



Flower-visiting and pollen-carrying arthropods of *Leucojum aestivum* L. (Amaryllidaceae) in wild, reintroduced and ex situ populations

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Abstract *Leucojum aestivum* L. is a wetland-dependent perennial geophyte occurring in Europe and western Asia. It is self-incompatible, with high level of fruit-set and seed-set. Yet, its pollinators are poorly known. Here, we present the most recent research on potential pollinators of *L. aestivum*. We collected data on flower-visiting and pollen-carrying arthropods in three populations of the species in the River Po plain (N-Italy), where *L. aestivum* occurs mainly in forests with *Alnus glutinosa* and *Fraxinus* sp. pl. close to rivers. We studied a wild population, a new reintroduced population and an ex situ population located at the Botanical Garden of the University of Pavia. Our study identified 18 arthropod taxa carrying *L. aestivum*

pollen; the soft-winged flower beetle *Dasytes plumbeus* (Coleoptera: Melyridae) and the sac-spider *Clubiona* sp. pl. (Araneae: Clubionidae) were the most frequent visitors. Hymenoptera only occasionally visited *L. aestivum* flowers (e.g. the sweat bee *Lasioglossum punctatissimum*). *D. plumbeus*, the long-horned beetle *Grammoptera ruficornis* (Coleoptera: Cerambycidae), *Clubiona* sp. pl. and *L. punctatissimum* resulted in the taxa with the highest average abundance of pollen grains on their body suggesting a potential role in *L. aestivum* pollination. Differences in flower-visiting and pollen-carrying arthropods were observed between the three populations, with a decreasing taxonomic diversity of visiting species from the wild population to the ex situ population. Our results, based on direct observation in the field, are partially in contrast with a previous study on *L. aestivum* pollinators that suggested diurnal and nocturnal Lepidoptera and occasionally bees as main pollinators for the species.

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Introduction

Leucojum aestivum L. subsp. *aestivum* (*L. aestivum* hereafter) is a geophyte in the Amaryllidaceae family (Parolo et al. 2011). It is an C-S-European/W-Asiatic plant species and its range extends from Ireland in the north to N-Italy in the South, and from Middle East (Iran and Caspian Sea) to W-France in the West (Parolo et al. 2011). *L. aestivum* is listed in the global IUCN red list as least concern (Lansdown et al. 2014), but it is locally threatened (e.g. VU in Italy, Orsenigo et al. 2021; see also Parolo et al. (2011) for an overview of the national status of *L. aestivum* in Europe) and thus a species of conservation interest, locally. It grows in wetland plant communities, like wet forests in the lowland river valleys and in wet meadows, near different types of water bodies such as rivers, swamps and lake shores, banks of canals and periodically inundated sites, from 0 to 350 m a.s.l. (Kasermann and Moser 1999; Parolo et al. 2011). In northern Italy, the wetlands where *L. aestivum* grows are island-like fragments of natural and semi-natural habitats in a landscape dominated by crops. The small size of suitable habitat has negative consequences on *L. aestivum* population size and density and on pollinator availability, also declining as a consequence of habitat fragmentation and pesticides (Brittain et al. 2010). Population isolation due to habitat fragmentation results in a low within population genetic diversity and a medium–high genetic differentiation among *L. aestivum* populations in northern Italy (Gentili et al. 2018). Although *L. aestivum* is scattered in northern Italy, river flooding and pollinators may have a role in maintaining the gene flow among populations (Gentili et al. 2018).

The perianth of *L. aestivum* is composed of six white tepals in two whorls, 13–22 mm long, with a green spot just below the tip of each tepal. Flowers are grouped in number of 2–8 in an apical inflorescence subtended by a spathe. Each plant can produce up to three inflorescences, but most commonly only one. *L. aestivum* is self-incompatible and there is no apomixis, thus the reproductive success of this plant depends entirely on pollinators (Parolo et al. 2011).

In their monograph on the species, Parolo et al. (2011) report a study by Knuth (1909), indicating diurnal and nocturnal Lepidoptera and Hymenoptera as the most likely pollinators of *L. aestivum*. Except

for this old study, no recent quantitative research has been performed on pollinators of this species.

In the only other species in the genus *Leucojum* (*L. vernum* L.), the floral morphology suggests pollination by Hymenoptera, Apidae in particular, buzz-pollination (Fishchuk and Odintsova 2020). *L. aestivum* has very similar flowers to *L. vernum*, thus it can be hypothesised a similar pollination mechanism is used in this species. However, pollinator visitations on *L. aestivum* are infrequent (Wisdom et al. 2019). Possible reasons for this may be reconducted to the early spring flowering period, when pollinator activity is still scarce, to the preference of pollinators for open habitats (Abeli et al. 2013; Gargano et al. 2017), to the lack of nectar as a reward (Parolo et al. 2011) and the lack of a scent. A recent study showed that *L. aestivum* does not produce any volatile organic compound (Abeli et al. 2016). Therefore, pollinator attraction in *L. aestivum* is mainly visual and probably deceptive, as the green marks in the tepals may suggest the presence of nectar, which occurrence is not demonstrated. Rather, these green marks may have a role in provisioning the seeds with photosynthetate like in *Galanthus nivalis* L. (Aschan and Pfanz 2006).

Such a generalist pollination system is expected to benefit early blooming species in habitat where pollinators are typically scarce, like the riparian woodlands where *L. aestivum* grows (Alonso 2004). Indeed, the study of fruit set highlighted that *L. aestivum* pollination system is quite effective, with values of fruit set close to 100% in most cases (Parolo et al. 2011). Nevertheless, *L. aestivum* seed set is often pollen limited (average seed set 33.8%), especially in low-density populations (Parolo et al. 2011). In a translocation of *L. aestivum* in N-Italy, Abeli et al. (2016) created two populations characterised by different densities, with the low-density population half-dense the high-density one. After five years from plant release, the latter (high-density) population showed higher fruit set and recruitment than the low-density population.

Seed set and density are often positively correlated, because higher numbers of flowering plant individuals per square metre are more attractive for pollinators, consequently, a greater quantity of pollen grains are available for the fertilisation of the ovules (Dauber et al. 2010).

Given the importance that pollinators have in the reproductive success of *L. aestivum*, in this study, we

investigated the flower-visiting and pollen-carrying arthropods at the southern range edge of the species, in the plain of the River Po (Northern Italy) in three different populations: a large wild population, a population recently reintroduced and an ex situ population cultivated in a botanical garden. The aim of the study is to identify potential pollinators of *L. aestivum* in Northern Italy by collecting the flower-visiting arthropods found on it and investigating the presence of pollen belonging to *L. aestivum* on their body.

Materials and methods

Study populations

Three spatially distinct populations were chosen for the present study in the Po Valley, in the Province of Pavia (Lombardy, N-Italy): a wild population of *L. aestivum* located within the special area of conservation (SAC) IT2080019 “Boschi di Vaccarizza” (Municipality of Linarolo), a reintroduced population located within the SAC IT2080007 “Garzaia di Bosco Basso” (Municipality of Breme and Sartirana Lomellina) and an ex situ population located at the Botanical Garden of the University of Pavia, inside the city of Pavia (Fig. 1).

The SAC IT2080019 site “Boschi di Vaccarizza” (45.14914 N; 9.25385 E; 61.3 m a.s.l.) is located along the course of the Po River, and it is characterised by a well-preserved wood of Black Alder (*Alnus glutinosa* (L.) Gaertn.) and White willow (*Salix alba* L.). Here, *L. aestivum* is dominant in the understorey and grows in a stable population at the edge of a small ditch bordering a farming land. During the flowering period (April–May) *L. aestivum* represents the only flowering species in the understorey.

The population of *L. aestivum* located at “Garzaia del Bosco Basso” (45.11840 N; 8.64288 E; 106.5 m a.s.l.) was established in 2016 in the context of a reintroduction programme of the species promoted by the local government of Pavia Province. This location is characterised by the presence of habitat 91E0 (see above), with Black alder (*A. glutinosa*), Goat willow (*Salix caprea* L.), thick reeds with dominance of the marsh reed (*Phragmites australis* (Cav.) Trin. Ex Steud.) and (*Typha latifolia* L.) growing around a

spring locally called “fontanile”, and it is entirely surrounded by cultivated areas and poplar groves.

Compared to Vaccarizza, the alder grove is mainly formed by low- and medium-height trees which ensure a very dense vegetation cover, also in the understorey. Vaccarizza is characterised by tall trees and an almost total absence of shrubs in the understorey.

The Botanical Garden of the University of Pavia (45.18572 N; 9.16349 E; 75 m a.s.l.) is located in the city centre of Pavia and it covers about two hectares and hosts a gymnosperm arboretum, an angiosperm arboretum, officinal plants and many other threatened species of conservation interest (Clauser and Pavone 2016). In the botanical garden, about 1,000 plants of *L. aestivum* from about 15 populations from N-Italy are grown in a small nursery. Plants are grown in groups of 10–20 individuals in plastic containers (ca. 50 cm³ volume), without holes in the bottom to maintain the soil fully wet during the growing season, from February to June. During the flowering period of *L. aestivum*, other co-flowering species in the botanical garden include *Prunus* spp., *Quercus ilex* L., *Castanea sativa* Mill., *Laurus nobilis* L., *Hyacinthoides hispanica* (Mill.) Rothm., *Malus domestica* Borkh., *Diospyros kaki* L.

Sampling of the flower-visiting arthropods

L. aestivum populations were investigated in 2017 and 2018.

In 2017, we visited the study populations during the flowering period in April–May twice a week; we captured all arthropods visiting or found near (i.e. a few cm) the flowers of *L. aestivum* with an entomological net or directly with plastic test tubes. The tubes containing the entomological samples, were dated and coded. In detail, in each day, five 30-min sampling sessions were performed in the morning (10 am), at noon (12 am), in the afternoon (3 pm), at twilight (8 pm) and at night (9 pm). Temperature during the sampling sessions were measured by using a DataLogger Geoprecision at 10' intervals. In year 2017, sampling was done during the entire *L. aestivum* flowering period, while in 2018 it was decided to focus on April only and on the time slots with the greatest presence of arthropods based on the results of 2017. Specifically, in the following time slots: in the morning (10 am), at noon (12 am), in the afternoon (3 pm).



Fig. 1 Map of the study populations in northern Italy. 1. Boschi di Vaccarizza; 2. Bosco Basso; 3. Botanic Garden of the University of Pavia

All collected samples were placed in plastic tubes and frozen waiting to be identified at MUSE-Science Museum of Trento (MUSE). About 70% of the caught specimens was identified to the species level, ca. 8% to the genus level, the rest to higher taxonomic levels (family or order). Specimens were identified by using entomological collections available at MUSE and thanks to the cooperation of skilled taxonomists (Supplementary File 1).

Pollen extraction and comparison with *Leucojum aestivum* pollen grains

At first, we tried to extract, under stereomicroscope, pollen grains directly from the insect's body, but we had little success. Subsequently, we tried the extraction of the pollen attached to the plastic walls of the test tubes by using a few drops of alcohol 99% v/v for detaching pollen from the tubes. After extraction,

pollen grains were placed on a slide for identification by using the optic microscope.

The samples were examined one by one and compared with a sample of *L. aestivum* pollen, taken directly from the flower. The collected arthropods were characterised in terms of feeding habits according to the literature available for each taxon (Gobbi and Latella 2011; Speight 2013; Hoebcke and Wheeler 2013; Losapio et al. 2016; Malmusi et al. 2017) and by the involved skilled taxonomists (Supplementary File 1), then sealed, coded and stored in alcohol 99% v/v, at the Department of Earth and Environmental Sciences of the University of Pavia.

Statistical analysis

A sample rarefaction analysis was performed using Mao's Tao function in PAST 4.03 (Hammer et al. 2001) to assess the efficacy of the sampling effort in

detecting arthropods visiting *L. aestivum* in the study area (Colwell et al. 2012; Gargano et al. 2017).

Two generalised linear models (GLMs) were performed to test the relative importance of the different orders and feeding habits of arthropods found to carry pollen of *L. aestivum* in the pollination process. Orders were included in the analysis instead of other taxonomic levels because for some specimens the identification could not reach more details. In the GLMs, the quantity of pollen found on each specimen was treated as a dependent variable and “Order” or “Feeding habits” as independent variables.

A specimen containing 200 pollen grains was removed from the analysis because it was considered an outlier. Orders and feeding habits represented by a single specimen were also removed.

The analyses were performed using R-software (R Core Team 2018; Fox and Weisenberg 2019).

Results

The analysis of the catches has returned a high number of different arthropod taxa (i.e. insects and arachnids), direct visitors of *L. aestivum* or present very close to the flowering plants. In total, 65 catches were made in two years: 39 in 2017 and 26 in 2018.

Overall, catches included 8 orders (plus an unidentified gastropod), 23 families, 20 genera and 18 identified species (Supplementary material 1).

The rarefaction analysis suggested that the sampling effort provided a partial estimation of the

arthropods visiting *L. aestivum* flowers, as asymptote was not achieved (Fig. 2).

Leucojum aestivum visitors and pollen-carrying arthropods

Thirty three specimens (51% of the total catches) belonging to 18 taxa were found to carry *Leucojum* pollen (Table 1). Of these, 33% of the catches were represented by *Dasytes plumbeus* O.F. Müller, 1776 (Coleoptera, Melyridae). The specimen with the largest number of pollen grains of *L. aestivum* (> 200 pollen grains) belonged to *Lasioglossum punctatissimum* Schenck, 1853 (Hymenoptera, Halictidae) followed by *D. plumbeus* and *Grammoptera ruficornis* Fabricius, 1781 (Coleoptera: Cerambycidae) (Table 1).

Concerning the feeding habits, phytophagous *s.l.* (pollinivorous, antherophagous and nectarivores), predators and omnivorous were the prevalent groups among pollen carriers (Table 1). The GLMs revealed a significant effect of three orders (Coleoptera, Dermaptera and Hymenoptera: $\chi^2 = 199.55$; $df = 6$; $p < 0.0001$; Table 2) and a significant effect of two feeding habits (omnivorous and phytophagous *s.l.*: $\chi^2 = 147.45$; $df = 4$ $p < 0.0001$; Table 2) of arthropods visiting *L. aestivum*.

Positive catches of *L. aestivum* pollen were compared with catch days. Figure 3 shows a difference in the maximum number of catches between 2017 and 2018. During these days, there were different weather conditions. On 10/IV/2017, the weather was sunny with the absence of wind. On 05/IV/2017, the weather

Fig. 2 Result of the rarefaction curve showing the species accumulation curve (number of arthropod taxa as a function of the sampling effort). Dashed lines indicate the 95% confidence intervals of estimations

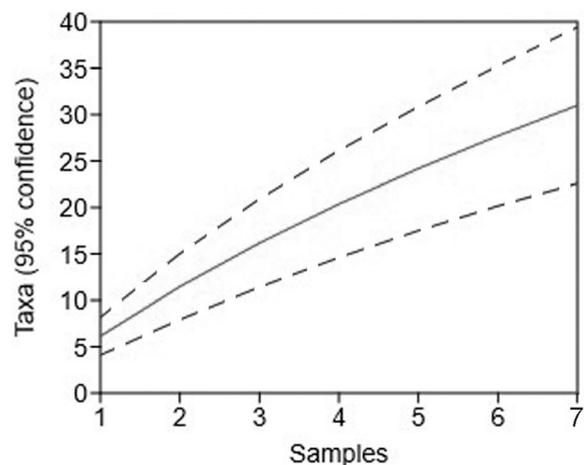


Table 1 Taxa carrying pollen of *Leucojum aestivum*, number of catches and mean number of pollen grains of *L. aestivum* found on each taxon

Order	Family	Species carrying pollen of <i>Leucojum aestivum</i>	No. of catches	No. of pollen grains (mean \pm st. dev.)	Feeding habits
Coleoptera	Melyridae	<i>Dasytes plumbeus</i> Müller, O.F., 1776	11	12.5 \pm 14.77	Antherophagous
Araneae	Clubionidae	<i>Clubiona</i> sp. Wagner, 1887	2	3 \pm 1.41	Predator
Araneae	Clubionidae	<i>Clubiona pallidula</i> Clerck, 1757	2	3.5 \pm 0.71	Predator
Coleoptera	Cerambycidae	<i>Grammoptera ruficornis</i> Fabricius, 1781	2	10 \pm 11.31	Antherophagous
Coleoptera	Nitidulidae	<i>Lamiogethes pedicularius</i> Gyllenhal, 1808	2	1.5 \pm 0.71	Antherophagous
Hymenoptera	Halictidae	<i>Lasioglossum punctatissimum</i> Schenck, 1853	2	101 \pm 140.01	Nectarivorous
Araneae	Theridiidae	<i>Enoplognatha</i> cfr. <i>ovata</i> Clerck, 1757	1	1	Predator
Araneae	Clubionidae	<i>Clubiona terrestris</i> Westring, 1851	1	1	Predator
Coleoptera	Melyridae	<i>Clanoptilus</i> sp. Motschulsky, 1853	1	1	Antherophagous
Dermaptera	Forficulidae	<i>Apterigyda albipennis</i> von Muhlfield, 1825	1	8	Omnivorous
Dermaptera	N.D.	N.D.	1	5	Omnivorous
Diptera	Syrphidae	<i>Epistrophe eligans</i> Harris, 1780	1	2	Nectarivorous
Diptera	Mycetophilidae	N.D.	1	2	Fungivorous
Diptera	Syrphidae	<i>Platycheirus scutatus</i> Meigen, 1822	1	1	Nectarivorous
Hymenoptera	Braconidae	N.D.	1	6	Glycyphagous
Hymenoptera	Apidae	<i>Bombus</i> cfr. <i>runderatus</i> Fabricius, 1775	1	2	Nectarivorous
Lepidoptera	Geometridae	<i>Ematurga atomaria</i> Linnaeus, 1758	1	2	Nectarivorous
Orthoptera	N.D.	N.D.	1	5	

was cloudy with wind. On 27/IV/2018, the weather was partly cloudy with light wind (See also Supplementary material 2).

Differences among sites

The most abundant catches of specimens carrying *Leucojum* pollen were made in the wild population of Vaccarizza, followed by the reintroduced population of Bosco Basso. No *Leucojum* pollen carriers were found at the Botanical Garden of Pavia (Table 3; Supplementary material 1). Moreover, the site of Vaccarizza resulted to be much richer from a taxonomic point of view than Bosco Basso (Table 3; Supplementary material 1), in which only three specimens, belonging to three different orders, carrying *Leucojum* pollen were identified (Table 3).

Interestingly, the only shared taxa between the two sites Vaccarizza and Bosco Basso were *L. punctatissimum* (Hymenoptera, Halictidae) and *Enoplognatha* cfr. *ovata* (Araneae, Theridiidae) (Table 3).

Discussion and conclusion

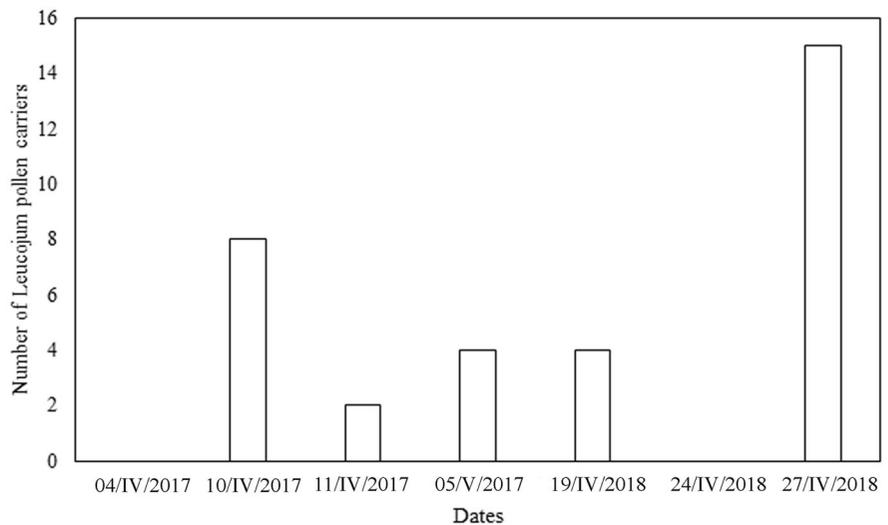
In this study, we present the most recent account of flower-visiting and pollen-carrying arthropods of *L. aestivum* subsp. *aestivum*. Among the several visitors caught on *L. aestivum* plants, we identified 18 taxa that carried *Leucojum* pollen and that surely have a role as pollinivorous and consequently may have a role as pollinators of this plant species. Interestingly, we highlighted that *L. aestivum* is visited not only by

Table 2 Results of the generalised linear model

		Estimates	St. Error	Z value	p
A	Order				
	Araneae	− 0.06454	0.25820	− 0.250	0.80262
	Coleoptera	1.60944	0.09325	17.259	< 0.001
	Dermaptera	0.77319	0.27735	2.788	0.00531
	Diptera	− 0.58779	0.44721	− 1.314	0.18873
	Hymenoptera	0.69315	0.31623	2.192	0.02839
B	Lepidoptera	− 0.69315	0.70711	− 0.980	0.32696
	Feeding habits				
	Omnivorous	0.77319	0.27735	2.788	0.00531
	Phytophagous s.l	1.25662	0.08771	14.328	< 0.001
	Predators	− 0.12516	0.25820	− 0.485	0.62785
Fungivorous	− 0.40547	0.70711	− 0.573	0.56636	

(A) role of “Orders” in the transportation of pollen of *Leucojum aestivum*. Dependent variable is the number of pollen grain found on carriers; (B) role of “Feeding habit” in the transportation of pollen of *L. aestivum*. Dependent variable is the number of pollen grain found on carriers. Phytophagous s.l. enclosed pollinivorous, nectarivorous, antherophagous and glycyphagous species. Statistically significant effects are in bold

Fig. 3 Frequency of carriers of *Leucojum aestivum* pollen during the sampling period. Dates are in the format dd/mm/yy



insects, but also by a network of arthropods with different feeding habits (Supplementary material 1).

A diverse array of wild flower visitors can transport viable pollen from a pollen source; data recorded by Rader et al. (2011) demonstrated that two bee genera (*Bombus* and *Lasioglossum*) carried pollen at least 300 m away, while hoverflies carried pollen at least 400 m.

Among the *Leucojum* pollen carriers, the most commonly found is the melyrid beetle *D. plumbeus*. Hoebeke and Wheeler (2013) report that this species

has, on its body, dense setae and pubescence able to trap pollen grains that are transported to other flowers during feeding; adults of *D. plumbeus* are facultative pollen and nectar feeders on various flowers and shrubs and adults are associated with wood (*Quercus*, *Acer*, *Betula* and *Picea*) in various stages of decomposition and larvae are predators in rotten wood. Its high frequency of occurrence on *L. aestivum* can be explained by the abundant presence of *Quercus robur* L. trees on Vaccarizza site which act as source of food for *D. plumbeus* larvae as well as oviposition sites. An

Table 3 *Leucojum aestivum* pollen carriers collecting sites (at the family level)

Order	Family	Vaccarizza (wild)	Bosco Basso (reintroduced)	Botanic Garden (ex situ)
Araneae	Clubionidae	1	0	0
Araneae	Theridiidae	1	1	0
Coleoptera	Cerambycidae	1	0	0
Coleoptera	Melyridae	1	0	0
Coleoptera	Nitidulidae	0	1	0
Dermaptera	Forficulidae	1	0	0
Dermaptera	N.D.	1	0	0
Diptera	Syrphidae	1	0	0
Diptera	Mycetophilidae	1	0	0
Hymenoptera	Apidae	1	0	0
Hymenoptera	Braconidae	1	0	0
Hymenoptera	Halictidae	1	1	0
Lepidoptera	Geometridae	1	0	0
Orthoptera	N.D.	1	0	0
Total observations		29	4	0

1 = Specimen collected at a site

additional “local” pollinivorous can be considered the long-horned beetle *G. ruficornis*. Similarly to *D. plumbeus*, *G. ruficornis* has the larval stages linked to some of the species of trees found in Vaccarizza site, for instance *Hedera helix* L., *Robinia pseudoacacia* L., *Q. robur* L., *Ulmus campestris* Mill., *Acer campestre* L. (Malmusi et al. 2017).

Other taxa had a significant effect on pollen transport as well as earwigs (Dermaptera) and wild bees (Hymenoptera). Representatives of the latter order were rarely seen foraging on *L. aestivum*. Most hymenoptera, including bumblebees (*Bombus* spp.) and carpenter bees (*Xylocopa violacea* Linnaeus, 1758), observed nearby *L. aestivum* made quick visits to the flowers probably when searching for nesting places or during foraging activity, as with other deceptive flowers (Tuomi et al. 2015). Indeed, the highest amount of pollen grains was found on *L. punctatissimum* (Hymenoptera), suggesting that this group may be quite efficient in pollination despite the low visitation rate. *L. punctatissimum* can be considered a good candidate for *L. aestivum* pollination due to the amount of transported pollen and the quite long distance able to perform that may permit to reach spatially separated populations of *L. aestivum*. Conversely, to the best of our knowledge, no data on the

distance performed by the rest of the sampled taxa are available (Rader et al. 2011).

The potential role of Arachnids in pollination of *L. aestivum* needs further studies. Interestingly, our data highlight the presence of pollen on *Clubiona* sacspiders (Clubionidae). *Clubiona pallidula* Clerck, 1757, the spider carrying the highest number of *Leucojum* pollen grains, is known to find refuge in convolute leaves (Nentwig et al. 2021). Probably, this predator forages and/or finds repair inside the bell-shaped *Leucojum* flowers and if pollination is mediated by arachnids, this should be considered accidental. This hypothesis is supported by the absence, or very low number, of pollen grains in the rest of spider species sampled on the flowers (Table 1).

Visiting arthropods of *L. aestivum* in our study area are mainly pollen-feeding beetles, as suggested by the GLMs. Interestingly, in contrast to Knuth (1909), we did not observe nocturnal visitations of flowers in our populations. However, we cannot exclude that *L. aestivum* is pollinated also by other taxa (i.e. moths, bees and bumblebees) as reported by Knuth (1909), as we made our observation in a few populations. Visiting arthropod assemblages can greatly differ across the geographic areas and habitat composition where a plant species is found (Péres-Barrales et al. 2007; Gargano et al. 2017). Moreover, a temporal

decline in spring pollinator assemblage and/or availability (see. Hallmann et al. 2017; Fitzgerald et al. 2021) may also have occurred since Knuth's study.

In the Amaryllidaceae family, different pollinator types were observed, from very specialised species (Geerts and Pauw 2012), with moths (Lepidoptera: Sphingidae) as main pollinators (Manning and Snijman 2002), to less specialised flowers such as in the genus *Leucojum* or *Galanthus*. In general, arthropods visitation in *L. aestivum* is less frequent as in other early-flowering temperate Amaryllidaceae (Baker et al. 2000).

The broader visiting arthropods diversity was detected, as expected, in the wild population of Vaccarizza, followed by the translocated population of Bosco Basso. This pattern reflects the general abundance and diversity of invertebrates caught in the three sites (Supplementary material 1) and is consistent with the characteristics of the sites, with Vaccarizza representing the optimum for the species in a well-preserved alder woodland. The lower number and diversity of pollinators found in the reintroduced population of Bosco Basso is consistent to the observation that pollinator visitations are less frequent in fragmented landscapes (Ward and Johnson 2005). In particular, this site is a quite small and relict alder wood in an intensive farming landscape that may negatively affect the presence of pollinators (Richards 2001). Another difference between Vaccarizza and Bosco Basso is that the former occurs near a farm in the process of converting to organic farming, while the latter is surrounded by conventional farms. Other differences between the two populations such as total population size and density of flowering plants may affect pollinator visitation. Indeed, the entomofauna of the two sites is completely different, with only two shared species. This is an interesting point that should be considered when planning future translocation, i.e. the occurrence in the recipient site of potential pollinator for the translocated species (Draper Munt et al. 2016).

The ex situ population, located in the Botanical Garden, is completely out of context in terms of pollinator availability and assemblage because it is located into a city. However, the lack of observation of *L. aestivum* pollen carriers is difficult to be explained, considering that this population do set seed every year (T.A. personal observation). It is possible that our observation protocol failed to recognise some

pollinators in this specific artificial habitat, also considering that the collected sample was only partially representative of the potential pollinator assemblage (Fig. 2). Alternatively, as observed by Geerts and Pauw (2012), we cannot exclude the presence of hawk moths (Sphingidae) as main pollinators in the Botanical Garden. Hawk moths are quite common in urban areas as visitors of city flower (Albu and Albu 2016); thus, probably a higher number of sampling sessions would have increased the probability to observe specimens belonging to this family.

In conclusion, we identified 18 visiting and pollen-carrying arthropods that can potentially act as pollinators of *L. aestivum* in N-Italy, with pollen-feeding Coleoptera the most frequent taxa involved in the pollination of this plant and mining bee *L. punctatissimum* as the species with the highest number of pollen grains stuck to its body. A recent paper published by Bonelli et al. (2020) demonstrated the importance and potentialities to combine manual sampling to video observations with the purpose to estimate the diversity of flower-visiting arthropods, to spot rare taxa and to evaluate which taxa could be involved in its pollination. Thus, a follow-up of this project, or more in general researches aimed at investigating pollination processes on plants with short flowering period, could be to apply both the sampling methods.

Although our study is limited to a small portion of the range of *L. aestivum*, and a standardised method for detecting pollen grains on the arthropod body parts has not been published, this is the first recent contribution to the study of pollinators in this species and in the genus *Leucojum*.

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Author contributions TA and GR conceived the experiment, LAV and TA carried out the field work, MG supervised the identification of the specimens. All authors wrote parts of the manuscript.

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Data availability Raw data used in this study are available in the Supplementary material 1 and 2.

Declarations

Conflict of interest The authors declared that they have no competing interest.

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