adelgid in its region of origin.

In this paper, we first summarize the published and unpublished information available on the natural enemies of *D. nordmannianae*; then, we describe our own observations on predators of the pest in its area of origin that were made during recent surveys in Turkey, Georgia and Russia. We also use DNA sequence data from *D. nordmannianae* specimens collected during these and other surveys to examine phylogenetic relationships within the European and western Asian *Dreyfusia* species complex. Finally, we discuss the potential for biological control of the adelgid in regions where it is invasive, with particular emphasis on Christmas tree plantations in Northern Europe.

Literature Review on Natural Enemies of *Dreyfusia nordmannianae*

Table 1 lists all the predator species recorded for *D. nordmannianae*, with their known distribution and approximate host range. The predator complex is

Table 1 List of the main predators reared from *Dreyfusia nordmannianae* in Europe and Turkey, on twigs and on stems, with approximate host range and distribution of observations

	Prey range[1]	Distribution on *D. nordmannianae*[2]	On twigs or on stems	References[3]
Diptera – Chamaemyiidae				
Cremifania nigrocellulata Czerny	DRE	CE, S	s	f, g
Leucopis hennigrata McAlpine	DRE	CE, T	t, (s)	a, d, i
Neoleucopis atratula (Ratzeburg)	DRE	CE,	s, (t)	c, f, g, i
Diptera – Cecidomyiidae				
Aphidoletes thompsoni Möhn	DRE	CE	s, (t)	c, g
Diptera – Syrphidae				
Episyrphus balteatus (De Geer),	POL	CE	t	a
Eupeodes lapponicus (Zetterstedt)	?	CE, T	t	a, d, i
Undetermined species[4]	?	CE, S	t	a, f
Coleoptera – Coccinellidae				
Adalia bipunctata (L.)	POL	T	t	d
Adalia decempunctata (L.)	POL	Sc	t	h
Anatis ocellata (L.)	POL	CE, Sc	t	e, h, i
Aphidecta obliterata (L.)	ADE	CE, T, S, Y	t, s	a, b, c, d, f, g, i
Coccinella septempunctata L.	POL	T	t	d
Brumus quadripustulatus (L.)	POL	CE, T	t	d, i
Harmonia quadripunctata (Pontoppidan)	POL	T	t	d
Myrrha octodecimguttata (L.)	POL	Sc	t	h
Scymnus impexus (Mulsant)	DRE	CE, Y	s, (t)	B, c, g, i
Hyppodamia undecimnotata (Schneider)	POL	T	t	d
Coleoptera – Derodontidae				
Laricobius erichsoni (Rosenhauer)	DRE	CE	s, (t)	c, g, i
Neuroptera – Chrysopidae				
Dichochrysa ventralis (Curtis)	POL	CE, S	s, t	c, g, i
Chrysopa vulgaris	POL	CE	t	a

[1] Approximate prey range: DRE: mainly or exclusively found on *Dreyfusia* spp. on fir; ADE: mainly or exclusively found on Adelgidae; POL: Polyphagous, that is, often found on other families; ?: Host range unclear.

[2] Regions where found on *D. nordmannianae*: CE: Central Europe, including Eastern France, Germany, Czech Republic, Switzerland; S: Sweden; Sc: Scotland; T: Turkey; Y: Former Yugoslavia.

[3] References: a: Eichhorn (1964); b: Eichhorn (1966); c: Eichhorn (1968); d: Eichhorn (1969a); e: Kucera (1955); f: Pschorn-Walcher and Kraus (1958); g: Pschorn-Walcher and Zwölfer (1956); h: Varty (1956); i: Wylie (1958).

[4] One or two undetermined species clearly different from the two species mentioned above.

dominated by polyphagous predators of sternorrhynchous Hemiptera but also contains several specialist predators of Adelgidae or *Dreyfusia*. While most species are probably native predators of European Adelgidae, it cannot be ruled out that some came more recently from the area of origin of *D. nordmannianae*. Pschorn-Walcher and Zwölfer (1956) and Eichhorn (1968) state that the predator complex of *D. nordmannianae* in Europe is less diverse than that of the closely related species *D. piceae* and *D. merkeri*, and that the twig-form of *D. nordmannianae* has an even poorer complex than the stem-form. Eichhorn (1968) suggested that the poorer predator complex may be due to the lack of a pronounced aestivosistens generation, which could be an obstacle for bi- and multivoltine predatory species. However, the fact that *D. piceae* is indigenous in Europe, whereas *D. nordmannianae* is exotic may also explain the difference in the predator complex. The main families of predators are discussed separately below.

Diptera – Chamaemyiidae

Leucopis spp. and related genera are important predators of aphids and adelgids. There has been some confusion in the identity of the species attacking *Dreyfusia* spp. in Europe and Turkey. However, at least two species, one univoltine and the other bivoltine, are reported from *D. nordmannianae*. *Neoleucopis atratula* (Ratzeburg) is a bivoltine species, specific on *D. piceae*, *D. merkeri* and *D. nordmannianae*, but found more often on stems than on twigs. In older publications (e.g. Pschorn-Walcher and Zwölfer 1956; Pschorn-Walcher and Kraus 1958; Wylie 1958), *N. atratula* was cited as *Leucopis* (or *Neoleucopis*, or *Leucopomyia*) *obscura* Haliday, and it was introduced in North America for the biological control of *D. piceae* as *L. obscura* (Eichhorn 1968). However, according to Eichhorn (1968) and Eichhorn et al. (1968), *Neoleucopis obscura* seems to be another, rare species, preying specifically on *D. prelli*. The biology and phenology of *N. atratula* is described in the study by Pschorn-Walcher and Zwölfer (1956) and Eichhorn (1968). It is well synchronized with the phenology of *D. piceae*, because larvae are active during the egg-laying period of both hiemosistens in spring and the aestivosistens in late summer. Overwintering occurs as larvae or pupae.

The second chamaemyiid species occurring on *D. nordmannianae*, *Leucopis hennigrata* McAlpine, is also known from *D. piceae* and *D. merkeri*, but seems to be far more common on twigs than on stems (Eichhorn 1968). Prior to its description (McAlpine 1978), it was cited as *L. griseola* Fallèn (e.g. Pschorn-Walcher and Zwölfer 1956), *L. melanopus* Tanas. (e.g. Eichhorn 1964) and *Leucopis* sp. (e.g. Eichhorn 1969a). In contrast to *N. atratula*, *L. hennigrata* is univoltine and therefore better adapted to the phenology of *D. nordmannianae*. According to Eichhorn (1969a), it was by far the most important predator of the adelgid in Turkey, where it was considered to be the main cause of the low abundance of *D. nordmannianae*. It was also observed on the same prey in France (Wylie 1958; Eichhorn 1964), Central Europe (Eichhorn et al. 1962) and Greece (Eichhorn 1966), but was not found in Sweden (Pschorn-Walcher and Kraus 1958). It feeds on adults, eggs and larvae in spring, pupates in May, emerges in August–September and probably overwinters as an adult.

Diptera – Cecidomyiidae

Only one cecidomyiid fly is recorded from *D. nordmannianae*, *Aphidoletes thompsoni* Möhn. It is a common predator of *Dreyfusia* spp. on fir, especially *D. piceae* (Pschorn-Walcher and Zwölfer 1956; Eichhorn 1968). Predatory larvae occur rather late in the season (i.e. rarely before the end of May), and are more commonly found on the aestivosistens than on the hiemosistens generation, suggesting that it would not be a good predator of *D. nordmannianae* because most *D. nordmannianae* population growth and damage to the host has already taken place when this predator is present. Eichhorn (1968) also recorded it on *D. nordmannianae* in Germany, but on the stem-form. It may be much less abundant or even absent on the twig-form, as suggested by the absence of records in France (Wylie 1958) or Turkey (Eichhorn 1969b). Pschorn-Walcher and Kraus (1958) found two specimens in Sweden, but they did not specify whether it was on stems or on twigs. It is not clear whether the low number of records on *D. nordmannianae*, especially the twig-form, is due to a lower preference for this species or form or due to a lack of sampling efforts later in the season when *Aphidoletes thompsoni* is more abundant. In any case, this fly is not a promising control agent for *D. nordmannianae*.

An unknown species of *Lestodiplosis* was found to be a common predator of the twig adelgid *D. prelli* in the Balkans (Eichhorn 1966), and it was also frequently found in stem colonies of undetermined *Dreyfusia* spp. It was never mentioned preying on the twig-form of *D. nordmannianae*.

Diptera – Syrphidae

Several syrphid species have been found preying on *Dreyfusia* spp., including *D. nordmannianae*. In Turkey, *Eupeodes lapponicus* (Zetterstedt) was one of the two major predators of *D. nordmannianae* in the spring and was partly responsible for the high predation rates in this region (Eichhorn 1969b; as *Syrphus lapponicus*). Larvae pupated in mid-May, and adults emerged in late May. *Eupeodes lapponicus* was also recorded [as *Syrphus arcuatus* (Fallèn)] on fir twigs attacked by *D. nordmannianae* in France (Wylie 1958), Central Europe (Eichhorn 1964) and Sweden (Pschorn-Walcher and Kraus 1958). In the latter, it was much less abundant than an undetermined syrphid species, preying frequently and exclusively on the twig-form. Mature larvae entered into an obligatory diapause and thus may be univoltine. Pschorn-Walcher and Kraus (1958) suspected the most abundant species could be *Parasyrphus* (=*Syrphus*) *punctulatus* (Verrall). Eichhorn (1964) provided some data on the biology of three syrphids found in Central Europe on twigs infested by *D. nordmannianae*: *E. lapponicus*, *Episyrphus* (=*Epistrophe*) *balteatus* (De Geer), a polyphagous species known to prey on many other sternorryncha and an undetermined species [perhaps *Melangyna* (=*Syrphus*) *lasiophthalma* (Zetterstedt)]. All three species were rather abundant, but only on twigs with heavy attacks. They all appeared early in the season. The first two species emerged for a second generation later in the season, whereas the undetermined species was univoltine with an obligatory diapause in the larval stage. This may be the same species as the one found in Sweden. Interestingly, surveys of stem-forms of *Dreyfusia* spp. in Germany and Switzerland (Eichhorn 1968) provided very few syrphids, and none were reported from *D. nordmannianae* on stems. In Denmark, larvae of syrphids only rarely are found on *A. nordmanniana* (Hans Peter Ravn, personal obs.). The only syrphid known to feed on *Dreyfusia* is *Neocnemodon vitripennis* (Meigen), a predator of *D. piceae* (Delucchi and Zwölfer 1957).

Coleoptera – Coccinellidae

Many ladybird species prey on *D. nordmannianae*; however, the majority of them are generalist predators only occasionally collected with the adelgid. Examples are *Adalia decempunctata* (L.), *Adalia bipunctata* (L.), *Harmonia quadripunctata* (Pontoppidan), *Coccinella septempunctata* L. and *Anatis ocellata* (L.), which has been studied on *D. nordmannianae* by Kucera (1955). *Brumus* (=*Exochomus*) *quadripustulatus* (L.) is also polyphagous; however, it is significant because it was found in abundance on *D. nordmannianae* in Turkey (Eichhorn 1969b).

Other coccinellids are more specifically associated with Adelgidae. *Scymnus* (*Pullus*) *impexus* (Mulsant) is a univoltine species nearly always found in association with *D. piceae*, of which it is one of the most abundant predators. It was introduced into North America for the biological control of *D. piceae*. It was occasionally recorded from *D. nordmannianae* in France (Wylie 1958) and Germany (Eichhorn 1968) but in most cases on stem-forms. It was not found in Sweden and Turkey. In contrast, *Aphidecta obliterata* (L.) is more commonly associated with *D. nordmannianae*, especially on twigs, than with *D. piceae* (Pschorn-Walcher and Zwölfer 1956; Pschorn-Walcher and Kraus 1958). It was found, usually in high numbers, in all studies of *D. nordmannianae* (Herting 1972) in France (Wylie 1958), Sweden (Pschorn-Walcher and Kraus 1958), Turkey (Eichhorn 1969a), Scotland (Varty 1956) and Switzerland and Germany (Eichhorn 1964, 1968). The larvae and adults feed on all stages of the adelgid. Adults appear in early April, and larvae are found in May. Pupation occurs in June and the adults do not reappear until the following spring. *Aphidecta obliterata* is known for its ability to find very scattered adelgid populations (Pschorn-Walcher and Kraus 1958; Eichhorn 1969a). In Turkey, larvae of *Aphidecta obliterata* complemented predation by *L. hennigrata* because they occurred mainly when the fly had started to pupate; consuming what remained (Eichhorn 1969a). *Aphidecta obliterata* is an oligophagous species. It is also found feeding on other Adelgidae and on aphids that feed on other conifer species (Pschorn-Walcher and Zwölfer 1956; Eichhorn et al. 1962).

Coleoptera – Derodontidae

Members of the derodontid genus *Laricobius* are only known to feed on Adelgidae (Leschen 2011), which makes them good candidates as biological controls. *Laricobius erichsoni* (Rosenhauer) shares many characteristics with the coccinellid *Scymnus impexus* as an important, rather specific predator of *D. piceae*. It has been introduced into North America as a biological control agent against this adelgid. It has been found occasionally on *D. nordmannianae* in Europe, but almost exclusively on stems (Wylie 1958; Eichhorn 1968). It was not found on twig populations in Sweden and Turkey. Therefore, this species is of little interest for the control of *D. nordmannianae*. *Laricobius caucasicus* Rost is described as having a distribution in the western Caucasus (Rost 1893). One of the authors (MK) collected one adult and one larval *Laricobius* from *D. nordmannianae* on 9 June in Guria Province, Georgia, that were tentatively identified as *L. caucasicus* by Leschen (2011). *Laricobius* tend to be most active early in the spring and in the autumn, so the impact of this predator on *Abies nordmannianae* in the Caucasus may not have been observed. It could therefore merit further study.

Neuroptera – Chrysopidae

The lacewing *Dichochrysa ventralis* (Curtis) is a polyphagous predator occasionally recorded from *Dreyfusia* spp., including *D. nordmannianae*, for example in France (Wylie 1958) or Sweden (Pschorn-Walcher and Kraus 1958). The latter study states that it is more abundant on *D. piceae* than on *D. nordmannianae*. Wylie (1958) summarizes its biology. Lacewing larvae feed on nymphs, adults and eggs of adelgids in spring and pupate in May–June. Adults emerge in summer and lay eggs, and the second larval instar overwinters on the infested trees. In addition, *Chrysopa vulgaris* L. was observed in twig attack of *D. nordmannianae* by Eichhorn (1964) who also reared this species on the adelgid in a cage.

Other predators

Other predators of *D. nordmannianae* are listed in Herting's review (1972), that is, anthocorid Heteroptera and trombidiid mites. Mites may play a role as predators, but their biology and ecology in relation to the Adelgidae are largely unknown.

Entomopathogens

Pathogens of *D. nordmannianae* have been very poorly studied. Schimitschek (1952) reports on an unidentified fungus, probably a Hypocreacea, which has been observed to reduce populations of *D. nordmannianae*. Information of pathogens of other Adelgidae is also scarce. An exception is the attempt to use entomopathogenic fungi isolated from *Adelges tsugae* to control this pest in North America (Cheah et al. 2004; Reid et al. 2010).

Impact of predators on populations of *Dreyfusia nordmannianae*

Only two publications provide data on the impact of predators on populations of *D. nordmannianae* and their role in population dynamics. Eichhorn (1968) studied the factors related to the population dynamics of *Dreyfusia* spp. in Germany and Switzerland, with emphasis on predators. However, he focused on adelgids feeding on *Abies* stems. Thus, only the stem-form of *D. nordmannianae* was included in the analyses. Furthermore, he separated the three investigated adelgid species (*D. piceae*, *D. merkeri* and *D. nordmannianae*) when considering mass attacks, whereas in spot attacks the three species were treated together. In general, the impact of the predators was found to be (i) higher in spot attacks than in mass attacks, suggesting that these predators are not density dependent, and (ii) higher in the aestivosistens generations than in the hiemosistens generations (no aestivosistens generation was found for *D. nordmannianae*). In mass attack of *D. nordmannianae*, 0.7% of the egg clusters contained a predator and 3–8% of the adults were killed by predators. These numbers are rather similar to those found for mass attacks of *D. piceae* and *D. merkeri* of the hiemosistens generation. In spot attacks of the three species pooled together, the average percentage of preyed adults varied from 25% to 51% in the hiemosistens generation.

The study of Eichhorn (1969a) in Turkey provides data on the impact of predators in the region of origin of *D. nordmannianae*. Populations are low in this region, and the adelgid is not considered to be a pest. Eichhorn (1969a) showed that the predators, especially *L. hennigrata*, are likely responsible for maintaining adelgid populations at low, uneconomic damaging levels. Between 76% and 93% of the adult hiemosistens were preyed upon in natural stands, and there was one predator per 6.5 egg masses (see the preceding paragraph for comparison with the situation in Central Europe). The predators appeared to be particularly efficient in locating every single egg cluster. The role of predators in natural stands was emphasized by the fact that the only site where high populations of adelgids were found was a nursery, where no predators were found.

New Surveys in the Region of Origin

Surveys were carried out in Turkey, Georgia and Russia in 2007 to investigate the predatory complex of *D. nordmannianae* in its region of origin and, in particular, to (i) confirm that Chamaemyiidae are important natural enemies of the adelgid throughout its native range; (ii) clarify the identity of *Leucopis* spp. in their region of origin; and (iii) make preliminary observations on the impact and specificity of the predators under field conditions in the area of origin. In addition, we collected *D. nordmannianae* in the three countries to clarify the genetic relationship between

D. nordmannianae found in its place of origin and in Central and Northern Europe.

Material and Methods

Surveys

Surveys in Turkey were organized to coincide in space and the time of year with those of Eichhorn (1969a, 2000) to confirm observations from the 1960s, showing that *D. nordmannianae* populations occur below levels that are considered to be of economic importance for Christmas trees and that *Leucopis* spp. are important predators of egg masses and females. All surveys were made in the Province of Bolu, in Northern Anatolia on 9–16 May 2007. Twelve sites between 900–1400 m above sea level were investigated for adelgids and predators. All adelgid colonies, mainly females and eggs, were found on Uludağ fir, *Abies nordmanniana* subsp. *bornmülleriana* (Mattf.). Uludağ fir was dominant or co-dominant at all investigated sites. *P. orientalis*, the primary host of *D. nordmannianae*, does not grow naturally in the area and is only rarely planted. At all sites, several (>1000 for all sites) young trees were carefully visually examined for the presence of *D. nordmannianae* and predators. Old damage (deformed and discoloured needles, missing wax stripes on needles) was searched on previous years' branches as well. Twigs with females and egg masses were collected in bags and counted and individually examined in the laboratory under a stereomicroscope. All predators found in the egg masses and on the twigs were counted. *Leucopis* abundance was roughly estimated by dividing the number of eggs and larvae by the number of adelgid egg masses examined. *Leucopis* larvae were kept in plastic vials and boxes to obtain pupae and adults for identification.

Surveys were also made on *A. nordmanniana* in Georgia and Russia, from where most seeds used for Christmas tree production in Europe originate. One of the authors (MK) visited two regions in Georgia on 4–12 June 2007: the Shuakhevi District in Ajara province (two sites sampled at 1600–1700 m a.s.l.) and the Bakhmaro district in Guria Province (three sites sampled at 1700–1950 m a.s.l.). In both regions, young *A. nordmanniana* were found in conifer forests dominated by *P. orientalis*. Several localities were investigated on 11–21 June in the Russian part of Caucasus (1.000–1.852 m a.s.l.) from Arkhyz in the East to Guzeripl in the West. Global positioning system (GPS) coordinates for the surveyed locations are shown in table 2. Although surveys in Georgia and Russia were carried out 1 month later than in Turkey, adelgids were in a similar developmental stage (i.e. females with eggs) because of the colder climatic conditions at these higher altitude and more northern sites. Surveys and examination were carried out as in Turkey, but local conditions for the examination of infested twigs and predators were less optimal because no laboratory was available close to the field sites and material had to be examined with a magnifying glass. A part of the samples were taken to Switzerland and Denmark for further examination.

Twenty-three specimens of *Leucopis* spp. adults obtained from larvae collected in Turkey (13 specimens, two sites) and Georgia (10 specimens, two sites) were sent for identification to Dr. Stephen Gaimari (California Department of Food and Agriculture,

Table 2 List of GPS coordinates for the sites visited during investigation 2007

Country	Site	Name	Date	Altitude (m)	GPS coordinates
Turkey	1–12	Bolu*	9–16 May	900–1400	40°44′19″N, 31°36′42″E
Georgia	1	Tselati	4–12 June	1600	41°29′13″N, 42°24′50″E
	2	Chirukhi	4–12 June	1600	41°28′48″N, 42°25′52″E
	3	Bakhmaro	4–12 June	1700	41°88′17″N, 42°35′44″E
	4	Bakhmaro	4–12 June	1950	41°86′11″N, 42°34′66″E
	5	Bakhmaro	4–12 June	1950	41°85′81″N, 42°32′72″E
Russia	1	Arkhuz	13 June	1639	43°32′17″N, 41°12′31″E
	2	Arkhuz	13 June	1852	43°34′22″N, 41°06′16″E
	3	Arkhuz	13 June	1853	43°33′52″N, 41°06′41″E
	4	Arkhuz	14 June	1689	43°30′35″N, 41°13′35″E
	5	Arkhuz	15 June	1677	43°32′17″N, 41°12′11″E
	6	Nikitina	16 June	685	43°59′30″N, 40°07′50″S
	7	Guzeripl	17 June	682	43°59′17″N, 40°07′33″E
	8	Guzeripl	17 June	682	43°59′17″N, 40°07′33″E

*All sites were within a radius of 40 km from Bolu.

USA), a specialist in Chamaemyiidae taxonomy. In addition, 12 specimens from the CABI Europe-Switzerland collection (Delémont, Switzerland) collected in 1969 on *D. piceae* in Germany by O. Eichhorn, and identified at that time as *L. hennigrata*, were sent for comparison.

Molecular systematics of *Dreyfusia nordmannianae*

Samples of *D. nordmannianae* from the Caucasus region, as well as *D. nordmannianae* and *D. piceae* from Europe and North America, were processed for molecular analyses (Appendix). DNA sequences for COI, cytochrome *b* (cyt*b*) and elongation factor-1 alpha (EF1α) were generated using the methods described in the study by Havill et al. (2006, 2007). In addition to the samples that we processed, COI and EF1α sequences associated with the studies described by Zurovcova et al. (2010) and Toenshoff et al. (2012) were obtained from GenBank. All new sequences generated for this study were deposited in GenBank, and voucher specimens were deposited at the Canadian National Collection of Insects, Ottawa, Canada.

Evolutionary relationships between COI and cyt*b* haplotypes were examined by constructing parsimony networks (Templeton et al. 1992), using the software TCS 1.20 (Clement et al. 2000). One sample, HEM061821, had an incomplete COI sequence and was therefore not included in the network analysis for this gene.

Phylogenetic analysis was performed using a combined data set with all three genes by using MrBayes 3.1 (Ronquist and Huelsenbeck 2003). Bayesian analysis was performed using separate unlinked GTR+I+G models for each gene, default priors, four incrementally heated Markov chains and two concurrent runs of 2 000 000 generations each, sampled every 1000 generations. A plot of log-likelihood scores vs. generation time was used to determine the number of 'burn-in' trees to discard. *Adelges cooleyi* (Gillette) and *Adelges pectinatae* (Cholodkovsky) were included as outgroups.

Results and Discussion

Surveys

In Turkey, the population density of *D. nordmannianae* was very low. Adelgid females and egg masses were found at nine of the twelve sites, but young trees with heavily infested single branches (>10% of the shoots with adelgids) were found at one site only. On some trees, signs of old damage were observed on branches of the previous year, but damage was not found to occur on the same tree in two consecutive years. In contrast to Eichhorn (1969a), we did not observe dead or dying young firs during the surveys.

A total of 1006 *D. nordmannianae* females with eggs were found and examined for predation. Within the egg masses, 134 *Leucopis* larvae or eggs were found, that is, an average of 0.15 *Leucopis* per egg mass per site (SD = 0.11, N = 9 sites). These numbers clearly underestimate *Leucopis* abundance as, at the low-altitude sites, most *Leucopis* larvae were large and several adelgid egg masses were found totally eaten and with a similar appearance as those known to have been preyed on by *Leucopis*. This suggests that some larvae may have already pupated. In contrast, at the highest altitude sites, adelgid oviposition had just started and only a few *Leucopis* eggs were found, suggesting that the fly oviposition period was not terminated. When the three highest sites are excluded, an average of 0.22 *Leucopis* was found per egg mass (SD = 0.07, N = 6 sites), which is similar to what Eichhorn (1969a) observed in the 1960s. Considering that a *Leucopis* larva may eat three to five egg masses during its lifetime (Eichhorn 1969a), it can be concluded that the predator probably has a severe impact on adelgid populations, although this impact could not be quantified during this survey. No other predator was found in Turkey, with the exception of one cecidomyiid larva and two syrphid pupae.

In Georgia, the density of *D. nordmannianae* and its predators was very different between the two regions. In the Shuakhevi District, adelgid populations were rather low, that is, similar to those observed in Turkey, with single branches occasionally heavily infested (>10% of twigs with adelgids). Only 23 *Leucopis* larvae were found among approximately 600 egg masses examined. However, all *Leucopis* found during collection were mature larvae and many *D. nordmannianae* females and egg masses showed obvious signs of predation, suggesting that most *Leucopis* had pupated prior to the collection. In Bakhmaro, many young firs were found heavily infested (i.e. with egg masses on nearly all twigs) and, on some trees, significant damage seemed to occur year after year, resulting in the formation of dwarf trees. From the approximately 1600 egg masses examined, only two empty *Leucopis* eggs were found, with hardly any sign of damage by predators in the egg masses. A few other predators were observed feeding on *D. nordmannianae* ovisacs in Georgia, including 27 cecydomyiids, five syrphids, one *Laricobius caucasicus* adult and six coleopteran larvae, possibly of *Laricobius*.

In Russia, no important damage by *D. nordmannianae* was observed on *A. nordmanniana,* and although some symptoms of feeding by adelgids were found on twigs of previous years, it seems that damage does not occur on the same tree or branch 2 years in a row. Colonies of *D. nordmannianae* and symptoms of their presence were most often found on small trees in suboptimal, shaded conditions. Only four *Leucopis* larvae were found in 902 egg masses at the two easternmost localities at the highest altitudes (1639 and 1852 m above sea level). However, ovisacs showing damage similar to that observed on ovisacs attacked by *Leucopis* were observed at all sites, which suggests that the timing of the visit was too late to make a proper assessment of *Leucopis* populations. Other predator species found in egg masses in Russia include nine cecidomyiids, two syrphids, three beetle larvae (possibly *Laricobius* sp.) and some mites.

No morphological differences were found between *Leucopis* adults collected from *D. nordmannianae* in Turkey and Georgia and from *D. piceae* in Germany. They were all identified as *L. hennigrata* by Dr. Stephen Gaimari (Plant Pest Diagnostics Branch, California Department of Food & Agriculture).

Molecular systematics of *Dreyfusia nordmannianae*

Cytochrome c oxidase I sequences were obtained for all analyses, and cyt*b* and EF1α were obtained for 42 and 34 samples, respectively. The single EF1α intron was removed for the outgroup taxa because it was not possible to align them. The final, concatenated alignment was 1699 bp long. Sequence divergence within the three genes ranged from 0% to 1.07%, 0% to 1.05% and 0% to 1.21% for COI, cyt*b* and EF1α, respectively.

Consistent with Havill et al. (2007), Foottit et al. (2009), Zurovcova et al. (2010) and Toenshoff et al. (2012), there was very little resolution among *Dreyfusia* samples (fig. 1). For example, the most common COI haplotype (Hap 1) was found in samples identified as all three species and in all three geographic regions. Cyt*b* sequences showed a similar pattern but with less resolution. Likewise, phylogenetic analyses using all three gene regions combined failed to resolve any clades within the group. The Bayesian tree (not shown) did not resolve any clades with a posterior probability >50%. The neighbour-joining tree shown in fig. 2 therefore should not be interpreted as showing support for phylogenetic relationships within *Dreyfusia* but does illustrate the limited genetic variation and lack of pattern associated with species or geography. These results are in sharp contrast to the strong phylogenetic signal shown by these same genes in *Adelges tsugae*, where there was clear differentiation

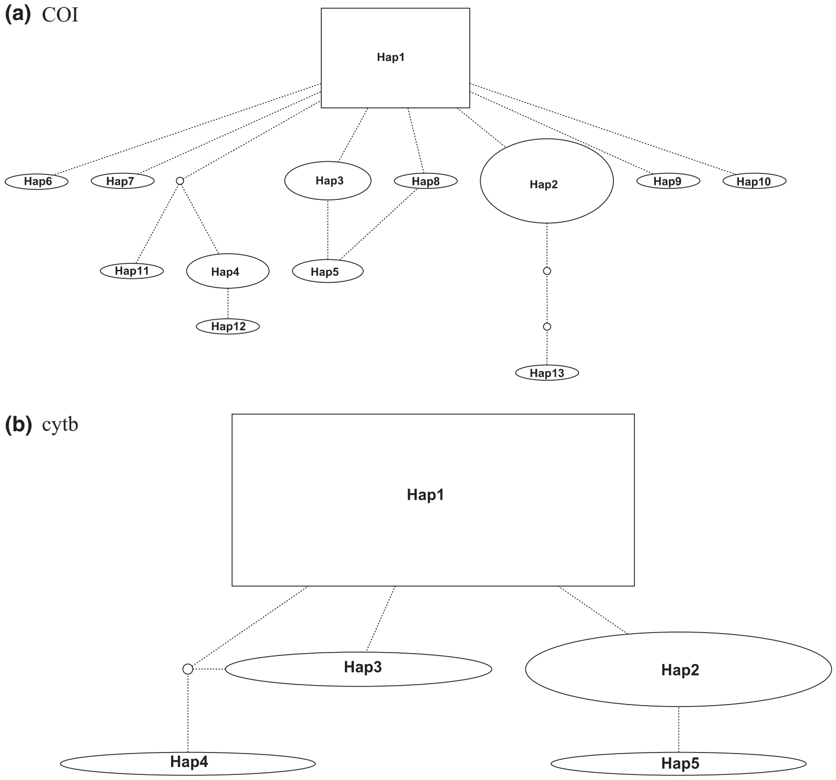

Fig. 1 Network showing relationships between (a) cytochrome c oxidase I and (b) *cytb* mitochondrial haplotypes for *Dreyfusia nordmannianae*, *Dreyfusia piceae* and *Dreyfusia prelli* samples. The size of each shape is proportional to the frequency of the haplotype. Haplotype names are shown inside the shapes, and their association with each sample are included in Appendix. Small open circles represent unsampled mutations that separate observed haplotypes. The rectangular haplotype is inferred as ancestral based on the frequency in the population and the number of connections to other haplotypes (Clement et al. 2000).

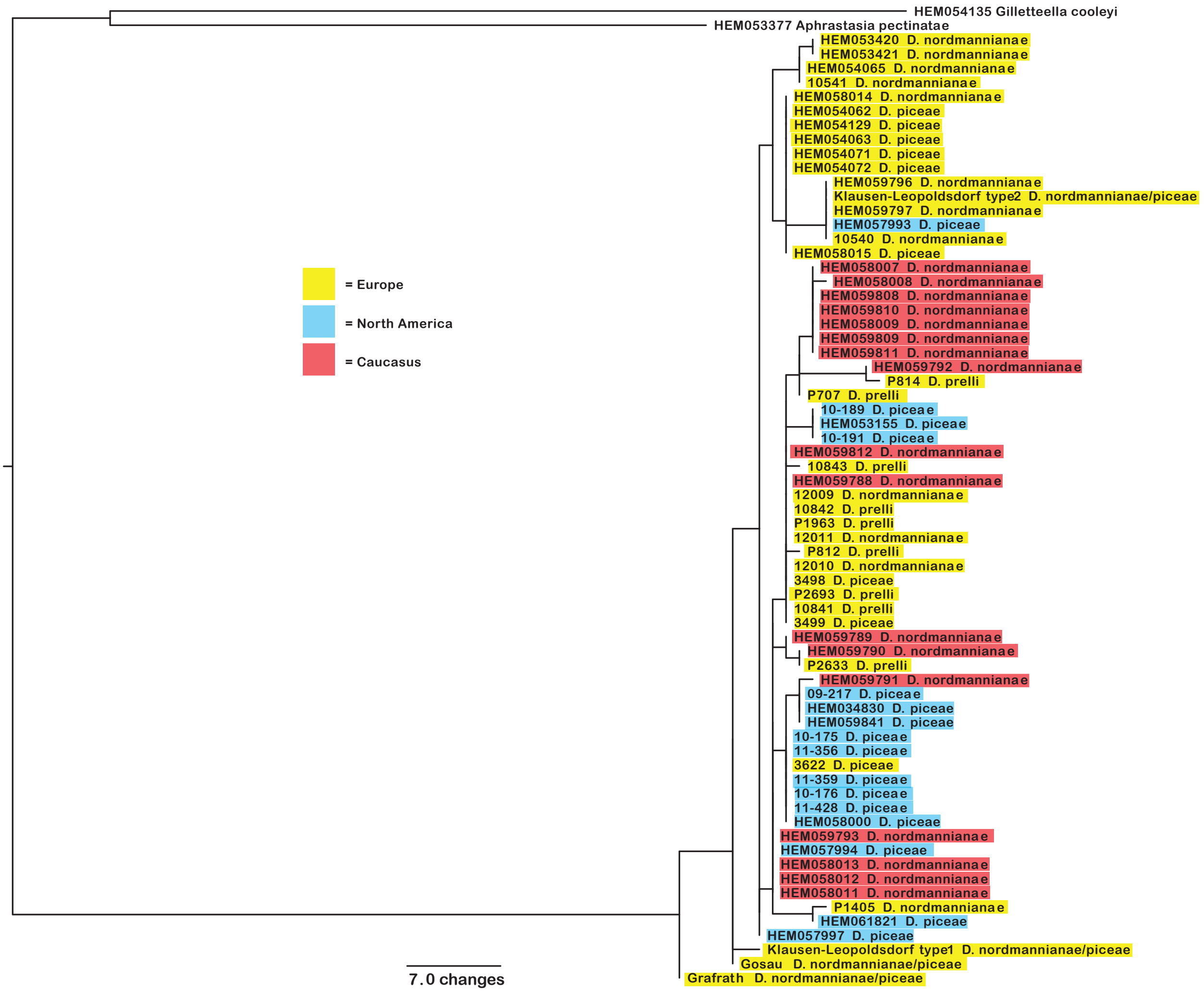

Fig. 2 Neighbour-joining tree for *Dreyfusia* spp. generated using DNA sequence data from the mitochondrial cytochrome c oxidase I and *cytb* genes and the nuclear EF1α gene. The geographic origin of each sample is designated with colour. *Adelges cooleyi* and *Adelges pectinatae* are included as outgroup taxa.

among lineages in different regions and associated with different host tree species in the same region (Havill et al. 2007).

While it is not possible to determine the invasion history of *D. nordmannianae* and *D. piceae* from these results, it does confirm that samples from Europe and the Caucasus are from the same species complex. The Caucasus region is still interpreted as the area of origin because both the primary and secondary hosts are native to this area and the host alternating life cycle is the ancestral life cycle in Adelgidae with non-host-alternating populations being derived from this (Havill and Foottit 2007).

These results indicate either that the *Dreyfusia* species included in this study are not isolated biological units, or that they are distinct but very recently diverged. A thorough analysis comparing morphological, molecular and biological characteristics in relation to phylogenetic pattern is required. Also required are population-level molecular analyses, such as with microsatellites (e.g. Ross et al. 2010), of each species in order to form informative hierarchies of relationships among populations in relation to the currently recognized species concepts. This kind of critical taxonomic examination at the genus and species level would allow for accurate interpretation of biological characteristics of importance for effective management such as differences in predation, phenology, life cycle, host association and impacts on tree health.

Potential for Biological Control in Northern Europe

Biological control activities are usually classified into three categories (Van Driesche and Bellows 1996). (i) *Classical biological control* is the introduction and establishment of an exotic natural enemy into a new region, to provide permanent control of a pest that is usually exotic itself. (ii) *Biological control by augmentation* implies inoculative or inundative releases of a natural enemy for an immediate control. (iii) *Biological control by conservation* includes any method that favours and enhances the abundance and impact of natural enemies that are already present in the system. The three categories of biological control could be considered against *D. nordmannianae* in Northern Europe. They are discussed separately herein.

Classical biological control

Dreyfusia nordmannianae is an exotic pest in Central and Northern Europe. Consequently, a classical biological control programme involving the introduction of an exotic natural enemy, probably a predator, from its region of origin seems a judicious strategy. This species is not considered a pest in its region of origin, suggesting that it may be controlled by biotic factors, some of which may be lacking in Europe. The information available on the predators in the region of origin of the adelgid is limited to a single study in Turkey in the 1960s and our preliminary surveys in Turkey, Georgia and Russia, but these observations suggest a much higher impact by predators there than in Europe. The reason for this difference is not clear because the predator complex in the region of origin seems rather similar to that found in Central Europe. However, *L. hennigrata*, which is apparently a key predator in Turkey, is much less abundant in Central Europe and not recorded from Northern Europe (Fauna Europaea 2011; Thomas Pape, Zoological Museum, Copenhagen, personal communication 2011). Furthermore, the taxonomy of some of these predators, particularly the Chamaemyiidae, is confusing and different cryptic species may be involved.

The possible non-target effect of introduced natural enemies is increasingly becoming a concern in biological control (Babendreier 2007). Nowadays, polyphagous natural enemies are excluded from biological control programmes, and only natural enemies that are unlikely to prey or parasitize non-target organisms are chosen for introduction. This criterion may undoubtedly exclude some predators that could be found in the region of origin of *D. nordmannianae*. However, several predators of Adelgidae are specific at the family, genus or even species level. In particular, the Chamaemyiidae seem to show a great level of specificity, such as *N. obscura* which has been found only on *D. prelli* (Eichhorn 1968; Eichhorn et al. 1968). Specificity at the genus level would probably be acceptable, because all *Dreyfusia* spp. in Europe are abundant and considered as minor or major pests of fir.

Classical biological control has already been attempted against adelgids with various levels of success. Mills (1990) provides a review of biological control attempts against Adelgidae. The biological control programme against the European *D. piceae* in North America is considered a failure, despite the establishment of six predators from Europe. So far, the recent introductions of predators to control the hemlock woolly adelgid, *Adelges tsugae*, have not resulted in the control of the pest (Cheah et al. 2004). In contrast, the pine adelgids (*Pineus* spp.) have been controlled successfully in New Zealand, Chile and Hawaii by various species of *Leucopis*. There has been much confusion in these biological control projects and subsequent literature regarding the identity of both the prey (it seems that, finally, all introduced populations may belong to *Pineus boerneri* Annand) and the predators (at least two species, probably *Leucopis tapiae* Blanchard and *Leucopis nigraluna* McAlpine, were involved in these successes) (Mills 1990; Greathead 1995; Day et al. 2003). On the other hand, the anthocorid predator *Teraphelps raoi* Ghauri, introduced against *Pineus boerneri* in eastern Africa, failed to control the pest.

The success in the control of *Pineus boerneri*, Eichhorn's work and our surveys show that Chamaemyiidae should be important predators to consider in a biological control programme against *D. nordmannianae*. The first choice for further investigations should be *L. hennigrata*, which appeared an important predator in Turkey and possibly, the Caucasus. High predation at sites with very low prey density in Turkey suggests high ability to locate hosts and maintain prey populations at low density. Molecular tools should be used to verify that the same species occur in Turkey, the Caucasus and Central Europe and can be used to identify larvae without having to rear them to the adult stage. Specificity should be further investigated by laboratory testing and by sampling other adelgid and aphid species where and when *L. hennigrata* is known to occur. Different geographic populations should be tested in their behaviour, life cycle and efficacy to select the best populations to introduce in

Northern Europe. Studies should particularly focus on Turkish populations in the region of Bolu because it is in this region that *L. hennigrata* is most abundant. Also, the phenology of the adelgid in this area is very similar to that observed in Christmas tree plantations in Denmark, with eggs being laid in early May, while oviposition occurs much later in the Caucasus Mountains. Thus, geographic populations of the predators may also vary in their ecology and reactions to climatic factors. However, in this region, the host tree is Uludağ fir, a subspecies of the *A. nordmanniana*, while those firs used in Christmas tree plantations in Northern Europe originally come from the Caucasus. Therefore, Caucasian populations of *L. hennigrata* should not be neglected. Furthermore, both Eichhorn's and our surveys in the region of origin were focused on predators of *D. nordmannianae* eggs and adults, and predation on nymphs should be further investigated. Finally, it must be noted that the natural enemy complex of *D. nordmannianae* has been only partially studied in Northern Europe (Pschorn-Walcher and Kraus 1958; Ravn and Riis-Nielsen 2006), where damage is highest and biological control is most desirable. Thus, a classical biological control programme should start with a proper investigation on natural enemies and their impact both in the regions of origin and in the introduced range.

Biological control by augmentation

Inundative releases of parasitoids and predators are often considered non-economic against forest pests except for high-value crop trees such as seed orchards or Christmas trees (Dahlsten and Mills 1999). Some of the predators of *D. nordmannianae* found in Europe, such as species of Chrysopidae and Coccinellidae, belong to groups that are commonly mass reared for use in augmentative biological control, and mass production techniques could be adapted to the local chrysopid and coccinellid species already present on *D. nordmannianae* in Denmark. Recent investigations have shown that chrysopid and especially coccinellid species are abundant in Danish Christmas tree plantations (Ravn and Riis-Nielsen 2006).

Pathogens are commonly used as biopesticides against forest pests (Dahlsten and Mills 1999). Of particular interest is the development of biopesticides based on entomopathogenic fungi against *Adelges tsugae* in North America (Reid et al. 2010). Should these studies result in the development of an efficient biopesticide, a similar approach could be considered for the control of *D. nordmannianae* in Denmark and elsewhere.

Biological control by conservation

Silvicultural practices to conserve and enhance the action of natural enemies present in the system can be developed only when the natural enemy complex is known. Therefore, it is of high importance to carry out a study in fir plantations in Denmark that would list the predators already present in the plantations, assess their abundance and impact and, if possible, identify the constraints that prevent them from being more efficient. If exotic predators are introduced into Europe, their action may also be enhanced by conservation strategies. Methods to favour predators in fir plantations are numerous and may include, for example, a better use of insecticide treatments (Ruberson et al. 1998). A more specific insecticide, lower concentration or a slight modification of the dates of spray may have a strong impact on predator populations. The planting of flower strips in Christmas tree plantations may provide nectar and pollen for adult predators or support aphid species as alternate hosts for the predators (Pickett and Bugg 1998). A study comparing abundance of all arthropod groups in organic and conventional Danish Christmas tree plantations (Ravn and Riis-Nielsen 2006) could provide a foundation on which specific studies on the use of predators in conservation biological control could be built.

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Appendix: Sample information for molecular phylogeny

			Haplotype/GenBank accession No.		
Species	Sample ID	Collection information	COI	cytb	EF1α
Adelges cooleyi	HEM054135	USA; Alaska; Sitka; 8.6.2005; coll. M. Schultz; ex. *Pseudotsuga menziesii*	EF073065[1]	EF073185[1]	EF073224[1]
Adelges pectinatae	HEM053377	JAPAN; Kamitakara-mura; 10.6.2004; coll. N.P. Havill; G. Yu; S. Shiyake; ex. *Abies veitchii*	EF073081[1]	EF073191[1]	EF073236[1]
Dreyfusia nordmannianae	HEM053420	Slovakia; Spania Dolina; 05.11.2004; coll. Josef Vakula; ex. *Abies alba*	Hap2/EF073080[1]	Hap5/EF073190[1]	EF073235[1]
Dreyfusia nordmannianae	HEM053421	Slovakia; Spania Dolina; 05.11.2004; coll. Josef Vakula; ex. *Abies alba*	Hap2/FJ502448[2]	–	–
Dreyfusia nordmannianae	HEM054065	Switzerland; Delemont; 13.6.2005; coll. N.Havill, M. Kenis; ex. *Picea orientalis*	Hap2/FJ502447[2]	Hap2/JX266986	JX266957
Dreyfusia nordmannianae	HEM058007	Turkey; Sultankoy-Yemisa; 11.5.2007; coll. M. Kenis, H.P. Ravn; ex. *Abies nordmanniana*	Hap4/FJ502460[2]	Hap1/JX266987	JX266958

(continued)

Table (continued)

			Haplotype/GenBank accession No.		
Species	Sample ID	Collection information	COI	cytb	EF1α
Dreyfusia nordmannianae	HEM058008	Turkey; Gölkuk Lake; 12.5.2007; coll. M. Kenis, H.P. Ravn; ex. *Abies nordmanniana*	Hap12/FJ502459[2]	Hap1/JX266988	JX266959
Dreyfusia nordmannianae	HEM058009	Turkey; Atyalasi; 14.5.2007; coll. M. Kenis, H.P. Ravn; ex. *Abies nordmanniana*	Hap4/FJ502458[2]	Hap1/JX266989	JX266960
Dreyfusia nordmannianae	HEM058011	Georgia; Tselati; 6.6.2007; coll. M. Kenis; ex. *Abies nordmanniana*	Hap1/FJ502457[2]	Hap1/JX266990	JX266961
Dreyfusia nordmannianae	HEM058012	Georgia; Chirukhi; 6.6.2007; coll. M. Kenis; ex. *Abies nordmanniana*	Hap1/FJ502456[2]	Hap1/JX266991	JX266962
Dreyfusia nordmannianae	HEM058013	Georgia; Bakhmaro; 9.6.2007; coll. M. Kenis; ex. *Abies nordmanniana*	Hap1/FJ502455[2]	Hap1/JX266992	JX266963
Dreyfusia nordmannianae	HEM058014	Switzerland; Delémont; 3.5.2007; coll. M. Kenis; ex. *Abies alba*	Hap2/FJ502454[2]	Hap2/JX266993	JX266964
Dreyfusia nordmannianae	HEM059788	Russia; Arkhyz; 13.6.2007; coll. H.P. Ravn; ex. *Abies nordmanniana*	Hap1/FJ502453[2]	Hap1/JX266994	JX266965
Dreyfusia nordmannianae	HEM059789	Russia; Arkhyz; 13.6.2007; coll. H.P. Ravn; ex. *Abies nordmanniana*	Hap1/JX267025	Hap1/JX266995	JX266966
Dreyfusia nordmannianae	HEM059790	Russia; Arkhyz; 15.6.2007; coll. H.P. Ravn; ex. *Abies nordmanniana*	Hap1/JX267026	Hap1/JX266996	–
Dreyfusia nordmannianae	HEM059791	Russia; Nikitina nr. Psebay; 16.6.2007; coll. H.P. Ravn; ex. *Abies nordmanniana*	Hap8/JX267027	Hap1/JX266997	–
Dreyfusia nordmannianae	HEM059792	Russia; Guzeripl nr. Lagunaki; 18.6.2007; coll. H.P. Ravn; ex. *Abies nordmanniana*	Hap11/JX267028	Hap4/JX266998	JX266967
Dreyfusia nordmannianae	HEM059793	Russia; Guzeripl; 19.6.2007; coll. H.P. Ravn; ex. *Abies nordmanniana*	Hap1/JX267029	Hap1/JX266999	–
Dreyfusia nordmannianae	HEM059796	Denmark; Hvalsoe; 4.10.2007; coll. H.P. Ravn; ex. *Abies nordmanniana*	Hap2/JX267030	Hap2/JX267000	–
Dreyfusia nordmannianae	HEM059797	Denmark; Snave; 11.2007; coll. H.P. Ravn; ex. ex. *Abies nordmanniana*	Hap2/FJ502452[2]	Hap2/JX267001	–
Dreyfusia nordmannianae	HEM059808	Turkey; Dereceören; 10.5.2007; coll. H.P. Ravn, M. Kenis; ex. *Abies nordmanniana*	Hap4/FJ502451[2]	Hap1/JX267002	JX266968
Dreyfusia nordmannianae	HEM059809	Turkey; Lake Abant; 10.5.2007; coll. H.P. Ravn, M. Kenis; ex. *Abies nordmanniana*	Hap4/FJ502450[2]	Hap1/JX267003	JX266969
Dreyfusia nordmannianae	HEM059810	Turkey; Dereceören; 13.5.2007; coll. H.P. Ravn, M. Kenis; ex. *Abies nordmanniana*	Hap4/FJ502449[2]	Hap1/JX267004	JX266970
Dreyfusia nordmannianae	HEM059811	Turkey; Dereceören; 13.5.2007; coll. H.P. Ravn, M. Kenis; ex. *Abies nordmanniana*	Hap4/JX267031	Hap1/JX267005	JX266971
Dreyfusia nordmannianae	HEM059812	Russia; Arkhyz; 13.6.2007; coll. H.P. Ravn; ex. *Abies nordmanniana*	Hap1/JX267032	Hap1/JX267006	JX266972
Dreyfusia nordmannianae	P1405	Czech Republic; Chvalčov; 13.4.2007 ex. *Abies alba*	Hap13/GU571016[3]	–	–
Dreyfusia nordmannianae	10540	Czech Republic; Libín; 11.3.2008; ex. *Abies alba*	Hap2/GU571017[3]	–	–
Dreyfusia nordmannianae	10541	Czech Republic; Libín; 11.3.2008; ex. *Abies alba*	Hap2/GU571018[3]	–	–

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Table (continued)

			Haplotype/GenBank accession No.		
Species	Sample ID	Collection information	COI	cytb	EF1α
Dreyfusia nordmannianae	12009	Czech Republic; Buchlovice; 15.8.2008; ex. *Picea orientalis*	Hap1/GU571019[3]	–	–
Dreyfusia nordmannianae	12010	Czech Republic; Buchlovice; 15.8.2008; ex. *Picea orientalis*	Hap1/GU571020[3]	–	–
Dreyfusia nordmannianae	12011	Czech Republic; Buchlovice; 15.8.2008; ex. *Picea orientalis*	Hap1/GU571021[3]	–	–
Dreyfusia nordmannianae/ piceae	Grafrath	Germany; Grafrath; ex. *Abies nordmanniana*	Hap1/HQ668157[4]	–	HQ668167[4]
Dreyfusia nordmannianae/ piceae	Klausen-Leopoldsdorf 1	AUSTRIA; Klausen-Leopoldsdorf; ex. *Abies alba*	Hap2/HQ668156[4]	–	HQ668165[4]
Dreyfusia nordmannianae/ piceae	Klausen-Leopoldsdorf 2	AUSTRIA; Klausen-Leopoldsdorf; ex. *Abies alba*	Hap2/HQ668156[4]	–	HQ668166[4]
Dreyfusia piceae	Gosau	AUSTRIA; Gosau; ex. *Abies alba*	Hap2/HQ668155[4]	–	HQ668164[4]
Dreyfusia piceae	ENT764291	USA; North Carolina; Mt. Mitchell State Park; 7.2009; coll. F. Hain; ex. *Abies fraseri*	Hap5/JX267033	Hap1/JX267007	–
Dreyfusia piceae	ENT764281	USA; Montana; Kootenai National Forest; 18.10.2010; coll. L. Pederson; ex. *Abies lasiocarpa*	Hap3/JX267034	Hap1/JX267008	JX266973
Dreyfusia piceae	ENT764285	USA; Idaho; Kamisku National Forest; 10. 9.2010; coll. L. Pederson; ex. *Abies lasiocarpa*	Hap3/JX267035	Hap1/JX267009	JX266974
Dreyfusia piceae	ENT764292	Canada; New Brunswick; Kingsclear; 10.2010; coll. R.D. Carleton; ex. *Abies balsamea*	Hap1/JX267036	Hap3/JX267010	JX266975
Dreyfusia piceae	ENT764299	CANADA; New Brunswick; Kingsclear; 10.2010; coll. R.D. Carleton; ex. *Abies balsamea*	Hap1/JX267037	Hap3/JX267011	JX266976
Dreyfusia piceae	ENT764302	USA; Washington; Olympic National Park; 5.8.2011; coll. K. Hutten; ex. *Abies lasiocarpa*	Hap3/JX267038	Hap1/JX267012	–
Dreyfusia piceae	ENT764303	USA; Washington; Olympic National Park; 24.8.2011; coll. S.R. Davies, S. F. Luxton; ex. *Abies lasiocarpa*	Hap3/JX267039	Hap1/JX267013	–
Dreyfusia piceae	ENT764304	USA; California; Fort Bragg; 8.12.2011; coll. K. Hutten; ex. *Abies grandis*	Hap3/JX267040	Hap1/JX267014	JX266977
Dreyfusia piceae	HEM034830	USA; North Carolina; Asheville; 13.5.1998; coll. R. Foottit; ex. *Abies fraseri*	Hap5/FJ502469[2]	–	–
Dreyfusia piceae	HEM053155	USA; Maine; Owlshead; 17.5.2002; coll. C. Donoghue; ex. *Abies balsamea*	Hap1/EF073085[1]	Hap3/EF073194[1]	EF073239[1]
Dreyfusia piceae	HEM054062	Switzerland; Delémont; 13.6.2005; coll. N. Havill, M. Kenis; ex. *Abies alba*	Hap2/JX267041	Hap2/JX267015	JX266978
Dreyfusia piceae	HEM054063	Switzerland; Delémont; 13.6.2005; coll. N. Havill, M. Kenis; ex. *Abies alba*	Hap2/EF073086[1]	Hap2/EF073195[1]	EF073240[1]
Dreyfusia piceae	HEM054071	Switzerland; Bourrignon; 13.6.2005; coll. N. Havill, M. Kenis; ex. *Abies alba*	Hap2/FJ502472[2]	Hap2/JX267016	JX266980
Dreyfusia piceae	HEM054072	Switzerland; Bourrignon; 13.6.2005; coll. N. Havill, M. Kenis; ex. *Abies alba*	Hap2/FJ502471[2]	Hap2/JX267017	JX266981
Dreyfusia piceae	HEM054129	Switzerland; Delémont; 13.6.2005; coll. N. Havill, M. Kenis; ex. *Abies alba*	Hap2/FJ502470[2]	Hap2/JX267018	JX266982

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Table (continued)

Species	Sample ID	Collection information	Haplotype/GenBank accession No.		
			COI	cytb	EF1α
Dreyfusia piceae	HEM057993	USA; Massachusetts; Jamaica Plain; Arnold Arboretum; 12.9.2007; coll. N. Havill; ex. *Abies nordmanniana*	Hap2/FJ502479[2]	Hap2/JX267019	–
Dreyfusia piceae	HEM057994	USA; Massachusetts; Jamaica Plain; Arnold Arboretum; 12.9.2007; coll. N. Havill; ex. *Abies cilicica*	Hap1/FJ502478[2]	Hap1/JX267020	–
Dreyfusia piceae	HEM057997	USA; Massachusetts; Jamaica Plain; Arnold Arboretum; 12.9.2007; coll. N. Havill; ex. *Abies alba*	Hap2/FJ502477[2]	Hap1/JX267021	–
Dreyfusia piceae	HEM058000	USA; Massachusetts; Jamaica Plain; Arnold Arboretum; 12.9.2007; coll. N. Havill; ex. *Abies lasiocarpa*	Hap3/FJ502476[2]	Hap1/JX267022	JX266983
Dreyfusia piceae	HEM058015	Switzerland; Develier; 7.5.2007 coll. M. Kenis; ex. *Abies alba*	Hap2/FJ502474[2]	–	–
Dreyfusia piceae	HEM059841	USA; North Carolina; Banner Elk; 16.4.2008; coll. M.E. Montgomey, R. McDonald; ex. *Abies fraseri*	Hap5/FJ502473[2]	Hap1/JX267023	JX266984
Dreyfusia piceae	HEM061821	USA; Washington; Tacoma; 12.5.2008; coll. M.E. Montgomey, R. McDonald; ex. *Abies* sp.	Hap1/JX267042	Hap1/JX267024	JX266985
Dreyfusia piceae	3498	Czech Republic; Bystřice pod Hostýnem; 17.9.2008; ex. *Abies alba*	Hap1/GU571022[3]	–	–
Dreyfusia piceae	3499	Czech Republic; Bystřice pod Hostýnem; 17.9.2008; ex. *Abies alba*	Hap1/GU571023[3]	–	–
Dreyfusia piceae	3622	Czech Republic; České Budějo-vice, Branišov; 9.11.2008; ex. *Abies alba*	Hap3/GU571024[3]	–	–
Dreyfusia prelli	P812	Czech Republic; Buchlovice; 28.6.2005; ex. *Picea orientalis*	Hap6/GU571025[3]	–	–
Dreyfusia prelli	P814	Czech Republic; Buchlovice; 28.6.2005; ex. *Picea orientalis*	Hap10/GU571026[3]	–	–
Dreyfusia prelli	P707	Czech Republic; Nový Dvůr; 29.6.2005	Hap1/GU571027[3]	–	–
Dreyfusia prelli	P1963	Czech Republic; Buchlovice; 17.7.2007; ex. *Picea orientalis*	Hap1/GU571028[3]	–	–
Dreyfusia prelli	P2693	Czech Republic; Buchlovice; 17.7.2007; ex. *Picea orientalis*	Hap1/GU571029[3]	–	–
Dreyfusia prelli	P2633	Czech Republic; Lednice; 28.6.2007; ex. *Picea orientalis*	Hap7/GU571030[3]	–	–
Dreyfusia prelli	10841	Czech Republic; Buchlovice; 20.6.2008; ex. *Picea orientalis*	Hap1/GU571031[3]	–	–
Dreyfusia prelli	10842	Czech Republic; Buchlovice; 20.6.2008; ex. *Picea orientalis*	Hap1/GU571032[3]	–	–
Dreyfusia prelli	10843	Czech Republic; Buchlovice; 20.6.2008; ex. *Picea orientalis*	Hap9/GU571033[3]	–	–

Haplotype designation is shown for mitochondrial regions.

[1] From Havill et al. (2007).

[2] From Foottit et al. (2009).

[3] From *Pineus*Zurovcova et al. (2010).

[4] From Toenshoff et al. (2012).