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Department of Environmental Science and Policy

Ph.D Thesis

Plant-arthropod interactions in mountain ecosystems

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Thesis overview

The present Ph.D. thesis is organized as a collection of research papers published, submitted, and in preparation.

Chapter 1. Here is introduced the knowledge about plant and arthropod interactions in mountain ecosystems and the aims of the Ph.D. project followed by an introduction to the two different study cases of my research. A brief overview of the two Ph.D. side projects is also reported in this chapter.

Chapter 2, "Plant-arthropod interactions: the case study of *Androsace brevis* (Hegetschweiler) Cesati (Primulaceae)". This chapter focuses on the characterization of flower-visiting arthropod community linked to the alpine plant species *Androsace brevis*, a narrow-endemic plant of Southern-Central Alps (Italy) characterized by a short (about two weeks) and early (May-June) flowering period. This species is particularly threatened by climate warming. The first study (paragraph 2.1) describes the floral and pollen morphology of *A. brevis,* which were useful in investigating the pollination biology of this species as well as its possible pollination strategies and vectors. The study reported in paragraph 2.2 focuses on the development and subsequent evaluation of two methodological approaches employed for the study of the flower-visiting arthropod communities of *A. brevis*: manual sampling and video-observations. Subsequently, a detailed characterization of the *A. brevis* flower-visiting arthropod community in relation with weather conditions, was carried out selecting two *A. brevis* populations (Cugn Peak, Como, Lepontine Alps, Italy; Benigni Hut, Bergamo, Orobic Alps, Italy) (paragraph 2.3). In paragraph 2.4, the pollen grains retrieved from *A. brevis* flower-visiting arthropod collected at Benigni Hut for three consecutive years have been analysed from both qualitative and quantitative point of view by light microscopy. Palynological analysis allowed to identify the pollination network that characterize the high-altitude ecosystem during a particular period of the year as the early season (May-June) in alpine environment, when the snow cover still present on the ground and few plants are flowering (work in preparation). Finally, in paragraph 2.5, a specific investigation on the high-altitude communities of Braconidae (Hymenoptera) was carried out for the same two sites previously reported, resulting in the discovery of four new species of Braconidae for the Italian fauna. Additionally, this study reports the first record of the alien species *Lysiphlebus testaceipes* (Cresson, 1880) in an Alpine context.

Chapter 3, "Plant and flower-visiting arthropod communities within the Stelvio National Park". This study applied the two methodological approaches developed with the previous project to a broader context. The study was conducted in the Martello Valley (Bolzano province, South Tyrol, Italy) within the Stelvio National Park. The effects of the altitudinal gradient (from 900 to 2700 m asl) and the different type of land management (from the natural grasslands to the apple orchards) were investigated on the flower-visiting arthropod abundance and community composition (paragraph 3.1, in preparation). Moreover, a molecular approach (ITS2 metabarcoding) has been developed as an alternative to the traditional palynological analysis conducted by light microscopy (paragraph 3.2, submitted).

Chapter 4, "Ph.D. Side projects". In this chapter, a new plant species (*Campanula bergomensis*, Campanulaceae) and a new insect species (*Oreonebria tresignore*, Carabidae) for Orobic Alps (Bergamo, Northen Italy) are characterized both morphologically and genetically (paragraphs 4.1, 4.2).

Chapter 5, "Conclusions and future prospects" of the research.

Chapter 6, "Ph.D. Activities". Here is presented the activities carried out during my Ph.D., comprehensive of all the papers published, submitted, in preparation, and future papers on data collected for the Ph.D. project; all the congresses and seminaries; teaching activities and participation in projects.

Chapter 1

Introduction, aims and scopes, Ph.D. side projects

1.1 Introduction

In terrestrial ecosystems, plant and arthropod communities interact with each other, creating networks that are still poorly known (Guzman et al., 2021). It is estimated that about 30% species of arthropod visit flowers (Wardhaugh, 2015). Flowers could provide optimal sites for arthropods to search for partners, find shelter or a safe place for oviposition, and rest for thermoregulation, as well as to find food resources (Woodcock et al., 2014; Inouye et al., 2015; Hernández-Vera et al., 2021). In the latter case, arthropods could search for both prey or a host to parasitise, as well as feed on floral resources such as petals, nectar, and pollen (Kirk, 1984; Krenn et al., 2005; Abbott, 2006; Wardhaugh, 2015). However, the most well-known and studied interactions are those related to the search of pollen and nectar by arthropods. During the search for these nutrients, pollen grains can remain attached to the bodies of visiting arthropods, which may act as pollinators if these pollen grains come into contact with the receptive pistil of a conspecific plant. Arthropod-mediated pollination is very important both ecologically and economically. About 90% of angiosperms, including many important crops, depend on pollinators (mostly insects) for their reproductive success, and conversely many arthropods depend on flowers for their survival (Klein et al., 2007; Aizen et al., 2009; Ollerton et al., 2011; Abrol et al., 2012; Inouye et al., 2015; Wardhaugh, 2015; Michez et al., 2019).

Given the importance of pollinators for the human economy (Klein et al., 2007), many studies on interactions between flowers and arthropods have been performed in agroecosystems. The taxa for which the important role in pollination is commonly recognised are Hymenoptera (Apoidea: Anthophila), Diptera (Syrphidae), Lepidoptera, and Coleoptera (Vanbergen et al., 2014; Lefebvre et al., 2018; Potts et al., 2021). Consequently, knowledge about pollinator species belonging to these taxa and their ecological needs is fairly good. On the other hand, the study of little-known flower visitors (e.g., Diptera non-syrphid, Hymenoptera non-anthophila), which could play an important role in pollination both in natural and seminatural ecosystems, has been neglected (Potts et al., 2010; Brock et al., 2021). Knowledge decreases further when mountain ecosystems are considered. Here, several neglected flower visitors are fundamental for the reproductive success of many plants (Orford et al., 2015; Brock et al., 2021). Moreover, in mountain environments, both ongoing climate change and other anthropogenic factors could alter the relationships between plants and flower visitors more severely than in low-altitudes ecosystems (Prather et al., 2023). Increasing temperatures potentially leads plants to anticipate flowering period and arthropods to anticipate the end of wintering (Bartomeus et. al 2011; Sevenello et al., 2020), and both could move to higher altitudes to find new suitable habitats (Chen et al., 2012). However, these phenological and altitudinal adaptations may occur differently between plant and flower visitors potentially resulting in phenological, temporal and spatial mismatches between them (Morton & Rafferty, 2017). The effects of these mismatches on the stability of biotic communities are still difficult to predict. For instance, an alteration of the mutualistic relationships established between plant and arthropods may lead to a decrease in the reproductive success of both parties or even to their local extinction (Kudo & Cooper, 2019; Inouye, 2020, Ohler et al., 2020; Richman et al., 2020).

On the other hand, agricultural practices are among the main causes of the decline in flower-visiting arthropods (Thapa, 2006; Siebold et al., 2019). The intensification of agriculture, with

changes in both the traditional agricultural landscape and agronomic practices, affects the biotic composition of both the lowland and mountain ecosystems (Schirpke et al. 2013). The use of herbicides and insecticides leads to a significant decrease in the arthropod biomass, including many flower-visitors. Consequently, serious effects may occur not only on crops production but also on ecosystems survival (Malaspina et al., 2008; Vanberger et al., 2013).

Since the interactions between plant and flower-visiting arthropod are important within biotic communities, it is important to increase the available data about both these interactions and the composition of plant and arthropod communities. In particular, it is fundamental to investigate which arthropod taxa are mostly closely associated with flowers for their own survival and which of them are primarily involved in pollination, especially in poorly studied mountain environments.

1.2 Aims and scopes

The Ph.D. project focused on the study of flower-visiting arthropod communities that characterize different high mountain ecosystems. In particular, the work aimed to assess the ecological role of flower visitors both through the study of botanical composition of pollen transported by them and through the analysis of their behaviour on flowers.

The work was divided into two case studies:

- The characterization of *Androsace brevis* (Hegetschw.) Ces. (Primulaceae) visiting arthropod community;
- The assessment of flower-visiting arthropod communities that characterise different types of land management and how they change along an altitudinal gradient within the Stelvio National Park (Eastern Alps, Italy).

The first case study was a pilot project. The aim was to assess the *Androsace brevis* flower-visiting arthropod community with a particular attention on the identification of taxa involved in the pollination of this plant species. This project involved the development of two methods for data collection: i) manual sampling of flower-visiting arthropods, with palynological analyses of pollen carried by collected arthropods; ii) video observations, with the analysis of arthropod behavior on flowers. *Androsace brevi*s was selected as a model species as it is a narrow endemic alpine plant particularly subjected to the threats induced by global warming.

The second case study used the methodology developed in the previous project in a broader context. In particular, the two approaches were used to study how the communities of flower visitor changed among different land use types and along an altitudinal gradient, from the most anthropized ecosystems (orchards), to semi-natural ecosystems (pastures and hay meadows), to the natural ones (high-altitude grasslands) within the Martello Valley (Stelvio National Park, Province of Bolzano, South Tyrol, Italy).

To study all the aspects related to the interactions between plants and arthropods, during the Ph.D. course, it became essential to develop and evaluate different methodologies both for data collection and palynological analysis. The first topic involved the two previously mentioned methodologies (manual sampling and video observations). In particular, the advantages and disadvantages presented by these methods in giving information about flower-visiting arthropods were evaluated. The second topic focused on the efficiency of

metabarcoding as a tool in analyze insect-carried pollen with particular interest in evaluating the accuracy of this technique in giving exhaustive quantitative results about pollen botanical composition. Results obtained by this molecular technique has been evaluated by comparing them with the results obtained with the traditional method as light microscopy.

Within the study context of mountain-related plant and arthropod species, considering the potential negative effects the ongoing climate change could have on their survival, during the Ph.D. course, there were the possibility to characterize morphologically and genetically two new species: *Campanula bergomensis* Mangili F. & Mangili L. sp. nov., (Campanulaceae) and *Oreonebria tresignore* (under description) (Coleoptera: Carabidae).

Both these two species are orophile endemisms, whose distribution range is included in the Orobic Prealps (Lombardy, Italy). This pre-Alpine sector, as well as the southern belt of the Alpine chain, is an important centre of endemism (Paowski, 1970; Aeschimann et al., 2004; Graf et al., 2014) probably due to the limited occurrence of ice cover during Pleistocene ice ages and the presence of many refugia (Bini et al., 2004).

Campanula bergomensis is related to dolomitic substrates, preferring debris cones, and generally poorly developed soils. Its state of preservation seems to have no critical issues (Valle et al., paper in proof).

Oreonebria tresignore is strongly related to the presence of glacier or permanent snowfield. This ecological need leads *Oreonebria tresignore* to follow the glacial and periglacial areas of the Orobic Alps resulting in a scattered distribution of its populations (Gobbi et al., 2018).

In a scenario where the effects of ongoing climate change are more and more evident in the natural landscape, it is essential to deepen the knowledge on the flora and fauna species. A good species ecological characterisation is fundamental if plans for species conservation will be required.

1.3 First case study: *Androsace brevis* (Hegetschw.) Ces. (Primulaceae)

The geographical origin of *Androsace* genus is located on the mountain chains of eastern Asia (Boucher et al., 2012) from which it radiated widely across the mountain ranges of the Northern Hemisphere, reaching Europe first and North America afterwards (Schönswetter et al., 2003; Roquet et al., 2013). According to the classification proposed by Smith & Lowe (1997), *Androsace brevis* belongs to the section Aretia, which also includes *A. alpina* (L.) Lam., *A. hausmanniii* Leybold, *A. helvetica* (L.) All, *A. pubescens* DC. In Lam. et DC, and *A. vandelli* (Turra) Chiov., *Androsace wulfeniana* Sieber.

Androsace brevis is a chamaephyte cushion plant which grows above 2000 m asl only on siliceous peaks and ridges characterized by both high sunlight and wind exposure (Provasi, 1922; Mangili et al., 2014). It is a narrow endemic species related to a limited area of Southern Central Alps (Aeschimann et al., 2004) and its distribution area encompasses three Alpine sectors: the Eastern Lepontine Alps (Como Province, Lombardy, Italy and Canton Ticino, Ticino, Switzerland), Western Orobic Alps (Bergamo and Lecco Province, Lombardy, Italy), and Western Rhaetian Alps (Sondrio Province, Lombardy, Italy) (Provasi, 1922; Pignatti, 1982; Mangili et al., 2014) (Figure1.a; 1b).

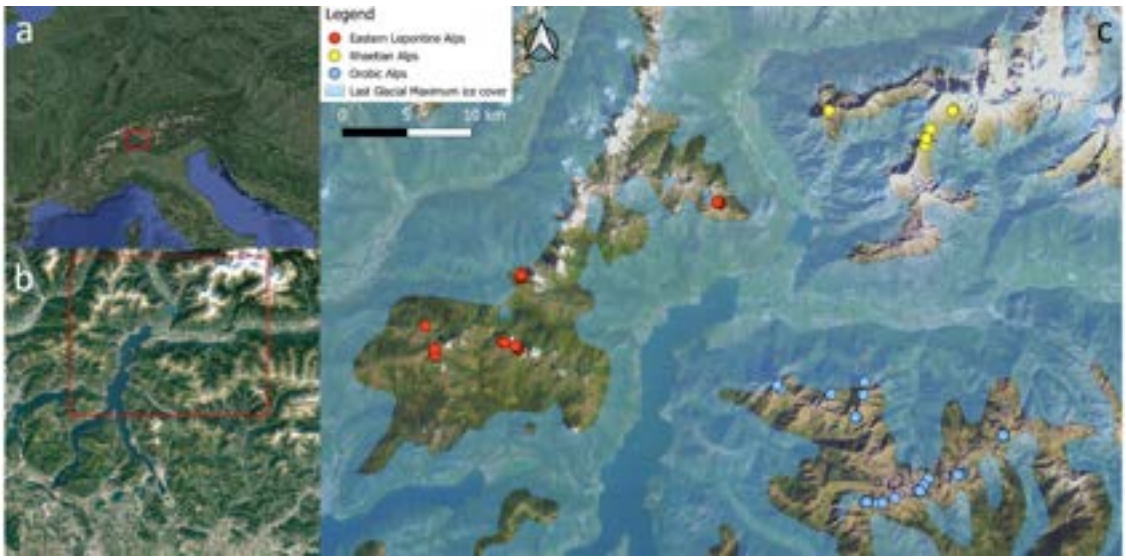

Figure 1. a: Location of *Androsace brevis* on the European Alps; **b**: *Androsace brevis* distribution range in the Southern Central Alps. **c**: *A. brevis* present distribution reflects the areas not covered by ice during the Last Glacial Maximum. LGM shapefile source: Quaternary glaciations - Extent and chronology (Editors: Ehlers, Gibbard, Hughes, 2011).

Androsace brevis current distribution area may be shaped by the numerous climatic changes occurred during the Pleistocene. During this period, many glacial and interglacial cycles occurred, culminating 23,000-18,000 years ago with the Last Glacial Maximum (LGM) (Mix et al., 2001). The presence of the ice sheet may have limited the distribution of *A. brevis* toward the inner Alps and may have led to the extinction of the populations already present there. On the other hand, the migration of the species southwards could have been limited by the presence of reliefs with a limestone/dolomitic substrate. Therefore, the species may have found refuge on the external siliceous peaks of the Southern Alps that were not covered by ice during the LGM (Figure1.c). Similarly, during the last 10,000 years, warmer periods than the present were registered several times on the Alps (Furlanetto et al., 2018). During these

phases, the Southern Alps tree line went from the current 1,800-2,000 m asl to higher altitudes. This suggest that the areas covered by ice during the glaciers advance were probably covered by forests during the climatic optimum. Also in this case, *A. brevis* probably had to cope with the formation of refuge island similarly to those present during the LGM.

The genetic variability of *Androsace brevis* populations is probably affected by their scattered distribution. The study conducted by Bonelli et al. (unpublished data) highlights how *A. brevis* populations located in the Rhaetian Alps sector as well as the northernmost populations located in the Lepontine Alps, show a poor genetic variability. This could be due to geographical isolation caused by glacial expansion. Indeed, glacial phenomena had a greater influence in these Alpine sectors than in the more southern ones (Schönswetter et al., 2003), leading to a limited gene flow and resulting in genetically uniform populations. On the other hand, *A. brevis* populations located in the Southern Lepontine Alps as well as those located in the Western Orobic Alps were poorly interested by the presence of glaciers. As a result, here *A. brevis* populations could have remained closer to each other than the more northern ones, thus allowing to maintain a high gene flow.

Nowadays this species faces many threats mainly related to global warming (Mangili et al 2014; Eustacchio et al., 2023). *Androsace brevis* seems to be extremely vulnerable to increasing temperatures as it already grows on mountain peaks and ridges, thus being not able to shift to higher altitudes. In addition, following the "Competitor, Stress-tolerator, Ruderal (CSR)" theory proposed by Grime (1977), *A. brevis* shows a low phenotypic plasticity and a Stress tolerator – Ruderal strategy, with the "S" trait more represented (Mangili, 2016). The total absence of Competitive "C" trait could become a serious problem if more competitive plant species move up from lower altitudes.

For the survival of the species, the reproductive success is a crucial issue. Molecular studies (Bonelli et al., unpublished data), as well as pollination exclusion experiments (Eustacchio et al., 2023), have highlighted that *A. brevis* prefers cross-pollination to self-fertilization, even where populations are highly isolated or present a limited number of cushions. This species is characterized by a very short flowering period which starts between late May and early June as soon as the snowmelt on outcrops occurs, and lasts about two weeks (Provasi, 1922; Pignatti, 1982; Bonelli et al., 2020, Bonelli et al., 2022). In this short time interval, *A. brevis* must ensure pollination which is carried out by insects (Bonelli et al., 2020). During early season, few taxa are active and can pollinate flowers but, at the same time, very few other floral resources are available for arthropods as a snow cover is usually still present. *Androsace brevis* flowers can thus represent an important source of food for many arthropods which can act as pollinators if they visit different plants (Bonelli et al., 2022). However, in mountain ecosystems, the early season is a critical moment for possible mismatches between plants and potential pollinators, as phenological and altitudinal shifts due to climate warming can occur more rapidly than later in the season (Ettinger et al., 2021). *Androsace brevis* is therefore an excellent model species for the identification of the fundamental relationships which link plants and arthropods in high altitude ecosystems.

Given the ongoing climate warming, to understand which flower visitors are responsible for *A. brevis* reproduction success could provide fundamental knowledge on how entomophilous species living in similar environmental contexts can adapt and survive in the future.

1.4 Second case study: Plant and flower-visiting arthropod communities within the Stelvio National Park

In mountain ecosystems, the composition and species richness of both arthropod and plant communities are influenced mainly by elevation and related decrease in temperatures (Moreira et al., 2018; Rasmann et al., 2016). For instance, in Alpine context, where vegetation cover is still continuous up to about 2800 – 3000 m asl (Nagy & Grabherr, 2009), beetles and bees usually increase their abundance and species richness in mid-elevations (1300 – 2000 m asl) (Kearns et al., 2017; Lefebvre et al., 2018; Pilar et al., 2020), while ants decline with decreasing temperature (Reymond et al., 2013). Conversely, flies are well adapted to cold environments, often dominating high-altitude ecosystems (Totland et al., 1993; Lefebvre et al., 2018). Generally, along a wide elevation range, mid-elevation shows the highest species diversity, as it shares species both with low and high altitudes (Kessler et al., 2000; Brehm et al., 2007). Additionally, both bees and butterflies as well as beetles are replaced by flies in high-mountain ecosystems (Lefebvre et al., 2018; Adedoja et al., 2020).

Another factor that influences the composition and species richness of biotic communities is the type of land use. For millennia, the mountain landscape has been subjected to many changes in favour of livestock farming and agriculture (Gingrich et al., 2015). These pratiques led to the formation and preservation of two of the richest ecosystems in the world in terms of biodiversity: pastures and meadows (Bonari et al., 2017; Faccioni et al., 2019). In the last decades, both livestock farming and agriculture shifted mainly to the low valley areas due to the mechanisation and intensification of production processes. Consequently, high-mountain semi-natural ecosystems are abandoned causing a decline in plant and arthropod species richness (Wesche et al., 2012; Jacquemyn et al., 2011; Niedrist et al., 2009; Fischer et al., 2008). Moreover, also an incorrect management of agroecosystems negatively impacts plant and arthropod diversity. Practices such as overgrazing, massive use of pesticides and herbicides as well as frequent mowing can effectively alter the availability of trophic resources, refuges, and potential nesting places (Marini et al., 2009, Bonari et al., 2017, Seibold et al., 2019).

The study of plant and flower-visiting arthropod communities and their relationships, considering both the altitudinal gradient and that of different land management types, is useful to better understand how these communities change and adapt under climatic conditions and anthropic factors (Körner, 2007; Rasmann et al., 2016; Galmán et al., 2018; Moreira et al., 2018).

Given its great variety of environments, the Stelvio National Park (Central Italian Alps) is a suitable area both for studying the network of interactions between plant and flower visiting arthropod communities, and for analysing how they change in relation to altitude and the type of land management.

The Stelvio National Park was instituted in 1935 and, with its 130,734 hectares, is the largest national park in the Italian Alps. It is located in Eastern Alps within the Rhaetian Alpine sector (Marazzi, 2005) encompassing Lombardy, Trentino and South Tyrol regions (Figure2.a). The park is characterised by a wide altitudinal range (from about 600 m to about 4000 m asl) and includes a high variety of natural, semi-natural and anthropic environments.

The Ph.D. project belongs to the EU-funded projects for the monitoring of pollinators within member States of the European Union. The project funding was provided by the Ministry of the Environment (Directive 2020: "Le api come bioindicatore della qualità ambientale - Insetti di valore conservazionistico, presenza, status e interazioni con specie di fitopatogeni"; "Bees as bio-indicator of environmental quality - Insects of conservation value, presence, status and interactions with phytopathogenic species") which commissioned the work to the Stelvio National Park. Subsequently, data collection and processing were assigned to two institutions: University of the Study of Milan (UniMi) and MuSE—Science Museum of Trento. In particular, this study is part of the project "Conoscere e proteggere gli impollinatori nel Parco Nazionale dello Stelvio: azioni di censimento, monitoraggio, valutazione e di sensibilizzazione" ("Knowing and protecting pollinators within the Stelvio National Park: census, monitoring, evaluation and awareness actions").

In agreement with the Stelvio National Park, UniMi and MuSE selected the Martello valley as study area (Figure2.b). This valley extends for about 27 km along an altitudinal gradient ranging from about 700 m asl of Laces town and 3,769 m asl of Cevedale Mount, including a wide type of natural and seminatural ecosystems.

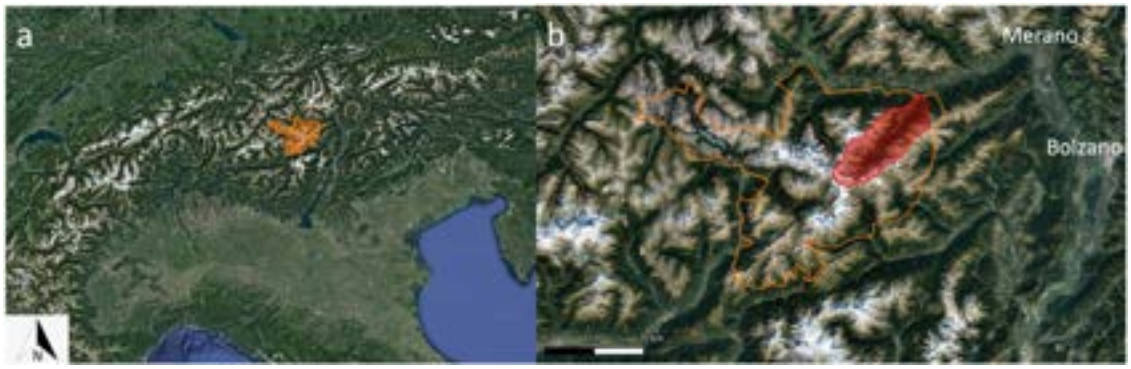

Figure 2. a. Location of Stelvio National Park in the European Alps; b. Location of Martello Valley within the Stelvio National Park.

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Chapter 2

Plant-arthropod interactions: the case study of *Androsace brevis* (Hegetschw.) Ces. (Primulaceae)

Chapter 2.1

Pollen and floral morphology of *Androsace brevis* (Hegetschw.) Ces. (Primulaceae), a vulnerable narrow endemic plant of the Southern European Alps

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Pollen and floral morphology of *Androsace brevis* (Hegetschw.) Ces. (Primulaceae), a vulnerable narrow endemic plant of the Southern European Alps

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ABSTRACT

Androsace brevis (Hegetschw.) Ces. (Primulaceae) is a narrow endemic plant of the Southern Central European Alps that lives only above 2000 m asl and flowers immediately after snowmelt for a very short period during which the plant must guarantee successful sexual reproduction. Despite the vulnerability of this species, mainly due to increasing temperature, competition with plant communities shifting to higher altitudes, and possible mismatches with pollinators, nothing is known about its pollen and floral morphology. In this study we aim to provide a detailed description of these traits and to investigate how they might be related to possible pollination strategies and vectors. Pollen and flower samples were collected from the Lepontine and Ovobic Alps (Northern Italy) populations. The pollen grains are small (polar axis: 19.17 μm ± 0.09; equatorial axis: 10.84 μm ± 0.1) with a prolate shape. The morphology of the flower, with the location of both stamens and pistil inside the corolla tube, and the coloured corolla mouths, ranging from yellow to purple, suggest that *A. brevis* requires insect-mediated pollination. Moreover, floral morphology does not show a particular insect-selective pattern in that the presence of both stamens and pistil less than 1 mm below the corolla mouth (0: 0.76 mm ± 0.05) might allow many high-altitude flower-visiting insects to reach both nectar and pollen. A generalist pollination strategy could be fundamental in contexts characterized by harsh environmental conditions as it could counteract potential plant-pollinator mismatches due to increase in temperatures.

1. Introduction

The genus *Androsace* L. (Primulaceae) includes 153 species distributed in temperate and cold regions of the world, with mostly high mountain or arctic species (Smith et al., 1997; Kelso, 1991). The biogeographic reconstruction suggests that this genus originated from an Asian ancestor about 35 million years ago (Boucher et al., 2012) from which it radiated widely across the mountain ranges of the Northern Hemisphere, reaching Europe first and North America afterwards (Schönswetter et al., 2003; Roquet et al., 2013).

In the European Alps, the *Androsace* genus consists of 17 species found mainly in prairies, rocky debris, and overhanging cliffs of the subalpine and alpine altitudinal belt (Aeschimann et al., 2004) and distributed up to 4200 m asl (Giacomini and Fenaroli, 1958). Among these species, eight are endemic to the Alps (Aeschimann et al., 2004): some of them are widely distributed along the chain (Conti et al., 2005), while others are only present in a very restricted area. *Androsace brevis* (Hegetschw.) Ces. (Fig. 1) belongs to the latter group, a narrow endemic species that is present in the Southern Central Alps (Aeschimann et al., 2004). In particular, the extent of its occurrence encompasses three Alpine sections: the Eastern Lepontine Alps (Como Province, Lombardy, Italy and Canton Ticino, Switzerland), Western Ovobic Alps (Bergamo and Lecco Province, Lombardy, Italy), and Western Rhaetian Alps (Sondrio Province, Lombardy, Italy) (Pirovani, 1922; Pignatti, 1982;

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Mangili et al., 2014). The species grows only on siliceous peaks and ridges above 2000 m asl, with an estimated area of occupancy of 92 km^2 (Mangili et al., 2014).

Androsace brevis is a chamaephyte cushion plant (Fig. 1) characterized by a very short flowering period, starting as soon as the snow melts on the rocky outcrops and lasting about two weeks between late May and early June (Pignatti, 1982; Bonelli et al., 2020, 2022). During this short period, the plant must guarantee successful sexual reproduction, which is the most common strategy for *A. brevis* (unpublished data) as well for many other alpine species living in cold alpine environments, compared to vegetative propagation (Körner, 1999; Wirth et al., 2010; Hörandl et al., 2011; Brutovi et al., 2019; Pegoraro et al., 2020).

Nowadays this species faces many threats: indeed, as reported by Mangili et al. (2014), *A. brevis* seems to be extremely vulnerable to increasing temperatures as it already grows on mountain peaks and ridges, thus being not able to shift to higher altitudes. This renders the species vulnerable to the upward shift of more competitive species from lower altitudes which could represent a serious threat in that it shows low competitive ability. Furthermore, populations of *A. brevis* are often located very close to mountain paths and huts as well as to high-altitude pastures, subjecting them to frequent trampling by hikers and grazing animals. In addition, the species has a very low seed/flower ratio which could lower fitness (Mangili et al., 2014). In this context, reproductive success could also be negatively impacted considering that climate change might also threaten its interactions with possible pollinators (Bonelli et al., 2020, 2022); indeed, the early season represents a very critical moment for plant-pollinator mismatches (Rodo and Cooper, 2019). Finally, *A. brevis* is classified as vulnerable ("VU") both in Italy and Switzerland (Mangili et al., 2014; Bornand et al., 2016), according to IUCN criteria "B" (B1 – extent of occurrence; B2 – Area of occupancy) and "C" (C2a(i) – no subpopulation estimated to contain more than 50 mature individuals), respectively.

Although the ecology of *A. brevis* is documented (Mangili et al., 2014), little is known about its pollen and floral morphology. Indeed, the pollen morphology of *A. brevis* has never been studied, even though Xu et al. (2016) described the pollen morphotypes of five species of the section Aretia (*A. alpina* (L.) Lam., *A. carnea* L., *A. obtusifolia* All., *A. wulfeniana* Steud. & Hochst.), to which *A. brevis* belongs. Regarding the floral morphology, only the external conformation of the flower has been described (Hegetschweiler, 1840; Cesati, 1844; Pignatti, 1982; Mangili et al., 2014): *A. brevis* corolla has a diameter of about 5–8 mm; it is gamopetalous and presents five limbs slightly fringed at the apex. The flower is pink with a yellow or purple mouth wrapped by a calyx about 3.5–4.5 mm long, which develops from a stem of 9–15 mm in length. Nothing has been reported about the inner morphology of the flower, i.e., the position of stigma and anthers, their distance from the mouth of the flower, and their implications for reproduction.

It is important to study both pollen and floral morphology as they are correlated with pollination vectors (Hesse, 2000; Wang et al., 2009; Zhao et al., 2016; Parisi and Franchi, 2020). In particular, pollen morphology could be the result of adaptations to specific pollinators (Ferguson and Skvarla, 1982; Proctor et al., 1997; Tanaka et al., 2004) while the morphology of the flower not only could derive from the need to protect pollen and nectar from nonoptimal weather conditions but also to attract specific insects for pollination (Armbruster, 1996; Aizen, 2003; You et al., 2017; Lawson and Rands, 2019). In this study, therefore, we aim to describe both the pollen and floral morphology of this narrow endemic Alpine species.

2. Materials and methods

2.1. Study sites and flower sampling

In 2021, full-anthesis flowers were sampled from *A. brevis* plants belonging to two different populations in the Lepontine (Como, Lombardy, Italy) and Orobic Alps (Bergamo, Lombardy, Italy) (Fig. 2). The first site is located on the border between Italy and Switzerland, on the Cugn Peak (UTM WGS84–32T E 512330, N 5112905, 2193 m asl), while the second site was located near the Mountain Hut "Cesare Benigni" (UTM WGS84–32T E 543496, N 5096577, 2222 m asl). The two sites are described in detail by Bonelli et al. (2022).

2.2. Pollen morphology

Morphometric analysis of *A. brevis* pollen grains was performed using light microscopy (LM), while the sporopollenin ultrastructure was investigated by scanning electron microscopy (SEM) in according with Andrew et al. (2022). The pollen grains were obtained from 30 anthers of six flowers per site and subjected to acetolysis according to Erdtman (1960).

For LM observations, slides of pollen were mounted in glycerine jelly. At least 100 pollen grains in equatorial or in polar view were photographed for each population using the 100x immersion objective lens under a Leica DM – RD microscope equipped with a Leica MC170 HD camera (Leica Camera AG, Wetzlar, DE). To determine the pollen grain shape, the equatorial axis (E) and polar axis (P) were measured and the P/E ratio of 40 randomly selected pollen grains for each population was calculated. The measurements were performed with ImageJ software (Schneider et al., 2012). For SEM observations, acetolyzed and air-dried pollen was sputtered with a 25-nm layer of gold in argon plasma (AGAR Automatic Sputter Coater Mod. B7341, Scientific Limited, Stansted, UK) equipped with a quartz crystal thickness monitor. At least 30 pollen grains in equatorial and polar view were observed under the electron microscope (Model Leo-1430, Zeiss Inc., Thornwood, NY, USA).

Fig. 1. a. *Androsace brevis* on a rocky outcrop at San Jorio Pass – Cugn Peak (Como, Lombardy, Italy, 2200 m asl); b. Detail of *A. brevis* flowers, Scale bar: 1 cm.

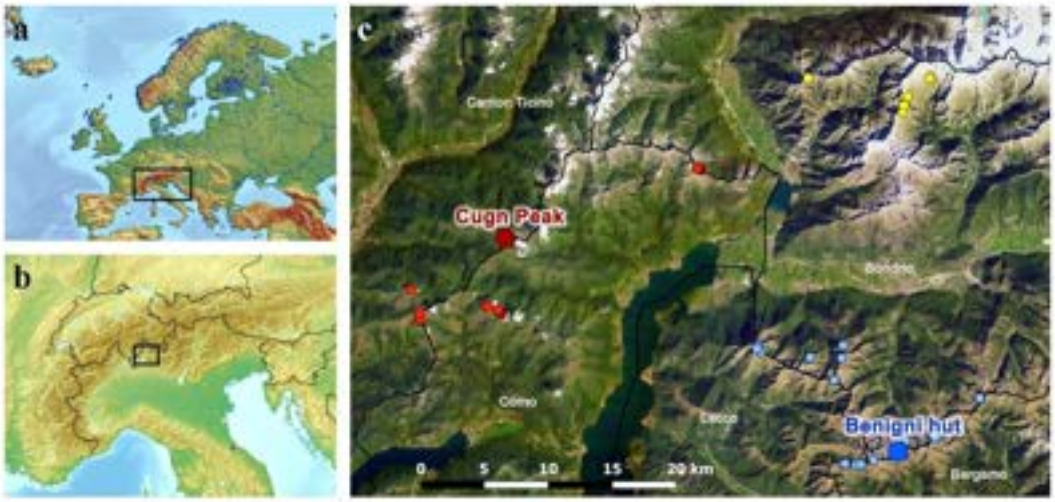

Fig. 2. a. Alps in Europe; b. *A. brevis* distribution range in the Southern Central Alps; c. Known populations of *A. brevis* (Red dots: Lepontine Alps; yellow dots: Rhaetian Alps; blue dots: Orobic Alps). The two sites chosen for this study (Cugn Peak and Benigni Hut) are highlighted with larger and darker dots.

We used palynological terminology according to Punt et al. (2007) and Heise et al. (2009).

2.3. Floral morphology and phenology

To assess the number of flowers produced by *A. brevis*, the number of buds, open flowers, and withered blossoms carried by a single plant were counted, considering 15 plants from each site examined in this study. To describe the floral morphology, three open full-anthesis flowers of *A. brevis* were collected from each site. Fresh flowers were placed on graph paper, and corollas were photographed with Olympus Tough TG-4 (Olympus Corporation, Tokio, JP) camera. Then, longitudinal sections from the corolla mouth to the calyx of the flower were prepared, and pictures were taken with a stereomicroscope (Leica M50) equipped with a camera (Leica IC90) at 4x magnification. Floral parts were measured with ImageJ (Schneider, et al., 2012) software. In particular, the length and width of the limb and corolla tube, the diameter of mouth and corolla (Fig. 3a,b), the length and width of calyx limb, the length of calyx and calyx tube (Fig. 3c), the length and the width of style, stigma, and anther, the length of stamen filament, the diameter of ovules, and the length of ovary (Fig. 3d,e) were measured. Moreover, the following distances were considered: anther – stigma, mouth – stigma, mouth – ovary, anther – ovary, and anther – anther (Fig. 3f). Based on these measurements, a scale drawing of the flower was rendered by using the software Adobe Photoshop CS6 (Adobe Inc., San Jose, CA, USA). A transverse section was also prepared to describe the structure and content of the ovary. In particular, three fresh flowers from each site were dissected along the upper wall of the ovary to observe the placentation and count the number of ovules. Moreover, the upper wall of the ovary was observed at 40x magnification under light microscope to evaluate the presence of structures involved in the nectar secretion.

Finally, to determine whether the different colors of the flower mouth are linked to the ripening stage of the anthers, three plants from each site bearing at least 10 flowers were observed from the beginning of anthesis to withering.

3. Results

3.1. Pollen morphology

The color of fresh *A. brevis* pollen is brownish-yellow. The mature pollen grains are released as free monads and have a prolate shape (mean ± SE: 10.04 μm ± 0.1 large and 19.17 μm ± 0.09 long) (Supplementary material S1). Grains are radially symmetric, isopolar, and zonocolporate with three colpori (tricolporate pollen grains) that are not fused at the polar edge (Fig. 4a,b,c). The tectum is nearly continuous on the apocolpium and perforated on the mesocolpium. Puncta (diameter < 0.5 μm) have different shapes and sizes (Fig. 4d,e). In many cases, the mesocolpium is not completely perforated by the puncta (Fig. 4f).

3.2. Floral morphology and phenology

Androsace brevis produces from two to about 200 (mean ± SE: 45 ± 9, Supplementary material S2) solitary, hermaphrodite, homostylous flowers. Flowers are held by erect pedicels and the corolla tube is enveloped by a gamosepalous, persistent calyx with five teeth. Inside the corolla tube, ca. 0.5 mm below the mouth, the androecium consists of one cycle of five yellow green anthers held by a green filament inserted around the mouth of the corolla tube at the opposite side of the corolla lobes and surrounding the stigma. The gynoecium is simple: the stigma develops from a linear yellow-green style which starts from the ovary located at the base of the corolla tube. The ovary presents a free central placentation and contains five ovules which are attached to the central axis without any partition between them (Fig. 5). Nectaries were not observed, but drops of a viscous substance were present both on the surface of the ovary and the inner side of the corolla tube.

The size (mean ± SE) of each trait of the *A. brevis* flower is reported below (Table 1), while the raw data are reported in Supplementary material S3.

Finally, it was observed that the color of the corolla mouth changes gradually from yellow to purple during the anthesis period. In particular, three main color shades can be distinguished (Fig. 6): 1) yellow, at the beginning of the flowering period, the moment when the anthers are brought straight close to the mouth; 2) yellow-purple, after the main

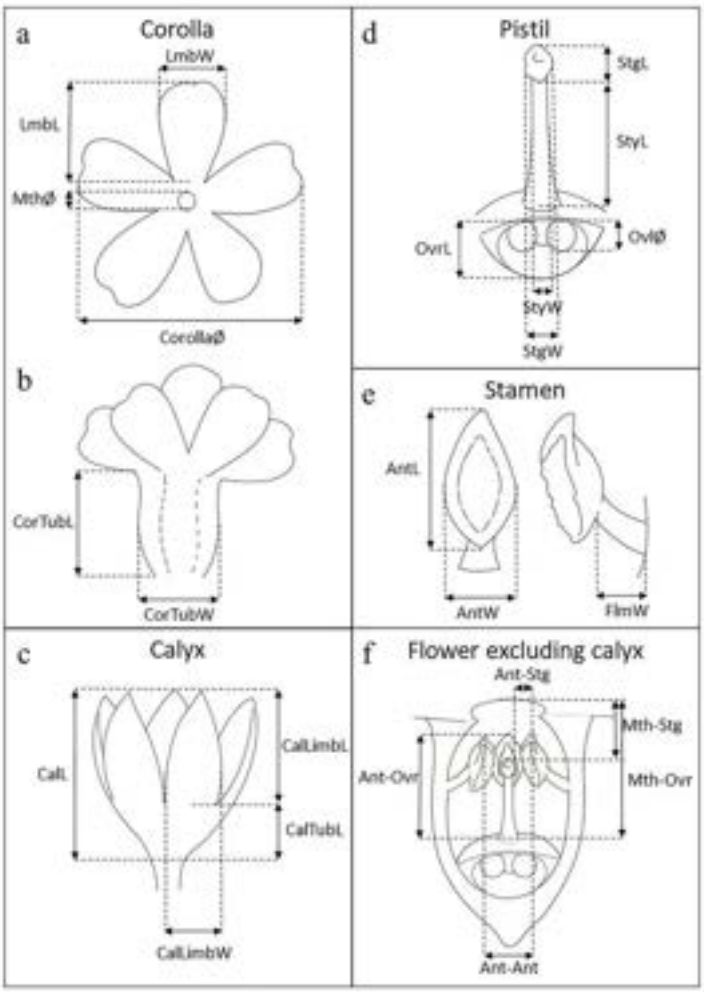

Fig. 3. Measurements of the flower parts (L – Length, W – Width, Ø – Diameter): **a.** Limb (Lmb), mouth (Mth), and corolla; **b.** Corolla tube (CorTub); **c.** Calyx (Cal), calyx limb (CalLimb), and calyx tube (CalTub); **d.** Style (Sty), stigma (Stg) and pistil (Pst), ovule (Ovl), and ovary (Ovr); **e.** Anther (Ant), filament (Flm); **f.** Distance anther – stigma (Ant-Stg), distance mouth – stigma (Mth-Stg), distance mouth – ovary (Mth-Ovr), distance anther – ovary (Ant-Ovr), and distance between anthers (Ant-Ant). Drawing by E. Bastacchio.

flowering period, when stamens begin to descend, revealing the corolla tube; and 3) purple, at the end of flowering period, when stamens are completely lowered, leaving the corolla tube visible.

4. Discussion

In relation to the six categories by which pollen grains can be classified according to their longest axis (Erdtman, 1952, 1969; Hesse et al., 2009), *A. brevis* produces small pollen grains (longest axis length ranging from 10 to 25 μm). Shape and exine ornamentation of the pollen grains are quite similar to those of other species belonging to the genus *Androsace* section Aretia described by Xu et al. (2016). However, *A. brevis* presents a larger polar axis but a similar equatorial one, resulting in a higher P/E ratio than other species reported by those authors. Although the small size of the pollen grains represents a feature of anemophilous pollen (Lu et al., 2022), the anthers are placed completely inside the corolla tube and the morphology and color of the corolla exclude anemophilous pollination. This was confirmed by pollinator exclusion experiments conducted in 2016 (Supplementary material S4): even though flowers do not present heterostyly, as known for *Primula* genus (Lloyd and Webb, 1992; Barrett and Shore, 2008), and anthers are located very close to the stigma (less than 0.5 mm apart), this species does not produce seeds if it is not visited by pollinators. This could suggest the occurrence of a mechanism which avoids self-fertilization, although the latter strategy is often advantageous in high-altitude environments because of low pollinator abundance (Kelso, 1987, 1991; Gugerli, 1998; Torres-Díaz et al., 2011; Goodwillie and Weber, 2018), especially for species that are characterized by small and fragmented populations (Van Rossum et al., 2006; Shao et al., 2008). However, plants that naturally live in small and scattered populations may have developed strategies to cope with long-term genetic isolation (Gonzalo-Turpin and Hazard, 2009; Becker et al., 2011; Hirao et al., 2019), as a

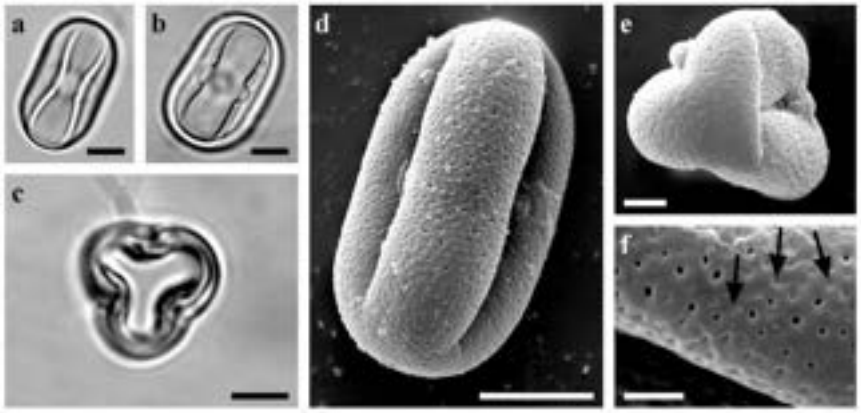

Fig. 4. **a–c**: LM micrographs. Scale bars: 5 μm. **a, b** Pollen grain in equatorial view at two different focal planes showing the equatorial pores; **c**. Pollen grain in polar view showing a triangular equatorial outline; **d–f**: SEM micrographs. Scale bars: d = 5 μm; e = 2 μm; f = 1 μm; **d**. Pollen grain in equatorial view showing a prolate shape and a perforate ornamentation; **e**. Pollen grain in polar view, showing a reduction in density of puncta on the apocolpium; **f**. In some cases, the tectum is not completely perforated by the puncta (arrows).

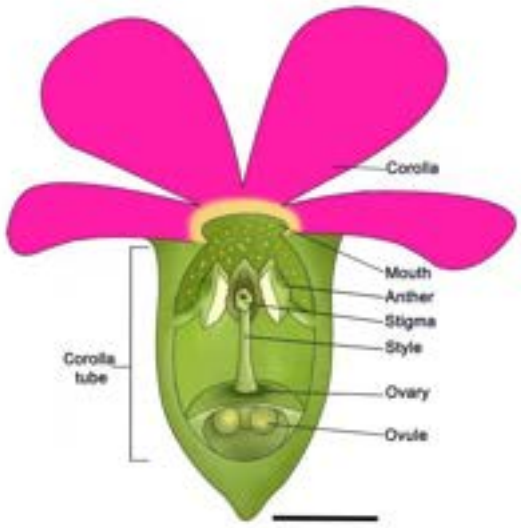

Fig. 5. Scale drawing of the longitudinal section of *A. brevis* flower based on data reported in Table 1. Scale bar: 1 mm. Drawing by E. Eustacchio.

Table 1
Mean value of the size of each trait of *A. brevis* flower with respective standard error (SE) and the number of measurements taken for each trait (n).

Section	Mean (mm)	SE	n
Anther length	0.58	0.02	6
Anther width	0.25	0.03	6
Anther-ovary distance	1.29	0.08	6
Anther-stigma distance	0.14	0.01	6
Anther-anther distance	0.27	0.05	6
Calyx length	3.36	0.20	6
Corolla diameter	8.78	0.40	6
Flower length	3.93	0.06	6
Median calyx limb length	2.01	0.08	25
Median calyx limb width	0.96	0.05	25
Median calyx tube length	1.61	0.09	25
Median limb length	4.00	0.06	30
Median limb width	2.54	0.05	30
Mouth diameter	0.76	0.05	6
Mouth-anther distance	0.68	0.03	18
Mouth-ovary distance	2.14	0.10	6
Mouth-stigma distance	1.10	0.03	6
Ovary length	0.72	0.04	6
Ovule diameter	0.29	0.02	6
Stamen filament length	0.27	0.02	6
Stigma length	0.16	0.01	6
Stigma width	0.20	0.02	6
Style length	0.85	0.04	6
Style width	0.17	0.02	6

high outcrossing ability even in conditions of low pollinator activity (Rodo, 2022). In support of this, also other cushion plant species that live in alpine environments, such as *Silene acaulis* (L.) Jacq., *Saxifraga oppositifolia* L. and *Eritrichium nanum* (Vill.) Schrad. ex Gaudin, show an increase in seeds production when cross-pollination occurs (Swanton and Molau, 1992; Hermanutz and Innes, 1994; Delf and Carroll, 2001; Zoller et al., 2002). In addition to produce a high amount of nectar to be more attractive for pollinators (Hocking, 1968; Bowles, 1979; Zoller et al., 2002), these species develop different strategies to limit self-pollination. For example, *S. acaulis* develops a variable number of hermaphrodite and female flowers within a population improving fitness in seeds production (Darwin, 1877; Hermanutz and Innes, 1994; Delf and Carroll, 2001), while anthers and pistil of *E. nanum* ripen at different times (Zoller et al., 2002).

Given the low availability of pollinators in high-altitude environments (Holsinger, 1991; Barrett, 2002; Kalisz et al., 2004; Torres-Díaz et al., 2011; Goodwillie and Weber, 2018), plants often develop larger flowers to be more visible by insects (Proctor et al., 1997; Harder and Johnson, 2009). Indeed, as reported by Schinzel (1995), the size of entomophilous plants tend to shrink with an increase in altitude since they invest more energy in reproductive structures. *Androsace brevis* develops relatively small solitary flowers whose diameter (~8.70 mm) is similar to those reported by Asterhiazara et al. (2004) for *A. alpina* (L.) Lam and *A. wulfeniana* Steud. & Hochst (respectively $\bar{O}$ = ~8 mm; $\bar{O}$ = ~10 mm) while it is larger than that of other species belonging to section Aretia distributed along the Alpine chain (*A. hausmannii* Leyb., $\bar{O}$ = ~4

Fig. 6. Three different color shades of *A. brevis* corolla mouth within the same plant: 1: yellow, 2: yellow-purple, 3: purple.

mm; *A. helvetica* (L.) All., $\varnothing = -5$ mm; *A. pubescens* DC., $\varnothing = -5$ mm, *A. wulfeli* Stemb., $\varnothing = -5$ mm). In alpine environments, many cushion plants develop small-sized flowers which potentially would hardly be visible by possible pollinators compared with those of other species flowering in the same area and period (Parachnowitsch and Kessler, 2010). For instance, in sites where *A. brevis* grows, other species that show a larger corolla surface (i.e., *Gentiana acaulis* L., *Primula hirsuta* All., *Pulsatilla alpina* (L.) Delarbre, and *Potentilla aurea* L.) are present and flower in the same period (Bonelli et al., 2022). However, *A. brevis* produces, on average, 50 flowers located very close to each other, and flowers have patent corolla limbs whose length is more than twice that of the calyx creating a homogeneous pink carpet with dots ranging from yellow to red. This pattern of a carpet of flowers with dots is very common in cushion alpine species as it helps them to compete with other alpine plants with larger flowers (Zoller et al., 2002; Parachnowitsch and Kessler, 2010). Showing a carpet of flowers leads pollinators to spend less flight energy moving from one flower to another searching for nectar and pollen and make greater gains in food rewards (Willson and Price, 1977; Heinrich, 1979; Kunin, 1993; Cartar et al., 2004; Dicht et al., 2020). The study of *A. brevis* floral morphology did not show the presence of nectaries above the gynoecium. Nevertheless, we observed a substance adherent to the inner wall of the corolla tube, above and under the stamens, allowing us to hypothesize that nectar is produced, but not secreted by specific structures as observed for *Androsace vitaliana* (L.) Lapeyr. by Abrahamczyk et al. (2017). In support of this, within the Primulaceae family, it is common that nectar is secreted by a nectariferous tissue which release a sugary substance through stomata located on the gynoecium (Percival, 1961; Vogel, 1966; Stevens, 2001; Soodersen, 2000). Moreover, the presence of nectar is supported by the different color shade of the corolla mouth observed in different flowers within the same cushion, which may give a signal about the quality and quantity of floral rewards throughout the flowering period for potential pollinators (Melendez-Ackerman et al., 1997; Aragón and Ackerman, 2004). Color changes of the corolla mouth during anthesis were observed also in other *Androsace* species (Pohuin, 1969; Zhang, 1982; Pohuin and Stainton, 1984; Stainton and Pohuin, 1988; Weiss, 1995; Sefidi, 2021) as well as in *E. nivalis*. The latter species, in particular, presents small ($\varnothing = -6$–7 mm) blue flowers with five yellow epipetalous fornices that become visible when anthers begin to shed pollen and when nectaries at the base of the ovary start to produce fair amounts of nectar. When the pollination is concluded, the yellow color of the fornices become pale, and fructification takes place (Zoller et al., 2002).

Regarding the content of the ovary, species belonging to Primulaceae usually develop from few tens to hundreds of ovules and potentially the same number of seeds per flower (Gustavsen, 1938; Anderberg and Stahl, 1995; Anderberg, 2004). For *Androsace* species, to the best of our knowledge, only Douglas (1936) reported the presence of six ovules in *A. primuloides* Duby. *A. brevis* similarly shows five relatively large ovules ($\varnothing = -0.29$ mm). They usually lead to the development of three or four seeds about 2.1 mm long, 1.4 mm wide and 0.5 mm thick (unpublished data), but one of them is frequently smaller than the others (Mangili et al., 2014) and probably not fertile. Investing a lot of energy to develop big seeds with a lot of storage could mean that *A. brevis* uses a K-strategy to cope with the harsh environmental conditions in which it lives. In fact, in alpine context larger and heavier seeds increase the species fitness, giving a better chance to recruit and to withstand hazards (Jakobsson and Eriksson, 2003; Coomes and Grubb, 2003; Stöcklin et al., 2009).

Finally, floral morphology does not show a particular insect-selective pattern. The presence of both stamens and pistil less than 1 mm below the corolla mouth and the diameter of the mouth ($\varnothing = -0.8$ mm) might allow many flower-visiting insects to reach both nectar and pollen (Conner et al., 1995) and possibly pollinate. Indeed, different flower-visiting taxa, such as families belonging to Diptera Brachycera and Hymenoptera Apoidea Anthophila, are known to visit *A. brevis* (Bonelli et al., 2022). According to the observations performed by Bonelli et al. (2022), our results confirm that *A. brevis* is a generalist pollinated species, with a possible plasticity in pollination. Indeed, if pollinator specificity can be advantageous for plants showing scattered populations (Wei et al., 2021), a generalist strategy may allows sharing of pollinators among different species that flower at the same time in a critical moment of the season. This strategy can be useful also to counteract potential plant-pollinator mismatches (Calloway et al., 2002; Kudrides et al., 2005; Ortman, 2015; Gérard et al., 2020), particularly in the context of climate change (Inouye, 2019).

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CRediT authorship contribution statement

Elena Eustacchio: Conceptualization, Methodology, Validation, Formal analysis, Visualization, Writing – original draft, Investigation. **Marco Bonelli:** Conceptualization, Methodology, Validation, Formal analysis, Supervision, Writing – review & editing, Investigation. **Mario Beretta:** Conceptualization, Methodology, Validation. **Irene Monti:** Investigation. **Mauro Gobbi:** Conceptualization, Funding acquisition. **Morena Casartelli:** Conceptualization, Resources. **Marco Caccianiga:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Supplementary material

S1: Measurement of the polar axis (P) and equatorial axis (E) taken by LM at 100x magnifications and the ratio between P and E of 40 pollen grains obtained from acetolysis of 30 anthers of *A. brevis* flowers collected from Benigni Hut (Orobic Alps) and Cugn Peak (Lepontine Alps) populations.

	Benigni Hut (Orobic Alps)			**Cugn Peak (Lepontine Alps)**		
	P (µm)	E (µm)	P/E	P (µm)	E (µm)	P/E
1	16.82	9.80	1.72	20.96	10.15	2.07
2	18.21	11.13	1.64	19.71	9.62	2.05
3	19.91	12.88	1.55	20.63	9.87	2.09
4	17.90	10.86	1.65	17.41	10.63	1.64
5	18.99	10.92	1.74	18.86	10.81	1.75
6	19.66	11.48	1.71	18.66	10.67	1.75
7	19.42	11.26	1.72	18.82	10.62	1.77
8	18.72	10.62	1.76	18.52	9.86	1.88
9	18.23	11.06	1.65	19.13	9.82	1.95
10	19.49	11.93	1.63	19.83	10.63	1.86
11	19.64	12.00	1.64	19.92	10.27	1.94
12	20.51	11.65	1.76	18.65	9.56	1.95
13	20.53	11.51	1.78	18.35	10.88	1.69
14	20.49	11.64	1.76	18.16	9.54	1.90
15	18.82	12.08	1.56	20.57	10.79	1.91
16	19.38	11.24	1.72	17.84	9.21	1.94
17	19.18	11.45	1.67	18.54	10.69	1.73
18	19.49	12.09	1.61	18.06	11.33	1.59
19	17.75	11.61	1.53	19.63	10.03	1.96
20	19.03	10.98	1.73	19.76	9.86	2.00
21	18.69	11.73	1.59	20.97	9.95	2.11
22	18.32	11.94	1.53	19.66	9.79	2.01
23	19.62	12.41	1.58	19.17	10.36	1.85
24	18.99	10.88	1.74	17.98	10.12	1.78
25	19.39	12.37	1.57	18.73	9.73	1.92
26	18.24	11.96	1.53	18.04	9.67	1.87
27	20.78	11.96	1.74	19.27	10.37	1.86
28	18.83	11.87	1.59	18.96	9.92	1.91
29	19.34	11.87	1.63	19.51	9.93	1.96
30	19.38	11.34	1.71	19.80	10.14	1.95
31	17.55	11.55	1.52	19.41	11.15	1.74
32	20.09	11.79	1.70	19.73	8.58	2.30
33	18.46	11.28	1.64	18.87	10.81	1.74
34	18.99	11.48	1.65	19.52	10.12	1.93
35	19.70	11.32	1.74	18.29	10.61	1.72
36	19.25	12.33	1.56	19.65	9.94	1.98
37	19.67	11.19	1.76	19.65	8.87	2.22
38	19.26	11.80	1.63	19.42	9.35	2.08
39	20.43	12.18	1.68	19.23	10.29	1.87
40	19.87	11.26	1.76	19.03	10.28	1.85
Mean	19.18	11.57	1.66	19.17	10.12	1.90
Minimum Value	16.82	9.75	1.52	17.41	8.58	1.59
Maximum Value	20.78	12.87	1.78	20.97	11.33	2.30
STD	0.86	0.57	0.08	0.83	0.59	0.15
SE	0.14	0.09	0.01	0.13	0.09	0.02

S2: Number of flowers held by each *A. brevis* plants. For each population considered (Benigni Hut, Orobic Alps; Cugn Peak, Lepontine Alps), flowers held by 15 plants were counted.

	Benigni Hut (Orobic Alps)	**Cugn Peak (Lepontine Alps)**
1	2	8
2	15	20
3	142	95
4	43	24
5	4	130
6	52	14
7	33	13
8	31	9
9	57	98
10	74	205
11	43	24
12	31	11
13	47	6
14	76	8
15	31	13
Mean	45.4	45.2
SE	8.9	15.3

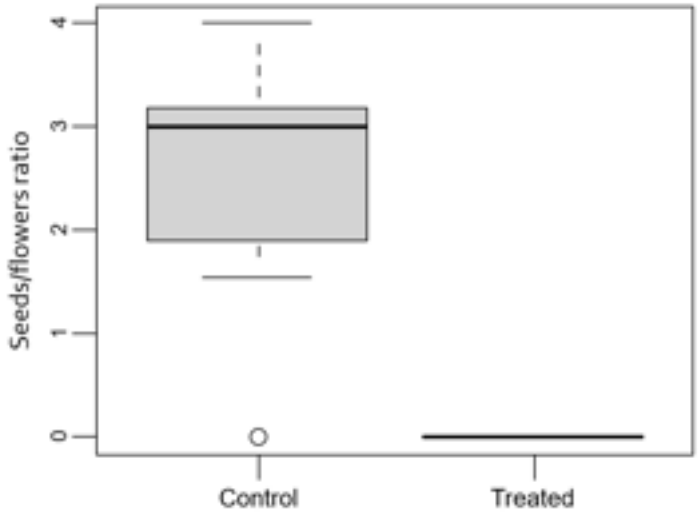
S3: Raw data of traits for each of *A. brevis* flower measured.

Section	Benigni Hut (mm)	Benigni Hut (mm)	Benigni Hut (mm)	Cugn Peak (mm)	Cugn Peak (mm)	Cugn Peak (mm)
Anther length	0.615	0.602	0.522	0.540	0.642	0.557
Anther width	0.180	0.305	0.202	0.381	0.253	0.203
Anther-ovary distance	1.499	1.186	1.203	1.561	1.055	1.221
Anther-stigma distance	0.085	0.122	0.135	0.168	0.163	0.169
Anther-anther distance	0.482	0.192	0.246	0.241	0.416	0.199
Calix length	3.632	2.931	2.811	3.473	3.395	4.124
Corolla diameter	7.860	9.228	9.449	7.618	8.379	10.142
Flower length	4.135	3.775	3.968	3.933	3.999	3.773
Median calix limb length	1.606	2.132	2.608	2.245	1.624	1.932
	1.332	2.585	2.518	2.165	1.723	1.852
	1.613	2.622	2.245	2.373	1.769	1.886
	1.387	2.577	2.593	*	1.659	1.686
	*	*	*	*	1.756	1.806
Median calix limb width	1.179	0.814	1.086	0.869	0.998	1.023
	0.752	1.025	0.878	0.957	0.785	0.794
	0.751	0.595	1.197	1.074	0.852	0.839
	1.179	1.083	1.807	*	0.977	0.972
	*	*	*	*	0.884	0.732
Median calix tube length	1.300	1.058	1.859	1.908	1.673	2.154
	1.114	1.271	1.776	2.388	1.064	1.963
	0.961	1.288	1.922	2.295	1.397	1.941
	1.179	1.225	1.507	*	1.37	2.085
	*	*	*	*	1.516	2.108
Median limb length	3.774	3.639	3.711	4.010	4.531	4.076
	3.749	4.069	4.183	3.859	4.591	4.088
	3.88	4.116	3.727	2.774	4.133	4.485
	3.648	3.863	4.155	3.184	4.793	4.396
	3.998	4.057	3.894	3.811	4.508	4.328
Median limb width	2.621	2.263	2.359	2.558	2.896	3.327
	2.046	2.642	2.701	2.393	2.272	3.027
	2.681	2.497	2.680	2.337	2.529	2.803
	2.924	2.555	2.524	2.002	2.144	2.711
	2.344	2.596	2.469	2.504	2.293	2.610
Mouth diameter	0.933	0.767	0.675	0.644	0.854	0.673
Mouth-anther distance	0.638	0.711	0.711	0.482	0.874	0.737
	0.589	0.667	0.717	0.496	0.795	0.720
	0.639	0.671	0.637	0.463	0.837	0.831
Mouth-ovary distance	2.013	2.122	2.189	1.763	2.466	2.278
Mouth-stigma distance	1.059	1.158	1.026	1.229	1.106	1.047
Ovary length	0.589	0.830	0.788	0.716	0.614	0.784
Ovule diameter	0.230	0.315	0.334	0.302	0.260	0.304
Stamen filament length	0.243	0.325	0.256	0.201	0.274	0.319
Stigma length	0.125	0.159	0.170	0.165	0.161	0.201
Stigma width	0.157	0.240	0.205	0.145	0.238	0.208
Style width	0.195	0.144	0.163	0.222	0.170	0.116
Style length	0.749	0.935	0.870	0.729	0.917	0.880

*Damaged section, unable to measure it accurately.

S4: Exclusion of pollinators effect.

To evaluate whether *A. brevis* needs flower visitors for reproduction, the effect of pollinator exclusion on fruit production was tested. On Cugn Peak, in May 2016, before *A. brevis* anthesis, we caged 7 cushions, nailing organza to the soil around the plant; moreover, 7 control cushions were prepared in the same way, but we cut the top of the organza, thus allowing possible pollinators access to the flowers.
The treated and control cushions did not differ significantly in number of flower buds (treated mean ± SE = 10.4 ± 5.3, control mean ± SE = 8.4 ± 2.8; Wilcoxon rank-sum test *p*-value = 0.063). At the end of the season, in September 2016, these structures were removed, and we counted the seeds produced per cushion. Then, the seed/flower ratio of control and pollinator-excluded plants were compared, showing that without pollinator access no seeds were produced (treated mean ± SE = 0.0 ± 0.0, control mean ± SE = 2.5 ± 0.5; Linear model *p*-value <0.001, Fig. S4.1).



S4.1 Boxplot representing the ratio between the seeds and the number of flowers produced by the treated and the control *A. brevis* plants.

Chapter 2.2

Manual sampling and video observations: an integrated approach to studying flower-visiting arthropods in high-mountain environments

insects

MDPI

Article

Manual Sampling and Video Observations: An Integrated Approach to Studying Flower-Visiting Arthropods in High-Mountain Environments

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Simple Summary: Our study compared two different methods to identify the arthropods visiting the flowers of the vulnerable endemic alpine species *Androsace brevis* (Primulaceae) and investigate their behavior. Using the traditional method of manual sampling we could taxonomically identify visiting arthropods on a fine scale and determine which taxa carry pollen. Conversely, video observations provided information on arthropod behavior and activity. By integrating the results obtained from these two approaches, we estimated the diversity of *A. brevis* flower-visiting arthropods and evaluated which taxa could be involved in its pollination. Our results, in addition to providing new insights on flowering plant–arthropod interactions in early season in the Alps, might be useful in developing effective methods of studying the ecological relationships in high-mountain ecosystems.

Abstract: Despite the rising interest in biotic interactions in mountain ecosystems, little is known about high-altitude flower-visiting arthropods. In particular, since the research in these environment can be limited or undermined by harsh conditions and logistical difficulties, it is mandatory to develop effective approaches that maximize possibilities to gather high-quality data. Here we compared two different methods, manual sampling and video observations, to investigate the interactions between the high-mountain arthropod community and flowers of *Androsace brevis* (Primulaceae), a vulnerable endemic alpine species with a short flowering period occurring in early season. We manually sampled flower-visiting arthropods according to the timed-observations method and recorded their activity on video. We assessed differences and effectiveness of the two approaches to estimate flower-visiting arthropod diversity and to identify potential taxa involved in *A. brevis* pollination. Both methods proved to be effective and comparable in describing the diversity of flower visitors at a high taxonomic level. However, with manual sampling we were able to obtain a fine taxonomic resolution for sampled arthropods and to evaluate which taxa actually carry *A. brevis* pollen, while video observations were less invasive and allowed us to assess arthropod behavior and to spot rare taxa. By combining the data obtained with these two approaches we could accurately identify flower-visiting arthropods, characterize their behavior, and hypothesize a role of Hymenoptera Apoidea and Diptera Brachycera in *A. brevis* pollination. Therefore, we propose integrating the two approaches as a powerful instrument to unravel interactions between flowering plants and associated fauna that can provide crucial information for the conservation of vulnerable environments such as high-mountain ecosystems.

Keywords: Alps; *Androsace brevis*; endemism; flowering plants; insect behavior; plant–arthropod interactions; mountain ecology; pollination biology; pollinators; video

1. Introduction

Plant–arthropod interactions are an essential component of terrestrial ecosystems, sustaining life on Earth and providing vital ecosystem services. About 88% of existing flowering species, including many crops, are pollinated by animals, mostly insects [1–3]. Conversely, many arthropods depend on flowers for their existence. Almost all bees (Hymenoptera: Apoidea: Anthophila) are completely dependent on flowering plants for their life cycle [4], and many other anthophilous arthropods—e.g., flies (Diptera), thrips (Thysanoptera), and spiders (Araneae)—exploit flowers for multiple purposes, such as foraging, hunting, sheltering, mating, and oviposition [5–8]. Moreover, plant–arthropod interactions can even shape ecosystems, having cascading effects on their components [9–12]. However, these interactions are exposed to both natural and anthropogenic-related threats [13,14]. In particular, an emerging concern is the ongoing change in climate, which can affect flowering plants, arthropods, and their relationships in several ways [15–19]. For instance, climate change can alter the frequency and the intensity of extreme meteorological and hydrological events, leading to habitat loss and fragmentation; moreover, variations in temperature can alter the timing of key stages both in plants (e.g., flowering) and arthropods (e.g., emergence period) or lead some species to migrate according to their ecological needs, while the climate niche for species that cannot migrate can shrink or disappear [14,20].

In mountain ecosystems, climate warming is expected to cause differential phenological and altitudinal shifts in plants and anthophilous species, leading to modifications of biotic interactions and potential mismatches between flowering and flower-visiting arthropods [21–25]. Knowledge of the actors involved is essential to assess the precise impact of climate change on flowering plant–arthropod interactions and to predict its effects on mountain ecosystems, as well as to hypothesize habitat management strategies or develop effective conservation plans. However, despite the rising interest in such interactions in mountain ecosystems [26,29,30], there is still a lack of knowledge about flower-visiting arthropods in these rich and fragile ecosystems [31]. For instance, apart from some early works [32,33], only a few recent studies have dealt with flower-visiting species in the Alps [31,34–41]. This knowledge gap might partly exist because mountain environments are often erroneously considered unpolluted and undisturbed and because of a perceived lack of short-term economic interest in this topic. Moreover, mountain environments are generally characterized by harsh and unpredictable conditions that can give rise to several logistical difficulties during field work, often limiting or undermining research. For instance, working under extreme conditions, such as microclimatic instability and remoteness, entails facing multiple constraints, such as restricted time-windows or limited opportunities for sampling that can severely affect the outcomes of data collection in the field. Thus, when investigating ecological relationships in high-mountain ecosystems, it is mandatory to employ effective approaches that maximize possibilities to gather high-quality data.

Flower-visiting arthropods have traditionally been monitored by direct observations and manual sampling, where timed observations have been proposed and used as a standard, repeatable, and effective method [38,42–48]. Nonetheless, an emerging technique is video observation, which has been employed with various goals and approaches to study the flower-visiting arthropod communities [39,49–53]. Although the arthropods collected by manual sampling can be taxonomically identified on a fine scale and in some cases may offer the possibility to perform investigations on the nature of their interactions with the focal plant (e.g., assessing the presence of pollen on sampled specimens), arthropod behavior and activity on plants cannot be observed or described in detail using this method. Conversely, fine-scale information can be obtained by monitoring arthropod behavior and activity with noninvasive and nondisruptive methods, such as recording observations by video. Both of these approaches can provide insight concerning pollination ecology but, to our knowledge, no study has compared their effectiveness in the context of high-mountain environments.

In this study we therefore compare two different methodological approaches (i.e., traditional manual sampling and video observations) to investigate interactions between the high-mountain arthropod community and the flowers of a plant species during the anthesis (i.e., blooming period). We employed *Androsace brevis* (Hegetschw.) Cesati (Primulaceae) as model species, an alpine plant with a very early and

short flowering period. The flowering occurs immediately after snowmelt, typically lasting about two weeks for single plants and ranging from two to three weeks for the entire population. We made this choice because in this moment of the season—when few trophic resources are available for arthropods and few taxa are active and can pollinate flowers—the potential impact of mismatches between plants and arthropods could be particularly relevant [24].

The present study aims to assess differences and relative effectiveness of manual sampling and video observations in (i) estimating the diversity of *A. brevis* flower-visiting arthropods and (ii) inferring arthropod interactions with *A. brevis*, identifying potential taxa involved in its pollination by means of both palynological analyses of pollen found on sampled arthropods and video analysis of their behavior. Finally, (iii) we discuss costs and benefits of the two methodologies and highlight advantages of an integrated approach in the context of the high-mountain environment.

2. Materials and Methods

2.1. Study Species

A. brevis is a narrow endemic cushion plant that grows above 2000 m asl on rocky ridges and peaks in a restricted area in the Southern Alps of Northern Italy (Lombardy) and adjacent Switzerland. The extent of occurrence is estimated at 907 km², the area of occupancy at 92 km². Populations of this species are scattered and of limited size and, following IUCN criteria, its conservation status is Vulnerable (VU) [54].

A. brevis usually flowers between the end of May and the beginning of June. Each cushion can produce from a few to about 200 solitary flowers held by 5- to 20-mm erect pedicels. The flower (Figure 1A) is about 4 mm long, characterized by a pink corolla (ca. 8 mm in diameter) with a yellow mouth (ca. 0.9 mm in diameter). The species is homostylous (i.e., flowers with styles of uniform length) and both stigma and anthers are located inside the corolla tube. The anthers are located slightly above the stigma, about 0.5 mm from the mouth of the corolla tube.

2.2. Study Site

The study was conducted in the Orobie Bergamasche Regional Park, a protected mountain area of the Orobic Alps (South-Eastern Alps) in the Alpine biogeographical region [55], in the vicinity of the Mountain Hut "Cesare Benigni" (Northern Italy, Lombardy, Province of Bergamo, UTM WGS84—32T E 543,496 N 5096577, 2222 m asl). The study area (Figure 1B) is characterized by the presence of a small glacial lake surrounded by rocky slopes with chasmophytic vegetation (EU Habitats Directive, Annex I habitat type, code 8220), including *A. brevis*. The population we studied occupies an area of approximatively 70,000 m², which includes the plateau surrounding the hut, eastward to the lake, and nearby rocky outcrops and ridges, southward to the lake.

The climatic regime in the Orobic Alps is typically alpine, with oceanic climate influences promoting abundant rainfall distributed throughout the year. In particular, the mean annual temperature at the study site is 2.8 °C, while on average the precipitation regime amounts to 2190 mm per year (extrapolated from data of climatic stations: "Gerola Alta Pescegallo", 1875 m asl, 1.5 km from the study site, observation period 1995–2018; "Mezzoldo Passo San Marco", 1824 m asl, 5.5 km from the study site, observation period 2004–2018; and "Valtorta", 982 m asl, 5 km from the study site, observation period 2012–2018).

The snow cover here usually lasts from October/November to May/June. Snow cover was monitored daily by checking the Mountain Hut webcam (available at https://orobiemeteo.com/) both to assess site accessibility and estimate the beginning of the *A. brevis* flowering season. The study was performed immediately after snowmelt, for three days between 8 and 16 June 2019, concurrently with the period of maximum flowering.

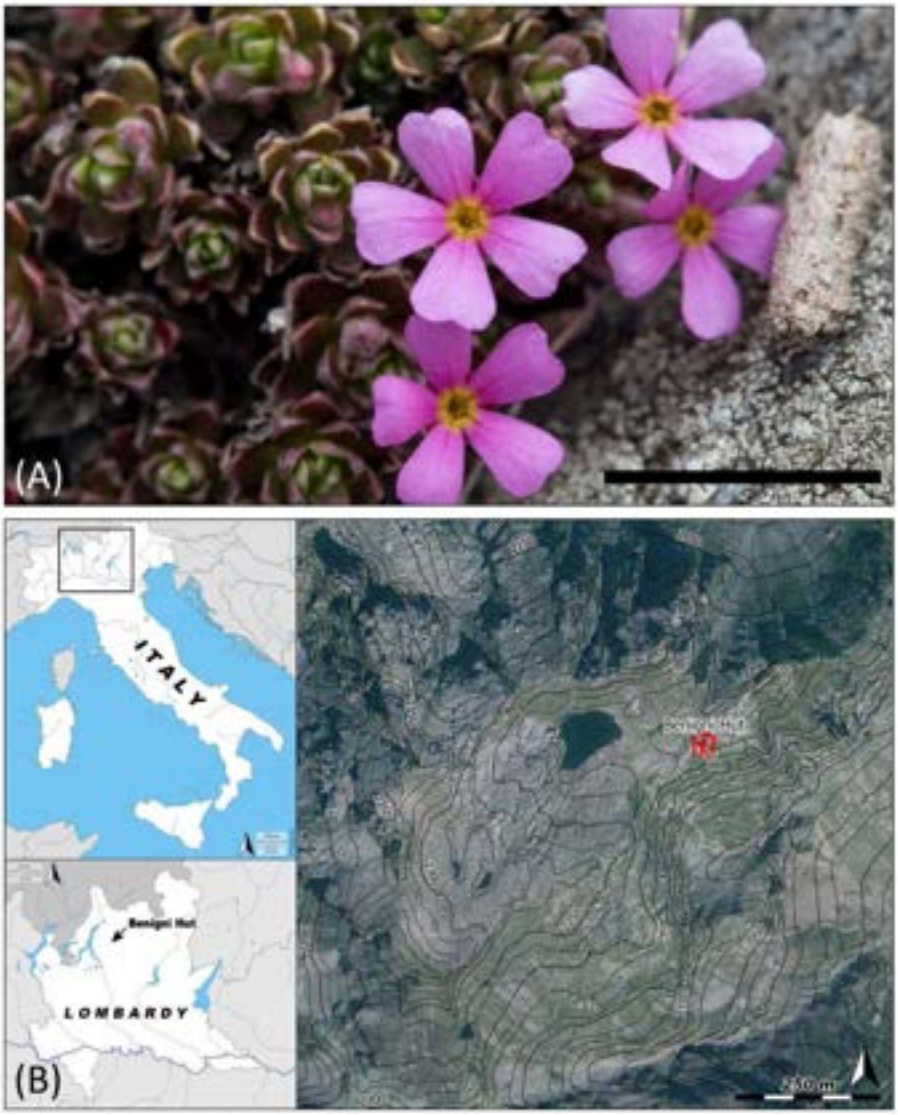


Figure 1. Study species (**A**) and site (**B**). *Androsace brevis* flowers, bar: 1 cm (**A**); and location of the Mountain Hut "Cesare Benigni" (**B**). Lombardy and Italy maps: www.d-maps.com, study area: Ortofoto AGEA 2012.

2.3. Manual Sampling

We manually sampled *A. brevis* flower-visiting arthropods according to the timed-observations method [42]. Each sampling session involved two researchers watching a single *A. brevis* cushion for one hour and collecting all flower-visiting arthropods (i.e., individuals touching at least one flower of the cushion). The

researchers were crouched at opposite sides of the cushion, 50 cm apart, wearing grey clothes. This position both ensured a clear view of the flowers and allowed a prompt intervention, while minimizing disturbances for arthropods and avoiding restriction of their access to the flowers. We collected arthropods by mouth aspirator or, for flying insects, by placing a 50-mL tube with the opening downward over the individual and then tapping the tube with the insect inside. Equipment for net sampling was also available, but we did not have to use it since we were able to capture all the flower-visiting arthropods by using the aforementioned two methods. We placed each sampled arthropod in a 1.5-mL tube with 70% ethanol, immediately after the capture. As focal plants, we selected *A. brevis* cushions bearing at least five flowers at anthesis (mean ± standard error of the mean (SEM): 15.8 ± 4.3 flowers per cushion, ranging from 5 to 30 flowers). During each sampling day, two different pairs of researchers observed two cushions at the same time. We conducted sampling sessions between 10:00 and 18:00, but not under adverse circumstances, e.g., during rainfall (Table 1). Overall, during the three sampling days, we conducted 18 sampling sessions involving six different plants (Table 1).

Table 1. Time frame and effort of manual sampling. Day of sampling, time window, plant identity, the number of flowers at anthesis present on the cushion, and the number of samplings performed are reported.

Manual Sampling				
Day	**Time Window**	**Plant**	**N Flowers at Anthesis**	**N Sampling Sessions**
8 June 2019	10:00–11:00	M1	30	4
	12:00–13:00			
	14:00–15:00	M2	28	4
	15:30–16:30			
15 June 2019	15:00–16:00	M3	5	3
	16:00–17:00	M4	10	3
	17:00–18:00			
16 June 2019	10:00–11:00	M5	8	2
	12:00–13:00	M6	14	2

2.4. Palynological Analyses

We performed quantitative palynological analyses of pollen grains found on sampled arthropods to assess which taxa can carry *A. brevis* pollen.

We vortexed each tube containing an ethanol-preserved specimen three times for 30 s to remove pollen grains from the arthropod body and to suspend pollen in the ethanol. Then, we removed the specimen from the tube and carefully observed it under a stereomicroscope to ensure that no pollen grains remained attached to the arthropod body. We repeated this process until no pollen grains were found on any specimen. Then, we placed the tubes containing the suspended pollen in a thermoblock at 65 °C to facilitate ethanol evaporation and concentrate pollen, while we shipped arthropods to expert taxonomists for morphological identification (Table S1).

We subjected the pollen samples to acetolysis [56], to make identification easier and more accurate. We poured 1 mL of the acetolysis mixture (acetic anhydride:sulfuric acid solution 9:1) into each tube containing pollen samples, we heated samples at 100 °C for 10 min and then placed in cold water to stop the acetolysis process. Subsequently, we centrifuged samples at 13,000× *g* for 20 min at 4 °C. We discarded supernatants and resuspended each pellet in distilled water. We repeated the last three steps (centrifugation, supernatant discarding, and pellet resuspension) until the supernatant was completely clean. Finally, after the last centrifugation cycle and removing the supernatant, we resuspended the precipitated pollen in 250 µL of a glycerol and distilled water mixture (1:1 v:v) and mounted it on microscope slides.

Moreover, during the field work, we collected in ethanol pollen samples from all plant species in flower within a radius of 500 m and within a difference in altitude of +/−200 m from the study site, in order to create a reference pollen library. We subjected these pollen samples to the same protocol described above, obtaining a library including 19 species (Table S2).

We observed pollen samples found on arthropods under an optical microscope (Leica DMRB) and identified pollen grains belonging to *A. brevis* with the support of the pollen reference library and pollen guides [57–59].

2.5. Video Observations

We video recorded the activity of flower-visiting arthropods on *A. brevis* cushions during the same three days of the manual sampling (Table 2). During video observations, we simultaneously recorded arthropod activity on three *A. brevis* cushions by means of three cameras (Olympus Tough TG-4, Olympus Tough TG-5, and Olympus OM-D E-M5 equipped with Olympus M.Zuiko Digital ED 12–50 mm f/3.5–6.3 EZ), ensuring the same video quality in macro-mode. Each video session lasted 1 h and included three 15-min videos per cushion. We selected this timing so as to have 5 min between each consecutive video to check camera function and change the batteries. We mounted each camera on a small tripod (h = 40 cm) placed 40 cm from the focal cushion. At this distance we could obtain high-definition videos, while minimizing the disturbance for arthropods. During video recordings, researchers were 10 m away from the cameras and intervened only to check cameras or stop the ongoing observation and start a new one. As focal plants, we selected *A. brevis* cushions bearing at least five flowers at anthesis (mean ± SEM: 15.4 ± 3.1 flowers per cushion, ranging from 5 to 29 flowers). In total, we recorded 123 videos on nine different *A. brevis* plants.

Table 2. Time frame and effort of video observations. Day of sampling, time window, plant identity, the number of flowers at anthesis present on the cushion, and the number of samplings performed are reported. The camera used for video recording is also indicated.

Video Observations					
Day	**Time Window**	**Plant**	**N Flowers at Anthesis**	**N Videos**	**Camera**
8 June 2019	10:00–18:00	V1	14	23	Olympus Tough TG-5
		V2	24	23	Olympus Tough TG-4
		V3	29	20	Olympus OM-D E-M5
15 June 2019	15:00–18:00	V4	8	9	Olympus Tough TG-5
		V5	11	9	Olympus Tough TG-4
		V6	14	9	Olympus OM-D E-M5
16 June 2019	10:00–13:00	V7	28	10	Olympus Tough TG-5
		V8	5	10	Olympus Tough TG-4
		V9	6	10	Olympus OM-D E-M5

2.6. Video Analysis and Behavioral Observations

We conducted video analysis using the software BORIS (version 7.7.3). BORIS is an open-source event-logging software, with which an ethogram of displays of interest can be built and the behavior of multiple subjects tracked on the basis of the codified displays [60]. The same researcher analyzed all the videos, observing each video multiple times to ensure replicability of display evaluation and to avoid missing observations. As for the manual sampling, for the video observations, too, we considered as flower-visiting arthropods the individuals touching at least one flower per cushion. Flower-visiting arthropod activity was monitored from the arrival on the first flower until the visitor left the field of view. As it was not possible to assign individual identification to visiting arthropods, we considered every individual entering the video as a new subject. Thus, during videos, we determined the number of observations of different subjects for each taxon, rather than the actual number of individuals, as the possibility that the same individual entered the video multiple times could not be excluded. The term "taxa" defined the lowest taxonomic levels that could be distinguished with certainty in all videos, and "undetermined" was associated with those arthropods that could not be identified with certainty (i.e., animals smaller than 1 mm, Figure S1). For most of the arthropods the lowest identifiable category was the order, while in some cases taxon was distinguishable at the suborder or superfamily level.

To infer the type of interaction existing among arthropods and *A. brevis*, we quantified the presence of flower-visiting arthropods (i.e., the number of subjects per taxon) and, for each subject, we recorded the

occurrence of every entrance event inside the corolla tube of the flowers (hereafter "entrance"), a behavior that increases the probability of pollination. Only when arthropods entered the corolla tube or inserted their head or mouthparts for at least 1 s we did consider the behavior as "entrance". On the basis of these data, we calculated two different dependent variables describing arthropod activity on an *A. brevis* cushion: the percentage of entering subjects among taxa during the video (calculated as the ratio of subjects performing at least one entrance over the total subjects observed), and the percentage of flowers entered per plant among taxa (the number of entered flowers by subjects over the number of flowers at anthesis on the cushion).

2.7. Data Analysis

We assessed behavioral differences among arthropods using ANOVA. We built separate models using the percentage of entering subjects or the percentage of flowers entered as a dependent variable and taxa as a fixed factor. We excluded from the analyses the taxa with an insufficient number of observations (i.e., Acari, Araneae, Hemiptera, Diptera Nematocera, and Lepidoptera—Figure 2A, Table S3). We used Tukey's post hoc test to perform pairwise comparisons among different taxa. Despite its likely taxonomic heterogeneity, the "undetermined" group was also considered in this analysis to assess whether taxa other than those identified are potentially involved in pollination.

To compare the number of taxa observed with the two methods (i.e., manual sampling and video observations), we built taxa accumulation curves for both approaches by plotting the cumulative number of sampled taxa (*y*-axis) against the time of sampling (*x*-axis) for manual sampling and observed taxa vs. monitoring time for video observations. We built these curves on the basis of taxa identified during videos, as they constituted the lowest taxonomic category common to both approaches. However, we excluded animals for which identification was unreliable (undetermined group) from the video curve (Figure S1). Conversely, for the sampling curve we included an additional taxon (Collembola), as it was uniquely found during manual sampling. Because during the first day of field work, the Olympus OM-D E-M5 failed to record some videos we excluded the observations made with that camera from this analysis to ensure comparability between the two approaches. Moreover, since on the first day of field work the manual sampling was suspended at 16:30, we did not consider the videos recorded after this time for the accumulation curve. As a result, we built the accumulation curves by merging two simultaneous sessions, both for manual sampling and video observations. Overall, for both approaches, the cumulative time effort was 9 h of sampling/observation on 6 different cushions.

The number of flowers at anthesis is known to influence plant attractiveness for pollinators [61–64]; therefore, the mean number of flowers between plants selected for manual sampling and video observations were compared. We did not find any significant difference in the number of flowers at anthesis between the cushions chosen for the two approaches, neither when considering all plants examined (unpaired *t*-test, $p = 0.943$) nor when comparing plants used for accumulation curves only (unpaired *t*-test, $p = 0.887$).

We conducted all statistical analyses in R environment (R version 3.6.0).

3. Results

By manual sampling, we collected a total of 12 flower-visiting arthropod individuals, capturing specimens in 39% of sessions, with a mean ± SEM of 0.67 ± 0.23 sampled specimens per session. All the arthropods were identified to family level, eight (67%) to genus level, and five (42%) to species level. The collected specimens belonged to 7 different orders (Araneae, Entomobryomorpha, Poduromorpha, Thysanoptera, Hemiptera, Hymenoptera, and Diptera) and 11 families (Table 3). All the sampled Diptera belonged to Brachycera (Drosophilidae, Sphaeroceridae, and Phoridae) and the sampled Hymenoptera belonged to Apoidea (Halictidae).

Table 3. Flower-visiting arthropods collected by manual sampling. Blank lines mean that no flower-visiting arthropods were collected. "na" means that taxonomic identification was not achieved. Nomenclature is according to Fauna Europaea [65]. * The *Lasioglossum* specimen is a female belonging to the subgenus *Dialictus*.

Day	Time Window	Plant	Class	Order	Family	Genus	Species
8 June 2019	10:00–11:00	M1					
		M2	Insecta	Hemiptera	Aphididae	*Cinara*	na
			Insecta	Diptera	Sphaeroceridae	*Leptocera*	*caenosa*
	12:00–13:00	M1	Entognatha	Entomobryomorpha (Collembola)	Entomobryidae	*Orchesella*	*arcuata*
			Insecta	Diptera	Drosophilidae	*Scaptomyza*	*pallida*
			Entognatha	Poduromorpha (Collembola)	Tullbergiidae	na	na
		M2	Insecta	Diptera	Pheridae	*Megaselia*	na
			Arachnida	Araneae	Linyphiidae	na	na
	14:00–15:00	M1	Insecta	Hymenoptera	Eulophidae	na	na
			Arachnida	Hemiptera	Aphididae	*Rhopalosiphum*	*maidis*
		M2					
	15:30–16:30	M1	Entognatha	Poduromorpha (Collembola)	Onychiuridae	na	na
		M2					
15 June 2019	15:00–16:00	M3					
		M4					
	16:00–17:00	M3					
		M4					
	17:00–18:00	M3					
		M4					
16 June 2019	10:00–11:00	M5	Insecta	Thysanoptera	Thripidae	*Thrips*	*vulgatissimus*
		M6					
	12:00–13:00	M5					
		M6	Insecta	Hymenoptera	Halictidae	*Lasioglossum* *	na

Although we observed pollen on all collected taxa, we detected *A. brevis* pollen only on Halictidae (Hymenoptera). The pollen of *A. brevis* could be clearly distinguished. It is radially symmetrical, isopolar, tricolporate, and prolate. The colpi are long and narrow with a distinct margin. The endoapertures are circular or slightly irregular. The ornamentation of the bexine is mainly micro-reticulated and the number of perforations at the apocolpium is reduced.

Regarding video observations, we observed flower-visiting arthropods in 64 videos (52% of the recorded videos). In total, we observed 484 subjects, belonging to at least 7 different orders (Figure 2A, Table S3): Thysanoptera, Hemiptera, Hymenoptera, Diptera, Lepidoptera, Araneae, and at least one order belonging to the subclass Acari. Moreover, according to video observations we could distinguish between different Diptera (suborder level: Brachycera vs. Nematocera) and Hymenoptera (superfamily Apoidea vs. other Hymenoptera). All non-Apoidea Hymenoptera observed were winged insects. In total 24 subjects (5% of the total observations) could not be identified with certainty and therefore we classified them as "undetermined" (Figure S1). Among the subjects observed, the vast majority was represented by Thripidae (78% of all observations, Figure 2A). However, their presence was highly variable among different cushions, with two of them hosting most of the subjects observed for this taxon (Table S3). Apart from Thripidae, the most frequent taxa were Hymenoptera (N = 44; 17 Apoidea and 27 other Hymenoptera) and Diptera (N = 28; 26 Brachycera and 2 Nematocera), accounting, respectively, for 9% and 6% of the subjects observed (Figure 2A). Lepidoptera, Hemiptera, Araneae, and Acari visited flowers in fewer than four cases (Figure 2A); therefore, these taxa, as well as Diptera Nematocera, were excluded from behavioral analyses. However, apart from Lepidoptera, we never observed these taxa entering *A. brevis* flowers (Table S4, Table S5).

Both percentage of entering subjects per video and percentage of flowers entered per plant varied significantly among taxa (ANOVA, respectively, $F_4 = 7.37$, p value < 0.001; $F_4 = 41.75$, p value < 0.001). In particular, post hoc pairwise comparisons revealed that the percentage of visiting subjects was higher in Hymenoptera Apoidea than in Thripidae and in both Hymenoptera Apoidea and Diptera Brachycera than in non-Apoidea Hymenoptera; moreover, when compared to the undetermined group, the percentage of visiting subjects was higher in all considered taxa, except for non-Apoidea Hymenoptera (Figure 2B, Table 4). We did not find any significant difference between other taxa from the paired comparisons considered (Figure 2B, Table 4).

The number of flowers entered per plant was significantly higher in Hymenoptera Apoidea or Diptera Brachycera than in Thripidae, non-Apoidea Hymenoptera, and the undetermined group (Figure 2C, Table 4). In contrast, we found no significant difference in the percentage of flowers entered per plant between Thripidae and the undetermined group, non-Apoidea and the undetermined group, or Thripidae and non-Apoidea (Figure 2C, Table 4).

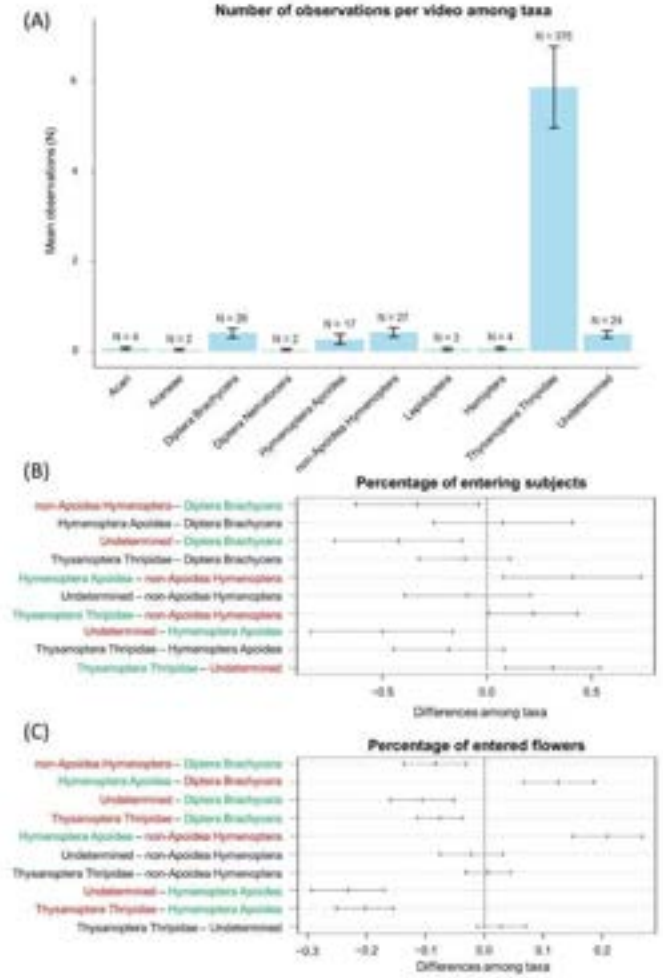


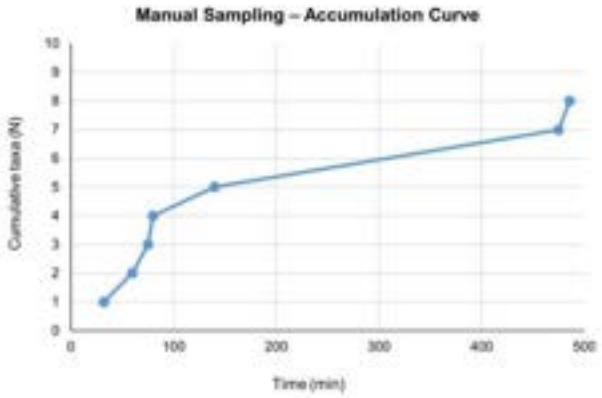
Figure 2. Results of video sampling (**A**) and behavioral analysis (**B**,**C**). Barplots (**A**) show the mean number of subjects observed per video for each taxon (the cumulative number of subjects observed is indicated above each bar). Error bars indicate standard error of the mean. Figure (**B**,**C**) show results of post hoc pairwise comparisons (Tukey's test) for the percentage of entering subjects per video and the percentage of flowers entered per plant, respectively. Segments represent confidence intervals on the differences between the means for behavioral parameters of taxa couples. Segments crossing the central vertical line indicate nonsignificant differences, while noncrossing segments stands for significant ones. Left-shifted segments indicate reduction in the mean value of the behavioral parameter in the first element of taxa couple, whereas right-shifted segments represent an increase in the mean in the first taxon compared to the second one. Taxa pairs with no significant differences in the mean value of the behavioral parameter are reported in black, while for significant differences, green stands for taxa showing a relative increase in the behavioral parameter and red stands for those showing a decrease.

Table 4. Difference in behavioral parameters among taxa visiting *A. ivensis* flowers. Results of post hoc pairwise comparison (Tukey's test) for all possible taxa pairs are reported. Taxa with an insufficient number of video observations were not included in these analyses (see Materials and Methods). For both the percentage of entering subjects per video and percentage of flowers entered per plant, the significance value and difference in mean values for all pairwise comparison are reported. Significant differences are in bold.

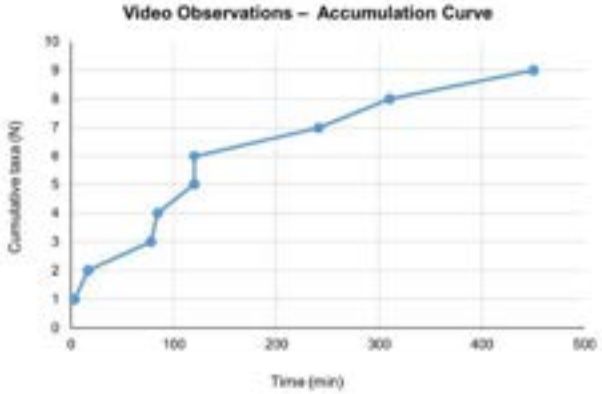
Taxa	Percentage of Entering Subjects		Percentage of Flowers Entered	
	p Value	Difference	*p* Value	Difference
Other Hymenoptera – Diptera Brachycera	**0.018**	**−0.33**	**<0.001**	**−0.08**
Hymenoptera Apoidea – Diptera Brachycera	0.970	0.08	**<0.001**	**0.13**
Undetermined – Diptera Brachycera	**0.001**	**−0.42**	**<0.001**	**−0.11**
Thripidae – Diptera Brachycera	0.658	−0.11	**<0.001**	**−0.08**
Hymenoptera Apoidea – Other Hymenoptera	**0.007**	**0.41**	**<0.001**	**0.21**
Undetermined – Other Hymenoptera	0.916	−0.09	0.781	−0.02
Thripidae – Other Hymenoptera	**0.034**	**0.22**	0.992	0.01
Undetermined – Hymenoptera Apoidea	**0.001**	**−0.50**	**<0.001**	**−0.23**
Thripidae – Hymenoptera Apoidea	0.318	−0.18	**<0.001**	**−0.20**
Thripidae – Undetermined	**0.001**	**0.32**	0.290	0.03

The accumulation curves built for manual sampling (Figure 3A) and for video observations (Figure 3B) showed a similar trend, with a decrease in the rate of acquiring new taxa at about 100 min, and a maximum accumulation (7 for manual sampling and 9 for video observations) between 400 and 500 min.

(A)



(B)



(C)

Manual Sampling			Video Observations		
Time (min)	Taxa (n)	Sampled taxon	Time (min)	Taxa (n)	Observed taxon
32	1	Hemiptera	3	1	Thysanoptera Thripidae
60	2	Diptera Brachycera	17	2	Hemiptera
75	3	Collembola	78	3	Diptera Brachycera
80	4	Araneae	85	4	Other Hymenoptera
140	5	Other Hymenoptera	120	5	Araneae
475	6	Thysanoptera Thripidae	120	6	Diptera Nematocera
486	7	Hymenoptera Apoidea	241	7	Acari
			311	8	Lepidoptera
			450	9	Hymenoptera Apoidea

Figure 3. Accumulation curves for manual sampling (**A**) and video observations (**B**). Cumulative number of sampled (**A**) or observed (**B**) taxa on *y*-axis, time in minutes of sampling (**A**) or monitoring (**B**) on *x*-axis. Timing and identities of taxa are reported in (**C**).

4. Discussion

Our study offers new insights into the relationships between a vulnerable alpine flowering plant and the flower-visiting arthropod community and provides evidence of the importance of an integrated approach when investigating plant–arthropod interactions in the field, in particular in mountain environments.

First, we compared the information about the diversity of arthropods visiting *A. brevis* provided by two approaches, i.e., traditional manual sampling and video observations. Although there was a pronounced difference in the number of arthropods observed—possibly, at least in part, because of multiple observations of the same individuals during videos—the two approaches yielded accumulation curves with a similar trend, revealing a similar arthropod diversity at order level. Thus, the two methods proved effective and comparable in describing the flower-visitors' diversity at a high taxonomic level. However, when looking at a finer scale, differences between the information provided by the two approaches were evident.

On the one hand, by manual sampling we could identify the flower-visiting arthropods at a taxonomic level that could not be achieved with video observations. For instance, we sampled a specimen belonging to Hymenoptera Apoidea, the best globally known and studied pollinators. It was possible to identify this individual as a Halictidae of the genus *Lasioglossum* Curtis, 1833, subgenus *Dialictus* Robertson, 1902. We were not able to determine the species of the specimen, however, because the species belonging to this subgenus are notoriously difficult to identify.

Furthermore, the barcoding approach can hardly be applied: *Dialictus* is regarded as a "nightmare taxon" due to the absence of a well-defined barcode gap [66]. However, the level of information gained is far greater than that achievable with video observation, allowing us to identify the subgenus and sex of the specimen. Moreover, by manual sampling we were able to determine family and sometimes species of very small arthropods such as Collembola, which cannot be identified by video observations. The finer taxonomic resolution of sampled arthropods obtained with this approach made it possible to establish the basis for characterizing the flower-visiting community in depth, while the taxonomic level that could be achieved by video observation provides strongly simplified ecological information, not considering the large variety existing within the identified taxa.

On the other hand, it is important to note that the video observation approach may be less of a disturbance for visiting arthropods. Although we could not quantify visiting arthropods by employing this approach, the large number of subjects observed in videos (average of 15.74 per hour per plant) compared to individuals collected with manual sampling (average of 0.67 per hour per plant), and the simultaneous observations of multiple subjects during videos, never reached during manual samplings, indicate that video recording may have a minor impact on arthropod visitors compared to manual sampling. Moreover, with manual sampling we did not collect any Diptera Nematocera or Lepidoptera; however, these taxa were observed in videos, albeit with a low incidence. In conclusion, the video approach could be useful to spot even rare or elusive taxa. This could be particularly convenient in mountain environments, where there are only few sampling occasions and where, especially in early season, some taxa might be present but numerically not very abundant. Therefore, the integrated use of video observations and manual sampling can provide a complete description of the community of flower-visiting arthropods, avoiding loss of significant information.

Another important aspect that deserves to be highlighted is the evidence that the two approaches provided different and relevant insights about arthropods' relationships with *A. brevis* flowers. In particular, we focused on identifying possible pollinators according to their behavior since pollination can be considered as one of the most critical biotic interactions in the alpine ecosystem, where most plants depend on insects for pollination, forming complex networks of dependencies among species [31]. One of the main advantages of manual sampling is the possibility to identify arthropod taxa carrying the pollen of the species of interest since, obviously, not all flower-visitors are pollinators. Nevertheless, many studies about pollination networks are based on observations of flower visits and few have tested whether the flower visitors actually carry pollen [67,68]. In the present study, we demonstrated that the pollen of *A. brevis* can be carried by Hymenoptera Apoidea. This result suggests that this taxon can play a role in the pollination of this species. However, due to the relatively small dimension of our sample, we cannot exclude that other taxa may be involved in the pollination. Conversely, video observation allowed us to characterize the behavioral ecology of the flower-visiting arthropods, monitoring their activity and time budget on flowers. Video analysis showed Hymenoptera Apoidea having a high percentage of entering subjects and the highest percentage of entered flowers, a further indication that these insects are good candidates in mediating *A. brevis* pollination. Similar to Hymenoptera Apoidea, Diptera Brachycera also showed high percentages of entering subjects and entered flowers. Indeed, these insects are alleged to play a major role as pollinators in the alpine environment [31,32,39], and according to their behavior we can hypothesize a potential role of Diptera Brachycera even in the absence of *A. brevis* pollen on the few individuals we sampled. Arthropods belonging to other taxa, such as arachnids and non-Apoidea Hymenoptera, entered flowers only occasionally, a behavior that, as expected for these taxa, makes them unlikely as pollinators. Thrips showed a relatively high percentage of entering subjects, similar to Brachycera dipterans and Apoidea hymenopterans, but with a significantly lower number of entered flowers. This behavior seems to suggest a certain fidelity to single flowers, which can potentially reflect territoriality, also supported by some apparently aggressive interactions between individuals of this taxon observed during video analyses. Territoriality and fighting were already observed in this taxon, presumably associated with mating, food, and guarding eggs [69], but this topic is still largely unexplored: video observations could be applied as a tool to shed light on these

behaviors. Finally, some taxa were rarely observed approaching *A. brevis* flowers during videos and we could report their activity on this plant only anecdotally. Among them, lepidopterans always entered flowers multiple times, and thus they can potentially play a relevant role for *A. brevis* pollination. However, we cannot discriminate whether this limited occurrence is due to their behavior, mainly exploiting other flowering plants, for instance, or if it might depend on the low abundance in the study area and/or on a reduced overlap between *A. brevis* flowering and their activity.

In conclusion, although further research is needed to better elucidate the potential role and contribution of rarely sampled and observed taxa as pollinators of *A. brevis*; we could maximize data collection efficiency in a limited time frame by integrating the two approaches proposed in this study.

5. Conclusions

Despite their pros and cons, both investigated approaches, i.e., manual sampling and video observations, have proven to be suitable and useful for studying plant–arthropod interactions in a mountain environment, providing different but complementary data. Even in the restricted sampling period imposed by the very brief *A. brevis* flowering period and by mountain environmental conditions, we could both accurately identify arthropods visiting *A. brevis* flowers and also characterize their behavior by combining our data, revealing variability among taxa and allowing us to hypothesize their ecological roles. The integration of the two methods results in a synergistic approach that represents a powerful instrument to unravel relationships between flowering plants and associated fauna and can provide crucial knowledge for the conservation of vulnerable environments such as mountain ecosystems.

Supplementary Materials: The following are available online at www.mdpi.com/2075-4450/11/12/881/s1, Table S1: Taxonomists who identified the sampled arthropods, Table S2: List of plant species in flower present within a radius of 500 m and within a difference in altitude of +/−200 m from the study site, Table S3: Distribution among taxa of the total number of observed subjects on each *A. brevis* plant during video observations, Table S4: Variation in behavioral parameters among taxa visiting *A. brevis* flowers, Table S5: Variation in flower entrance among taxa visiting *A. brevis* flowers, Figure S1: Undetermined arthropods, whose taxon was not clearly identifiable from video recordings.

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Table S1. Taxonomists who identified the sampled arthropods.

Taxon	Taxonomist (affiliation)
Araneae	Paolo Pantini (Museo Civico di Scienze Naturali Enrico Caffi di Bergamo – Sezione di Zoologia degli Invertebrati, Italy)
Collembola	Barbara Valle (Università degli Studi di Milano – Dipartimento di Bioscienze, Italy)
Thysanoptera	Barbara Conti (Università di Pisa – Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Italy)
Hemiptera	Alice Casiraghi (Centro Mixto Universidad de Valencia – Instituto de Biología Integrativa de Sistemas, Spain; Universitat de Barcelona – Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Spain). Nicolas Pérez Hidalgo (Centro Mixto Universidad de Valencia – Instituto de Biología Integrativa de Sistemas, Spain; Museo de Ciencias Naturales de Barcelona – Departamento de Artrópodos, Spain)
Hymenoptera	Fabrizio Rigato (Museo Civico di Storia Naturale di Milano – Sezione di Entomologia, Italy)
Hymenoptera, Apoidea	Andree Cappellari (Università degli Studi di Padova – Dipartimento di Agronomia, Animali, Alimenti, Risorse naturali e Ambiente, Italy)
Diptera	Daniele Avesani (Museo di Storia Naturale di Verona – Sezione di Zoologia, Italy)
Diptera, Drosophilidae	Gerhard Bächli (Universität Zürich – Institut für Evolutionsbiologie und Umweltwissenschaften, Switzerland)
Diptera, Sphaeroceridae	Jindřich Roháček (Muzeum Śląskie – Entomologické oddělení, Czech Republic)
Diptera, Phoridae	Henry Disney (University of Cambridge – Department of Zoology, United Kingdom)

Table S2. List of plant species in flower present within a radius of 500 m and within a difference in altitude of +/- 200 m from the study site. These species were sampled to create a reference pollen library.

Species	Family
Androsace brevis	Primulaceae
Androsace vandellii	Primulaceae
Anthyllis alpicola	Fabaceae
Cardamine hirsuta	Brassicaceae
Carex curvula	Cyperaceae
Daphne striata	Thymelaeaceae
Draba aizoides	Brassicaceae
Erica carnea	Ericaceae
Geum montanum	Rosaceae
Lotus corniculatus	Fabaceae
Myosotis alpestris	Boraginaceae
Polygala chamaebuxus	Polygalaceae
Polygala vulgaris	Polygalaceae
Primula hirsuta	Primulaceae
Pulsatilla alpina	Ranunculaceae
Ranunculus sp.	Ranunculaceae
Soldanella alpina	Primulaceae
Viola biflora	Violaceae
Viola sp.	Violaceae

Table S3. Distribution among taxa of the total number of observed subjects on each *A. brevis* plant during video observations. The number of videos with flower-visiting arthropods per plant is also showed.

Plant	Day	N videos with active arthropods	Total observations							
			Diptera	Hymenoptera	Lepidoptera	Thripidae	Hemiptera	Araneae	Acari	Undetermined
V1	8 June 2019	19	6	8	0	66	1	1	1	2
V2	8 June 2019	23	19	16	2	305	2	0	0	12
V3	8 June 2019	2	1	0	1	0	0	0	0	0
V4	15 June 2019	3	1	0	0	0	0	0	3	2
V5	15 June 2019	5	1	3	0	0	1	0	0	2
V6	15 June 2019	1	0	0	0	0	0	0	0	1
V7	16 June 2019	4	0	10	0	2	0	1	0	0
V8	16 June 2019	5	0	5	0	2	0	0	0	5
V9	16 June 2019	2	0	2	0	0	0	0	0	0
TOTAL		64	28	44	3	375	4	2	4	24

Table S4. Variation in behavioral parameters among taxa visiting *A. brevis* flowers. The total number of subjects observed together with mean value and standard error of the mean calculated for the percentage of entering subjects per video and for the percentage of flowers entered per plant are reported.

Taxa	Total observed subjects	Percentage of entering subjects		Percentage of flowers entered	
		mean	SEM	mean	SEM
Diptera Brachycera	26	0.92	0.05	0.14	0.02
Diptera Nematocera	2	0	0	0	0
Hymenoptera Apoidea	17	1.00	0	0.26	0.06
Other Hymenoptera	27	0.59	0.10	0.05	0.01
Lepidoptera	3	1.00	0	0.24	0.15
Thripidae	375	0.82	0.02	0.06	0.00
Heniptera	4	0	0	0	0
Araneae	2	0	0	0	0
Acari	4	0	0	0	0
Undetermined	24	0.50	0.10	0.03	0.01

Table S5. Variation in flower entrance among taxa visiting *A. brevis* flowers. For all taxa the total number of subjects observed is reported along with the total number of flowers entered during all observations and the number of entrances per subject (mean ± SEM).

Taxa	Total observed subjects	Number of flowers entered		
		total	mean	SEM
Diptera Brachycera	26	70	2.69	0.34
Diptera Nematocera	2	0	0	0
Hymenoptera Apoidea	17	53	3.12	0.76
Other Hymenoptera	27	31	1.15	0.26
Lepidoptera	3	18	6.00	3.51
Thripidae	375	496	1.32	0.06
Hemiptera	4	0	0	0
Araneae	2	0	0	0
Acari	4	0	0	0
Undetermined	24	12	0.50	0.10

Figure S1. Undetermined arthropods, whose taxon was not clearly identifiable from video recordings. The figure represents the maximum-sized individual (inside the red circle) among the observed undetermined arthropods. These arthropods were very small (less than 1mm) and often only detectable when moving (23 out 24 subjects).

Chapter 2.3

The early season community of flower-visiting arthropods in high-altitude alpine environment

insects

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Article

The Early Season Community of Flower-Visiting Arthropods in a High-Altitude Alpine Environment

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Simple Summary: The knowledge about the flower-visiting arthropods in high-altitude environments is limited, in particular about those occurring on early flowering plants. We characterised the flower visitor community of an early flowering high-altitude Alpine species: *Androsace brevis*, a vulnerable endemic plant belonging to the Primulaceae family, which grows in the Alps above 2000 m asl and flowers for a very short period immediately after snowmelt. In addition, we tested the effect of temperature, wind speed, and other variables on flower-visiting arthropod activity. We identified dipterans (in particular, anthomyiid flies) and hymenopterans (in particular, ants and parasitoid wasps) as the main flower visitors. Moreover, we assessed that temperature and time (hour of the day) affect the flower visitors' activity. Our study contributes to defining the composition of high-altitude Alpine flower-visiting arthropod communities and sets the stage for future evaluation of climate change effects on flower-visiting arthropods in high-altitude environments in the early season.

Abstract: In mountain ecosystems, climate change can cause spatiotemporal shifts, impacting the composition of communities and altering fundamental biotic interactions, such as those involving flower-visiting arthropods. On of the main problems in assessing the effects of climate change on arthropods in these environments is the lack of baseline data. In particular, the arthropod communities on early flowering high-altitude plants are poorly investigated, although the early season is a critical moment for possible mismatches. In this study, we characterised the flower-visiting arthropod community on the early flowering high-altitude Alpine plant, *Androsace brevis* (Primulaceae). In addition, we tested the effect of abiotic factors (temperature and wind speed) and other variables (time, i.e., hour of the day, and number of flowers per plant) on the occurrence, abundance, and diversity of this community. *A. brevis* is a vulnerable endemic species growing in the Central Alps above 2000 m asl and flowering for a very short period immediately after snowmelt, thus representing a possible focal plant for arthropods in this particular moment of the season. Diptera and Hymenoptera were the main flower visitors, and three major features of the community emerged: an evident predominance of anthomyiid flies among Diptera, a rare presence of bees, and a relevant share of parasitoid wasps. Temperature and time (hour of the day), but not wind speed and number of flowers per plant, affected the flower visitors' activity. Our study contributes to (1) defining the composition of high-altitude Alpine flower-visiting arthropod communities in the early season, (2) establishing how these communities are affected by environmental variables, and (3) setting the stage for future evaluation of climate change effects on flower-visiting arthropods in high-altitude environments in the early season.

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1. Introduction

Climate change constitutes a key threat to biodiversity [1–5] and can strongly impact mountain ecosystems [6,7], affecting single species but also causing altitudinal and phenological shifts that can alter both the composition of communities and biotic interactions [8–10]. In particular, some recent studies focussed on potential mismatches between flowering plants and flower-visiting arthropods in mountain environments [11–14]. Moreover, climate-change-driven mismatches can impact the interactions among arthropods, such as those between host and parasitoid or prey and predator [15], which frequently occur on flowers [16–21], but to the best of our knowledge, no studies investigated potential mismatches among flower-visiting arthropods in mountain ecosystems. One of the main problems in assessing the effects of climate change on biodiversity is the lack of robust data about species occurrence and diversity [22]. Furthermore, the available information comes mostly from human-altered ecosystems and thus is not useful for drawing conclusions about natural ones [22,23]. Therefore, it is essential to collect precise, current data to build a solid platform of knowledge that can help develop further comparative studies and proper conservation plans in the near future.

The Alps are the highest and longest mountain range that lies entirely in Europe, and they host peculiar biodiversity, comprising several endemic plants and arthropods and threatened species [24–29]. While a significant body of literature deals with plant diversity, species occurrence, and phenology in the Alps (e.g., [30–35]), not many studies have addressed the topic of flower-visiting arthropod communities and abiotic factors impacting their activity in this environment [36–45]. The paucity of information on arthropods is a global issue in studying mountain ecosystems [46,47], although arthropods are the most abundant and diverse group of organisms [48,49] and play crucial roles in terrestrial ecosystem functioning, especially through interactions with plants [50–56]. In addition, many faunistic surveys about flower-visiting arthropods are based on data that were collected without applying standardised and repeatable methods, making it difficult or even impossible to use them for future analyses and evaluations [22,36]. Finally, although environmental variables such as temperature and wind speed can impact flower visitors' activity [57,58], only a few studies reported the micrometeorological conditions occurring during the survey, thus often preventing any evaluation of the context in which the data were collected. This lack of information can be particularly problematic in mountain environments where extreme weather changes and unstable conditions can be observed even in the same period of the season. Therefore, there is a need to collect reliable data on arthropods in mountain ecosystems, which will be integral for future studies assessing their response to climate change [59,60], as well as to increase our knowledge of high-altitude flower-visiting arthropods

These considerations prompted us to perform a first assessment of the flower-visiting arthropod community in a high-altitude Alpine environment, using the narrow-endemic plant *Androsace brevis* (Hegetschw.) Cesati (Primulaceae) as a model species. Climate warming could represent a serious threat for this species, as it is almost impossible to shift its range upward since the plant already lives on mountain ridges and tops. Moreover, *A. brevis* shows low competitive ability, and the upward shift of more competitive species from a lower altitude could represent a serious threat [61]. The flowering period of *A. brevis* is very short and occurs in the early season, when snow cover is still present, except for ridges and outcrops, and very few other floral resources are available for arthropods [45]. In this context, *A. brevis* flowers can thus represent a possible focal species for arthropods. Moreover, the early season is particularly interesting because it is a critical moment for pos-

sible mismatches between plants and flower-visiting arthropods [62,63] and phenological shifts in early spring can occur more rapidly than later in the season [64].

Here, we present data collected over four years on *A. brevis* flower-visiting arthropods, together with the micrometeorological conditions that occurred during sampling.

The goal of our study was to shed light on the composition and response to environmental variables of flower-visiting arthropods of an early flowering high-altitude Alpine plant. We performed an omni-comprehensive assessment considering all flower-visiting-arthropods, not only those having a possible role as pollinators and/or interacting with flowers for feeding activity but also those present for other purposes (e.g., for mating, sheltering, basking, or finding a prey) or by chance, highlighting possible biotic interactions in which these arthropods are involved. Our data can be useful to develop a platform of knowledge suitable for future evaluation of the effects of climate change on early season Alpine flower-visiting communities.

2. Materials and Methods

2.1. Study Species

Androsace brevis (Figure 1a) is a narrow-endemic cushion plant that grows above 2000 m asl on rocky ridges and outcrops in a limited area in the southern Alps of northern Italy (Lombardy) and neighbouring Switzerland, in few and scattered populations of limited size. Its conservation status in Italy is vulnerable (VU) according to IUCN criteria [61]. The flowering period is very short, typically lasting about 2 weeks between the end of May and the beginning of June. It occurs where the snow has just melted, while in the immediate vicinity, it is still present in patches (Figure 1b). Each plant carries from a few to about 200 solitary flowers (ca. 4 mm long) with a pink corolla (ca. 8 mm in diameter) and a yellow mouth (ca. 0.9 mm in diameter); flowers are held by 0.5–2 cm erect pedicels [45,61].

2.2. Study Sites

The present study was conducted in the Alpine biogeographical region [65] in the southern Alps of northern Italy. Two sites were selected across the *A. brevis* distribution range: San Jorio Pass—Cima di Cugn (SJP) in the Lepontine Alps (Como, Lombardy) and Mountain Hut 'Cesare Benigni' (BEN) in the Orobic Alps (Bergamo, Lombardy) (Figure 2).

At the first site (SJP), the fieldwork was conducted on an *A. brevis* population located along the ridgeline north of the San Jorio Pass and southwest of Cugn Peak (UTM WGS84—32T E 512338 N 5112905, 2193 m asl), close to the Mountain Huts 'Rifugio San Jorio' and 'Capanna delle Aquile'. The site is characterised by a continental climate without a dry season [66]. In particular, the mean annual temperature is 6.2 °C with a minimum of −1.9 °C in February and a maximum of 16.0 °C in August. The average annual rainfall amounts to about 1800 mm, mostly concentrated in the equinoctial months (extrapolated from data of the climatic stations: 'Cavargna', 1100 m asl, 8.9 km from the study site, observation period 2012–2020; 'Porlezza', 280 m asl, 13.4 km from the study site, observation period 2014–2020; 'Garzeno', 688 m asl, 8.1 km from the study site, observation period 2013–2020).

At the second site (BEN), located within the Orobie Bergamasche Regional Park, the fieldwork was conducted near the Mountain Hut 'Cesare Benigni' (UTM WGS84—32T E 543496 N 5096577, 2222 m asl). The site is characterised by a temperate climate with a humid summer [66]. In particular, the mean annual temperature is 3.3 °C, with a minimum of −4.2 °C in February and a maximum of 11.4 °C in July; the average annual rainfall amounts to about 1800 mm, distributed throughout the year (extrapolated from data of the climatic stations: 'Gerola Alta Pescegallo', 1875 m asl, 1.5 km from the study site, observation period 2012–2020; 'Mezzoldo Passo San Marco', 1824 m asl, 5.5 km from the study site, observation period 2012–2020; 'Valtorta', 982 m asl, 5 km from the study site, observation period 2012–2020).

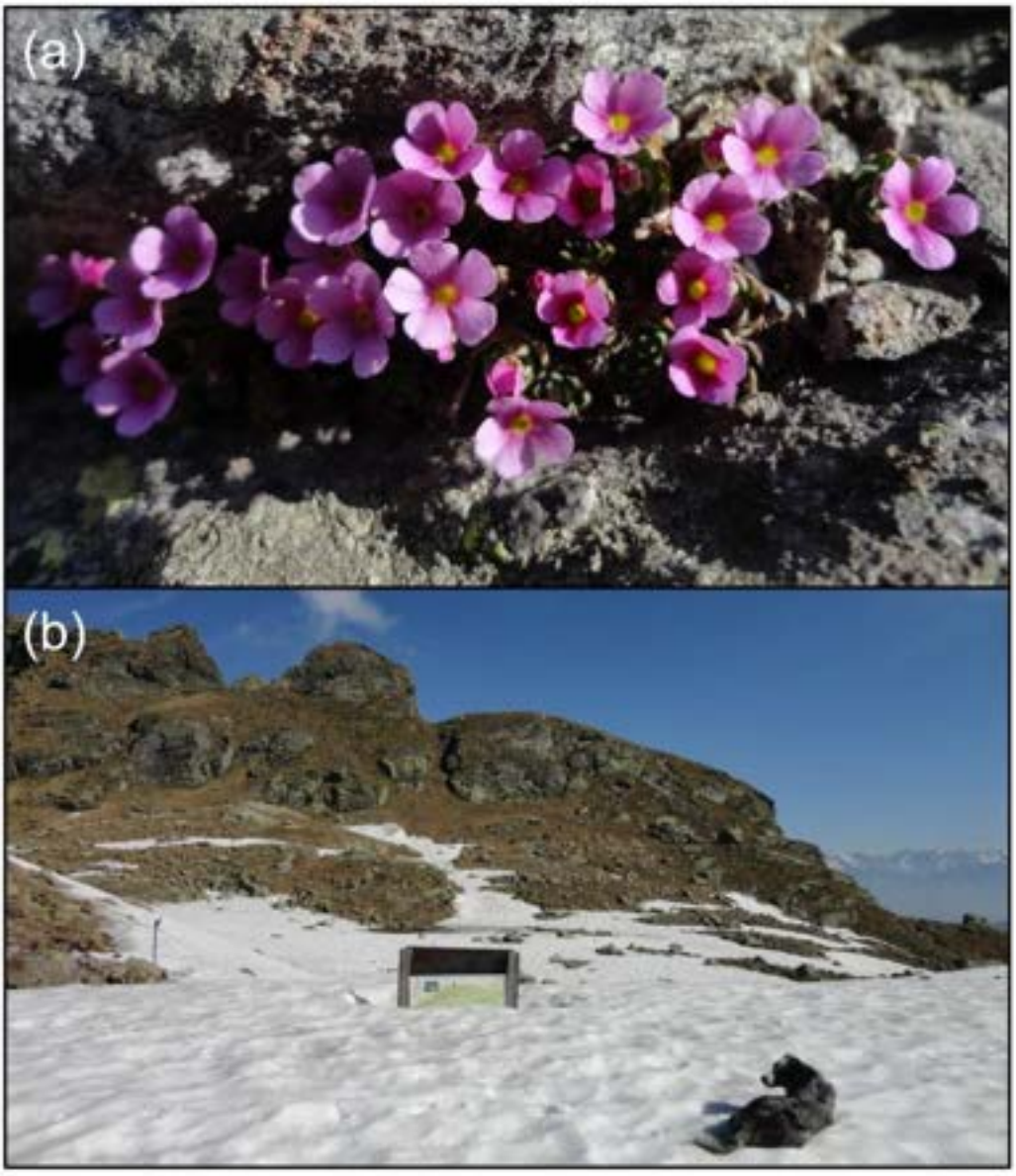

Figure 1. *Androsace brevis* (**a**) and snow cover during its flowering at the Mountain Hut 'Cesare Benigni' in the Orobic Alps (Bergamo, Lombardy) on 31 May 2017 (**b**).

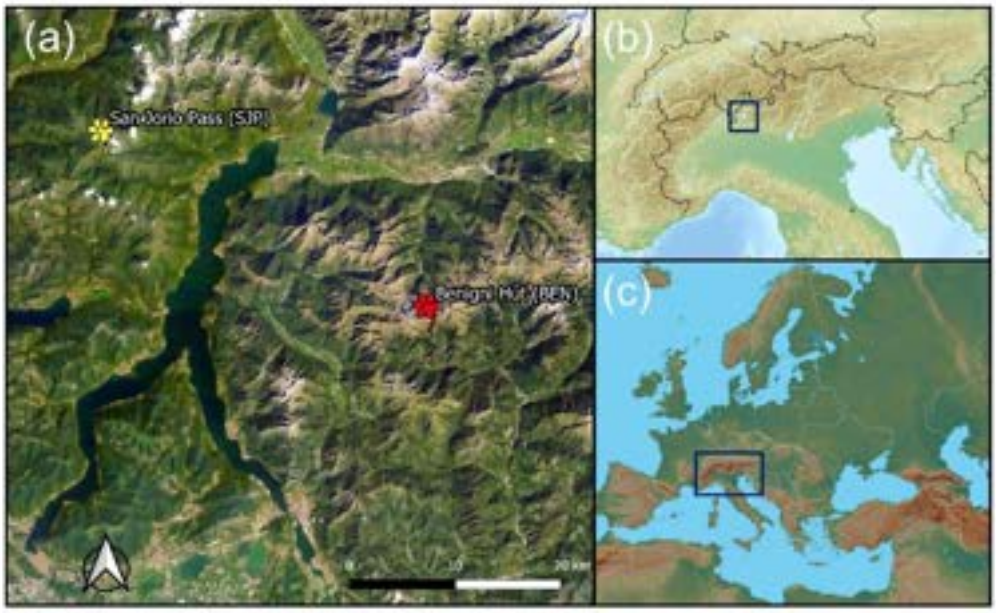

Figure 2. Study sites (**a**) and their location in the Alps (**b**) and in Europe (**c**).

Snow cover usually lasts from October/November to May/June at both sites, although the snow generally melts in SJP about 1 week sooner than in BEN. To evaluate the accessibility of both sites and estimate the full flowering period of *A. brevis*, snow cover was monitored daily by the Mountain Hut 'Cesare Benigni' webcam (available at https://orobiemeteo.com/ (accessed on 1 April 2022)).

The fieldwork was conducted for 2 years at each site (SJP: 2016 and 2019, BEN: 2017 and 2018) during the *A. brevis* flowering period. During the fieldwork period, a maximum of 13 entomophilous plants other than *A. brevis* were flowering at the study sites.

2.3. Sampling of Flower-Visiting Arthropods

For each year and site, two focal plants of *A. brevis*, more than 5 m apart and bringing more than 20 flowers at anthesis each, were randomly selected. On these plants, flower-visiting arthropods were sampled according to the timed-observation method [67], as described in Bonelli et al. [45]. Briefly, two simultaneous sampling sessions, lasting 1 h, were conducted, with two different pairs of operators observing the two focal plants during the same time windows (Table 1). Time windows were interspersed with a break lasting from 30 to 60 min, to reduce the disturbance to flower-visiting arthropods and to ensure the independence of data points obtained from the same plant on the same day. The operators collected in 70% ethanol all flower-visiting arthropods (i.e., individuals touching at least one flower of the plant). The operators were crouched at opposite sides of the plant, 50 cm apart, thus having a clear view of the flowers while minimising disturbances for the flower visitors. Each year, we conducted two days of sampling, with four pairs of sampling sessions per day, for a total of 16 h of timed observations per year, except for 2019 at SJP, when the extreme meteorological conditions (i.e., hailstorm) hampered the fieldwork. In total, we performed 54 sampling sessions, corresponding to 54 h of flower-visiting arthropod sampling by timed observations (Table 1). Achieving these total cumulative hours of timed observations should be considered relevant since the amount of time available for sampling is constrained by multiple factors: the very short flowering

period, occurring when snow cover is still partially present and microclimatic instability is possible, and the remoteness of the sites.

Table 1. Flower-visiting arthropod sampling by timed observations. Year, site, day, time windows, number (N) of sampling sessions, hours (h) of timed observations per year, and number (N) of flowers at anthesis present per plant (letters indicate the identity of the plant) are reported.

Year	Site	Day	Time Windows	N of Sampling Sessions	h of Timed Observations per Year	N of Flowers at Anthesis (per Plant)
2016	SJP	25 May 2016	11.30–12.30 13.30–14.30 15.30–16.30 17.30–18.30	8	16	69 (A); 109 (B)
		26 May 2016	11.30–12.30 13.30–14.30 15.30–16.30 17.30–18.30	8		
2017	BEN	31 May 2017	11.00–12.00 12.30–13.30 14.00–15.00 15.30–16.30	8	16	37 (C); 25 (E)
		1 June 2017	11.00–12.00 12.30–13.30 14.00–15.00 15.30–16.30	8		
2018	BEN	9 June 2018	13.00–14.00 14.30–15.30 16.00–17.00 17.30–18.30	8	16	33 (F); 36 (G)
		10 June 2018	10.15–11.15 11.45–12.45 13.15–14.15 14.45–15.45	8		
2019	SJP	4 June 2019	10.30–11.30 12.00–13.00 15.00–16.00	6	6	20 (H); 25 (I)

During the timed observations, we recorded the air temperature at ground level near the selected plants and the wind speed at 50 cm from the ground using data loggers (Tinytag Plus 2) and thermo-anemometers (LaCrosse Technology EA-3010U), respectively. Moreover, to increase the volume of data on flower-visiting arthropods, free observation sampling was conducted on the same days and sites of timed observation sampling but at least 10 m away from focal plants that were considered for the timed observations. In this case, operators walked freely in the study site, observing many different *A. brevis* plants, and collecting in 70% ethanol all the arthropods they saw on flowers. These data were not considered in any statistical analysis and were only used to provide a broader description of the flower visitors' diversity.

2.4. Identification of Flower-Visiting Arthropods

The ethanol-preserved specimens were shipped for morphological identification to expert taxonomists (Table S1), who were allowed to keep them for private or institutional collections. For insects whose morphological identification was particularly challenging or not possible (Table S2), molecular identification was performed through COI barcoding. The arthropods were rinsed in phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4), and the genomic DNA was extracted using

the CTAB protocol [68], as adapted to insect samples by Bonelli et al. [69]; then, a fragment of the mitochondrial COI gene was amplified. The primer sets used and their references, PCR conditions, and the size of the amplicons are reported in Table S2. A commercial service provider (Eurofins Genomics, Vimodrone, Italy) purified and sequenced the PCR products. All sequences were uploaded to GenBank (Accession numbers are provided in Table S2). The resulting sequences were queried against the GenBank database (NCBI) using the basic local alignment search tool (BLAST) and against the Barcode of Life Data System (BOLD) using the identification engine with a species-level option. Species identity was assigned when the similarity statistic (the number of nucleotide identities between the query and reference) was > 99%.

2.5. Statistical Analyses

Differences in the mean of the micrometeorological data among the four years of sampling were assessed with ANOVA for wind speed and Kruskal–Wallis test for temperature (as for temperature, ANOVA assumptions were not fulfilled: residuals not normally distributed, Shapiro–Wilk test $p < 0.05$). The influence of micrometeorological conditions (i.e., temperature and wind speed, as means of the values recorded during the sampling session) on flower visitors' presence (i.e., presence/absence of sampled specimens during the sampling session), diversity (i.e., the total number of different families sampled during the sampling session) and abundance (i.e., the total number of sampled specimens during the sampling session) was tested using generalised linear models (GLMs) with binomial (for presence) and Poisson (for diversity and abundance) distribution. The time (both linear and quadratic terms) and the number of flowers per plant were included as covariates in the model, while the site was included as a fixed factor. Time was indicated as the hour of the day, expressed in minutes lasting from midnight to the middle of the considered sampling session (e.g., for a sampling session performed in the time window 11.30–12.30, time was indicated in the model as 720 min, that is time in minutes from midnight to the middle of the time window—i.e., 12.00). Each data point corresponds to a single sampling session (Table 1); therefore, all dependent and independent variables were calculated for each sampling session. All continuous independent variables were standardised before analyses with a mean of 0 and a standard deviation of 1. The same analyses were performed considering both all the flower visitors sampled during the timed observations and only the flying visitors (defined as the arthropods with functional wings); the analyses on flying arthropods were performed to test whether the variables (e.g., wind) affect their activity specifically. The *'glm'* and *'drop1'* functions from the *stats* package [70] were used to perform the analysis and obtain the *p*-value for each independent variable considered, and the *visreg* package [71] was used to generate plots. Before running the models, we calculated Pearson's correlation coefficient between all pairwise combinations of independent variables using the *'cor'* function from the *stats* package [70]. Correlation coefficients were always $<|0.7|$; hence, all the independent variables were kept in the models. Model performances were evaluated through a likelihood ratio test and by calculating the pseudo R^2, performed with the *'anova'* function from the *stats* package [70] and with the *'r2'* function from the *performance* package, respectively [72]. All statistical analyses were performed in an R environment (R version 4.1.0).

3. Results

All sampled specimens were identified to order level, 99% to family, 84% to genus, and 71% to species level (Tables S3 and S4).

During the timed observations, 140 arthropods were sampled, with 2.6 ± 0.3 captures per hour (mean ± SEM), for a total of 9 orders and 33 families (Table 2 and Table S3), with 26 identified species (Table S3).

Table 2. Arthropods sampled by timed observations: number (*N*) of sampled specimens, captures per hour (mean ± SEM) and number of orders and families, reported for each year and site and overall.

	SJP 2016	BEN 2017	BEN 2018	SJP 2019	Total
N of sampled specimens	58	14	35	33	140
Captures per hour (mean ± SEM)	3.6 ± 0.5	0.9 ± 0.3	2.2 ± 0.6	5.5 ± 1.3	2.6 ± 0.3
N of orders	5	6	5	7	9
N of families	17	9	9	17	33

The two most abundant taxa sampled during timed observations were Diptera (43%) and Hymenoptera (38%), followed by Hemiptera (8%), Thysanoptera (5%), and Coleoptera (3%); other arthropod orders were each represented by about 1% of the total individuals sampled (Figure 3a, Table S3). In particular, Diptera and Hymenoptera represented more than half of the flower-visiting arthropods for all years and sites (Figure 3b).

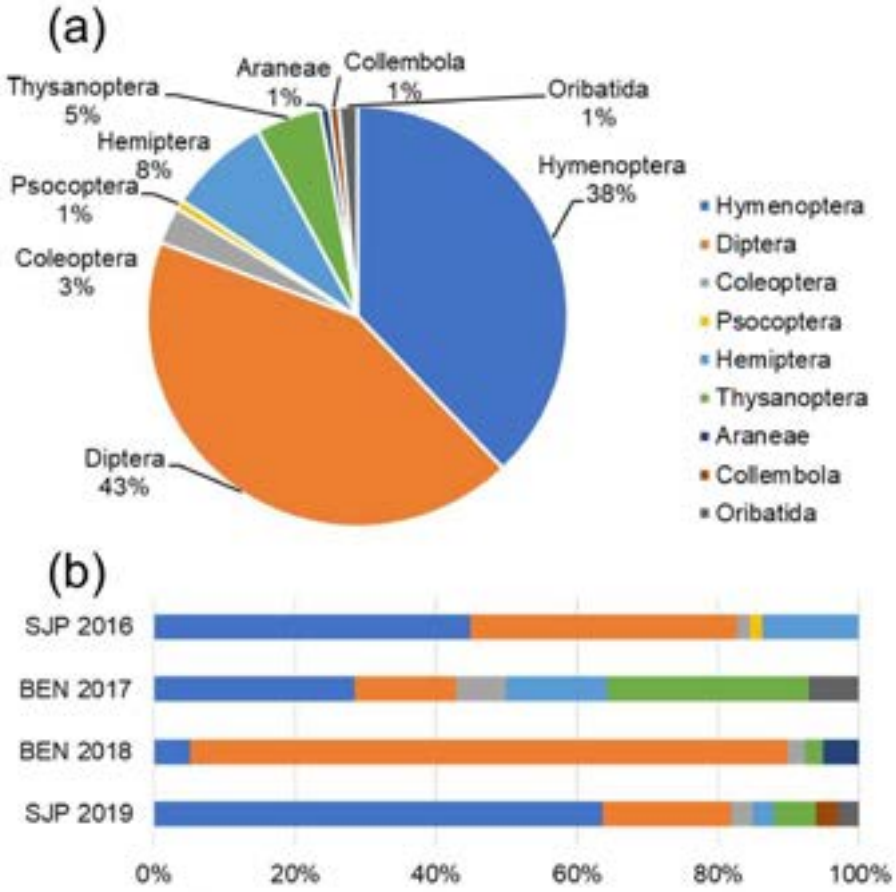

Figure 3. Arthropods sampled by timed observations: overall (**a**) and yearly composition at the two sites (**b**) of the flower-visiting arthropod community to order level.

Among Diptera, 45% of the species belonged to the family Anthomyiidae, followed by Sphaeroceridae (13%), Sciaridae (12%), Chironomidae (10%), and Chloropidae (5%), while Agromyzidae, Anthomyzidae, Cecidomyiidae, Drosophilidae, Lonchopteridae, Phoridae, and Sepsidae were each represented by less than 5% of the sampled Diptera (Table S3). Regarding Hymenoptera, the sampled specimens belonged to the families Formicidae (45%), Braconidae (26%), and Eulophidae (6%); the sample rates for Ceraphronidae, Encyrtidae, Figitidae, Ichneumonidae, Megaspilidae, Mymaridae, Pteromalidae, Scelionidae, and Torymidae were all less than 5%. All the identified Hemiptera belonged to Aphididae, and all Thysanoptera to Thripidae. Coleoptera were represented by Chrysomelidae, Coccinellidae, Meloidae, and Staphylinidae (25% each, one specimen each family). For Collembola, Psocoptera, and Araneae, a single specimen was sampled for each order, belonging to the families of Entomobryidae, Ectopsocidae, and Linyphiidae, respectively.

In addition to the arthropods sampled during timed observation, more than 100 flower visitors were sampled during free observations (24 in 2016 at SJP, 25 in 2017 at BEN, 61 in 2018 at BEN, and four in 2019 at SJP) (Table S4). They belonged to eight orders (Araneae, Collembola, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Thysanoptera), and 30 families, with 28 identified species (Table S4). By applying this method, we could identify 1 order (Lepidoptera) and 13 families of flower visitors not detected during timed observations: Diptera Ephydridae, Muscidae, Syrphidae, and Scathophagidae; Hymenoptera Andrenidae, Apidae, Halictidae, and Tenthredinidae; Coleoptera Cantharidae and Curculionidae; Lepidoptera Nymphalidae; Hemiptera Cicadellidae; Araneae Theridiidae.

Overall, considering both timed and free observations, 254 flower-visiting arthropods belonging to 10 orders and 47 families were sampled.

The daily patterns of temperature (Figure S1a,c,e,g) and wind speed (Figure S1b,d,f,h) varied between samplings; however, no significant difference was observed in mean temperature (Kruskal–Wallis test, $p = 0.1295$; Figure S1i) and mean wind speed (ANOVA, $p = 0.0561$; Figure S1j) among the four years.

The models relating micrometeorological conditions to flower visitors' presence, abundance, and diversity showed better performance than null models and good R-squared values (Table S5).

The temperature had a significant, positive effect on the presence ($\chi^2_1 = 17.88$, $p < 0.001$), diversity ($\chi^2_1 = 10.38$, $p < 0.01$) and abundance ($\chi^2_1 = 20.49$, $p < 0.001$) of flower visitors (Figure 4a–c), while the wind speed did not significantly affect any of the dependent variables considered (presence: $\chi^2_1 = 0.08$, $p = 0.775$; diversity: $\chi^2_1 = 0.14$, $p = 0.705$; abundance: $\chi^2_1 = 0.19$, $p = 0.666$) (Figure 4d–f). Regarding covariates, the quadratic time showed a significant effect on the presence ($\chi^2_1 = 4.81$, $p < 0.05$) and abundance ($\chi^2_1 = 5.43$, $p < 0.05$) of flower visitors but not on their diversity ($\chi^2_1 = 0.96$, $p < 0.326$) (Figure 4g–i), whereas the number of flowers did not have any significant effect on the presence ($\chi^2_1 = 0.65$, $p = 0.421$), diversity ($\chi^2_1 = 0.00$, $p = 0.999$), and abundance ($\chi^2_1 = 0.09$, $p = 0.764$) of flower visitors (Figure 4j–l). The site significantly affected their presence ($\chi^2_1 = 3.88$, $p < 0.05$), diversity ($\chi^2_1 = 28.57$, $p < 0.001$), and abundance ($\chi^2_1 = 30.32$, $p < 0.001$). Also considering only the flying flower visitors, temperature had a significant effect on the dependent variables considered (presence, diversity and abundance), whereas wind did not have any significant effect (Table S6).

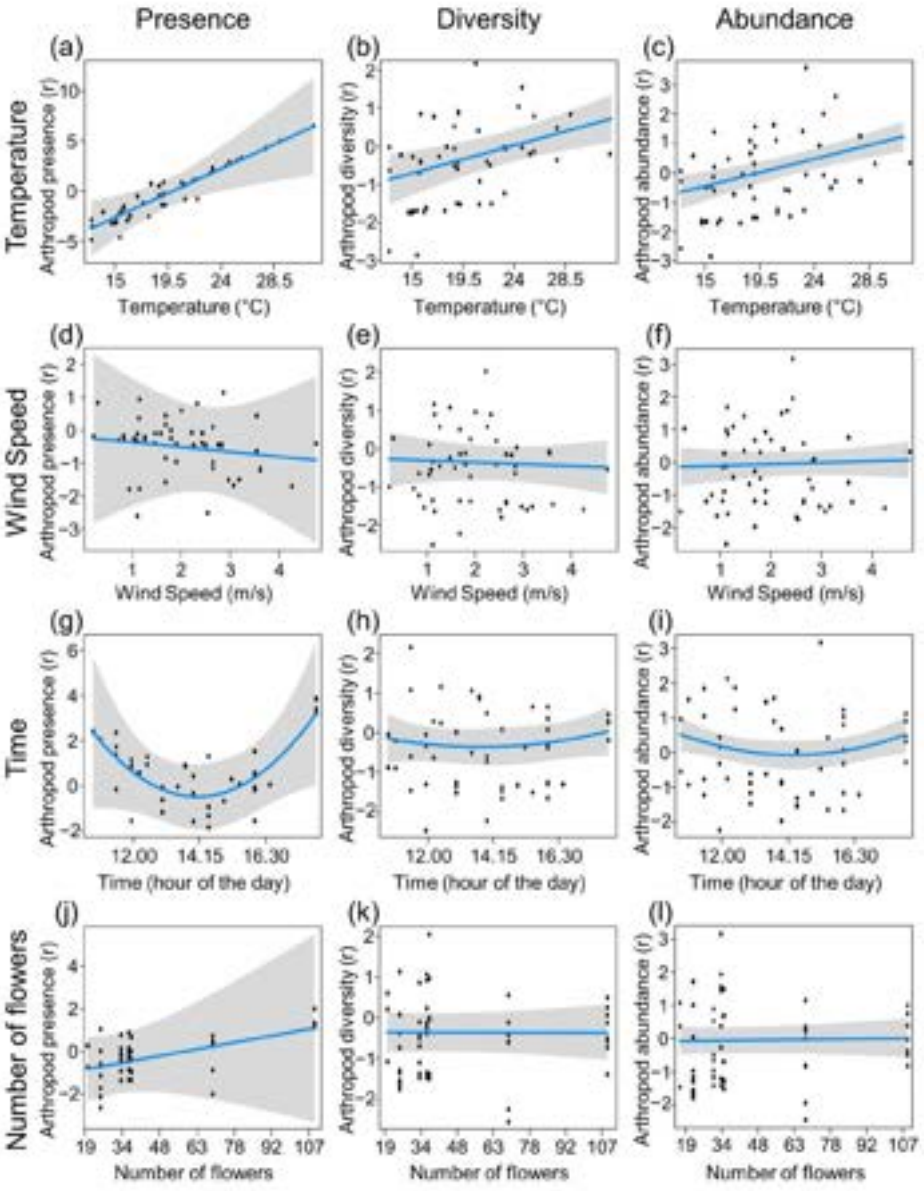

Figure 4. Partial residual plots showing the influence of temperature (**a**–**c**), wind speed (**d**–**f**), time (quadratic) (**g**–**i**), and number of flowers (**j**–**l**) on the presence (**a**,**d**,**g**,**j**), diversity (**b**,**e**,**h**,**k**) and abundance (**c**,**f**,**i**,**l**) of flower-visiting arthropods. Each black dot represents a sampling session. Blue lines indicate the mean value obtained using GLMs, while shaded areas represent 95% confidence bands; *r* = residuals.

4. Discussion

This research provides new insights into the flower-visiting arthropods of an early flowering high-altitude Alpine plant (*Androsace brevis*), taking into consideration the effect of abiotic factors (temperature and wind speed) and other variables (time and number of flowers) on their occurrence, abundance, and diversity.

The most abundant order of flower visitors (i.e., all the arthropods occurring on *A. brevis* flowers) was Diptera, nearly half of which were flies belonging to Anthomyiidae. This family was represented almost entirely by *Paregle coerulescens* (Strobl, 1893) and *Delia platura* (Meigen, 1826). The first insect is a common high-altitude species in central and southern Europe; the second one lives in temperate areas throughout the world and is known to feed on flowers [73]. Therefore, the flower visits by Anthomyiidae can be due to trophic activity, as actually observed on *A. brevis*, and in previous reports on other plant species [74,75]. Moreover, anthomyiid flies may visit flowers for thermoregulation, spendingtime on sun-exposed flowers, as reported in the literature for other anthophilous Diptera [76,77]. This thermoregulatory behaviour can be particularly relevant in cold environments [78], and climate change might negatively impact it, leading to fewer visits to flowers in the case of higher temperatures, thus reducing the pollination that Anthomyiidae can provide [42]. The other dipteran families were represented at lower percentages (<15%); among them, Sphaeroceridae were the most abundant, and all specimens but one belonged to two species: *Leptocera caenosa* (Rondani, 1880) and *Spelobia* cf. *clunipes* (Meigen, 1830). The former is mainly considered a synanthropic species, and its presence could be due to mountain huts at the sampling sites; however, both species were observed in burrows of mammals [79]. Here, too, however, their presence on flowers could be due to basking or trophic activity, even though the latter seems to be less likely as they prefer to feed on decaying organic matter of animal or plant origin [80]. The second most abundant order was Hymenoptera, with species belonging to two groups: ants and parasitoid wasps. Among them, the most represented family was Formicidae, and in particular *Formica lemani* Bondroit, 1917, an active predacious, aphidicolous, and nectarivorous species, whose workers forage on the ground, flowers, and plants [43,81,82]. In the Alps, this species is common in the grasslands above the tree line, where carbohydrates are a limiting resource, and therefore, ants can visit flowers to look for these important nutrients [83,84]. We also detected many families of parasitoid wasps, whose adults can visit flowers to feed on nectar [85,86]. Moreover, considering that all specimens identified at sex level were females, it is possible that they were searching for a host. In particular, the most abundant family was Braconidae, with almost all species belonging to the subfamily Aphidiinae, which are exclusive parasitoids of aphids [87]. Among Hemiptera, we found only winged forms of aphids, most of which belonged to the genus *Cinara* Curtis, 1835. Owing to the very early moment of the season, it is likely that aphids reached these mountain sites as aeroplankton, as demonstrated in other Alpine environments [88]. In our case, aphids could represent an important feeding resource for predators such as *F. lemani* [84]. Regarding Thysanoptera, all samples belonged to Thripidae, which may use flowers as shelter and mating sites, as well as food resources [89–91]. Although their presence and abundance seem to indicate a relevant role of this taxon in the context of this Alpine environment, it is difficult to draw any conclusions about the biotic interactions in which they might be involved. Indeed, very few studies have investigated their role in natural ecosystems, but they are well known as economically significant crop pests, and, in this context, it was suggested that their presence on plants can impact the behaviour and performance of other visiting insects [92]. The presence of Coleoptera on *A. brevis* flowers was very limited and therefore probably accidental in most cases. Therefore, the arthropod community associated with *A. brevis* flowers seems quite complex, despite the early flowering season, with potentially interesting biotic interactions with the plant as well as among insects.

Considering the general abundance pattern, our research highlights the dominance of flies among the high-altitude flower visitors, as already found in other studies on the Alps [36,37,41,42,93,94] and hypothesised as a global feature [95]. However, three peculiar

aspects emerge from our work. Firstly, among flies, we found an evident predominance of Anthomyiidae. This pattern was already observed at higher altitudes [36,37,93,96] but not at the same elevation at which we operated, where Muscidae and Empididae seem to predominate [36]. However, we did not detect these two latter families, except for a single specimen of Muscidae. This result highlights the peculiarity of the early season flower-visiting community. Secondly, bees (Hymenoptera: Apoidea: Anthophila) were not collected during timed observations, but only a few specimens were found during the free observations. Their presence is, therefore, rare in this period, while later in the season, they are more common at these altitudes, though not as abundant as flies [36]. Even for bees, our early season pattern seems to recall what happens at higher altitudes, where the presence of these insects is very limited [36]. Indeed, a global switch from bees to flies along elevation gradients suggests that temperature may be a limiting factor for bees in high-altitude habitats, a pattern that, in the future, could be impacted by climate change, with unknown effects on fly communities already present at higher elevations [95]. Finally, we identified a considerable share of parasitoid wasps. This finding did not emerge from previous studies in the Alpine environment. Indeed, most of the studies did not look for this taxon, and even when parasitoid wasps were considered, they were not detected in large numbers [37], with the partial exception of Ichneumonidae [42,93].

These findings underline the importance of focussing future research efforts also on neglected taxa such as flies and parasitoid wasps and not only on well-investigated taxa such as Hymenoptera Apoidea Anthophila, Diptera Syrphidae, and Lepidoptera, which certainly play a fundamental role in mountain ecosystems, but which in some contexts—such as ours—seem to be poorly represented. Although bees, even when numerically limited, can play an important role as pollinators due to their efficiency in pollen dispersal, other taxa might also represent keystones of biotic interactions and be strongly threatened by climate change. For instance, despite the importance of flies as pollinators, especially in mountain environments [95], these insects have received little consideration in the literature [77,97]. Moreover, little is known about parasitoid wasp–flower interactions and their interdependence, as only very few observations of parasitoid–flower interactions have been reported [86], although they might have crucial roles in ecosystem functioning that could be affected by climate change [98].

In addition to describing the flower-visiting arthropod community of an early flowering high-altitude Alpine plant, our study provides new insights into how micrometeorological conditions (i.e., temperature and wind speed) and other variables (i.e., time and number of flowers per plant) affect the presence, diversity, and abundance of flower-visiting arthropods. As expected in a cold environment during the early season, where temperature constitutes a limiting factor for flower-visitor activity, this variable had a significant, positive effect on the presence, diversity, and abundance of flower-visiting arthropods. However, although in the present context higher temperatures can increase the activity of the flower-visiting arthropods, climate warming could impose new physiological constraints in the future [99], limiting their performance at elevated temperatures currently not occurring in the mountain environment during the early season. We did not observe any significant effect of wind speed on the presence, diversity, and abundance of flower-visiting arthropods. To the best of our knowledge, our study is the first in which the effect of wind on flower visitors in the Alps was investigated, although the effect of wind speed on the activity and flight of insects has been evaluated in some previous papers, with mixed results [57,100–106]. Even though each taxon and species might respond differently to microclimatic conditions, it is possible to hypothesise that arthropods capable of living in an extreme environment might be somehow adapted to the wind. However, during our fieldwork, the wind speed was always lower than 4.4 m/s, and no extreme wind conditions occurred. Therefore, we cannot rule out that higher wind speed could affect the flower visitors' activity since a nonlinear response of arthropods to wind speed cannot be excluded [100]. Moreover, as the wind speed can change rapidly, with gusts and sudden variations, especially in extreme environments such as mountain ridges, it might be inter-

esting to more accurately evaluate its effect on flower visitors' behaviour through video observations [45]. The number of flowers per plant did not affect any of the dependent variables considered; this might seem quite surprising, as other studies highlighted its positive effect on flower visits [107–109]. However, owing to the number of flowers in the plants studied (always $\geq$ 20), we could not detect whether plants with fewer flowers would receive fewer visits; indeed, the positive effect of the number of flowers on the number of visits per plant might be more pronounced for low flower numbers and less marked for plants with many flowers [110]. Regarding the daily pattern of flower-visiting arthropod activity, we observed a significant effect of quadratic time on both flower visitors' presence and abundance but not on their diversity. This bimodal pattern, in our case with a reduction in presence and abundance in the central hours of the day, has also already been observed in some other studies in different environments for many brachyceran flies (the group to which most of the *A. brevis* dipteran flower visitors belonged), and also for some bees, ants, and beetles [77,111–114]. However, climate conditions can influence this pattern [77], which might, therefore, potentially be affected by climate change. The lack of effect of quadratic time on diversity might suggest that the time influences the activity of arthropods, but it does not have a differential effect on the detected flower-visiting taxa. These considerations, although preliminary, could represent a starting point for assessing the variables that can impact the activity of Alpine early season flower-visiting communities.

In conclusion, this study contributes to our understanding of the composition and response to environmental variables of high-altitude Alpine flower-visiting arthropod communities in the early season. These communities can impact ecosystem function and stability not simply due to pollination but also because arthropods are part of complex biotic interactions that, if modified, can lead to secondary extinctions [115], with effects also later in the season [116]. This research also provides baseline data, collected using replicable and standardised methods, that can be useful for further studies on the effects of climate change at high altitudes, the impact of which on arthropods can be considerable but is still largely unclear and unknown [11,60,117].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/insects13040393/s1, Figure S1: Boxplots of the micrometeorological conditions recorded during the timed observations, Table S1: Taxonomists who identified the sampled arthropods and their affiliation, Table S2: Molecular identification through COI barcoding, Table S3: Flower-visiting arthropods sampled during the timed observations, Table S4: Flower-visiting arthropods sampled during the free observations, Table S5: Model performances, Table S6: Effect of micrometeorological and context variables on flying flower visitors. References [118–120] are cited in the supplementary materials.

Author Contributions: Conceptualisation, M.B., M.C. (Marco Caccianiga), M.G. and M.C. (Morena Casartelli); methodology, M.B. and E.E.; formal analysis, M.B., E.E. and M.F.; investigation, M.B., E.E., D.A., V.M., M.C. (Marco Caccianiga), M.G. and M.C. (Morena Casartelli); resources, M.C. (Marco Caccianiga), M.G. and M.C. (Morena Casartelli); data curation, M.B. and E.E.; writing—original draft preparation, M.B.; writing—review and editing, E.E., M.C. (Marco Caccianiga), M.G. and M.C. (Morena Casartelli); supervision, M.C. (Marco Caccianiga), M.G. and M.C. (Morena Casartelli); project administration, M.C. (Marco Caccianiga), M.G. and M.C. (Morena Casartelli); funding acquisition, M.C. (Marco Caccianiga), M.G. and M.C. (Morena Casartelli). All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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

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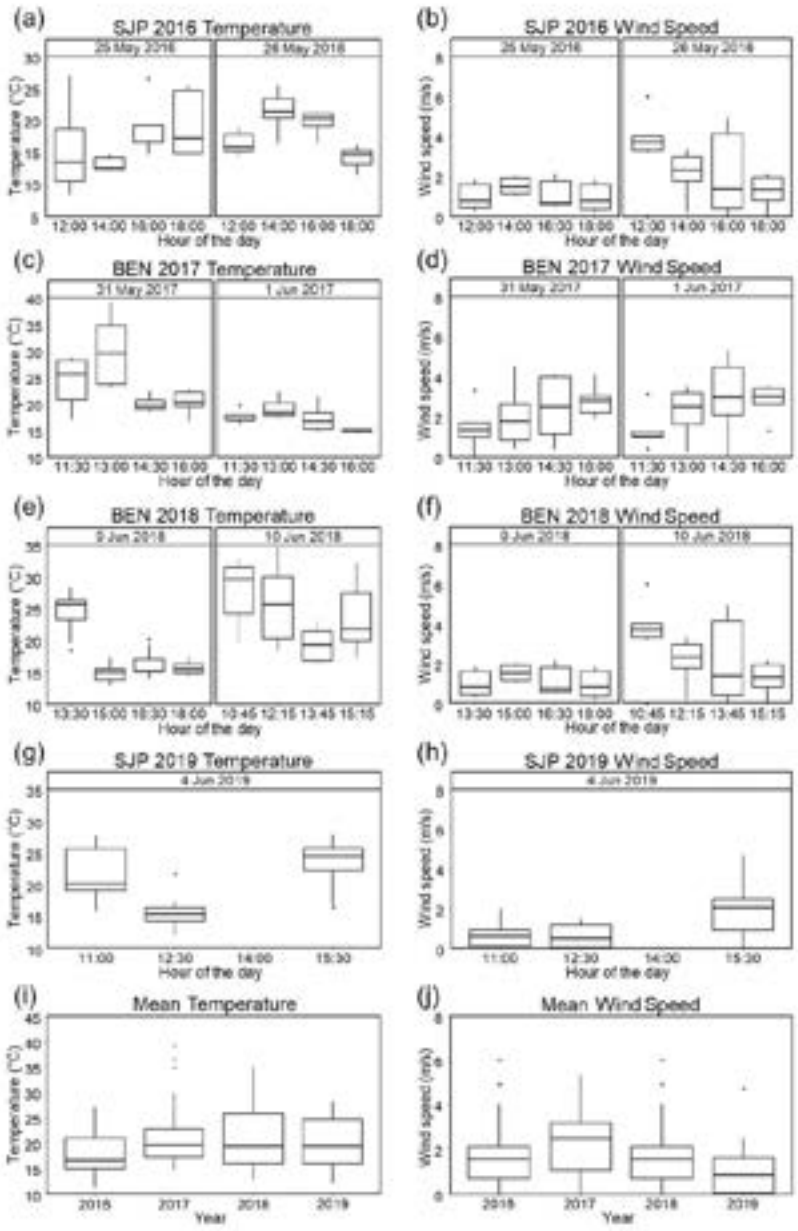

Figure S1. Boxplots of the micrometeorological conditions recorded during the timed observations: temperature and wind speed at SJP 2016 (a, b), BEN 2017 (c, d), BEN 2018 (e, f) and SJP 2019 (g, h) and mean temperature (i) and wind speed (j) through all years and sites.

Table S1. Taxonomists who identified the sampled arthropods and their affiliation.

Taxon	Taxonomist (affiliation)
ARACHNIDA	
Araneae	Paolo Pantini (Zoology of Invertebrate Section, Civic Museum of Natural Science Enrico Caffi, Bergamo, Italy)
Oribatida	Massimo Plumari (Museo Civico di Lentate sul Seveso, Lentate sul Seveso, Italy)
HEXAPODA	
Coleoptera	Mauro Gobbi
Coleoptera, Chrysomelidae	Matteo Montagna (Department of Agricultural and Environmental Sciences - Production Landscape Agroenergy, University of Milan, Milan, Italy)
Coleoptera, Meloidae	Marco Alberto Bologna (Department of Science, Roma Tre University, Rome, Italy)
Collembola	Pietro Paolo Fanciulli (Department of Life Sciences, University of Siena, Siena, Italy) Barbara Valle (Department of Biosciences, University of Milan, Milan, Italy)
Diptera	Daniele Avesani
Diptera, Agromyzidae	Miloš Černý (Independent Taxonomist, Halenkovice, Czech Republic)
Diptera, Anthomyiidae	Verner Michelsen
Diptera, Chironomidae	Valeria Lencioni (Research and Museum Collections Office, Climate and Ecology Unit, MUSE - Science Museum, Trento, Italy)
Diptera, Chloropidae	Emilia Petrovna Nartshuk (Zoological Institute, Russian Academy of Sciences, Saint Petersburg, Russia)
Diptera, Drosophilidae	Gerhard Bächli (Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland)
Diptera, Phoridae	Ronald Henry Lambert Disney (Department of Zoology, University of Cambridge, Cambridge, United Kingdom)
Diptera, Scathophagidae	Andrey Ozerov (Zoological Museum, Moscow State University, Moscow, Russia)
Diptera, Sphaeroceridae	Jindřich Roháček (Department of Entomology, Silesian Museum, Opava, Czech Republic)
Diptera, Syrphidae	Daniele Sommaggio (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy)
Hemiptera, Auchenorrhyncha	Davide Scaccini (Department of Agronomy Food Natural Resources Animals and Environment, University of Padua, Padua, Italy)
Hemiptera, Sternorrhyncha (2016, 2017, 2018)	Lidia Limonta (Department of Food Environmental and Nutritional Sciences, University of Milan, Italy)
Hemiptera, Sternorrhyncha (2019)	Alice Casiraghi (Department of Evolutionary Biology Ecology and Environmental Sciences, University of Barcelona, Spain; Institute for Integrative Systems Biology, University of Valencia, Spain) Nicolas Pérez Hidalgo (Institute for Integrative Systems Biology, University of Valencia, Spain; Arthropod Department, Museum of Natural Sciences of Barcelona, Spain)
Hymenoptera (2016)	Davide Dal Pos (Department of Biology, University of Central Florida, Orlando, United States)
Hymenoptera (2017, 2018, 2019)	Fabrizio Rigato (Sezione di Entomologia, Museo Civico di Storia Naturale, Milan, Italy)
Hymenoptera, Apoidea (2016)	Marco Bonelli Mauro Gobbi
Hymenoptera, Apoidea (2017, 2019)	Andrea Cappellari (Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padua, Padua, Italy)
Hymenoptera, Braconidae	Vladimir Žikić (Department of Biology and Ecology, University of Niš, Niš, Serbia) Željko Tomanović (Department of Invertebrate Zoology and Entomology, University of Belgrade, Belgrade, Serbia)
Hymenoptera, Eulophidae	Alex Gumovsky (Department of Taxonomy of Entomophagous Insects and Ecological Principles of Biocontrol, National Academy of Sciences of Ukraine, Kyiv, Ukraine)
Hymenoptera, Formicidae	Fabrizio Rigato (Entomology Section, Milan Natural History Museum, Milan, Italy)
Hymenoptera, Ichneumonidae	Filippo Di Giovanni (Department of Agriculture Food and Environment, University of Pisa, Pisa, Italy)
Hymenoptera, Mymaridae	Riccardo Jesu (Department of Agriculture, University of Naples Federico II, Italy)
Hymenoptera, Torymidae	Adriano Cazzuoli (Independent Taxonomist, Modena, Italy)
Lepidoptera	Marco Bonelli
Psocoptera	Rinaldo Nicoli Aldini (Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Piacenza-Cremona, Italy)
Thysanoptera	Barbara Conti (Department of Agriculture Food and Environment, University of Pisa, Pisa, Italy)

Table S2. Molecular identification through COI barcoding. Taxa and sexes, specimen ID and GenBank accession number, primer sets (names, sequences, lengths) and their references, PCR conditions, and the size of the amplicons obtained are reported.

Taxon (sex)	Specimen ID (GenBank accession number)	Primer set (names, sequences, lengths)	Reference	PCR protocol	Amplicon size
Hymenoptera Apidae *Bombus* sp. (male)	2017V02 (OM220090)	Bombus F GGATCNCCWGATATAGCWTTTCC (23) Bombus R TGCAGANGTAAAATAAGCTCGTG (23)	designed with Primer-BLAST [118] and adapted	94°C for 3', followed by 35 cycles at 94°C for 30", 51°C for 30", and 72°C for 45", and then 10' at 72°C	387 bp
Thysanoptera Thripidae (unknown)	G02* (OM220091)	LCO1490 GGTCAACAAATCATAAAGATATTGG (25) HCO2198 TAAACTTCAGGGTGACCAAAAAATCA (25)	[119]	94°C for 3', followed by 35 cycles at 94°C for 30", 53°C for 30", and 72°C for 45", and then 10' at 72°C	649 bp
Diptera Sciaridae (females)	H04 (OM220092) H06 (OM220093) 2018VV18 (OM220094) 2018VV19 (OM220095) 2018VV27 (OM220096)				

* Damaged specimen.

Table S3. Flower-visiting arthropods sampled during the timed observations. Site and year, specimen ID, order, family, genus, species, stage (A=adult, J=juvenile) and repository are reported. Blank lines mean that taxonomic identification was not achieved. Nomenclature is according to Fauna Europaea [120].

Site and Year	ID	Order	Family	Genus	Species	Author	Stage	Repository*
SJP2016	A01	Psocoptera	Ectopsocidae	*Ectopsocus*		McLachlan, 1899	A	CSCU
SJP2016	A02	Diptera	Drosophilidae				A	MUSE
SJP2016	A03	Diptera	Sphaeroceridae	*Leptocera*	*Leptocera caenosa*	(Rondani, 1880)	A	SM
SJP2016	A04	Diptera	Sphaeroceridae	*Leptocera*	*Leptocera caenosa*	(Rondani, 1880)	A	SM
SJP2016	A05	Diptera	Sciaridae				A	MUSE
SJP2016	A06	Diptera	Anthomyzidae				A	MUSE
SJP2016	A07	Diptera	Sphaeroceridae	*Spelobia*	*Spelobia* cf. *clunipes*	(Meigen, 1830)	A	MUSE
SJP2016	A08	Diptera	Chironomidae				A	MUSE
SJP2016	A10	Diptera	Phoridae	*Megaselia*	*Megaselia rufipes*	(Meigen, 1804)	A	MUSE
SJP2016	A11	Hemiptera	Aphididae	*Chaitophorus*		Koch, 1854	A	UNIMI A
SJP2016	AA01	Hymenoptera	Eulophidae	*Baryscapus*		Forster, 1856	A	UNIMI B
SJP2016	AA02	Hymenoptera	Braconidae	*Aphidius*		Nees, 1818	A	UN
SJP2016	AA03	Hymenoptera	Braconidae	*Aphidius*	*Aphidius ervi*	Haliday, 1834	A	UNIMI B
SJP2016	AA04	Hymenoptera	Eulophidae	*Baryscapus*		Forster, 1856	A	UNIMI B
SJP2016	AA05	Hymenoptera	Braconidae	*Aphidius*	*Aphidius ervi*	Haliday, 1834	A	UN
SJP2016	AA06	Hemiptera	Aphididae	*Cinara*		Curtis, 1835	A	UNIMI A
SJP2016	AA07	Hymenoptera	Formicidae	*Formica*	*Formica lugubris*	Zetterstedt, 1838	A*#	UNIMI B
SJP2016	AA08	Diptera	Sepsidae				A	MUSE
SJP2016	AA09	Diptera	Sphaeroceridae	*Spelobia*	*Spelobia* cf. *clunipes*	(Meigen, 1830)	A	MUSE
SJP2016	AA10	Hymenoptera	Braconidae	*Aphidius*	*Aphidius ervi*	Haliday, 1834	A	UN
SJP2016	AA11	Diptera	Sphaeroceridae	*Leptocera*	*Leptocera caenosa*	(Rondani, 1880)	A	SM
SJP2016	AA12	Hymenoptera	Ichneumonidae	*Aclastus*		Forster, 1869	A	UNIPI
SJP2016	AA13	Hymenoptera	Eulophidae	*Baryscapus*		Forster, 1856	A	UNIMI B
SJP2016	AA14	Diptera	Sciaridae				A	MUSE
SJP2016	AA15	Diptera	Sciaridae				A	MUSE
SJP2016	B01a	Hymenoptera	Formicidae	*Formica*	*Formica* (*Serviformica*) *lemani*	Bondroit, 1917	A	UNIMI B
SJP2016	B01b	Hymenoptera	Formicidae	*Formica*	*Formica* (*Serviformica*) *lemani*	Bondroit, 1917	A	UNIMI B
SJP2016	B01c	Hymenoptera	Formicidae	*Formica*	*Formica* (*Serviformica*) *lemani*	Bondroit, 1917	A	UNIMI B
SJP2016	B02	Hemiptera	Aphididae	*Cinara*		Curtis, 1835	A	UNIMI A
SJP2016	B03	Diptera	Lonchopteridae				A	MUSE
SJP2016	B04	Hymenoptera	Formicidae	*Formica*	*Formica* (*Serviformica*) *lemani*	Bondroit, 1917	A	UNIMI B
SJP2016	B05	Hymenoptera	Formicidae	*Formica*	*Formica* (*Serviformica*) *lemani*	Bondroit, 1917	A	UNIMI B
SJP2016	B06	Diptera	Chironomidae				A	MUSE
SJP2016	B07	Hemiptera	Aphididae	*Chaitophorus*		Koch, 1854	A	UNIMI A
SJP2016	B08	Diptera	Sphaeroceridae	*Leptocera*	*Leptocera caenosa*	(Rondani, 1880)	A	SM
SJP2016	B09	Hemiptera	Aphididae	*Cinara*		Curtis, 1835	A	UNIMI A
SJP2016	B10	Hemiptera	Aphididae	*Cinara*		Curtis, 1835	A	UNIMI A
SJP2016	B11	Hymenoptera	Formicidae	*Formica*	*Formica* (*Serviformica*) *lemani*	Bondroit, 1917	A	UNIMI B
SJP2016	B12	Hymenoptera	Formicidae	*Formica*	*Formica* (*Serviformica*) *lemani*	Bondroit, 1917	A	UNIMI B
SJP2016	B13	Hymenoptera	Braconidae	*Chorebus*		Haliday, 1833	A	UN

SJP2016	B14	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
SJP2016	B801	Hymenoptera	Braconidae	*Aphidius*	*Aphidius ervi*	Haliday, 1834	A	UN
SJP2016	B802	Diptera	Chloropidae				A	MUSE
SJP2016	B803	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
SJP2016	B804	Diptera	Sphaeroceridae	*Leptocera*	*Leptocera caenosa*	(Rondani, 1880)	A	SM
SJP2016	B805	Hymenoptera	Braconidae	*Diaeretiella*		Stary, 1960	A	UN
SJP2016	B806	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
SJP2016	B807	Hemiptera	Aphididae	*Cinara*		Curtis, 1835	A	UNIMI A
SJP2016	B808	Hymenoptera	Braconidae	*Aphidius*		Nees, 1818	A	UN
SJP2016	B809	Hymenoptera	Torymidae	*Torymus*	*Torymus baudysi*	Boucek, 1954	A	AC – pc
SJP2016	B810	Diptera	Sciaridae				A	MUSE
SJP2016	B811	Hemiptera	Aphididae	*Chaitophorus*		Koch, 1854	A	UNIMI A
SJP2016	B812	Diptera	Chloropidae				A	MUSE
SJP2016	B813	Hymenoptera	Braconidae	*Aphidius*	*Aphidius ervi*	Haliday, 1834	A	UN
SJP2016	B814	Diptera	Sciaridae				A	MUSE
SJP2016	B815	Hymenoptera	Braconidae	*Aphidius*	*Aphidius ervi*	Haliday, 1834	A	UN
SJP2016	B815bis	Coleoptera	Staphylinidae	*Amphichroum*	*Amphichroum canaliculatum*	(Erichson, 1840)	A	MUSE
SJP2016	B816	Diptera	Sphaeroceridae	*Opalimosina*	*Opalimosina (Opalimosina) mirabilis*	(Collin, 1902)	A	SM
BEN2017	C01	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	Haliday, 1836	A	UNIPI
BEN2017	C02	Diptera	Chironomidae	*Smittia*		Holmgren, 1869	A	MUSE
BEN2017	C03	Hymenoptera	Mymaridae	*Gonatocerus*		Nees, 1834	A	MSN
BEN2017	C04	Diptera	Agromyzidae	*Chromatomyia*	*Chromatomyia fuscula*	(Zetterstedt, 1838)	A	MC – pc
BEN2017	C05	Coleoptera	Meloidae	*Meloe*	*Meloe (Meloe) violaceus*	Marsham, 1802	J	UNIMI B
BEN2017	C06	Oribatida					A	UNIMI B
BEN2017	C07	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	Haliday, 1836	A	UNIPI
BEN2017	C08	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	Haliday, 1836	A	UNIPI
BEN2017	E01	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
BEN2017	E02	Hemiptera	Aphididae				A	UNIMI A
BEN2017	E03	Hemiptera	Aphididae	*Cinara*		Curtis, 1835	A	UNIMI A
BEN2017	CC01	Hymenoptera	Formicidae	*Lasius*	*Lasius (Dendrolasius) fuliginosus*	(Latreille, 1798)	A**	UNIMI B
BEN2017	CC02	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	Haliday, 1836	A	UNIPI
BEN2017	EE01	Hymenoptera	Braconidae	*Aphidius*	*Aphidius avenae*	Haliday, 1834	A	UNIMI B
BEN2018	F01	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	F02	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	F03	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	G01	Araneae	Linyphiidae	*Agyneta*		Hull, 1911	A	UNIMI B
BEN2018	G02	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	Haliday, 1836	A	BARCODING
BEN2018	G03	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	G04	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	G05	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	FF05	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	FF06	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	FF07	Diptera	Chloropidae	*Oscinella*	*Oscinella (Oscinella) frit*	(Linnaeus, 1758)	A	UNIMI B

BEN2018	FF09	Coleoptera	Coccinellidae	*Coccinella*	*Coccinella septempunctata*	Linnaeus, 1758	A	MUSE
BEN2018	FF10	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	MSN
BEN2018	FF11	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	GG01	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	GG02	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	GG03	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	UNIMI B
BEN2018	GG04	Hymenoptera	Mymaridae				A	MSN
BEN2018	GG05	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	GG06	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	GG07	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	GG08	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	GG09	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	GG10	Diptera	Chironomidae	*Camptocladius*	*Camptocladius cf. stercorarius*	(De Geer, 1776)	A	MUSE
BEN2018	GG11	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	GG12	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	GG13	Diptera	Anthomyiidae	*Pegoplata*	*Pegoplata aestiva*	Meigen, 1826	A	MUSE
BEN2018	GG14	Diptera	Drosophilidae	*Scaptomyza*	*Scaptomyza pallida*	(Zetterstedt, 1847)	A	UZ
BEN2018	GG15	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	GG16	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	GG17	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	GG18	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	GG19	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	GG20	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	GG21	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
SJP2019	I01	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
SJP2019	I02	Diptera	Cecidomyiidae				A	MS – pc
SJP2019	I03	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
SJP2019	I04	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
SJP2019	I05	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
SJP2019	I06	Hymenoptera	Formicidae	*Leptothorax*	*Leptothorax acervorum*	(Fabricius, 1793)	A	UNIMI B
SJP2019	I07	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
SJP2019	I08	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
SJP2019	I09	Diptera	Chironomidae	*Pararthocladius*	*Pararthocladius cf. nudipennis*	(Kieffer, 1908)	A	MUSE
SJP2019	I10	Hymenoptera	Braconidae				A	MSN
SJP2019	I11	Coleoptera	Chrysomelidae	*Plagiosterna*	*Plagiosterna aenea*	(Linnaeus, 1758)	A	UNIMI B
SJP2019	I12	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	Haliday, 1836	A	UNIPI
SJP2019	I13	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
SJP2019	I14	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Bondroit, 1917	A	UNIMI B
SJP2019	I15	Oribatida					A	UNIMI B
SJP2019	I16	Collembola	Entomobryidae	*Orchesella*	*Orchesella arcuata*	Lindenmann, 1950	A	UNIMI B
SJP2019	I17	Hymenoptera	Scelionidae				A	UNIMI B
SJP2019	I18	Hymenoptera	Formicidae	*Formica*	*Formica (Serviformica) lemani*	Linnaeus, 1758	A	UNIMI B
SJP2019	I19	Hymenoptera	Encyrtidae				A	UNIMI B

SJP2019	H01	Diptera	Phoridae	*Phora*		Latreille, 1796	A	UC
SJP2019	H02	Hymenoptera	Braconidae				A	MSN
SJP2019	H03	Hymenoptera	Megaspilidae				A	MSN
SJP2019	H04	Diptera	Sciaridae	*Austrosciara*	*Austrosciara hyalipennis*	(Meigen, 1804)	A	BARCODING
SJP2019	H05	Hemiptera	Aphididae	*Brachycaudus*		Van Der Goot, 1913	A	NPH – pc
SJP2019	H06	Diptera	Sciaridae	*Austrosciara*	*Austrosciara hyalipennis*	(Meigen, 1804)	A	BARCODING
SJP2019	H07	Diptera	Chironomidae	*Paratrichocladius*	*Paratrichocladius* cf. *nudipennis*	(Kieffer, 1908)	A	MUSE
SJP2019	H08	Hymenoptera	Braconidae				A	MSN
SJP2019	H09	Hymenoptera	Figitidae				A	MSN
SJP2019	H10	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	Haliday, 1836	A	UNIPI
SJP2019	H11	Hymenoptera	Encyrtidae				A	MSN
SJP2019	H12	Hymenoptera	Pteromalidae				A	MSN
SJP2019	H13	Hymenoptera	Ceraphronidae				A	MSN
SJP2019	H14	Hymenoptera	Megaspilidae				A	MSN

* Repository acronyms: AC – pc: Adriano Cazzuoli – Personal Collection (Italy); CSCU: Department of Sustainable Crop Production, Catholic University of the Sacred Heart (Italy); MC – pc: Miloš Černý – Personal Collection (Czech Republic); MS – pc: Marcela Skuhravá – Personal collection (Czech Republic); MSN: Entomology Section, Milan Natural History Museum (Italy); MUSE: Research and Museum Collections Office Climate and Ecology Unit, MUSE – Science Museum (Italy); NPH – pc: Nicolás Pérez Hidalgo – Personal Collection (Spain); SM: Department of Entomology – Silesian Museum (Czech Republic); UC: Department of Zoology, University of Cambridge (United Kingdom); UN: Department of Biology and Ecology, University of Niš (Serbia); UNIMI A: Department of Food Environmental and Nutritional Sciences, University of Milan (Italy); UNIMI B: Department of Biosciences, University of Milan (Italy); UNIPI: Department of Agriculture Food and Environment, University of Pisa (Italy); UZ: Department of Evolutionary Biology and Environmental Studies, University of Zurich (Switzerland). ^{wf} = winged gyne

Table S4. Flower-visiting arthropods sampled during the free observations. Site and year, specimen ID, order, family, genus, species, stage (A=adult, J=juvenile) and repository are reported. Blank lines mean that taxonomic identification was not achieved. Nomenclature is according to Fauna Europaea [120].

Site and Year	ID	Order	Family	Genus	Species	Author	Stage	Repository
SPI2016	2016V01	Hymenoptera	Ichneumonidae	*Probles*	*Probles (Microdiaporsis) cauliculatus*	Khalaim, 2007	A	UNIPI
SPI2016	2016V02	Diptera	Phoridae	*Megaselia*	*Megaselia rufipes*		A	MUSE
SPI2016	2016V03	Diptera	Ephydridae				A	MUSE
SPI2016	2016V04	Hymenoptera	Braconidae	*Aphidius*	*Aphidius ervi*	Haliday, 1834	A	UN
SPI2016	2016V05	Diptera	Anthomyiidae				A	MUSE
SPI2016	2016V06	Hymenoptera	Torymidae	*Torymus*	*Torymus baudysi*	Boucek, 1954	A	AC – pc
SPI2016	2016V07	Hemiptera	Aphididae	*Cinara*		Curtis, 1835	A	UNIMI A
SPI2016	2016V08	Hymenoptera	Braconidae	*Aphidius*	*Aphidius ervi*	Haliday, 1834	A	UN
SPI2016	2016V09	Hymenoptera	Apidae	*Bombus*	*Bombus lapidarius*	(Linnaeus, 1758)	A	MUSE
SPI2016	2016V10	Diptera	Muscidae	*Helina*		Robineau-Desvoidy, 1830	A	MUSE
SPI2016	2016V11	Hymenoptera	Torymidae	*Torymus*	*Torymus baudysi*	Boucek, 1954	A	AC – pc
SPI2016	2016V12	Diptera	Sciaridae				A	MUSE
SPI2016	2016VV01	Lepidoptera	Nymphalidae	*Aglais*	*Aglais urticae*	(Linnaeus, 1758)	A	UNIMI B
SPI2016	2016VV02	Hymenoptera	Eulophidae	*Necremnus*	*Necremnus cf. cosconius*	(Walker, 1839)	A	UNIMI B
SPI2016	2016VV03	Diptera	Anthomyiidae				A	MUSE
SPI2016	2016VV04	Hymenoptera	Apidae	*Bombus*	*Bombus lapidarius*	(Linnaeus, 1758)	A	MUSE
SPI2016	2016VV05	Diptera	Agromyzidae				A	MUSE
SPI2016	2016VV06	Hymenoptera	Torymidae	*Torymus*	*Torymus baudysi*	Boucek, 1954	A	AC – pc
SPI2016	2016VV07	Coleoptera	Curculionidae	*Orchestes*	*Orchestes (Salius) fagi*	(Linnaeus, 1758)	A	MUSE
SPI2016	2016VV08	Hymenoptera	Torymidae	*Torymus*	*Torymus baudysi*	Boucek, 1954	A	AC – pc
SPI2016	2016VV09	Hymenoptera	Torymidae	*Torymus*	*Torymus baudysi*	Boucek, 1954	A	AC – pc
SPI2016	2016VV10	Coleoptera	Staphylinidae	*Amphichroum*	*Amphichroum canaliculatum*	(Erichson, 1840)	A	MUSE
SPI2016	2016VV11	Coleoptera	Curculionidae	*Orchestes*	*Orchestes (Salius) fagi*	(Linnaeus, 1758)	A	MUSE
SPI2016	2016VV12	Hemiptera	Aphididae	*Cinara*		Curtis, 1835	A	UNIMI A
BEN2017	2017V01	Coleoptera	Meloidae	*Meloe*	*Meloe (Meloe) violaceus*	Marsham, 1802	J	UNIMI B
BEN2017	2017V02	Hymenoptera	Apidae	*Bombus*	*Bombus pratorum*	(Linnaeus, 1761)	A	BARCODING
BEN2017	2017V03	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	Haliday, 1836	A	UNIPI
BEN2017	2017V04	Hemiptera	Aphididae				A	UNIMI A
BEN2017	2017V05	Hymenoptera	Braconidae	*Aphidius*	*Aphidius avenae*	Haliday, 1834	A	UNIMI A
BEN2017	2017V06	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2017	2017V07	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	UNIMI B
BEN2017	2017V08	Hymenoptera	Halictidae	*Lasioglossum*		Curtis, 1833	A	UNIPD
BEN2017	2017V09	Hymenoptera	Halictidae	*Lasioglossum*		Curtis, 1833	A	UNIPD
BEN2017	2017V10	Diptera	Anthomyiidae	*Pegoplata*	*Pegoplata aestiva*	(Meigen, 1826)	A	MUSE
BEN2017	2017V10	Diptera	Anthomyiidae	*Pegoplata*	*Pegoplata aestiva*	Meigen, 1826	A	MUSE
BEN2017	2017V11	Diptera	Anthomyiidae	*Pegoplata*	*Pegoplata aestiva*	(Meigen, 1826)	A	MUSE
BEN2017	2017V12	Diptera	Anthomyiidae				A	MUSE
BEN2017	2017V13	Diptera	Anthomyiidae	*Pegoplata*	*Pegoplata aestiva*	(Meigen, 1826)	A	MUSE
BEN2017	2017V14	Diptera	Anthomyiidae	*Pegoplata*	*Pegoplata aestiva*	(Meigen, 1826)	A	MUSE
BEN2017	2017V15	Diptera	Anthomyiidae	*Zaphne*	*Zaphne frontata*	(Zetterstedt, 1838)	A	MUSE

BEN2017	2017VV01	Hymenoptera	Braconidae	*Aphidius*	*Aphidius avenae*	Haliday, 1834	A	UN
BEN2017	2017VV02	Hemiptera	Aphididae	*Eulachnus*		Del Guercio, 1909	A	UNIMI A
BEN2017	2017VV03	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	Haliday, 1836	A	UNIPI
BEN2017	2017VV04	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	Haliday, 1836	A	UNIPI
BEN2017	2017VV05	Thysanoptera	Thripidae	*Thrips*	*Thrips tabaci*	Lindeman, 1889	A	UNIPI
BEN2017	2017VV06	Diptera	Chloropidae	*Oscinella*	*Oscinella (Oscinella) frit*	(Linnaeus, 1758)	A	UNIMI B
BEN2017	2017VV07	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	Haliday, 1836	A	UNIPI
BEN2017	2017VV08	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2017	2017VV09	Hymenoptera	Braconidae	*Aphidius*	*Aphidius ervi*	Haliday, 1834	A	UNIMI B
BEN2018	2018V01	Diptera	Anthomyiidae	*Zaphne*	*Zaphne frontata*	(Zetterstedt, 1838)	A	MUSE
BEN2018	2018V02	Hymenoptera	Figitidae				A	MSN
BEN2018	2018V03	Hymenoptera	Formicidae	*Myrmica*	*Myrmica sulcinodis*	Nylander, 1846	A	MSN
BEN2018	2018V04	Araneae	Linyphiidae	*Bathyphantes*	*Bathyphantes setiger*	F.O. Pickard-Cambridge, 1894	A	UNIMI B
BEN2018	2018V05	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018V06	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	2018V07	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	2018V08	Araneae	Linyphiidae	*Agyneta*			J	UNIMI B
BEN2018	2018V10	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	2018VV01	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV02	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV03	Hymenoptera	Ichneumonidae				A	MSN
BEN2018	2018VV04	Diptera	Syrphidae	*Scaeva*	*Scaeva dignota*	(Rondani, 1857)	A	DS – pc
BEN2018	2018VV05	Hymenoptera	Formicidae	*Myrmica*	*Myrmica sulcinodis*	Nylander, 1846	A	MSN
BEN2018	2018VV06	Araneae	Linyphiidae	*Agyneta*			J	UNIMI B
BEN2018	2018VV07	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV08	Diptera	Scathophagidae	*Scathophaga*	*Scathophaga stercoraria*	(Linnaeus, 1758)	A	UNIMI B
BEN2018	2018VV09	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV10	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV11	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV12	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV13	Coleoptera	Staphylinidae	*Philonthus*	*Philonthus (Philonthus) frigidus*	Markel & Kiesenwetter, 1848	A	MUSE
BEN2018	2018VV14	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	2018VV15	Hymenoptera	Ichneumonidae				A	MSN
BEN2018	2018VV16	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV17	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV18	Diptera	Sciaridae	*Corynoptera*	*Corynoptera trepida*	(Winnertz, 1867)	A	BARCODING
BEN2018	2018VV19	Diptera	Sciaridae	*Corynoptera*	*Corynoptera trepida*	(Winnertz, 1867)	A	BARCODING
BEN2018	2018VV20	Diptera	Anthomyiidae	*Zaphne*	*Zaphne frontata*	(Zetterstedt, 1838)	A	MUSE
BEN2018	2018VV21	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV22	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	2018VV23	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	2018VV24	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	2018VV25	Collembola	Entomobryidae	*Orchesella*		Templeton, 1835	A	UNIMI B

BEN2018	2018VV26	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV27	Diptera	Sciaridae				A	BARCODING
BEN2018	2018VV28	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	2018VV29	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	2018VV30	Araneae	Theridiidae				J	UNIMI B
BEN2018	2018VV31	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV32	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VV33	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS01	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS02	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS03	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS04	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS05	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS06	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS07	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS08	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS09	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS10	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS11	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS12	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS13	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS15	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
BEN2018	2018VIS16	Diptera	Syrphidae	*Eristalis*	*Eristalis similis*	(Fallen, 1817)	A	DS – pc
BEN2018	2018VIS17	Hemiptera	Cicadellidae				J	UNIMI A
BEN2018	2018VIS18	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS19	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	MUSE
BEN2018	2018VIS20	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	(Strobl, 1893)	A	MUSE
SJP2019	2019V01	Coleoptera	Cantharidae	*Cantharis*	*Cantharis (Cantharis) nigricans*	Müller, 1766	A	MUSE
SJP2019	2019V02	Hymenoptera	Tenthredinidae	*Dolerus*		Panzer, 1801	A	MSN
SJP2019	2019V03	Hymenoptera	Andrenidae	*Andrena*	*Andrena lapponica*	Zetterstedt, 1838	A	UNIPD
SJP2019	2019V04	Diptera	Anthomyiidae	*Delia*	*Delia platura*	(Meigen, 1826)	A	UNIMI B

* Repository acronyms: AC – pc: Adriano Cazzuoli – Personal Collection (Italy); DS – pc: Daniele Sommaggio – Personal Collection (Italy) MSN: Marcela Skuhravá – Personal collection (Czech Republic); MUSE: Research and Museum Collections Office Climate and Ecology Unit, MUSE – Science Museum (Italy); UN: Department of Biology and Ecology, University of Nis (Serbia); UNIMI A: Department of Food Environmental and Nutritional Sciences, University of Milan (Italy); UNIMI B: Department of Biosciences, University of Milan (Italy); UNIPD: Department of Agronomy Food Natural Resources Animals and Environment, University of Padova (Italy); UNIPI: Department of Agriculture Food and Environment, University of Pisa (Italy).

Table S5. Model performances. Likelihood ratio test and pseudo R^2.

Model	Likelihood ratio test			pseudo R^2	
	df	Explained deviance	p	pseudo R^2 value	Method
Presence	6	31.230	< 0.001	0.515	Tjur's R^2
Diversity	6	48.709	< 0.001	0.732	Nagelkerke's R^2
Abundance	6	60.923	< 0.001	0.711	Nagelkerke's R^2

Table S6. Effect of micrometeorological and context variables on flying flower visitors. Asterisk indicates statistically significant effects.

	Presence	Diversity	Abundance
Temperature	$\chi^2_1 = 9.66$, $p < 0.001$ *	$\chi^2_1 = 9.54$, $p < 0.01$ *	$\chi^2_1 = 19.82$, $p < 0.001$ *
Wind Speed	$\chi^2_1 = 0.22$, $p = 0.635$	$\chi^2_1 = 0.00$, $p = 0.965$	$\chi^2_1 = 0.89$, $p = 0.345$
Time (quadratic)	$\chi^2_1 = 2.35$, $p = 0.125$	$\chi^2_1 = 2.56$, $p = 0.109$	$\chi^2_1 = 5.20$, $p < 0.05$ *
Number of flowers	$\chi^2_1 = 0.02$, $p = 0.885$	$\chi^2_1 = 0.09$, $p = 0.761$	$\chi^2_1 = 0.07$, $p = 0.789$
Site	$\chi^2_1 = 5.55$, $p < 0.05$ *	$\chi^2_1 = 25.24$, $p < 0.001$ *	$\chi^2_1 = 17.03$, $p < 0.001$ *

Chapter 2.4

Plant-pollinator interactions during early season in Alpine ecosystem: a three-year study focusing the narrow endemic plant *Androsace brevis* (Hegetschw.) Ces. (Primulaceae)

Work in preparation

Plant-pollinator interactions during early season in Alpine ecosystem: A three-year study focusing the narrow endemic plant *Androsace brevis* (Hegetschw.) Ces. (Primulaceae)

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Abstract

Despite the importance of plant-pollinator interactions for ecosystem survival, many knowledge gaps occur, particularly for mountain environments. Here, the ongoing climate warming is expected to cause phenological and spatial shifts in many species, leading to mismatches between plants and pollinators, potentially negatively impacting their fitness.

To increase the knowledge about plant-pollination interaction, qualitative and quantitative analysis of pollen carried by flower visitors are essential to provide comprehensive information on arthropod taxa involved in pollination and the importance of different plant species as trophic sources for flower-visitors.

This three-year study aimed to identify the flower-visiting arthropod community and pollinators of the early-flowering alpine species *Androsace brevis* (Hegetschw.) Ces. (Primulaceae), and to evaluate how they change over years. *Androsace brevis* was selected as its flowering period is closely related to snowmelt and it is vulnerable to climate warming due to its inability to shift to higher elevations making critical the mismatches with pollinators.

Fieldwork was conducted within the Orobic Alps (Northern Italy, Lombardy) using *A. brevis* population located near the Benigni hut at 2.222 m asl. Using the timed-observation method, all the *A. brevis* flower-visiting arthropods were collected and stored in ethanol. Pollen grains from each specimen were retrieved from their body, identified, and counted using a light microscope to establish the plant-pollination networks for each year.

Results highlight that *Androsace brevis* flower-visiting arthropod community shows a high taxonomic diversity, but it changes over years both in taxa composition and arthropod abundance. Among the three years, Diptera Anthomyiidae and Hymenoptera Apoidea Anthophila resulted to be the primary pollinators of this plant species, as well of most of the other flowering species observed within the study area.

Further long-term study could be useful to better understand the web of interaction between plants and pollinators and how they evolve under climate warming.

1. Introduction

Many arthropods depend on flowers for their survival and most of the flowering plants depend on animals – mostly insects – for their pollination (Ollerton et al., 2011; Abrol et al., 2012; Inouye et al., 2015; Wardhaugh, 2015; Ollerton, 2017). The effects of the dependences between plants and pollinators are not limited only to the actors directly involved in these interactions, but can indirectly affect the broader ecological network, impacting on the whole ecosystem survival (Vázquez et al., 2012). Indeed, plant-arthropod interactions play a crucial ecological role in supporting both natural ecosystems as well as human food security (Fontaine et al., 2006; Brodie et al., 2014; Lucas-Barbosa, 2016; Ollerton, 2021; Benvenuti, 2022).

Despite the global importance of plant-pollinator interactions, the knowledge about their complexity is not homogeneous among different ecosystems, and many knowledge gaps still occur, particularly in mountain ecosystems. Indeed, most pollination-networks were studied in agricultural context, while mountain environments, such as Alpine ones, have been poorly described (Vizentin-Bugoni et al., 2018). This knowledge gap might partly exist because mountain environments are often erroneously considered both unpolluted and undisturbed as well as because of the probable perceived lack of short-term economic interest in this topic (Bonelli et al., 2022). Moreover, Alpine research presents logistical constraints, as the limited duration of the growing season, the difficult access to study sites, and the occurrence of extreme meteorological phenomena (Inouye, 2020).

However, Alpine ecosystems contain peculiar plant and arthropod communities characterized by the presence of also endemic species (Fenaroli, 1973; Aeschimann et al., 2011, Martini et al., 2012; Graf et al., 2014) which contribute to provide fundamental services both for ecosystems survival as well as aesthetic and cultural values (Tomitaka et al. 2021). In these environments, about 78% of plant species depend on arthropods for pollination (Ollerton et al., 2011; Lefebvre et al., 2018), and the interactions which link plants and pollinators to each other, even if poorly known, are complex (Bascompte et al., 2003).

In alpine areas, the high variability in environmental variables (such as variation in temperatures, humidity, wind speed, length of light period, and persistence of snow

cover), highly influence the length of the vegetative season. Many entomophilous plant species develop few large flowers or many small flowers to be as attractive as possible to potential pollinators (Proctor et al., 1997; Zoller et al., 2002; Cartar et al., 2004; Harder & Johnson, 2009; Parachnowitsch & Kessler, 2010; Dixit et al., 2020). However, the unpredictable occurrence of anomalous weather phenomena can alter the length both of plants flowering and the activities of flower visitors, especially if these extreme phenomena become more and more frequent over years, as it is occurring for increasing temperatures in the context of climate warming (Rottler et al., 2019). Increasing in seasonal average temperatures leads plants and flower-visiting arthropods to change their phenology or to migrate toward higher altitudes searching for more suitable habitats (Inouye, 2020; Gérard et al., 2020). Both phenological and spatial adaptations to new environmental conditions may not be equal between plants and flower visitors, thus leading to potential serious mismatches between the two parties with a decrease in their fitness (Morton & Rafferty, 2017). These mismatches are expected to mostly occur in particular times of the years, such as the early season. In this period, flowering of entomophilous plants is closely related to the snowmelt and, with higher spring temperature, they are expected to flower earlier than now (Inouye, 2000; Augspurger, 2013). Increasing temperatures could lead also to alterations in the period of activity of pollinators, potentially bringing to deleterious consequences on the plants reproductive success. By the same way, changes in flowering period could represent a serious threat for the arthropods that depend on flowers for their survival (Morton & Rafferty, 2017).

In this context, the identification of pollination networks could be used as tool to understand plant–pollinator associations, and so to evaluate the ecological service provided by flower-visiting arthropods in the ecosystems survival. The identification of pollination networks is nowadays mainly based on recording flower-visitation rate of arthropods, rather than recording clearly defined effective pollination event (Ballantyne et al., 2015; Cuartas-Hernández & Medel, 2015; Traveset et al., 2016; De Manincor et al., 2020). However, qualitative and quantitative palynological analysis of pollen grains transported by flower visitors are fundamental to provide comprehensive information about both the importance of different arthropod taxa in plants pollination and the

importance of different plant species as trophic resource for flower visitors (De Manincor et al., 2020; Bonelli et al., 2020).

In this work, we aim to characterize the main pollinators of an early season flowering species in an Alpine context through a three-year study, using the narrow endemic plant species *Androsace brevis* (Hegetschw.) Ces. (Primulaceae) as model species. Moreover, we aim to assess how the structure of pollination-networks established between the early season flowering plants and flower-visiting arthropods varies over the years.

2. Materials and methods

2.1 Model species

Androsace brevis (Hegetschw.) Ces. (Primulaceae) is an Alpine perennial cushion plant narrow-endemic to the Southern European Alps. This species grows only above 2000 m asl in a limited area of Northern Italy and Switzerland. According to IUCN criteria, its conservation status is vulnerable (Mangili et al. 2014, Bornand et al., 2016). The choice of *A. brevis* as model species for this study, is based on its phenology. *Androsace brevis* flowering occurs immediately after snowmelt and lasts about two weeks between the end of May and the beginning of June, being among the first species flowering in its geographic-altitudinal context (Bonelli et al., 2022; Eustacchio et al., 2023). Moreover, this species depends on insect pollination for reproduction (Eustacchio et al., 2023). The floral morphology of this species has been described in detail by Eustacchio et al. (2023).

2.2 Retrieving and palynological analyses of pollen carried by flower-visiting arthropods

Quali-quantitative palynological analyses of pollen grains carried by *A. brevis* flower visiting arthropods were performed to investigate which of these visitors can act as pollinator.

The pollen was retrieved from the body of flower-visiting arthropods collected in 2017 and 2018 on *A. brevis* reported in Bonelli et al. (2020) and from flower-visiting arthropods collected in 2019 reported in Bonelli et al. (2022). For this study, only pollen

retrieved from specimens sampled in *A. brevis* population located in the vicinity of the Mountain Hut "Cesare Benigni" (UTM WGS83 – 32T E 543496 N 5096577, 2222 m asl) in the Orobic Alps (Bergamo, Lombardy, Northern Italy) were analyzed. The site is described in detail in Bonelli et al. (2020) and Bonelli et al. (2022). Flower-visiting arthropods were sampled on flowers according to timed and free observation methods (Gibson et al., 2011; Bonelli et al., 2022) (Table1).

Table 1. Timed observations conducted for flower-visiting arthropod sampling. Year, day, *A. brevis* cushion code, number of flowers in anthesis, and time window are reported.

Year	Day	Cushion code	Number of flowers in anthesis	Timed window
2017	31 May 2017	C; E	37; 25	11.30-12.00 12.30-13.30 14.00-15.00 15.30-16.30
	1 June 2017	CC; EE	37; 25	11.30-12.00 12.30-13.30 14.00-15.00 15.30-16.30
2018	9 June 2018	F; G	33; 36	13.00-14.00 14.30-15.30 16.00-17.00 17.30-18.30
	10 June 2018	FF; GG	33; 36	8.45-9.45 10.15-11.15 11.45-12.45 13.15-14.15 14.45-15.45
2019	8 June 2019	J; K	30; 28	10.00-11.00 12.00-13.00 14.00-15.00 15.30-16.30
	15 June 2019	L; M	5, 10	15.00-16.00 16.00-17.00 17.00-18.00
	16 June 2019	N; O	8, 14	10.00-11.00 12.00-13.00

The samples collected during free observations performed in 2019 are described in Supplementary Material S1 as they are not reported in Bonelli et al. (2020). Arthropod sampling was conducted as reported in Bonelli et al. (2022). The collected arthropods were immediately placed individually in 1.5 mL safe-lock tubes with 70% ethanol, thus preserving the pollen carried by arthropods. All the collected samples subdivided at family level are reported in Supplementary Material S2. Each tube containing the ethanol-preserved specimen was vortexed three times for 30 s to remove pollen grains from the arthropod body and to suspend pollen in the ethanol. Then, the specimen was

removed from the tube and observed under a stereomicroscope to check that no pollen grains remained attached to the body. If necessary, the process was repeated until no pollen grains was found on the specimen. Then, the tubes containing the suspended pollen were put in a thermoblock at 65 °C to accelerate ethanol evaporation and concentrate pollen.

Finally, pollen retrieved from the same arthropod taxon were merged according to Table2, thus obtaining 27 samples. In detail, the pollen material was merged at order level for orders represented by less than 15 specimens. For two orders (Diptera and Hymenoptera) a finer level of detail was applied. Diptera were divided considering separately the pollen from Anthomyiidae (as they were the most abundant family and represented the vast majority of collected Diptera), while pollen from other families (each represented by equal or less than 3 specimens) was merged. Regarding Hymenoptera, Apoidea were divided at family level due to their well-known role in pollination. Moreover, Formicidae were kept separated because of their peculiar morphology and ecology compared to "other" Hymenoptera (all winged parasitoid wasps).

Table 2. Flower-visiting arthropod specimens from which the carried pollen was retrieved, reported per taxon and year.

Taxon	2017	2018	2019	Total (per taxon)
Diptera Anthomyiidae	10	70	1	**81**
Diptera (other)	3	9	3	**15**
Hymenoptera Apoidea Apidae	1	0	0	**1**
Hymenoptera Apoidea Halictidae	2	0	2	**4**
Hymenoptera Formicidae	2	3	0	**5**
Hymenoptera (other)	5	4	2	**11**
Hemiptera	4	1	2	**7**
Coleoptera	2	2	0	**4**
Thysanoptera	9	1	3	**13**
Oribatida	1	0	0	**1**
Araneae	0	5	2	**7**
Collembola	0	1	3	**4**
Total (per year)	**39**	**96**	**18**	**153**

The pollen samples obtained for flower-visiting arthropods were subject to acetolysis to better observe the pollen morphology (Erdtman, 1960; Bonelli et al., 2020),

mounted on microscope slides in glycerol-water mounting medium and observed under an optical microscope (Leica DMRB). The pollen was counted and identified both by comparison with literature data (Moore et al., 1991; Beug, 2004; Eustacchio et al., 2023) and with a pollen library created ad-hoc. The latter was made by collecting in 70% ethanol pollen samples from all plant species observed in flower during the *A. brevis* flowering period (Supplementary Material S3), within a radius of 500 m and within a difference in altitude of +/−200 m from the study site. We subjected these pollen samples to the same protocol described above, obtaining a pollen library for reference use.

2.3 Pollination-network analysis

The data from qualitative and quantitative palynological analysis were analysed through bipartite pollination networks, with the functions implemented in the R package *igraph* (Csardi & Nepusz, 2006; R Core Team, 2018). The pollen counts were transformed in percentages, allowing to compare data from different years, sites, and number of sampled specimens among taxa. Plant-arthropod interactions were investigated in both directions, plotting two types of networks: from the perspective of arthropods, where the line thickness represents the percentage of pollen of different plant taxa found on an arthropod taxon, and from the perspective of plants, where the line thickness indicates the percentage of pollen from a plant taxon found on different arthropods taxa. Moreover, the percentage of entomophilous and anemophilous pollen carried by arthropods were evaluated, to better investigate the role of different taxa as pollinators.

3. Preliminary Results

A total of 3064 pollen grains were counted and identified (Supplementary Material S4). *Androsace brevis* pollen was observed only on Diptera Anthomyiidae family (2017 and 2018), Hymenoptera Apoidea (2017 and 2019), “other” Hymenoptera (2017), and “other” Diptera (2017) (Figure1).

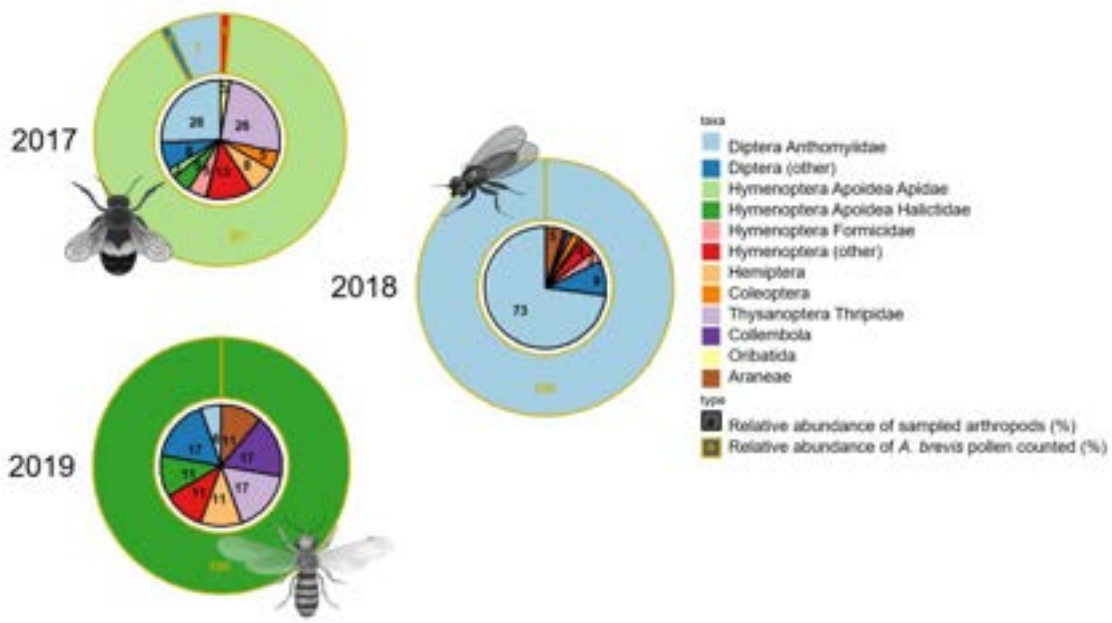

Figure 1. Relative abundance of *A. brevis* pollen grains (outer circle) observed for each arthropod taxon sampled during 2017, 2018, and 2019 (inner circle).

The pollination-networks are well-structured and arthropod taxa involved varied among the 3 years analysed. In 2017, Hymenoptera Apidae and Diptera Anthomyiidae showed the highest number of botanical taxa within the pollen transported (Figure2.A), among which pollen of *Primula hirsuta* was the most abundant. This species showed a high relative abundance also within the amount of pollen observed for Hymenoptera Halictidae. Looking at the network from the perspective of plants (Figure2.B), Apidae collects and transports the highest number of grains of almost all the plants observed within the pollen. Only *Leuchantemopsis alpina* entrusted its pollen exclusively to Anthomyiidae.

In 2018, no Hymenoptera Apoidea were sampled, and Anthomyiidae were the taxon with the highest number of grains, which were mainly represented by *Primula hirsuta* (Figure2.C). High variety of plant taxa also occurred within the sample "other Diptera" which included pollen retrieved from Sciaridae, Chironomidae, Scathophagidae, Drosophilidae, Syrphidae, and Chloropidae. Similar to what was observed for Apidae in 2017, in 2018 most plants were visited and pollinated by Anthomyiidae. Indeed, the network observed from the perspective of plants, showed that almost all the grains of the various botanical taxa were transported by this family of Diptera (Figure2.D).

In 2019, different taxa of arthropods (Anthomyiidae, Halictidae, Collembola –

Entomobryiidae, Tullbergidae, Onychiuridae –, and Thripidae) carried pollen from many different plant taxa. The taxa with the greatest number of interactions are the halictids, which carry mainly pollen of Rosaceae and *Androsace* (Figure2.E). Also in this case, Apoidea and Anthomyiidae seem to be fundamental to the reproductive success of many plants, compared to other arthropod taxa (Figure2.F).

Subdividing pollen in relation of the main pollination strategy adopted by the plant taxon to which pollen belongs, entomophilous (*e.g.*, *Androsace brevis*, *Primula hirsuta*, Fabaceae,) and entomophilous/anemophilous (*e.g.*, *Kalmia procumbens,* Polygonaceae) pollen type represent almost all the grains observed for Hymenoptera Apoidea (Apidae and Halictidae) and Diptera Anthomyiidae (Figure3). The relative abundance of these two types of pollen decreases considering other arthropod taxa (in order: "other" Hymenoptera, Hemiptera, Thysanoptera, "other" Diptera, Coleoptera, Araneae, Collembola, and Hymenoptera Formicidae) on which the anemophilous pollen type (*e.g.*, Poaceae, Pinaceae) increases.

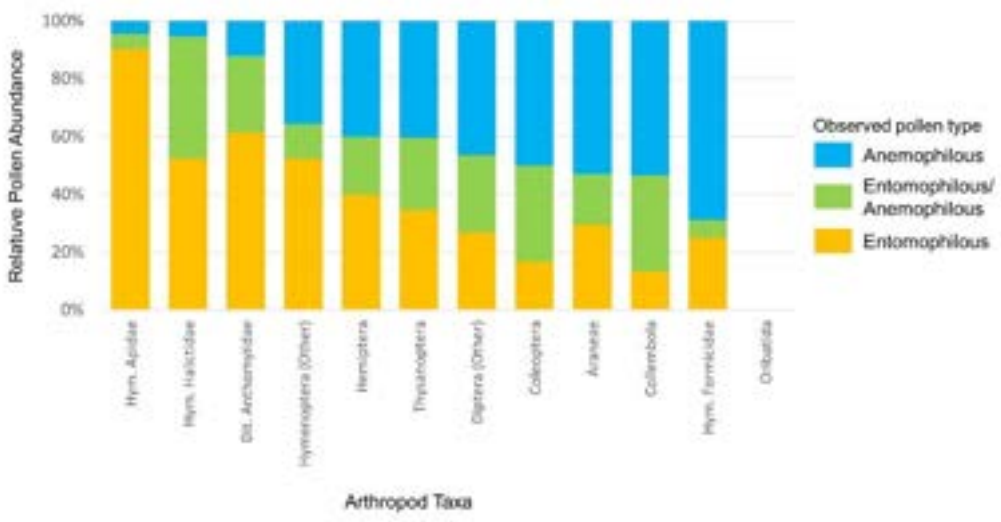

Figure 3. Relative abundance of pollen grains counted and identified for each arthropod taxon merging the data collected among the three years analysed. Pollen grains were divided in relation of the main pollination strategy of the plant taxa.

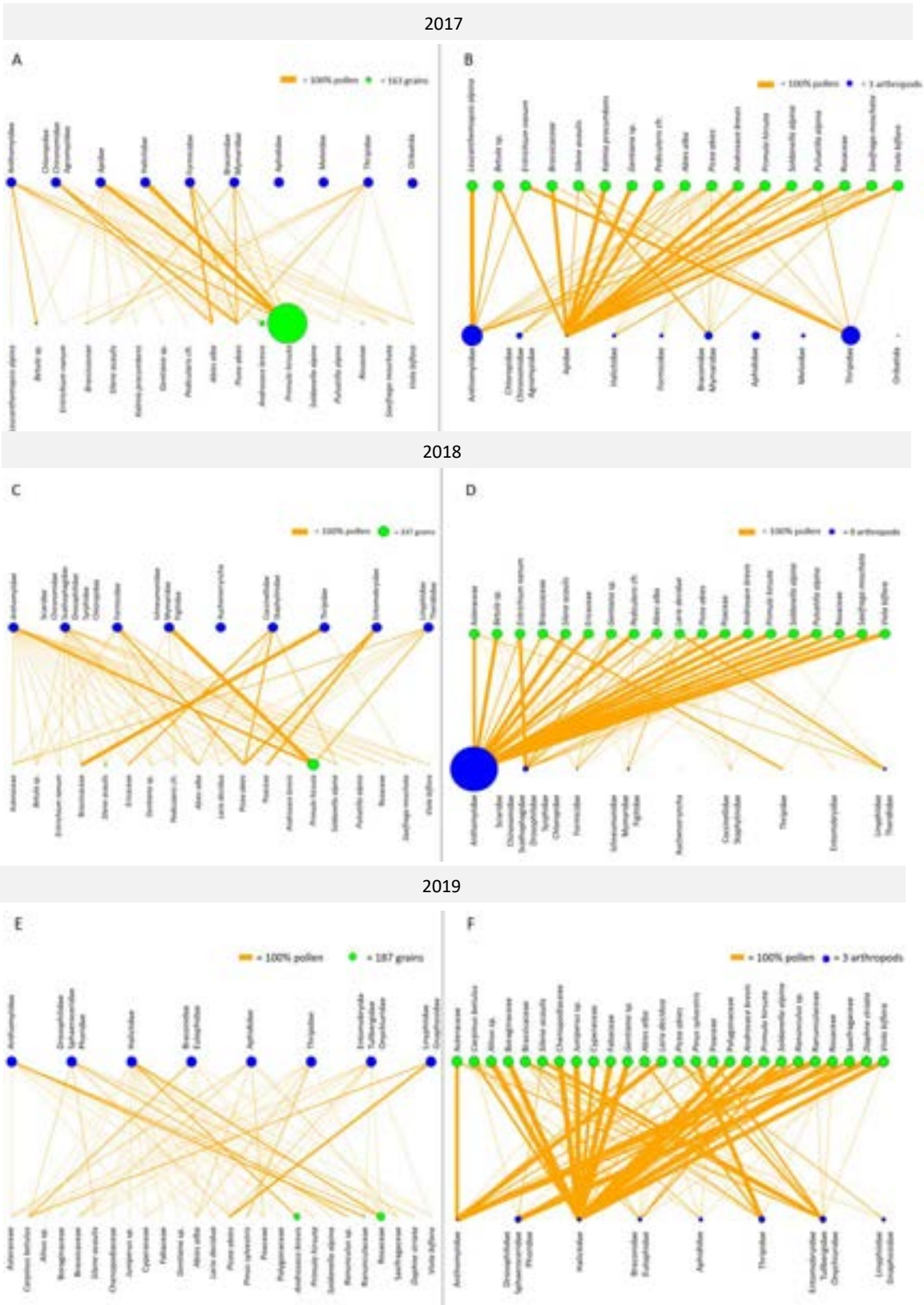

Figure 2. Resulting plant-pollinator networks of each analysed year represented from two different point of view. **A**;**C**;**E**: networks from the perspective of arthropods. The line thickness represents the percentage of pollen of different plant taxa found on an arthropod taxon; green dot size represents the annual abundance of pollen counted for each plant taxon. **B**;**D**;**F**: networks from the perspective of plants. The line thickness represents, as a percentage, on which arthropod taxa we found the pollen of each plant taxon; blue dot size represents the annual abundance of arthropod counted for each taxon.

4. Preliminary discussion

Although the sampling effort was focused on a single plant species during a particular moment of the season, resulting pollination-networks are well structured, with many arthropods carrying numerous pollen grains belonging to different plant taxa. The qualitative and quantitative information obtained through palynological analysis made it possible to observe which plant taxa were visited most by the different arthropod taxa sampled.

Characterizing the main *A. brevis* pollinators, it has been observed how the arthropod taxa that carry the highest amounts of its pollen, potentially acting as pollinators, are mainly Hymenoptera Apoidea (Apidae and Halictidae), represented by a male of *Bombus pratorum* (Linnaeus, 1761) and three female of *Lasioglossum* Curtis, 1833, and Diptera Brachycera (Anthomyiidae), represented mainly by *Paregle coerulescens* (Strobl, 1893) and *Delia platura* (Meigen, 1826), as reported in Bonelli et al. (2020; 2022). For both Apoidea and Anthomyiidae, *A. brevis* appears to be the second most visited species following *Primula hirsuta*, which may be subject to a higher visits-rate due to its greater frequency in the study area and the presence of a bigger floral display (Eustacchio et al., 2023). Overall, *Androsace brevis* and *Primula hirsuta* result to be the principal food resources on which the diet of Apoidea and Anthomyiidae is based during the early season. Moreover, both these two flower-visiting taxa are responsible of the transport of almost all the entomophilous plants identified, becoming therefore fundamental for the reproductive success of these plants. In particular, Apoidea collected also pollen of entomophilous plants not observed in bloom at the elevations where fieldwork was conducted, but probably were booming at lower altitudes (*e.g.*, *Pedicularis*). This observation highlights how much Apoidea, especially bumble bees, are able to move for longer distances respect to other arthropods (Maebe et al., 2021). Although the abundance and frequency of Apoidea tends to decrease with altitude (Lefebvre et al., 2018), their activity as floral visitors remain fundamental to the reproductive success of many entomophilous plants, even during the early season, given their ability to fly and forage when temperatures are still low (Lefebvre et al., 2018).

Regarding Anthomyiidae, *D. platura* and *P. coerulescens* are two common species related to high mountain ecosystems and to temperate areas of Northen Hemisphere and, occasionally, they feed on flowers searching for nectar and pollen (Boucher et al., 2021). Especially during the early season, the trophic resources could be limited within the environment and temperature could change repetitively in few minutes (Hågvar & Klanderud, 2009). Flower visiting by Anthomyiidae could therefore be linked both to the search for food and to thermoregulation activity. In the latter case, it is well known how dipterans are used to rest on flowers to thermoregulate, in a behaviour known as 'basking' (Faheem, 2004; Van der Kooi, 2019). Indeed, flowers maintain a constant warmer microclimate compared to the surrounding environment as it is essential for the plant both for the formation of nectar and pollen and for the optimal functioning of the flowers (Van der Kooi et al., 2019). Moreover, *A. brevis*, as many other Alpine plant species (e.g. *Silene acaulis*, *Eritrichium nanum*), produces an average of 50 flowers for plant creating a homogeneous apparent carpet (Eustacchio et al., 2023). This flowering pattern allows cushion plants to be highly attractive to flower-visitors, especially when temperature is low, as arthropods can search for nectar and pollen both spending less flight energy and obtaining greater profit in food rewards than in case of single-flower visits per plant (Kunin, 1993; Cartar et al., 2004; Dixit et al., 2020).

In “other” Diptera group, “other” Hymenoptera, Hemiptera, Thysanoptera, Araneae, Coleoptera, Hymenoptera Formicidae, and Collembola, *A. brevis* pollen was not observed (in exclusion of very few grains in “other” Diptera and “other” Hymenoptera taxa collected in 2017). For these taxa, the abundance of pollen belonging to entomophilous plants decreases becoming similar, or even lower, than the abundance of pollen grains of anemophilous plants. This result highlights how these taxa are not strictly interested in nectar or pollen seeking, but presumably visit flowers for other reasons. For example, Araneae, Coccinellidae, Formicidae, and “other” hymenopteran (parasitoid wasps), could search for a prey or for a host (*e.g*. aphids) in which to lay eggs (Žikic et al., 2017; Guariento et al., 2018a; 2018b); Thysanoptera may use flowers as mating site or as a nest for their colonies as well as a place where search for food (Kirk, 1984; 1984; McFarlane et al., 2015). These observations suggest that these taxa play a marginal role in pollination of alpine plants, and the pollen grains carried by

them were probably collected randomly walking on the ground or on the flowers.

Monitoring the same study area for three consecutive years, allowed us to observe how flower-visiting arthropod community change over years both in taxa composition and arthropod abundance. Only two families were recorded during all three years: Anthomyiidae (Diptera) and Thripidae (Thysanoptera); but their abundance was very different over years: Anthomyiidae abundance ranged from 1 specimen in 2019 to 70 specimens in 2018, while Thripidae abundance ranged from 1 specimen in 2018 to 9 specimens in 2017. These differences could be mainly due to the different persistence of snow cover on the ground observed among years, more than for anthropic environmental pressure as anthropic activities are very limited in the area. In 2017 and 2019 the surface of land covered by snow was larger than in 2018, potentially influencing the type and abundance of flower visitors. Anthomyiidae larvae, for example, develop in moist soils where excrements provided by cattle, sheeps and goats are present (Lefebvre et al, 2018). The persistence of snow on the ground, may inhibited the emergence and the activity of this taxon during 2017 and 2019. On the other hand, the higher abundance of Thripidae within flowers during the years with high snow cover could be linked to the use of flowers as shelter site and refugia as this taxon can wait for favourable temperatures inside the corolla tube of *A. brevis* (Woldemelak, 2008). The presence of Hymenoptera Apoidea only in 2017 and 2019, let us to hypothesise that this taxon increases its foraging area (till 9.9 kilometres in case of *Bombus terrestris* (Linnaeus, 1758)), when food resources surrounding the nest location are scarce or when bad weather conditions occur (Pyke et al., 2011). These reasons, together with the short alpine vegetative season, also lead bees to be more generalist in choosing floral resources than at lower elevations or warmer environments (Obeso, 1992; Pyke et al., 2011). This change in behaviour, that is fundamental to ensure bees own survival and the survival of their colonies, especially for Apidae, make this taxon very important not also in the reproductive success of plants living within the same population, but potentially also in creating and maintaining a pollen flow between displaced plant species populations (Dreisig, 1995; Westphal et al., 2006; Kremen et al., 2007; Cranmer et al., 2012).

Diptera Muscidae and Empididae were not observed in this study, despite these

families are usually very common flower-visitors in temperate high-altitude environments (Lefebre et al., 2014; Tiusanen et al., 2016; Lefebvre et al., 2018; Martínez-Díaz et al., 2023). Also Diptera Syrphidae were low represented in this work, as only two specimens belonging to *Scaeva dignota* Rodani, 1857 and *Eristalis similis* Fallen, 1817 were sampled in 2018. The low abundance of pollen grains retrieved on their bodies indicates that they are not central pollinators of *A. brevis,* neither of other plant taxa. This result highlights how during the early season the floral visitor community may be represented by a subset of taxa compared to those that can be observed later in the season. In support of this, our results are in accordance with the observations reported by Lefebre et al. (2018), where among the most common Alpine flower-visiting arthropods above 2000 m asl, Syprhidae are present but less abundant than Anthomyiidae in early season, while Muscidae and Empididae start their activity few weeks later. By the same way, Apidae are confirmed to be the most active flower visitors early in the season together, in our case, with Halictidae family.

During this particular time of the season, such a varied composition over the years in flower-visiting arthropod community, highlights how pollination is entrusted to "inconstant" and "fortuitous" arthropod taxa. To cope with this occurrence, *A. brevis*, as well as most of the plant species observed flowering during this period, shows different adaptations as: 1) a generalist strategy regarding pollinators (Eustacchio et al., 2023); 2) high longevity of plants (Östman, 2018). These strategies could be fundamental in a changing climate as they allow both to share pollinators with different plant species, potentially find new flower visitors in the future, and to cope with the possible absence or limited occurrence of flower visitors during some year, so helping to contrast critical plant-pollinator mismatches (Callaway et al., 2002; Kikvidze et al., 2005; Östman, 2018; Gerard et al., 2020; Inouye , 2020).

The possible consequences of climate warming may lead to a marked alteration of the type of floral resources and the floral visitor communities (D'Alò et al., 2021). The first issue is mainly represented by the migration of low-altitude plant species as well as by the alteration of soil composition characterizing alpine environments. In particular, higher temperatures increase the microbial activity, leading to a high release of micro- and macronutrients as nitrogen, phosphorus, sulphur, and other minerals, which in

high quantities, could represent a serious threat for alpine nutrient-poor soil adapted species (Margesin et al., 2014; Cavicchioli et al., 2019; Dorji et al., 2020; Xu et al., 2020; D'Alò et al., 2021). The second issue is mainly linked to the changing need of arthropods in visit flowers. The need in visiting flowers to thermoregulate as well as to supplement the diet with nectar and pollen, could decrease for those arthropods not closely related to floral resources to survive, such as Anthomyiidae and other Brachycera typical of high altitudes. Regarding Hymenoptera, increasing temperature could increase the abundance of Apoidea, leading both to an increase in competition for food resources and to increase the selectivity in choosing flower type (Galen, 1999). Moreover, a change in the flower composition and an increase in floral resources could decrease the visitation rate on each single plant.

In conclusion, in a scenario where composition of flower-visiting arthropod communities highly changes in function of environmental parameters mainly linked to weather conditions, the attractiveness of alpine-adapted plants could be different between years depending on the type of active arthropods. In this highly variable context, the effect of the ongoing climate warming on both the stability of alpine ecosystems and the survival of plant and arthropod communities, could be hardly predictable. However, a long-term study could be useful to better understand these complex interactions between plants and arthropods and how they evolve under climate change.

This work will include additional output as:

- analysis of pollination networks using parameters as "nestedness" and "weighted NODF" to describe the specialization of plant-arthropod interactions (Almeida-Neto & Ulrich, 2011; Almeida-Neto et al., 2008);
- Comparison of pollination networks identified among years and potential links with weather conditions using generalised linear models (GLMs)

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Supplementary Material

Supplementary Material S1. Flower-visiting arthropods sampled during the free observations during year 2019 in Orobic Alps population (Benigni Hut) of *Androsace brevis*. Specimen ID, order, family, genus, species, stage (A=adult, J=juvenile) and repository (UNIMI=UNIMI B: Department of Biosciences, University of Milan – Italy); MSN=Entomology Section, Milan Natural History Museum – Italy) reported. Blank lines mean that taxonomic identification was not achieved. Nomenclature is according to Fauna Europaea (De Jong et al., 2014). List of taxonomists*.

ID	Order	Family	Genus	Species	Author	Stage	Repository
VB01	Thysanoptera	Thripidae	*Thrips*	*Thrips vulgatissimus*	*Haliday, 1836*	A	UNIMI B
VB02	Diptera	Anthomyiidae	*Paregle*	*Paregle coerulescens*	Strobl, 1893	A	UNIMI B
VB03	Hymenoptera	Braconidae				A	MSN
VC01	Araneae	Gnaphosidae				J	UNIMI B
VD01	Hymenoptera	Halictidae	*Lasioglossum*		Curtis, 1833	A	UNIMI B
VD02	Thysanoptera	Thripidae	*Frankliniella*		Trybom, 1895	A	UNIMI B

*ARACHNIDA: Araneae=Paolo Pantini (Zoology of Invertebrate Section, Civic Museum of Natural Science Enrico Caffi, Bergamo, Italy); HEXAPODA: Collembola=Pietro Paolo Fanciulli (Department of Life Sciences, University of Siena, Siena, Italy), Barbara Valle (Department of Life Sciences, University of Siena, Siena, Italy; NBFC, National Biodiversity Future Center, Palermo, Italy); Diptera, Anthomyiidae=Verner Michelsen (Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark); Hymenoptera, Apoidea=Andree Cappellari (Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padua, Padua, Italy); Hymenoptera, Braconidae: Vladimir Žikić (Department of Biology and Ecology, University of Niš, Niš, Serbia), Željko Tomanović (Department of Invertebrate Zoology and Entomology, University of Belgrade, Belgrade, Serbia); Thysanoptera: Barbara Conti (Department of Agriculture Food and Environment, University of Pisa, Pisa, Italy).

Supplementary Material S2. Flower-visiting arthropods from which the carried pollen was retrieved, reported per family and year.

Order	Family	2017	2018	2019	Total (per family)
Hymenoptera	Braconidae	4		1	5
	Figitidae		1		1
	Mymaridae	1	1		2
	Eulophidae			1	1
	Ichneumonidae		2		2
	Formicidae	2	3		5
	Apidae	1			1
	Halictidae	2		2	4
Diptera	Sphaeroceridae			1	1
	Phoridae			1	1
	Chloropidae	1	1		2
	Drosophilidae		1	1	2
	Anthomyiidae	10	70	1	81
	Syrphidae		2		2
	Scathophagidae		1		1
	Agromyzidae	1			1
	Sciaridae		3		3
	Chironomidae	1	1		2
Coleoptera	Staphylinidae		1		1
	Meloidae	2			2
	Coccinellidae		1		1
Hemiptera	Aphididae	4		2	6
	Cicadellidae		1		1
Thysanoptera	Thripidae	9	1	3	13
Araneae	Linyphidae		4	1	5
	Gnaphosidae			1	1
	Theridiidae		1		1
Collembola	Tullbergidae			1	1
	Onychiuridae			1	1
	Entomobryidae		1	1	2
Oribatida		1			1

Supplementary Material S3. Flowering species observed both within a radius of 500 m and a difference in altitude of +/−200 m from the study site among 2017, 2018, and 2019. Nomenclature is referred to Pignatti, (2017)

Family	*Species*	Author
Asparagaceae	*Muscari* sp.	
Asteraceae	*Homogyne alpina*	(L.) Cass.
	Leucanthemopsis alpina	(L.) Heywood
Boraginaceae	*Eritrichium nanum*	(L.) Schrad. ex Gaudin
	Myosotis alpestris	F.W.Schmidt
Brassicaceae	*Arabis bellidifolia*	Crantz
	Cardamine hirsuta	L.
	Draba aizoides	L.
Caryophyllaceae	*Silene acaulis*	(L.) Jacq.
Cyperaceae	*Carex curvula*	All.
Ericaceae	*Erica carnea*	L., 1753
	Kalmia procumbens	(L.) Gift & Kron & P.F.Stevens ex Galasso, Banfi & F.Conti
Fabaceae	*Anthyllis alpicola*	Brügger
	Lotus corniculatus	L.
Gentianaceae	*Gentiana acaulis*	L.
	Gentiana verna	L.
Iridaceae	*Crocus vernus*	(L.) Hill
Liliaceae	*Gagea serotina*	(L.) Ker-Gawl.
Poaceae	*Festuca luedii*	(Markgr.-Dann.) Foggi, Gr. Rossi, Parolo et Wallossek
Polygalaceae	*Polygala chamaebuxus*	L.
	Polygala vulgaris	L.
Primulaceae	*Androsace vandellii*	(Turra) Chiov.
	Primula hirsuta	All.
	Soldanella alpina	L.
Ranuncolaceae	*Pulsatilla alpina*	(L.) Delarbre
	Ranunculus sp.	
Rosaceae	*Geum montanum*	L.
	Potentilla aurea	L.
Saxifragaceae	*Saxifraga moschata*	Wulfen
Thymelaeaceae	*Daphne striata*	Tratt.
Violaceae	*Viola biflora*	L.
	Viola hirta/thomasiana	
	Viola sp.	

Supplementary Material S4. Resulting qualitative and quantitative analysis of pollen retrieved from flower-visiting arthropods collected on *Androsace brevis* in 2017, 2018 and 2019. Family and lower taxonomic level reached in pollen identification is reported. Pollination strategy of plant to which pollen grains identified belong is also reported: E=Entomophylous strategy; A=Anemophilous strategy; B=Both Anemophilous and Enthomophylous strategy.

			Asteraceae		Betulaceae		Boraginaceae		Brassicaceae	Caryophyllaceae	Chenopodiaceae	Cupressaceae	Cyperaceae	Ericaceae		Fabaceae	Gentianaceae	Orobanchaceae	Pinaceae				Poaceae	Polygonaceae	Primulaceae			Ranunculaceae			Rosaceae	Saxifragaceae	Thymelaeaceae	Violaceae
			Leucanthemopsis alpina	\	*Betula* sp.	*Alnus* sp.	*Eritrichium nanum*	\	\	*Silene acaulis*	\	*Juniperus* sp.	\	\	*Kalmia procumbens*	\	*Gentiana* sp.	*Pedicularis* cfr.	*Abies alba*	*Larix decidua*	*Picea abies*	*Pinus sylvestris*	\	\	*Androsace brevis*	*Primula hirsuta*	*Soldanella alpina*	*Pulsatilla alpina*	*Ranunculus* sp.	\	\	*Saxifraga moschata*	*Daphne striata*	*Viola biflora*
	Pollination strategy		E	E	A	A	E	E	E	E	A	A	A	B	B	E	E	E	A	A	A	A	A	B	E	E	E	E	E	E	B	E	E	E
	Arthropod Order	Arthropod taxa																																
2017	Dip	Anthomyiidae	1	0	33	0	1	0	0	0	0	0	0	0	0	0	0	0	4	0	6	0	0	0	11	33	0	6	0	0	0	2	0	2
	Dip	Chloropidae; Chironomidae; Agromyzidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	9	0	0	0	2	0	0	0	0	0	0	0	0	1
	Hym	Apidae	0	0	56	0	0	0	32	2	0	0	0	0	25	0	26	2	5	0	6	0	0	0	148	1123	5	7	0	0	47	1	0	30
	Hym	Halictidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	32	0	0	0	0	0	2	0	0
	Hym	Formicidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
	Hym	Braconidae; Mymaridae	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	2	0	7	0	0	0	2	4	0	0	0	0	0	1	0	0
	Hym	Aphididae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Col	Meloidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Thy	Thripidae	0	0	0	0	3	0	6	2	0	0	0	0	0	0	0	0	3	0	7	0	0	0	0	2	0	4	0	0	0	0	0	2
	Cmb	Oribatida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2018	Dip	Anthomyiidae	0	10	17	0	1	0	6	76	0	0	0	17	0	0	1	32	8	0	10	0	8	0	29	321	9	24	0	0	9	12	0	39
	Dip	Sciaridae; Chironomidae; Scathophagidae; Drosophilidae;	0	0	3	0	3	0	1	3	0	0	0	1	0	0	2	2	5	1	1	0	0	0	0	0	3	2	0	0	3	1	0	2

		Syrphidae; Chloropidae																																
	Hym	Formicidae	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	5	0	0	0	0	2	0	0	0	0	0	0	0	0
	Hym	Ichneumonidae; Mymaridae; Figitidae	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	10	0	0	0	0	0	0	0	0
	Hem	Auchenorryncha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Col	Coccinellidae; Staphylinidae	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0
	Thy	Thripidae	0	1	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Cmb	Entomobryidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
	Ara	Linyphiidae; Theridiidae	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	2	2	0	0	0	0	4	0	0	0	0	0	0	0	0
2019	Dip	Anthomyiidae	0	5	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	9	0	0	2	0	0	1	0	0	47	2	21	6	0	0
	Dip	Drosophilidae; Sphaeroceridae; Phoridae	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	1	0
	Hym	Halictidae	0	0	4	8	0	2	0	0	1	1	5	0	0	6	10	0	1	1	2	0	1	5	187	3	2	0	0	1	204	5	0	15
	Hym	Braconidae; Eulophidae	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	1
	Hem	Aphididae	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
	Thy	Thripidae	0	0	0	2	0	0	1	1	0	0	1	0	0	0	0	0	3	0	1	4	0	0	0	0	0	0	0	0	2	0	0	0
	Cmb	Entomobryidae; Tullbergidae; Onychiuridae	0	2	0	2	0	0	2	3	0	0	1	0	0	0	0	0	0	1	6	1	3	0	0	0	0	0	3	0	2	0	0	2
	Ara	Linyphiidae; Gnaphosidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0

Chapter 2.5

Braconidae (Hymenoptera) in the Central Southern Alps: the first Alpine record of the alien parasitoid *Lysiphlebus testaceipes* (Cresson) and new species for Italy

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BRACONIDAE (HYMENOPTERA) IN THE CENTRAL SOUTHERN ALPS: THE FIRST ALPINE RECORD OF THE ALIEN PARASITOID *LYSIPHLEBUS TESTACEIPES* (CRESSON) AND NEW SPECIES FOR ITALY

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Tomanović Ž., Žikić V., Pietra F., Eustacchio E., Bonelli M. - Braconidae (Hymenoptera) in the Central Southern Alps: the first Alpine record of the alien parasitoid *Lysiphlebus testaceipes* (Cresson) and new species for Italy.

Despite the ecological importance of parasitoid wasps for the stability of Alps ecosystems, the available knowledge about braconid wasps for these environments is still very limited. Here, we explored the diversity of Alpine braconid parasitoids in the Southern Central Alps. We recorded 18 species of braconids, 12 of which are from the subfamily Aphidiinae. In particular, we detected for the first time the alien parasitoid species *Lysiphlebus testaceipes* (Cresson) in the Alps, providing information that could be considered to evaluate the invasiveness of this species. The second most diverse subfamily was Microgastrinae with two detected species, while one species was recorded from each of the subfamilies Alysiinae, Braconinae, Cheloninae and Euphorinae. Four braconid species from the subfamily Aphidiinae – *Aphidius hieraciorum* Starý, *Aphidius schmitzcheki* (Starý), *Harkeria angustivalvus* (Starý) and *Monoctonus crepidis* (Haliday) – are new records to the fauna of Italy. This work contributes to the knowledge of braconid diversity in the Alps. Moreover, it can be a starting point both to explore complex tritrophic interactions potentially threatened by climate change and the role of early flowering Alpine plants on braconid diversity.

KEY WORDS: Alps, Alpine ecosystems, biotic interactions, braconids, Italian fauna, parasitoids.

INTRODUCTION

Research on Alpine ecosystems is related to the conservation and natural protection of unique biodiversity at high altitudes. Exploring Alpine biodiversity can lead to an understanding of its ecosystem functions, and to predict possible threats from global climate changes (KÖRNER, 2001; ERNAKOVICH *et al.*, 2014). Parasitoid insects consist of the dominant component of terrestrial ecosystems belonging to tritrophic associations which include host plants and usually host insects feeding on those plants (GODFRAY, 1994). Due to the high degree of plant endemism in the European Alps (MENCHETTI *et al.*, 2021; PARISOD, 2022), Alpine ecosystems might consequently support unique tritrophic associations and parasitoid diversity (STARÝ *et al.*, 1971; PENNACCHIO and TREMBLAY, 1988).

In recent decades, many new species of braconid parasitoid wasps (Hymenoptera: Braconidae), mainly from the subfamily Aphidiinae, have been described or registered from Alpine and high mountains areas throughout Europe (STARÝ *et al.*, 1971 – western Europe; HALMØ, 1992 – northern Europe; STARÝ *et al.*, 1998, TOMANOVIĆ and KAVALLIERATOS, 2002, TOMANOVIĆ *et al.*, 2002, 2004, 2007; KAVALLIERATOS *et al.*, 2004 – south-eastern Europe; LOZAN *et al.*, 2010 – southern and central Europe; HAVELKA *et al.*, 2014). However, despite the ecological importance of parasitoids for the stability of Alps ecosystems (KENIS *et al.*, 2005), the available knowledge about braconid wasps on these mountains is still very limited (STARÝ *et al.*, 1971; KENIS *et al.*, 2005; BONELLI *et al.*, 2022).

The goal of our research is to detect new braconid species for Alpine ecosystems; this could lead to future conservation programs and to a deeper understanding of the biotic interactions occurring in these fragile environments, especially under pressure of climatic changes. Our research on braconid diversity focused on the Central Southern Alps and it is related to a broader research on the ecology of the vulnerable narrow endemic plant *Androsace brevis* (Hegetschw.) Cesati (Primulaceae). This plant is one of the few floral resources available for arthropods in the early season – when snow cover is still present – and braconids have been identified among its most abundant flower visitors (BONELLI *et al.*, 2020; BONELLI *et al.*, 2022), therefore it serves as a good starting point to search for braconid species and to investigate their role in high mountain ecological networks.

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MATERIALS AND METHODS

The two investigated sites host two different representative populations of *Androsace brevis*, within its distribution range (Eustacchio *et al.*, 2023). The first site is in the Lepontine Alps (Como, Lombardy, Italy) on the Cugn Peak (UTM WGS84–32T E 512338 N 5112905, 2193 m asl), while the second site is in the Orobic Alps (Bergamo, Lombardy, Italy) near the Mountain Hut "Cesare Benigni" (UTM WGS84–32T E 543496 N 5096577, 2222 m asl). A more detailed description of the two selected sites is given in Bonelli *et al.* (2020) and Bonelli *et al.* (2022). Timed-observation samplings on *A. brevis* flowers were performed in May-June from 2016 to 2021. More information about the sampling effort and methods are reported by Bonelli *et al.* (2022). Finally, to increase the amount of available data, a further period of sampling was performed by sweeping in June-July 2022 in both sites. As the Cugn Peak site is more easily accessible, in this site we also sampled plants infested with aphids, as they are possible hosts of Aphidiinae. Plants were identified on-site during fieldwork. Plant parts, mainly leaves infested with aphids, and aphid mummies were transferred to plastic containers with perforated lids and kept under laboratory conditions until the parasitoid emergence. Some collected winged and wingless adult aphids were preserved in 70% ethyl alcohol and later identified by a specialist in the laboratory. After emergence, parasitoids were fixed in 96% ethyl alcohol and preserved for later examinations. Braconids were identified using existing identification keys (Starý, 1973; Tobias *et al.*, 1986; Tomanović *et al.*, 2018). Additionally, morphological identification of some specimens has been confirmed by barcoding COI gene – *Aphidius ervi* Haliday (OQ029512), *A. schimitscheki* (Starý) (OQ029508), *A. uzbekistanicus* Luzhetzki (OQ029509, OQ029510), *Diaeretiella rapae* (M'Intosh) (OQ029511). The external morphology of the specimens was studied using a ZEISS Discovery V8 stereomicroscope. Aphidiinae specimens are deposited in the collection of the Institute of Zoology, Faculty of Biology, University of Belgrade, Serbia. The rest of the parasitoid specimens are stored at the Faculty of Sciences and Mathematics, University of Niš, Serbia.

RESULTS

A total of 18 parasitoid species were recorded, with four species new to the fauna of Italy and with the first record of the alien species *Lysiphlebus testaceipes* (Cresson) in the Alps.

The overview of the recorded braconids is given below. For each species, collection data and general biological distributions are reported. Records marked with an asterisk [*] represent new species for the Italian fauna. Abbreviations for legators: MB = Marco Bonelli, ŽT = Željko Tomanović.

Subfamily Alysiinae

Aspilota fuscicornis (Haliday, 1838)

Material examined: 1♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 01-02.VII.2022, leg. ŽT (sweeping).

Remarks: Recorded mainly as parasitoid of *Euleia heraclei* (L.) (Diptera: Tephritidae), as reported by Frost (1924), and *Spiniphora bergenstammi* (Mik) (Diptera: Phoridae) (Yu, 2019), but also of necrophagous dipterans (Frederickx *et al.*, 2013).

Distribution: Europe (van Achterberg, 2014).

Subfamily Aphidiinae

Aphidius avenae Haliday, 1834

Material examined: 3♀, Mountain Hut "Cesare Benigni" (Orobic Alps, Bergamo, Lombardy, Italy), 2222 m, 31.V-01.VI 2017, *leg.* MB (collected *ex A. brevis* flowers).

Remarks: Polyphagous parasitoid of various aphid species, of which the most important are pea aphid – *Acyrthosiphon pisum* (Harris), rose aphid – *Macrosiphum rosae* (L.), green peach aphid – *Myzus persicae* (Sulzer), and many species of cereal aphids e.g., *Rhopalosiphum padi* (L.), *Sitobion avenae* (F.) and *Schizaphis graminum* (Rondani) (Tomanović *et al.*, 2021).

Distribution: Palaearctic (van Achterberg, 2014); introduced to Africa (Burundi) and America (Brazil, Chile, United States of America) (PeSalver-Cruz *et al.*, 2017).

Aphidius ervi Haliday, 1834

Material examined: 8♀, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 25-26.V.2016, leg. MB (collected ex *A. brevis* flowers); 1♀, Mountain Hut "Cesare Benigni" (Orobic Alps, Bergamo, Lombardy, Italy), 2222 m, 01.VI.2017, leg. MB (collected ex *A. brevis* flowers); 1♀, Mountain Hut "Cesare Benigni" (Orobic Alps, Bergamo, Lombardy, Italy), 2222 m, 01-04.VI 2022, leg. MB (sweeping); 2♀ and 2♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 01-02.VII.2022, leg. ŽT (sweeping).

Remarks: This is a polyphagous species recorded on approximately 120 hosts worldwide. Among them, pea aphid – *A. pisum*, and English grain aphid – *S. avenae*, are very common hosts (Yu, 2019). The host range for this parasitoid is displayed in Žikić *et al.* (2017).

Distribution: cosmopolitan (van Achterberg, 2014).

Aphidius hieraciorum Starý, 1962

Material examined: 1♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 02.VII.2022, leg. ŽT (emerged from *Nasonovia* sp. collected on *Hieracium* sp.).

Remarks: A specialized aphid parasitoid of the genus *Nasonovia* Mordvilko, that mainly attacks *Hieracium* spp. (Tomanović *et al.*, 2008).

Distribution: Europe (van Achterberg, 2014).

Aphidius schimitscheki (Starý, 1960)

Material examined: 1♀ and 1♂, Mountain Hut "Cesare Benigni" (Orobic Alps, Bergamo, Lombardy, Italy), 2222 m, 01-04.VI.2022, leg. MB (sweeping).

Remarks: A monophagous parasitoid that develops in *Elatobium abietinum* (Walker) a spruce-feeding aphid on *Abies alba* Mill., *Picea abies* (L.) H. Karst., *P. pungens* Engelm., and *P. sitchensis* (Bong.) Carr. (Starý, 1973; Tomanović *et al.*, 2021).

Distribution: Europe (Czech Republic, Germany, Hungary, Serbia, Slovakia, United Kingdom), Asia (India, Pakistan) (van Achterberg, 2014; Das and Chakrabarti, 2023).

Aphidius urticae Haliday, 1834

Material examined: 1♀, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 26.V.2016, leg. MB (collected ex *A. brevis* flowers).

Remarks: An oligophagous parasitoid primarily related to the aphid hosts of the genera *Macrosiphum* Passerini, *Acyrthosiphon* Moedvilko, but also *Amphorophora* Buckton, *Microlophium* Mordvilko, etc. (Jamhour *et al.*, 2016).

Distribution: Holarctic, Oriental, Oceania (van Achterberg, 2014).

Aphidius uzbekistanicus Luzhetzki, 1960

Material examined: 1♀ and 1♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 04.VI.2021, leg. MB (collected ex *A. brevis* flowers).

Remarks: This oligophagous species attacks aphids that infest cereals, such as *Diuraphis* Aizenberg, *Metopolophium* Hille Ris Lambers, *Rhopalosiphum* Koch, *Schizaphis* Börner, *Sipha* Passerini, *Sitobion* Mordvilko (Kos *et al.*, 2011).

Distribution: Palaearctic, Nearctic, Neotropical, and Oriental (van Achterberg, 2014); introduced in Argentina, Brazil, Chile, and USA (Starý, 1993).

Diaeretiella rapae (M'Intosh, 1855)

Material examined: 1♀, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 26.V.2016, leg. MB (collected ex *A. brevis* flowers).

Remarks: The primary host for *D. rapae* is the aphid *Brevicoryne brassicae* (L.) from cultivated and uncultivated crucifers (Pike *et al.*, 1999; Kavallieratos *et al.*, 2004).

Distribution: Palaearctic, Oriental, Neotropical, Oceania, Afrotropical (van Achterberg, 2014); introduced to North America, and Australia (Baer *et al.*, 2004).

Harkeria angustivalvus (Starý, 1959)

Material examined: 6♀ and 1♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 02.VII.2022, leg. ŽT (emerged from *Nasonovia* sp. collected on *Hieracium* sp.).

Remarks: Like *Aphidius hieraciorum*, *H. angustivalvus* is a specialized aphid parasitoid of *Nasonovia* spp. (Tomanović *et al.*, 2008).

Distribution: Europe (van Achterberg, 2014).

Lysiphlebus confusus Tremblay *et* Eady, 1978

Material examined: 177♀ and 25♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 02.VII.2022, leg. ŽT (emerged from *Brachycaudus cardui* (L.) collected on *Cirsium spinosissimum* (L.) Scop.).

Remarks: Genetically and morphologically, this polyphagous species is very close to the below mentioned *Lysiphlebus fabarum* (Marshall). It has a largely overlapping host range with *L. fabarum*. European populations are mostly asexual; this finding is one of the few sexual populations of *L. confusus* in Europe (Tomanović *et al.*, 2018).

Distribution: Palaearctic, Oriental (van Achterberg, 2014; Tomanović *et al.*, 2018).

Lysiphlebus fabarum (Marshall, 1896)

Material examined: 206♀ and 41♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 02.VII.2022, leg. ŽT (emerged from *B. cardui* collected on *Carduus acanthoides* L.); 10♀, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 02.VII.2022, leg. ŽT (emerged from *B. cardui* collected on *C. spinosissimum*).

Remarks: This is one of the most polyphagous Aphidiinae, found on over 100 aphid hosts from many genera and the most common *Lysiphlebus* species in European crop and non-crop habitat. *L. fabarum* is very promising potential agent for the biological control of many agricultural pest aphids. Most important host of *L. fabarum*

in agroecosystems is *Aphis craccivora* Koch, on lucerne, clover, bean; *A. gossypii* Glover, on cucumber, potato; *A. fabae* Scopoli, on corn, bean, beet, parsnip; *A. idaei* van der Goot, on raspberry, blackberry; *A. pomi* De Geer and *Dysaphis plantaginea* (Passerini) on apple; *Brachycaudus helichrysi* (Kaltenbach) and *B. schwartzi* (Borner) on peach; *Myzus persicae* (Sulzer) on peppers (Tomanović *et al.*, 2018).

Distribution: Palaearctic, Australasia, Oceania, Oriental (van Achterberg, 2014; Tomanović *et al.*, 2018).

Lysiphlebus testaceipes (Cresson, 1880)

Material examined: 14♀ and 15♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 02.VII.2022, leg. ŽT (emerged from *B. cardui* collected on *C. acanthoides*); 196♀ and 93♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 02.VII. 2022, leg. ŽT (emerged from *B. cardui* collected on *C. spinosissimum*).

Remarks: It is already noted that this is an allochthonous and potentially invasive species in Europe, which started spreading on the east of the continent from the Mediterranean where competes with native *Lysiphlebus* species (Mitrović *et al.*, 2013; Žikić *et al.*, 2015) (Fig. I).

Distribution: Holarctic, Afrotropical, Neotropical (van Achterberg, 2014; Tomanović *et al.*, 2018).

Monoctonus crepidis (Haliday, 1834)

Material examined: 2♀ and 2♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 02.VII.2022, leg. ŽT (emerged from *Nasonovia* sp. collected on *Hieracium* sp.).

Remarks: Another species that is primarily related to representatives of the genus *Nasonovia* e.g., *N. ribisnigri* (Mosley), *N. pilosellae* (Borner) associated with *Hieracium* spp., *Crepis* spp. and several other plants from the family Asteraceae (Tomanović *et al.*, 2008).

Distribution: Palaearctic (van Achterberg, 2014).

Subfamily Braconinae

Bracon intercessor Nees, 1834

Material examined: 1♀, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 01-02.VII.2022, leg. ŽT (sweeping).

Remarks: This species is ectoparasitoid recorded as generalist on coleopterans such as *Rhynchites bacchus* L. (Attelabidae), many weevils: *Microlarinus* Hochhuth, several *Anthonomus* Germar, e.g., *A. pomorum* (L.), *A. pedicularius* (L.), *A. sorbi* Germar, *Apion* Herbst spp., *Lixus* F. spp. (Curculionidae), then some Tenthredinidae (Hymenoptera), from the genus *Pontania* Costa, also Torticidae, e.g., *Spargamothis* Hübner spp. (Yu, 2019).

Distribution: Palaearctic (van Achterberg, 2014).

Fig. I - Lateral view of a female *Lysiphlebus testaceipes* recorded from Lepontine Alps.

Subfamily Cheloninae

Ascogaster variipes Wesmael, 1835

Material examined: 1♀, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 01-02.VII.2022, leg. ŽT (sweeping).

Remarks: This egg-larval parasitoid attacks microlepidoptera, e.g., Tortricidae, primarily several species of the genus *Cydia* Hubner, but also *Endothenia* Stephens, and *Epinotia* Hubner, (Tobias, 1976; Čapek and Hofmann, 1997).

Distribution: Palaearctic (van Achterberg, 2014).

Subfamily Euphorinae

Pygostolus falcatus (Nees, 1834)

Material examined: 1♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 01-02.VII.2022, leg. ŽT (sweeping).

Remarks: *P. falcatus* parasitizes Coleoptera Curculionidae, primarily *Phyllobius* Germar, and *Sitona* Germar, e.g., *S. lineatus* (L.), *S. hispidulus* (F.), and *S. humeralis* Stephens (Loan and Holdaway, 1961).

Distribution: Holarctic (van Achterberg, 2014).

Subfamily Microgastrinae

Sathon falcatus (Nees, 1834)

Material examined: 1♀, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 01-02.VII.2022, leg. ŽT (sweeping).

Remarks: This species has been reared from lepidopteran larvae of *Eupithecia succenturiata* (L.) (Geometridae), *Hellinsia osteodactylus* (Zeller) (Pterophoridae), *Apamea lateritia* (Hufnagel), *A. monoglypha* (Hufnagel), *Noctua* (L.) (Noctuidae), *Synanthedon tipuliformis* (Clerck) (Sesiidae) (Williams, 1988).

DISTRIBUTION: Palaearctic, Oriental (VAN ACHTERBERG, 2014).

Glyptapanteles fulvipes (Haliday, 1834)

MATERIAL EXAMINED: 2♂, Cugn Peak (Lepontine Alps, Como, Lombardy, Italy), 2194 m, 01-02.VII.2022, leg. ŽT (sweeping).

REMARKS: Very important natural enemy of the gypsy moth, *Lymantria dispar* (L.) and the yellow tail, *Euproctis similis* (Füssli) (Erebidae) pests in orchards and forests. This parasitoid is also recorded from more than 30 noctuids (YU, 2019).

DISTRIBUTION: Holarctic (VAN ACHTERBERG, 2014).

DISCUSSION

We conducted our sample quite early in the season, which can be a critical moment for possible climate change-driven mismatches in biotic interactions (KUDO and COOPER, 2019; ETTINGER *et al.*, 2021), we found 18 species of braconid parasitoids from 6 subfamilies (Aphidiinae (12), Alysiinae (1), Braconinae (1), Cheloninae (1), Euphorinae (1), Microgastrinae (2).

With respect to Aphidiinae, *Lysiphlebus testaceipes* was introduced from Cuba to Mediterranean France in 1973, to control citrus aphids (STARÝ *et al.*, 1988a). In a relatively short period, *L. testaceipes* spread in the coastal area of the Mediterranean, including North Africa (STARÝ *et al.*, 1988a; SUAY CANO and MICHELENA SAVAL, 1997; KAVALLIERATOS *et al.*, 2004; HAVELKA *et al.*, 2011). It has been detected in many localities in southern and central Italy in citrus plantations (TREMBLAY *et al.*, 1978). *L. testaceipes* also started to invade the interior of the Iberian Peninsula and the Pyrenees (PONS *et al.*, 2004; STARÝ *et al.*, 2004), and later in South-eastern Europe (Serbia) along the river valleys (Nišava and Južna Morava) (ŽIKIĆ *et al.*, 2015) adapting to new aphid hosts. After its introduction in Europe, *L. testaceipes* has acquired over 30 aphid hosts (STARÝ *et al.*, 1988b; KAVALLIERATOS *et al.*, 2004; TOMANOVIĆ *et al.*, 2009). Due to the spread and adoption of new aphid hosts and competition with native parasitoids, despite the effectiveness of this species as biocontrol agent, the EPPO excluded it from the positive list of biological control agents (EPPO, 2009; EPPO, 2021). Here, to the best of our knowledge, we detect this species for the first time in the Alps, and this is the first record of *L. testaceipes* over 2000 m asl in Europe (STARÝ *et al.*, 2004; MITROVIĆ *et al.*, 2013; ŽIKIĆ *et al.*, 2015), providing new insights on the possible invasiveness of this species. In our samples, *L. testaceipes* dominates in the host range pattern of *B. cardui* aphids on *Carduus* and *Cirsium* plants in some samples over native *Lysiphlebus* parasitoids (*L. confusus* and *L. fabarum*). The spreading of *L. testaceipes* over fragile Alpine ecosystems could lead to a negative impact on the structure of native parasitoid complexes and consequently change ecosystems' functioning.

Regarding *L. confusus*, it is interesting to note that it consists predominantly of asexual populations throughout Europe, except for a few sexual populations in southeastern Europe (Greece) and northern Europe (Finland). We have detected additional sexual populations from the association of *B. cardui*/*Cirsium spinosissimum* in the Lepontine Alps. *L. fabarum* consists of asexual and sexual populations throughout the Palaearctic (TOMANOVIĆ *et al.*, 2018). In the Lepontine Alps, we also detected a sexual population of *L. fabarum* dominated by females. These findings may be helpful for future studies to evaluate the factors involved in determining the drivers of geographic parthenogenesis and sex determination process.

Additionally, we found nine other Aphidiinae species in the investigated area of Alpine ecosystems. According to Fauna Europea (VAN ACHTERBERG, 2014) and the available literature, we recorded four new Aphidiinae species to Italy – *Aphidius hieraciorum*, *A. schimitscheki*, *Harkeria angustivalvus*, and *Monoctonus crepidis*. According to STARÝ (1970) *A. hieraciorum*, *H. angustivalvus*, and *M. crepidis* belong to the faunal complexes of European deciduous forests and represent a member of the parasitoid guilds of *Nasonovia* aphids on *Hieracium* plants. *A. schimitscheki* belongs to West – Eurasian Coniferous Forest faunistic complexes (STARÝ, 1970). Based on literature data, we could assert that all four species are mountain faunistic elements in the southern Europe (KAVALLIERATOS *et al.*, 2004). It is worth noting that *A. schimitscheki* has so far been recorded in only a few European countries (VAN ACHTERBERG, 2014). It is a specific parasitoid of *Elatobium abietinum* aphids on *Picea* trees. Although these parasitoid species are not directly related to pest aphids in agroecosystems, it is known that *M. crepidis* parasitizes *Nasonovia ribisnigri* on lettuce (TOMANOVIĆ *et al.*, 2008). Other recorded species are widely distributed and cosmopolitan, parasitizing several aphid species in crop and non-crop habitats (e.g., *A. avenae*, *A. ervi* – cereal and legume aphids; *A. uzbekistanicus* – cereal aphids; *D. rapae* – aphids on Brassicaceae and vegetables; *L. fabarum* – aphids on various vegetables, legumes and orchards). All these parasitoid species can regulate aphid population density in agroecosystems to a greater or lesser extent (KAVALLIERATOS *et al.*, 2004; TOMANOVIĆ *et al.*, 2009).

In addition to Aphiidinae, five other species of Braconidae, known as widely distributed in Europe, were detected.

Aspilota fuscicornis (Alysiinae) is widely distributed on the European continent and belongs to the tribe Alysiini, as well as to the group of genera called the "*Aspilota*-group" (VAN ACHTERBERG, 1988; YU, 2019). This species is associated with cyclorrhaphous dipteran hosts, parasitizing their plant-feeding larvae (FISCHER, 1975; FISCHER *et al.*, 2014), as well as necrophagous dipterans in urban biotope (FRÉDERICKX *et al.*, 2013). *Bracon intercessor* (Braconinae), a generalist parasitoid, is one of about 80 *Bracon* species inhabiting Italy (VAN ACHTERBERG, 2014). *Ascogaster varipes* is a solitary en-

doparasitoid that, like other members of the subfamily Cheloninae, parasitizes the eggs of microlepidoptera, and leaves the host when it is in the larval stage. A parasitoid of weevils, *Pygostolus falcatus* (Euphorinae) is one of the four species of this genus registered in Europe. In addition to this species, *P. multiarticulatus* (Ratzeburg) and *P. stictiens* (F.) are also known to be present in the fauna of Italy. Microgastrinae are a very diverse subfamily of braconids both in Europe and in the world. They are solitary and gregarious parasitoids of exophytophagous and mining caterpillars. Therefore, some of them, such as *Cotesia glomerata* (L.) have been investigated as natural agents for the biological control of *Pieris* (Schrank) spp. Due to its long ovipositor, *Sathon falcatus* is specialized for parasitizing concealed microlepidopteran larvae, while *Glyptapanteles fulvipes*, like other members of this diverse genus, which have a relatively short ovipositor, is adapted to oviposition in exposed hosts such as many members of Erebidae, Geometridae, Lasiocampidae or Noctuidae (Yu, 2019). All recorded braconids not trophically connected with aphids discussed here are important members of parasitoid complexes, associated with their dipteran, lepidopteran and coleopteran hosts (Yu, 2019).

The detected species likely do not reflect the diversity along the whole season present in the researched area, although there is no recent checklist of Braconidae for Italy with which to compare it and especially there is not at all a checklist for the Alps. However, apart from the relatively well-studied subfamily Aphidiinae for the territory of Italy (Starý, 1965), the other subfamilies are scarce in data. Based on old reports that have not been verified, the checklist of Italian braconids consists of 866 species (Bergamasco *et al*., 1995), which is low, considering the geographical position of Italy and its diverse terrain.

This work contributes to the knowledge of braconid diversity in the Alps. Providing new data, especially for the poorly investigated early season, can be relevant to gain new faunistic knowledge about parasitoids which may have a main role in Alpine ecosystems. Moreover, it can provide a baseline to explore complex tritrophic interactions that may be threatened by climate change and the role of early flowering alpine plants on braconid diversity.

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Chapter 3

Plant and flower-visiting arthropod communities within the Stelvio National Park

Chapter 3.1

Impact of land management and elevation on composition and structure of alpine flower visiting communities

Work in preparation

Impact of land management and elevation on composition and structure of alpine flower visiting communities

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Abstract

Decline in pollinators due to anthropogenic activities is acknowledged in many ecosystems, including Alps. Here, seminatural habitats as meadows and pastures, which host a high plant and arthropod biodiversity and are recognized as biodiversity hotspots of pollinators as bees and hoverflies, are highly threatened mainly by changing in land use. Despite threats affecting flower-visiting arthropod communities, little is known about their ecology and how they are impacted by environmental changes, especially in mountain environments.

This two-year study (2021-2022) aims to investigate the impact of elevation and land use (orchards, hay-meadows, pastures, high-altitude grassland) on the composition, abundance, and diversity of flower-visitor communities in mountainous environments, including a focus on the impact on species richness of wild Hymenoptera Apoidea and Diptera Syrphidae.

Sampling plots were located along the Martello valley (Stelvio National Park, Italian Alps) along an altitudinal gradient (900-2700 m asl) and surveyed from May to August. Four plant families (Asteraceae, Fabaceae, Ranunculaceae/Rosaceae) were selected as models for sampling flower-visiting arthropods. Bayesian multivariate Generalised Linear Mixed Models were applied to assess the influence of environmental parameters (elevation, plant family, land use) both on abundance of all flower-visitors and species diversity of wild bees and hoverfly.

Results highlight a high abundance of Coleoptera and Thysanoptera in environments located above the tree line, suggesting that pollination may be performed by more taxa than those commonly studied. Diversity and abundance of Apoidea decreases with increasing elevation, being replaced by hoverflies and flies. Arthropod taxa are affected differently by land use types: Brachycera, Hymenoptera wasps, and Hemiptera are associated with high managed land uses (orchards and hay meadows), whereas wild Apoidea and Coleoptera are favoured by lower intensity management.

These results could give fundamental knowledge for conservation management organizations to maintain and promote a high biodiversity in plant and arthropods communities.

1 Introduction

Plants and arthropods share a long evolutionary history, during which many and varied interactions developed. In particular, the Angiosperm radiation offered to arthropods the possibility to exploit the resource constituted by the reproductive structure of this plant group, the flower. It has been estimated that about 30% of arthropods visit flowers for various purposes, such as foraging, hunting, sheltering, thermoregulating, mating, and laying eggs (Kevan & Backer, 1983; Cooley, 1995; Wardhaugh, 2015). The flower visiting arthropods can act as pollen vectors thus being involved in plant pollination, a fundamental process for the functioning of natural and agricultural ecosystems. It has been estimated that about 80% of wild plant species and 75% of crops are directly dependent by insect pollination for fruit set (Potts et al., 2010). Among the most common and studied arthropod flower visitors there are insects belonging to the orders Hymenoptera, Diptera, Coleoptera and Lepidoptera, but also other orders such as Thysanoptera, Hemiptera and Orthoptera comprehend flower visiting species even though their role in pollination is less investigated (Wardhaugh, 2015).

In the last years, insect population declines were reported worldwide (Sánchez-Bayo & Wyckhuys, 2019; Wagner, 2020) and particular concern is raised about the possible consequence of pollinator declines (Van der Sluijs, 2020; Chaptou et al., 2021; Janousek et al., 2023). Even if the knowledge about this topic is still incomplete and scattered (Montgomery et al., 2020; Wagner, 2020; Weisser et al., 2023), it is possible to identify the anthropogenic pressure as the origin of the main drivers of insect decline. These drivers include climate change, alien species, pests and pathogens, electromagnetic pollution, use of pesticides, land use intensification, and degradation, fragmentation, and loss of habitats (Potts et al., 2010; Sánchez-Bayo & Wyckhuys, 2019; Inouye, 2020; Wagner; 2020; Harvey et al., 2023). These diverse factors can interact and contribute to undermine the state of health of insect populations, and it is impossible to identify a sole cause of decline; however, it is widely recognised that the alterations in a land use practice is one of the main factors impacting on flower visiting communities (Sànchez-Bayo & Wyckhuys, 2019).

Considering Alpine ecosystems, agriculture and livestock farming/breeding have been practiced in the European Alps for millennia (Gingrich et al., 2015; Gilck & Poschlod, 2019). Traditionally, subsistence farming was combined with livestock breeding in this

region, often involving transhumance to pastures located above or at the tree line (Gingrich et al., 2015; Glick & Poschold, 2019). This agro-livestock system was characterized by traditional, low-input practices that led to the creation and maintenance of seminatural habitats such as meadows and pastures (Faccioni et al., 2019). Nowadays, these two semi-natural mountain ecosystems are the most common habitats in the Alps and are listed among the ecosystems with the highest biodiversity in the world (Bonari et al., 2017), also recognized as important hotspots for flower-visitors (Jones et al., 2019). Due to their important role in species conservation, these habitat types are protected by the European Union Habitats Directive (92/43/EEC).

In the Alps, grazed and mowed areas also exhibit the highest biodiversity of the two insect taxa widely recognized as major pollinators in mountain natural and semi-natural ecosystems: bees (Hymenoptera Apoidea Anthophila) and hoverflies (Diptera Syrphidae) (Vujić et al., 2022; Praz et al., 2023). Bees are the taxon that has developed the closest relationship with flowers, with almost all species totally depending on floral resources for their lifecycle (Michener, 2007), and they are considered the main pollinators on a global scale (Potts et al., 2010), although this may not be true in specific habitats and for particular plant species. The importance of hoverflies in mountain ecosystems is known (Lefebvre et al., 2018; Sommaggio et al., 2022) as they replace bees with increasing altitudes and play an important role as pollinators of flowering plants within high-mountain ecosystems.

However, starting from the latter half of the 20th century, many socio-economic and technical factors, including the expansion of urban areas, increased tourism, abandonment of less favourable areas, and agricultural intensification, contributed to altering the traditional alpine landscape (Faccioni et al., 2019). Particularly, agricultural intensification, which primarily occurred in the lowest valley areas, and the abandonment of more remote or less easily accessible areas (e.g., steep slopes) had a significant impact on the preservation of mountain ecosystems (Monteiro et al., 2011).

Despite the increasing threats to flower-visitor communities, especially in mountain environments, little is still known about their ecology and how these communities change or are impacted either by different land uses or altitude considered. For example, it is still unclear which taxonomic groups are most affected by the

anthropization of natural ecosystems and what consequences this decline will have on the ecosystem stability (Seibold et al., 2019). Due to variations in size, mobility, and functional traits among arthropod species, different land use types can have varying and at times contradictory impacts on different flower-visiting taxa (Tscharntke et al., 2005). Further studies investigating the influence of environmental conditions on flower-visiting arthropod communities are fundamental, particularly for developing effective conservation policies that aid in preserving Alpine vulnerable and increasingly endangered ecosystems, which are undeniably significant to humans as well. Indeed, the interest of pollinators is not purely conservationist, as the services provided by these arthropods are crucial for the health and sustainability of mountain agroecosystems.

The present study aims to investigate the impact of altitude increase and different land uses on the composition, abundance, and diversity of floral visitor communities in a mountainous environment. Special attention will be given to understanding how these variables affect the abundance and species richness of Hymenoptera Apoidea Anthophila and Diptera Syrphidae.

2. Material and methods

2.1 Study area and sampling sites

The study area is situated within the Stelvio National Park, which is renowned as one of the largest protected areas in Europe. With a total area of 130 km^2, it is also the largest protected area found in the Italian Alps. In particular, the Stelvio National Park is located in Eastern Alps within the Rhaetian Alpine sector (Marazzi, 2005) encompassing Lombardy, Trentino and South Tyrol regions. The Park is characterised by a wide altitudinal range (from about 600 m to about 4000 m asl) and includes a variety of natural, semi-natural and anthropogenic environments.

The study was conducted within the Martello valley (Bolzano, Trentino-Alto Adige, Italy). The valley faces north-northeast and extends for about 27 km along an altitudinal gradient ranging from about 700 m asl of Laces town and 3,769 m asl of Cevedale Mount. Following the Köppen-Geiger classification, four climate types can be distinguished from low to high altitudes of this valley: Dfb (warm-summer humid continental climate), Dfc (subarctic), ET (tundra), and EF (ice cap). Mean annual

precipitation ranges between 600-700 mm in the lowest part of the valley, to 1500 mm over 2000 m asl (Provincia Autonoma di Bolzano, 2008). The mean annual temperature ranges between 4 °C and -1 °C along the valley (Provincia Autonoma di Bolzano, 2008).

Excluding the portion of the valley without vegetation (rocky outcrops, debris areas, and ice-covered areas), most of the valley is occupied by forests and grasslands. Cultivated areas are concentrated in the lowest portion of the valley as well as urban areas which are low represented.

Within this valley, like in many other Alpine areas, the dominant forms of open land use are pastures, hay meadows, high-altitude grasslands, and apple orchards. Pastures can be found at different altitudes and are primarily grazed by cows and goats. Hay meadows are abundant between 900 m and 2000 m asl. They are mowed once to three times a year, depending on the weather conditions, and receive weekly irrigation. Above 2000 meters, high-altitude grasslands prevail. These are natural ecosystems grazed exclusively by wild animals. Apple orchards, particularly in the eastern Alps, are a widespread agricultural crop. Due to grafting, pruning, and training techniques employed in these orchards, they can be classified as "open" ecosystems, as grass cover is maintained between the rows and mowed approximately once a month. The use of phytochemical treatments in these crops adheres to the Agrios directive (https://www.agrios.it).

Taking into consideration the most representative vegetation types along the altitudinal gradient, excluding crops, meadows and pastures, the valley can be divided into three main zonation levels: montane (low valley: 700-1600 m asl), subalpine (middle valley: 1601-2300 m asl) and alpine (high valley: 2301-3000 m asl). Whitin the montane level, the vegetation cover consists of mixed deciduous (beech forests) and coniferous forest (spruce forest). The subalpine level is characterized by coniferous forest (spruce, larch, and Swiss stone pine forest). The alpine level is situated over the tree line, where the vegetation cover is represented by high altitude grasslands (Ufficio Pianificazione territoriale e cartografia – GeoCatalogo (http://www.buergernetz.bz.it)).

Considering this altitudinal zonation and the most common “open” land use types occurring within the valley, the fieldwork was conducted within 14 sites diversified for altitude and land use (Table1; Figure1):

- Low valley: 900 – 1600 m asl (2 apple orchards, 2 pastures, 2 hay meadows);

- Middle valley: 1600 – 2300 m asl (2 pastures, 2 hay meadows);

- High valley: 2300 – 2700 m asl (2 pastures, 2 alpine grasslands).

Table 1. Identification data of selected sites within the study area.

Sampling site ID	Sector	Land use	Elevation (m asl)	Longitude (E) (UTM WGS84 – 32T)	Latitude (N) (UTM WGS84 – 32T)
BME1	Low valley	Apple orchard	978	639099.747	5160372.847
BME2	Low valley	Apple orchard	1019	639088.817	5160005.165
BPA1	Low valley	Pasture	1000	639101.940	5160267.616
BPA2	Low valley	Pasture	1313	637206.532	5159232.948
BPR1	Low valley	Hay meadow	1007	639008.745	5160163.524
BPR2	Low valley	Hay meadow	1335	636515.198	5157903.354
MPR1	Middle valley	Hay meadow	1713	632678.709	5153213.361
MPR2	Middle valley	Hay meadow	1895	631112.586	5150456.005
MPA2	Middle valley	Pasture	2281	631006.136	5151523.120
MPA1	Middle valley	Pasture	2246	629529.646	5150562.143
VPA1	High valley	Pasture	2343	631355.137	5152310.066
VPA2	High valley	Pasture	2524	631080.304	5153022.357
VPR1	High valley	Alpine grassland	2611	630958.424	5153413.361
VPR2	High valley	Alpine grassland	2683	630850.650	5153557.103

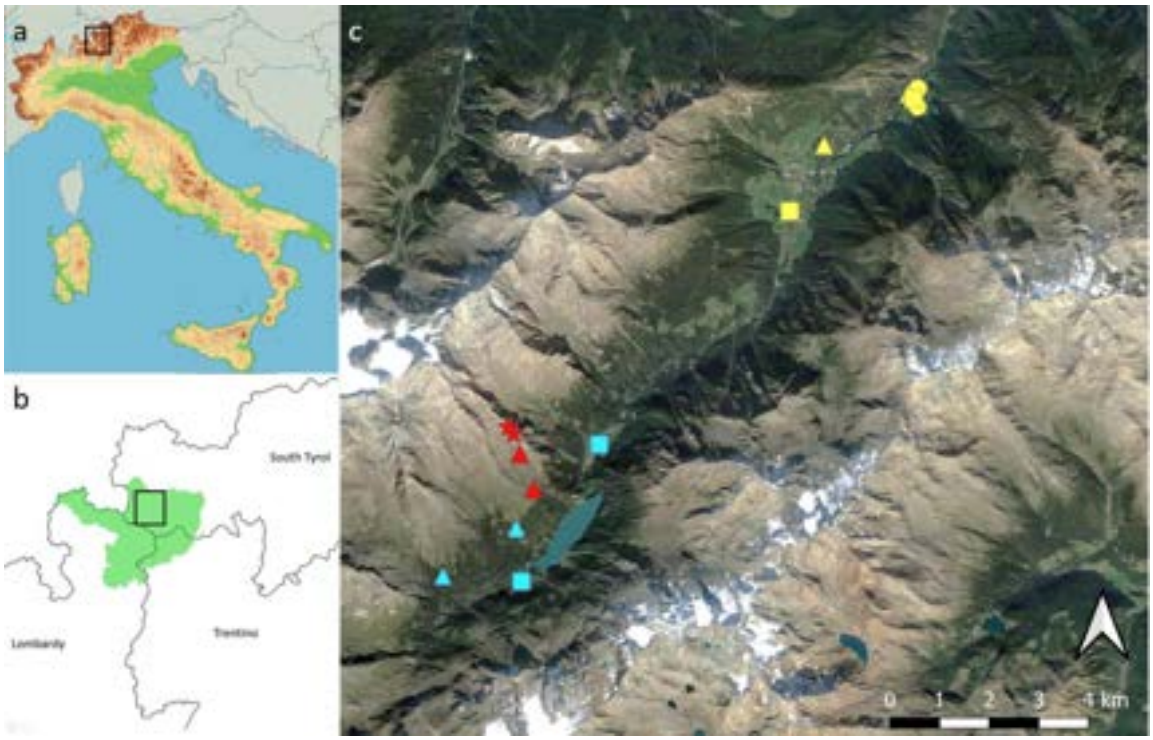

Figure 1. a. Stelvio National Park in the European Alps; **b.** Martello valley (black square within the Stelvio National Park (green area); **c.** Selected sampling sites along the Martello valley: sites located in the low valley (yellow), sites located in the mid valley (blue), sites located in the high valley (red). Dots: apple orchards; triangles: pastures; squares: hay meadows; stars: alpine grasslands.

2.2 Sampling of flower-visiting arthropods

Sampling of flower-visiting arthropods was conducted during the plants growing season (from May to August) of 2021 and 2022. A total of 11 fieldwork sessions were conducted (Table2). During each fieldwork session, sampling sites were surveyed once, if possible. Indeed, total number of surveys of sites located above 1600 m asl and sites managed as hay meadows is lower due to both the seasonal differences in the beginning of flowering and the frequency of summer mowing. In both cases, sampling of flower-visiting arthropods is impossible due to the absence of flowers. The surveys were performed between 9:00 and 18:00 in non-rainy days.

Table 2. Total of surveys conducted during the 11 field-work sessions "●": site surveyed; "X": site not surveyed because of mowing, "o": survey not conducted because flowering season was not started yet.

	2021						2022				
Sampling site ID	27/V-30/V	12/VI-14/VI	27/VI-05/VII	13/VII-21/VII	27/VII-02/VIII	24/VIII-28/VIII	24/V-26/V	13/VI-18/VI	5/VII- 10/VII	23/VII-28/VII	8/VIII-12/VIII
BME1	●	●	●	●	●	●	●	●	●	●	●
BME2	●	●	●	●	●	●	●	●	●	●	●
BPA1	●	●	●	●	●	●	●	●	●	●	●
BPA2	●	●	●	●	●	●	●	●	●	●	●
BPR1	●	X	●	●	●	X	●	X	●	●	●
BPR2	●	X	●	●	●	●	●	X	●	●	X
MPR1	o	●	●	●	●	X	●	●	X	●	●
MPR2	o	●	●	●	●	X	●	●	●	X	●
MPA1	o	o	●	●	●	●	o	●	●	●	●
MPA2	o	o	●	●	●	●	o	●	●	●	●
VPA1	o	o	●	●	●	●	o	●	●	●	●
VPA2	o	o	●	●	●	●	o	●	●	●	●
VPR1	o	o	●	●	●	●	o	●	●	●	●
VPR2	o	o	●	●	●	●	o	●	●	●	●

Flower-visiting sampling was conducted on the most common entomophilous plant families found at all the surveyed sites. Families that exhibited different flower morphology and phenology were also considered, as they could potentially influence the choice of flowers by arthropods (Ollerton, 1996; Waser, 1998). The selected families were Fabaceae, Asteraceae, and Ranunculaceae or Rosaceae. These last two families were chosen as substitutes for each other because they did not consistently flower throughout the entire vegetative season. However, the floral morphology of the

selected species within these two families was comparable, as Rosaceae and Ranunculaceae species with an actinomorphic symmetry were chosen.

During each survey, one species for each plant families was selected. The species selected was the most representative in terms of area covered by flowers:

- Asteraceae: *Aster alpinus*, *Cadruus nutans*, *Centaurea stoebe*, *Crepis vesicaria*, Jacobea *abrotanifolium*, *Leontodon hispidus*, *Leucanthemopsis alpina*, *Leucanthemum vulgare*, *Pilosella officinarum*; *Scorzoneroides helvetica*, *Taraxacum officinale*;

- Fabaceae: *Anthyllis vulneraria*, *Lotus corniculatus*, *Medicago lupulina*; *Trifolium alpinum*, *T. montanum*. *T. pratensis*, *T. repens*, Vicia *sepium*;

- Ranuncolaceae: *Ranunculus acris*, *R. bulbosus*; Rosaceae: *Geum montanum*, *Potentilla aurea*, *P. neumanniana.*

Flower-visiting arthropods were collected in according to the *timed-observation* method described by Gibson et al. (2011) and adapted by Bonelli et al. (2020). Two pairs of operators stood at least five metres apart from each other simultaneously conducted a 15-minute sampling on the same species. During this time window, all the flowers situated within a 20 cm diameter circle were observed and any arthropods that made contact with the flowers were collected. The operators were crouched about 50 cm away from the observed plants to minimize disturbance to the flower visitors and ensure clear visibility of the flowers.

At the end of the first 15-minute time window, the plant family was changed, and the sampling session was repeated. This process was then repeated for the last remaining family after another 15-minute time window. At the end of this first cycle, a second cycle was then repeated in the same manner (Figure2). The entire observation period for each plant family for each survey within each site, amounted to 1 hour.

Each collected arthropod was stored individually in collection tubes containing 70% ethanol and then preserved at 4 °C. The specimens preserved in ethanol were photographed using a stereoscope (Leica M50 equipped with the camera Leica IC90 E) and then identified by expert taxonomists to the lowest possible taxonomic level. The list of taxonomists is reported in Supplementary Material S1.

Figure 2. Schematic diagram of the sampling plan. For each plot, two pairs of operators simultaneously observed two groups of plants of the same species for a period of 15 minutes. At the and of the time window, a new 15-minutes period of sampling was conducted a second botanical family. At the end of the second time window, the sampling was replicated on the last botanical family. This cycle of observations was repeated twice for each survey.

2.3 Analyses of flower-visiting arthropod communities

The collected arthropods were subdivided in accordance with their taxonomical order. Hymenoptera and Diptera were further subdivided according to their phylogenetic position and different biology.

- Diptera: specimens were divided into three taxa: Diptera Nematocera, Diptera Brachycera, and Diptera Syrphidae. Brachycera and Nematocera were divided considering their phylogenetic position in according to literature (Bertone & Wiegmann, 2009). Additionally, since Syrphidae adults feed only on pollen and nectar, this family was kept separate from other Brachycera.

- Hymenoptera: specimens were divided into five taxa: wild Hymenoptera Apoidea Anthophila, *Apis mellifera* Linnaeus 1758, Hymenoptera Formicidae, Hymenoptera wasps, and Tenthredinoidea (Hymenoptera: Symphyta). *Apis mellifera* were kept separate from other bees as it is a manages species which could presents particular ecological and floral needs. Formicidae were kept separate from other Hymenoptera Aculeata as workers, mainly visiting flowers, are wingless (Hölldobler & Wilson, 1990). Wasps includede all those Hymenoptera Apocrita that are neither Formicidae nor Anthophila.

For all subsequent statistical analysis, only taxa presenting at least 50 collected specimens were considered, excluding Thysanoptera. This order was not taken into consideration as specimens behave differently from other flower visitors. Indeed, they commonly exhibit a gregarious habit and individuals spend long periods hidden on

flowers and inflorescences (Lewis, 1973). The presence of Thysanoptera on flowers can more correctly be referred to 'staying' behaviour rather than to 'visiting'. Therefore, the observed abundance on flowers of this order on flowers does not have the same ecological meaning as that observed for other flower-visiting taxa.

2.4 Factors related to abundance and diversity of arthropod communities

To assess the factors related to abundance and taxonomic diversity of different groups, we used two Bayesian multivariate Generalised Linear Mixed Models (GLMMs) (Bürkner, 2017).

In the first model, the counts of the eight most common flower-visiting arthropod taxa sampled on each plant family, for each survey, and for each site were employed as dependent variables ("Wild" Hymenoptera Apoidea, Coleoptera, Hymenoptera Formicidae, Hymenoptera wasps, Hemiptera, Diptera Nematocera, Diptera Brachycera, Diptera Syrphidae). Each dependent variable was assumed to follow a negative binomial distribution.

For the second model, we employed the Shannon entropy (package *'hillR'*, Chao et al., 2014) to calculate the taxonomic diversity of major pollinator groups (wild Hymenoptera Apoidea and Diptera Syrphidae). The diversity of wild bees and hoverflies obtained for each survey and for each site, were then used as dependent variables. Each dependent variable was assumed to follow a lognormal distribution.

In both models, we tested the effect of the following independent variables: management (expressed as "sum-to-zero" constraints: managed land vs alpine grassland; apple orchard vs grassland; hay meadow vs pasture), elevation (expressed as continuous variable), and the plant family considered. The number of flowers observed for each plant family during each survey and the day of sampling (expressed as number from the begin of the year) were included as covariates in the model, while the site and the year were considered by including them in the models as random factors.

Before running the models, all the continuous independent variables (i.e., elevation, number of flowers, and day of sampling) were scaled to a mean of 0 and a standard deviation of 1, to improve convergence and allow the comparison of effect sizes. Models

were built with Stan, using the R package '*brms'*. The Posterior probabilities of parameters were sampled by running three Markov chains Monte Carlo, each for 4,000 interactions. The first 2,000 iterations were discarded as burn-in, and a thinning of 2 was applied to obtain a total of 1,000 posterior samples for each chain.

3. Preliminary results

A total of 4538 specimens were sampled. All specimens were identified to order level, 38% to family, 26% to genus, and 25% to species level. The collected specimens belonged to 12 different orders. The most abundant order was Thysanoptera (57.35%) followed by Diptera (22.62%), Hymenoptera (10.38%), Coleoptera (7.29%), Hemiptera (4.04%), Lepidoptera (0.36%), Araneae (0.34%), Acari (0.20%), Orthoptera (0.20%), Collembola (0.18%), Dermaptera (0.05%), and Neuroptera (0.05%). The abundance of specimens belonging to each order sampled throughout the entire field seasons is reported in Supplementary material S2.

3.1 Distribution of the sampled flower-visiting arthropod

Taking into consideration the four types of land use distributed along the altitudinal gradient, in low valley Thysanoptera is the most abundant order, representing over 40% of specimens among all the types of land use (Figure3.a). Regarding the other most abundant orders, hymenopterans are well represented within pasture (20.9%) and hay meadows (13.6%), while their abundance decrease in apple orchards (4.8%). Beetles are well represented within pasture, where 15.1% of specimens belong to this order, while their relative abundance is low in apple orchards and hay meadows (1.0% and 2.1%, respectively). The relative abundance of dipteran is similar in both pastures and hay meadows, as well as in apple orchards (12.7%, 12.4%, and 10.1%, respectively). Regarding hemipteran, they are more abundant within pastures (9.3% of collected flower visiting arthropods) than in hay meadows and apple orchards (3.1% and 1.6%, respectively).

In mid valley, the abundance of dipteran prevails in both hay meadows and pastures, representing over 55% of the collected specimens (Figure3.b). In the first, dipterans are

followed by Thysanoptera, which represents 25% of the samples, while in pastures they are followed by hymenopteran which represent 12% of the collected specimens. Both beetles and hemipteran have similar relative abundances in pastures and hay meadows (2.9% and 4.9% for Coleoptera, respectively; 4.8% and 7.8% for Hemiptera, respectively).

In high valley, pastures show a high presence of Thysanoptera, representing over 50% of the collected flower visitors, followed by dipterans (23.8%), beetles (13.6%), and hymenopterans (6.0%) (Figure3.c). Whitin high altitude grasslands, beetles become the most common flower visiting arthropods, as more than 50% of the collected specimens belong to this order, followed by dipteran (23.8%), hemipteran (3.9%) and hymenopteran (1.3%).

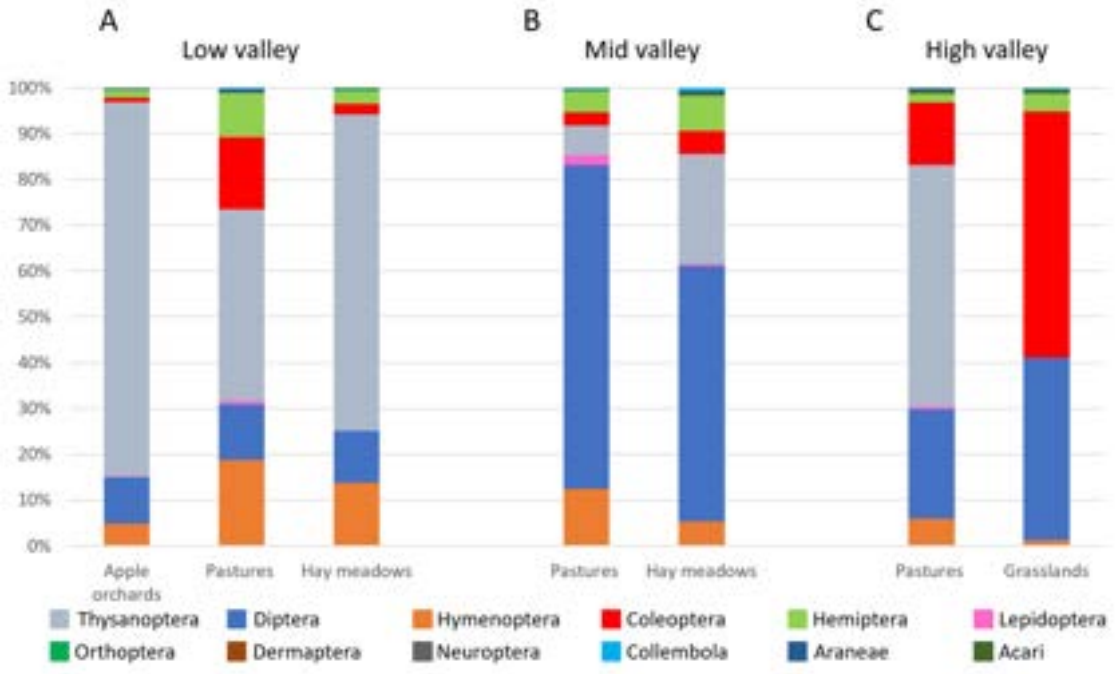

Figure 3. Abundance of the sampled orders divided for altitudinal gradient (A. low valley, B. mid valley, C. high valley) and for land use.

Observing the abundance of most representative arthropod orders (Thysanoptera, Diptera, Hymenoptera, Coleoptera, Hemiptera, and Lepidoptera) throughout the vegetative seasons of 2021 and 2022, it is evident that there is no consistent pattern between the two years (Supplementary Material S3).

Excluding Thysanoptera, which are currently being identified, 98.82% of the specimens belonging to the most abundant order of floral visitors (Coleoptera, Diptera, and Hymenoptera) were identified at family level (Supplementary Material S4), 71.97% of the specimens were identified at genus, and 71.27% at species level (Supplementary Material S5).

The most represented family of Coleoptera is Dasytidae, accounting for over 50% of the beetles, followed by Nitidulidae (15.65%). The first family was mainly observed in pastures and grasslands located in high valley, while the second one was predominantly observed in pastures of low valley.

Muscidae is the most abundant family of Diptera, comprising 39% of dipterans, followed by Syrphidae (24.06%) and Chloropidae (11.65%). The relative abundance of both Muscidae and Syrphidae remains consistent across different types of land use along the altitudinal gradient. Muscidae are most abundant in pastures of high valleys, while Syrphidae reach their peak in pastures of mid valleys. Chloropidae are highly represented in low valley led uses, particularly in apple orchards.

Regarding the Hymenoptera order, over 50% of the specimens belong to the family Formicidae, followed by Apidae (14.62%). Ants are primarily found in low valley land uses, especially in hay meadows, but their relative abundance among hymenopteran remain high also in mid valley. The presence of Apidae within the hymenopteran flower-visiting community is higher in low valley land uses than in higher valley, with a peak in apple orchards.

3.2 Influence of environmental parameters on flower-visiting arthropod abundance

Regarding the factors that influence the abundance of different flower-visiting arthropod taxa, the data revealed that increasing altitude has a negative influence primarily on the the abundance of wild Hymenoptera Apoidea (Est ± SE = -1.42 ± 0.27; 95% CI [-2.02, -0.91], 100% probability of being negative), Hemiptera (Est ± SE = -0.42 ± 0.20; 95% CI [-0.82, -0.05], 98.40% probability of being negative), and Hymenoptera Formicidae (Est ± SE = -1.08 ± 0.61; 95% CI [-2.41,+0.05], 97.17% probability of being negative). On the other hand, Diptera Brachycera non-Syrphidae abundance is influenced positively by

increasing altitude (Est ± SE = +0.27 ± 0.18; 95% CI [-0.09, +0.91], 93.33% probability of being positive) (Figure4, Supplementary Material S6).

Regarding the influence of different type of land use on the abundance of analysed taxa, managed environments (apple orchards, pastures and hay meadows) have a positive effect on the abundance of Diptera Brachycera (Est ± SE = +0.19 ± 0.12; 95% CI [-0.06, +0.42], 93.93% probability of being positive) and Diptera Syrphidae (Est ± SE = +0.23 ± 0.14; 95% CI [-0.06, +0.49], 95.07% probability of being positive). However, they have a negative influence on the abundance of Coleoptera (Est ± SE = -0.67 ± 0.25; 95% CI [-1.16, -0.19], 99.30% probability of being negative) compared to natural environments (high altitude grasslands).

Apple orchards positively affect the abundance of Diptera Brachycera (Est ± SE = +0.17 ± 0.13; 95% CI [-0.10, +0.42], 90.60% probability of being positive) and Diptera Syrphidae (Est ± SE = +0.28 ± 0.15; 95% CI [-0.03, +0.58], 96.40% probability of being positive). On the other hand, they appear to have a negative impact on the abundance of wild Hymenoptera Apoidea (Est ± SE = -0.39 ± 0.17; 95% CI [-0.75, -0.05], 98.23% probability of being negative), Coleoptera (Est ± SE = -0.37 ± 0.28; 95% CI [-0.94, +0.18], 92.47% probability of being negative) and Hemiptera (Est ± SE = -0.22 ± 0.15; 95% CI [-0.53, +0.08], 93.03% probability of being negative).

Hay meadows positively affect the abundance of Diptera Syrphidae (Est ± SE = +0.58 ± 0.27; 95% CI [+0.24, +0.92], 99.63% probability of being positive) and Diptera Nematocera (Est ± SE = +0.78 ± 0.56; 95% CI [-0.26, +1.97], 93.57% probability of being positive). On the other hand, they have a negative impact on the abundance of wild Hymenoptera Apoidea (Est ± SE = -0.46 ± 0.23; 95% CI [-0.95, -0.05], 98.10% probability of being negative).

Taking into consideration the abundances of the analysed taxa collected on each observed plant family, the Asteraceae family appears to be more attractive than Rosaceae/Ranunculaceae and Fabaceae for almost all flower visiting taxa.

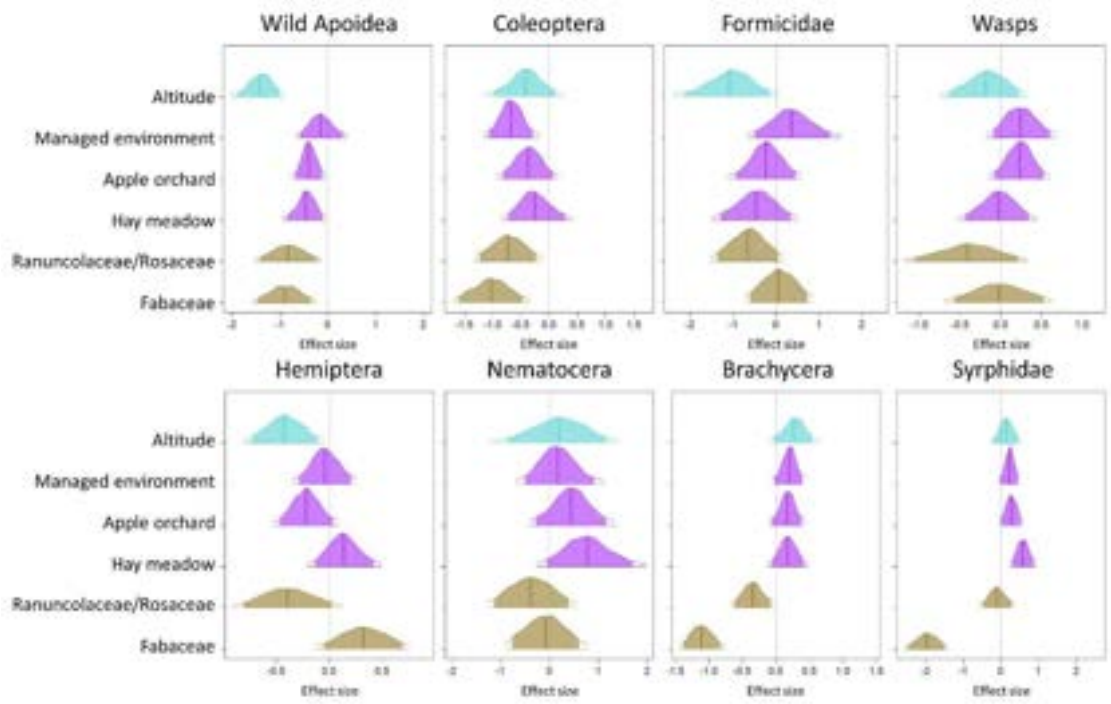

Figure 4. Visualisation of the probability of direction for the parameters of a Bayesian regression model for the abundance of each flower-visiting arthropod considered. Posterior distributions (light blue, violet and brown) of effects (the x-axis) with the illustration of indices of Null-Hypothesis Significance Testing (vertical dotted line). Left-shifted areas indicate a negative effect of the parameter considered, whereas right-shifted areas represent a positive effect of the parameter considered.

Altitude and the type of land use have different influences on the species diversity of wild Hymenoptera Apoidea and Diptera Syrphidae. The species diversity of wild Apoidea is negatively affected by both increasing altitude (Est ± SE = -0.11 ± 0.03; 95% CI [-0.16, -0.05], 100% probability of being negative) and environments with a high level of management (Est ± SE = -0.04 ± 0.02; 95% CI [-0.07, -0.00], 97.50%% probability of being negative for hay meadows; Est ± SE = -0.05 ± 0.02; 95% CI [-0.09, -0.01], 98.83% probability of being negative for apple orchard). On the other hand, the species diversity of syrphid seems to increase with a higher level of management in the environment (Est ± SE = +0.10 ± 0.03; 95% CI [+0.04, +0.15], 99.77% probability of being positive for hay meadows; Est ± SE = +0.04 ± 0.02; 95% CI [-0.01, +0.08], 95.50% probability of being positive for managed environments) (Figure5, Supplementary Material S7).

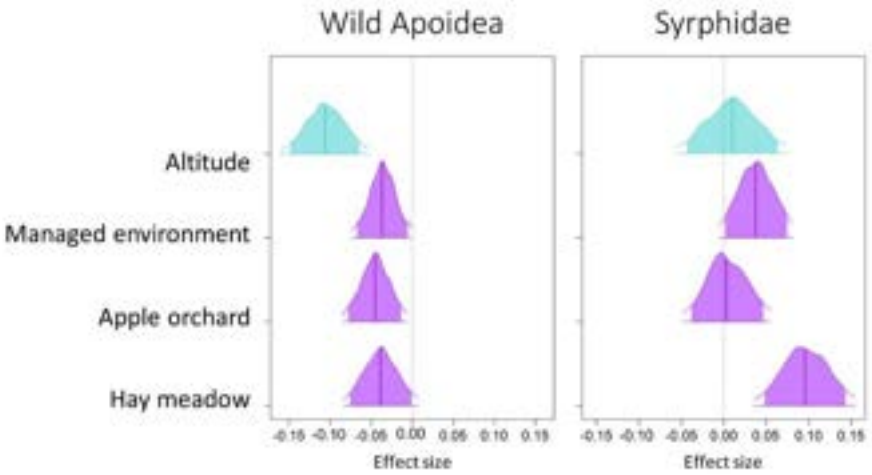

Figure 5. Visualisation of the probability of direction for the parameters of a Bayesian regression model for the species diversity of wild Hymenoptera Apoidea and Diptera Syrphidae. Posterior distributions (light blue, violet) of effects (the x-axis) with the illustration of indices of Null-Hypothesis Significance Testing (vertical dotted line). Left-shifted areas indicate a negative effect of the parameter considered, whereas right-shifted areas represent a positive effect of the parameter considered.

4. Preliminary discussion

This study aimed to evaluate how both land use type and elevation influence the composition of Alpine flower-visiting arthropod communities.

The observed composition of mountain flower-visiting arthropod communities in this study is consistent with findings from other studies conducted in Alpine contexts (Losapio et al., 2016; Wagner, 2016; Lefebvre et al., 2018; Bonelli et al., 2022). Indeed, Diptera, Hymenoptera, and Coleoptera, are the most common flower-visiting arthropods in Alpine environments, while Lepidoptera represent a very low percentage (0.5%) of mountain flower visitors (Wagner 2016; Lefebvre et al., 2018). In this study, over 50% of flower-visiting arthropods sampled were Thysanoptera. As observed by other authors (Pelikán, 1996; Steinwandter & Seeber, 2023), the highest relative abundance of this order was observed in low valley land areas, decreasing along the altitudinal gradient, and disappearing above 2400 m asl. The presence of Thysanoptera as alpine flower visitors is also reported in other studies conducted in high mountain environments located above 2000 m asl (Bonelli et al., 2020; Bonelli et al., 2022). However, there are no studies reporting their presence on flowers above this elevation, even though their altitudinal range reaches 3000 m asl, where they can be found as soil fauna near the glacier forehead (Sierka et al., 2008; Steinwandter & Seeber, 2023). Nevertheless, most ecological information about Thysanoptera focuses on their

pathogenic interest in agronomic contexts (Reitz et al., 2020; Gulzar et al., 2021), while knowledge about their ecology and ecosystem services in natural and mountain ecosystems is scarce. Generally, Thysanoptera abundance is strongly influenced by gregariousness phenomena, as they spend a long time on flowers exhibiting behavior that differs from other flower visitors. Many species use flowers as refuges for the entire colony, as well as for searching for a partner, oviposition, and feeding on floral products such as pollen and nectar (Kirk, 1984; Kirk, 1985; Varatharajan, 2016). The importance of Thysanoptera as pollinators is recognized for many tropical tree species in Africa, Australia, South-Eastern Asia, and Neotropics (Gottsberger et al., 1980; Hansman, 2001; Frame & Durou, 2001; Kato et al., 2008; Danieli-Silva & Varassin 2013). However, the potential role as pollinators also in Alpine context is still underestimated and poorly studied (Annand, 1926; Ananthakrishnan, 1993; Mound, 2005). The limited knowledge about the ecological role of Thysanoptera in Alpine natural and seminatural ecosystems highlights the importance of conducting more studies on this order to better understand its ecological role.

In relation to the increase in elevation, this study confirmed that Diptera Brachycera (Syrphidae and non-Syrphidae) tend to replace hymenopterans (Apoidea: Anthophila) as the most common flower-visitors, as reported also in other studies conducted in the Alps (Müller 1880; Zoller et al., 2002; Losapio et al., 2016; Wagner et al., 2016; Lefebvre et al., 2018; Sieber et al., 2018) and hypothesised as a pattern repeated at global scale (McCabe et al., 2021). This replacement is also reflected in the species richness. As observed in previous works, wild bee species richness decreases with altitude, as few species are adapted to the alpine climate. On the other hand, syrphids species richness is less affected by increasing altitude as there are many species that adapted to high mountain conditions (Lefebvre et al., 2018; Inouye, 2020; Sommaggio et al., 2022).

In this study an increase in beetles within ecosystems located above the tree line up to 2700 m asl was observed. Beetles became the most represented order within high-altitude grasslands, accounting for about 53% of the flower-visiting arthropods sampled, followed by dipterans (about 40%). This finding contradicts the results of many studies conducted in alpine contexts, which reported a decrease in flower-visiting beetles above 1400-1600 m asl (Arroyo et al., 1982; Warren et al., 1988; Gómez-Murillo & Cuartas-Hernández, 2016; Lefebvre et al., 2018; Zhao et al., 2019; Adedoja et al., 2020).

However, in high-altitude grasslands, all the specimens were identified as *Dasytes alpigradus* Kiesenwetter, 1863. This species is commonly found in alpine grasslands of the Central and Eastern Alps (Liberti & Focarile, 2005; Steinwandter et al., 2022). Both males and females of this species are known to mate as well as search for nectar and pollen on flowers. (Liberti, 2020). The presence of *Dasytes alpigradus* has also been recorded in another study conducted in an alpine environment (Lefebvre et al., 2018), albeit with limited abundance. This genus is recognized as an important pollinator in various environments (from low land to mountainous one) and is involved in the pollination of different plant species (Mawdsley, 2003; Hoebeke & Wheeler, 2013; Maggi et al., 2021), suggesting that this genus could play a fundamental role as pollinator even in alpine contexts. Among Diptera, the most common families were Muscidae and Syrphidae. Almost all the Muscidae specimens were identified as *Coenosia obscuricula* (Rondani, 1871), while most of Syrphidae were identified as *Eupeodes lapponicus* (Zetterstedt, 1838). Both species are common in alpine and temperate areas. Regarding *Coenosia obscuricula*, there is no literature available on its ecology to the best of our knowledge. As other species belonging to *Coenosia* genus, they should be predators and their diet should be based on many little arthropod species (Yohan et al., 2017). In high-mountain environments, they may also visit flowers to seek a warm place for thermoregulation and supplement their diet with high-nutrient nectar (Lefebvre et al., 2018). The second most observed dipteran species is typically found in coniferous forests (*Picea*/*Abies*) up to the *Larix* zone (above 2000 m asl in the Alps). Its presence is favoured when land management is limited or absent (Hussain et al., 2018).

Considering the flower-visiting arthropod community that characterizes apple orchard land use, it appears to have a positive influence on the presence of those taxa, such as hymenopteran wasps and dipteran syrphids, which rely on aphids either as hosts for larvae development or as a source of food (Hirose, 2006; Vollhardt et al., 2008; Speight, 2011). At the same time, apple orchards have a negative impact on the presence of Hemiptera. This pattern could be attributed to a balanced ecosystem management, probably facilitated by the presence of grassed inter-rows. As reported in previous studies (Herz et al., 2019; Cahenzli et al., 2019), a grassed inter-row can enhance the population of natural aphid enemies, thus providing biological control for this common crop pest. In this study for example, the species *Episyrphus balteus* (De Geer, 1776) was

found to be abundant among Syrphidae. This species could be considered both an important pollinator during the adult stage, and fundamental in the biological control of aphids during the larvae stage (Tenhumberg, 1995; Hong & Hung, 2010). Regarding hymenopteran wasps, specimens are currently being identified, which prevents a comprehensive evaluation of community composition. However, a grassed inter-row could also promote non-aphid crop pests, such as the beetle *Phyllopertha horticola* (Linnaeus, 1758) (Ruther, 2005). The studied apple orchards exhibited a high relative abundance of specimens belonging to this species. This presence was likely promoted by the abundance of mow-adapted plant genera such as *Taraxacum* and *Trifolium* within the inter-row. Especially *Trifolium,* is one of the most suitable plant genera for the development of *P. horticola*, as larvae primarily feed on its roots. Conversely, adults are phytophagous and are considered agricultural pest as it feed on leaves, flowers, and developing fruit of many crops, from lowland to mountain environments (Hann et al., 2015). Another phytophagous family well-represented in apple orchards, was the Chloropidae dipteran (Nartshuk, 2014). The collected specimens are currently being identified, preventing us from investigating the ecological role they have within different mountain ecosystems. The presence of wild Apoidea within apple orchards was found to be low. Wild bees, together with honeybees, are essential for the pollination of apple flowers, indeed, the abundance of these pollinators, increase the apple flowering period (Delaplane & Mayer, 2000). However, our work indagated the presence of wild bees within this land use after the flowering period of apple trees. During the two-year fieldwork, only eight wild bee specimens from seven species (*Dufourea dentiventris* (Nylander, 1848), *Bombus lapidarius* (Linnaeus 1758), *Lasioglossum cupromicans* group, *L. calceatum* (Scopoli, 1763), *Hylaeus annulatus* (Linnaeus, 1758), *Bombus lucorum*/*terrestris*) were collected. A comparison with a sampling conducted during the apple flowering period could be useful to better evaluate the impact of this crop on the wild bee community. Nonetheless, our results suggest that intensive management negatively affects both the abundance and species richness of wild Apoidea. One factor that could impact native pollinators and flower visitor species richness in highly managed agroecosystems is the decrease in plant diversity, which is crucial for the foraging activities of pollinators and an important food source for flower visitors (Richards, 2001; Steffan-Dewenter et al., 2005; Nicholls & Altieri, 2020). In addition to

low vegetation diversity, the use of pesticides, commonly employed in fruit crops, could also influence the composition of abundance and diversity of bee communities (McKerchar et al., 2020; Pardo & Borges, 2020; Gomez et al., 2022).

Finally, we compared the flower-visiting arthropod community of pasture and hay meadows. Some previous studies investigated the effect that grazing and mowing have on the composition of flower-visiting arthropod communities (Zurbrügg & Frank, 2006; Sjödin et al., 2008; Lázaro et al., 2016; Bussan et al., 2022), but the results are mixed. Moreover, the influence of land management in alpine contexts has rarely been investigated. Generally, overgrazing and frequent mowing are deleterious for the health of these ecosystems, leading to vegetation variability and availability to decrease, altering the type of arthropod community. However, within the Martello valley, this problem seems to not be present as pastures are not overgrazed (about 1.6-1.8 cattle/hectare (www.agricoltura.provincia.bz)), and meadows are mowed 2-3 times at yeas in the lowest sector of the valley (up to 1600 m asl), and 1-2 times in the middle sector (up to 2400 m asl). On one side, the two uses of land present some similarities. The most common taxa sampled in hay meadows and pastures are Diptera Muscidae (mainly represented by *Thricops* genus), Hymenoptera Formicidae (mainly represented by *Myrmica rubra* (Linnaeus, 1758) and *Forminca lemani* Bondroit, 1917 within pastures, and by *Lasius niger* (Linnaeus, 1758) within hay meadows), and Hemiptera. The genus *Thricops* is known to be very common in mountain ecosystems characterized by a boreal and temperate climate. Adults are known to be frequent flower visitors of various species as they feed on pollen and nectar (Pont, 1993; Dlusskii, 2002; Vikhrev & Sorokina, 2009) making them potential important pollinators in these environments. Regarding Formicidae, all the observed species are predators of a wide variety of invertebrate, especially aphids, but they are also frequently observed to feed on nectar (both from floral and extrafloral nectaries) (Petal, 1967; Schiestl & Glaser, 2012; Guariento et al., 2018a; Guariento et al., 2018b). Although ants were present in both land uses, they appear to be more common in pastures than in hay meadows. This could be related to the preferences of certain species for nesting in low covered or bare ground (Hutchinson & King, 1980; Pontin, 1963), as in the case of *Formica lemani*. This species is particularly common in open fields above the tree line, where vegetation cover is lower compared to meadows in the mid and low valley (Guariento et al., 2018a;

Guariento et al., 2018b). Regarding Hemiptera, a detailed analysis of the genera and species composition of pastures and hay meadows communities is not currently possible as the samples are still being identified. Generally, this order was observed in higher abundance in hay meadows than in pastures. As observed also by other authors, this could be due to their tallgrass prairies preferences (Morris, 1971, Morris, 1973; Körösi et al., 2012).

Excluding the most shared taxa, pastures and hay meadows present some differences in the composition of Coleoptera, wild Hymenoptera Apoidea, and Diptera Syrphidae communities. Regarding Coleoptera, the most abundant families observed in pastures are Dasitydae (*Dasytes alpigradus*) and Nitidulidae (*Meligethes*). In hay meadows, the most represented families are Apionidae, Chrysomelidae (mainly represented by *Neocrepidodera peirolerii* (Kutschera, 1860)), and Buprestidae (mainly represented by *Anthaxia quadripunctata* (Linnaeus, 1758)). Among Apoidea, the most represented family both in pastures and hay meadows is Halictidae, mainly represented by *Lasioglossum morio* (Fabricius, 1793), followed by Apidae (*Bombus pyraeneus* Pérez, 1879, and *Bombus lapidarius* (Linnaeus, 1758)) in pastures, and by Andrenidae (*Panurgus banksianus* (Kirby, 1802)) in hay meadows. About Syrphidae, the most represented genera in pastures are *Eupeodes* (*E. corollae* (Fabricius 1794), *E. lapponicus* (Zetterstedt, 1838)) and *Scaeva* (*S. selenitica* (Meigen,1822)), while within hay meadows, they are *Eristalis* (*E. tenax* (Linnaeus, 1758)) and *Parasyrphus* (*P. annulatus* (Zetterstedt, 1838)).

These differences in the composition of Coleoptera, wild Apoidea, and Syrphidae communities between pastures and hay meadows may be related to the sensitivity of these three taxa to the consequences of mowing and their ecological needs throughout their entire life cycle. For example, the most represented genus of Coleoptera within grazed areas of low valley was *Meligethes.* This genus relies on flowers for its entire life cycle, with both larval and adult stages inhabiting flowers. This potentially results in high population density on a single flower, where they mainly feed on pollen and occasionally on pistils and carpels (Freude et al., 1967; Utelli & Roy, 2001; Ouvrard et al., 2016; Piesik et al., 2013). *Meligethes*, along with many other beetle genera that feed on nectar and pollen, typically have low flying ability and activity (Kevan & Baker, 1983; Steinwandter et al., 2017). The removal of the aerial part of the vegetation can certainly cause many

issues for these types of flower visitors, especially during the larval stage when they are unable to quickly leave flowers. On the other hand, within hay meadows, the most represented beetles are not closely dependent on flowers for their survival, which may make them less influenced by mowing and the absence of flowers. For example, the most abundant species sampled within hay meadows primarily feed on petals, nectar, or leaves during the adult stage, while their larvae grow in the soil or in trees by feeding on roots or wood (Montagna, 2009, Kofler, 2011; Rheinheimer & Hassler, 2018; Ruesink et al., 2023). Wild Apoidea and Syrphidae, showed different land use preferences. On one side, wild Apoidea showed the highest abundance and species richness in low managed environments such as pastures. On the other hand, Syrphidae showed to be less influenced by the level of management (Persson et al., 2020), exhibiting, however, a preference for mowed grasslands. Despite both bees and hoverflies abundance and diversity being enhanced when plant species richness is high (Vellend, 2008; Fründ et al., 2010; Nicholls & Altieri, 2013), these differences could be due to their different ecological needs and tolerance to stress (Persson et al., 2020). For example, most wild bee species are used to nesting underground in the soil (Földesi et al., 2016; Liczner & Colla, 2019; Neumüller et al., 2022). Frequent passages of lawnmowers could impact the ability of wild bees to nest underground, as the soil becomes too hard or compacted (Sardiñas & Kremen, 2014; Antoine & Forrest, 2021). By choosing to nest in low-stressed environments, wild bees are likely to select foraging areas close to the nest. Except for bumblebee species capable of flying long distances, the maximum foraging area for most wild bee species ranges between a radius of 150 m and 600 m from the nest (Steffan-Dewenter et al., 2002; Jauker et al., 2013). Therefore, the type of foraging habitat is highly influenced by the nest's location. Additionally, over 50% of the collected bees within hay meadows were *Apis mellifera* Linnaeus,1758. Such a high abundance of honeybees compared to other bee species draws attention to the potential impact that this species may have on wild bees. As several authors have reported (Shavit et al., 2009; Mallinger et al., 2017; Henry & Rodet, 2018; Ropars et al., 2019; Valido et al., 2019; Angelella et al., 2021), honeybee can negatively affect the presence of wild bees, especially when the number of colonies in high. Therefore, it is important to carefully evaluate the beekeeping practices, especially within protected natural areas, to ensure the survival of wild Apoidea (Henry & Rodet, 2018; González-Varo & Geldmann, 2018;

Alaux et al., 2019). However, the limited number of honeybees sampled during the concluded field season does not allow for a sufficiently robust statistical analysis of the possible impact this insect may have on wild bee communities. Regarding Syrphidae, all the most abundant species collected in pastures and hay meadows, are characterized by an aphid-feeding larval stage (Speight, 2011). However, the higher abundance of Hemiptera within hay meadows, could have influenced both the species richness of syrphids and their abundance within this type of land use. Moreover, syrphids are common flower visitors, and during the adult stage, they usually do not show a preference for a particular habitat (Speight, 2011). Unlike bees, they are not limited to the nest position and can freely fly among different habitats in search of suitable place for feed and oviposition (Földesi et al., 2016; Walcher et al., 2020). In addition, many syrphid species prefer to visit white flowers belonging to Apiaceae family (Speight, 2011). Given the high abundance of Apiaceae observed in hay meadows during fieldwork (e.g. *Pimpinella major*, *Heracleum spondylium*, *Daucus carota*, *Carum carvi*, and *Aegopodium podagraria*) compared to pastures, it is possible that the high occurrence of Syrphidae in hay meadows could be influenced by the higher presence of this plant family here than in pastures.

Finally, all the most represented arthropod taxa analysed, preferred to visit Asteraceae family rather than Fabaceae, Ranunculaceae, or Rosaceae. This result could be related to the various advantages that the Asteraceae family provides to flower visitors. For example, Asteraceae species typically produce a substantial amount of nectar per flower and develop capitulum inflorescences, which offer a generous reward to the visitor (Wróblewska et al., 2016). The abundance of nectar encourages the visits of many species, such as Apoidea, which maximize their efforts in searching for food by choosing flowers closely located flowers. Additionally, Coleoptera and Syrphidae are also encouraged to visit due to the nectar availability (Freude et al., 1967; Utelli & Roy, 2001; Pleasants, 1989; Patterson et al., 2000; Speight, 2011). Furthermore, the floral morphology of the Asteraceae species in the study area does not show a selective trait thus favouring the visit of many insect species (Torres & Galetto, 2002).

In conclusion, this study contributes to provide new knowledge about the composition of flower-visiting arthropod communities within different land uses and along an altitudinal gradient in Alpine context. In this study, it was confirmed that as altitude

increases, the diversity and abundance of Apoidea tends to decrease, gradually being replaced by Brachycera, which play an important role in the pollination of alpine flowers. However, our study also observed a significant abundance of Coleoptera and Thysanoptera as floral visitors in environments located above the tree line. This highlights how the role of pollinators may be performed by more taxa than those commonly studied and investigated. Therefore, it is important to increase research towards these neglected taxa as they could be crucial in the pollination network of high-altitude ecosystems.

This study highlighted that different levels of ecosystem management have varying effects on different arthropod taxa. Specifically, Diptera Brachycera, Hymenoptera wasps, and Hemiptera are more closely associated with land uses that have a high level of management, such as orchards and hay meadows, whereas wild Apoidea and Coleoptera are primarily favoured by lower intensity management. This study provided new knowledge necessary for an assessment of how mountain environment management can impact flower-visiting communities. Given the great variability in the management practices of meadow areas adopted in different alpine regions, it is important that conservation management organizations, such as natural parks, strive to maintain a high degree of environmental diversity to promote a high biodiversity of both animal and plant species. Finally, the presence of honeybees will need to be investigated to evaluate the possible negative effects that this domestic species may have on wild bee communities.

This work will include additional output as:

- Principal Coordinate Analysis (PCoA) of flower visiting arthropod community in relation to different land uses and elevations considered in the study using the R package *vegan* (Oksanen et al., 2022).

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Supplementary material

Supplementary material S1. Taxonomists who identified the sampled arthropods and their affiliation.

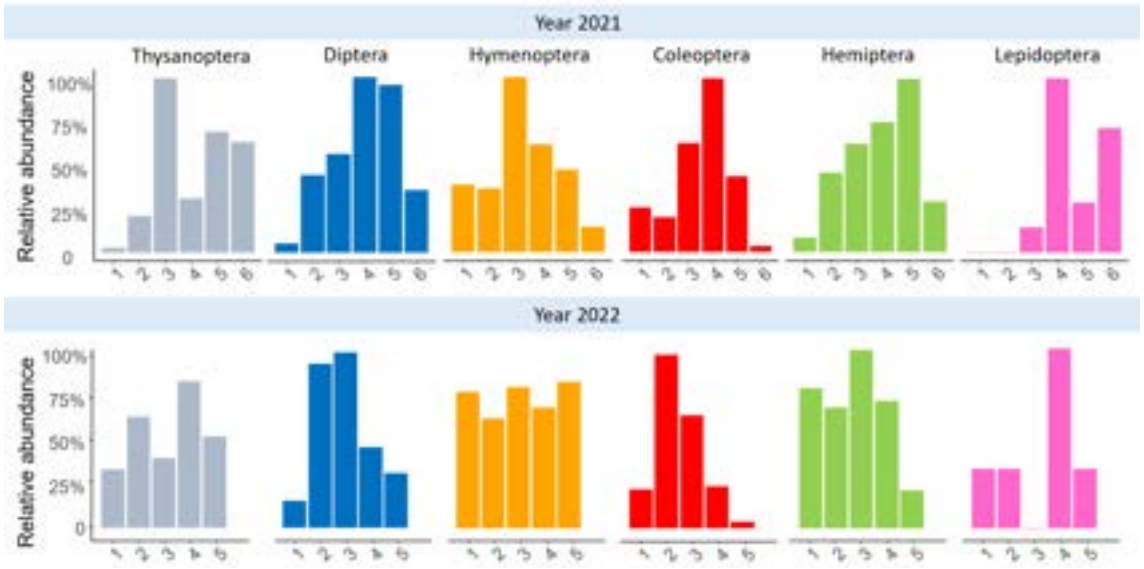
Taxon	Taxonomist (affiliation)
ARACHNIDA	
Araneae	Paolo Pantini (Zoology of Invertebrate Section, Civic Museum of Natural Science Enrico Caffi, Bergamo, Italy)
Oribatida	Massimo Plumari (Museo Civico di Lentate sul Seveso, Lentate sul Seveso, Italy)
HEXAPODA	
Collembola	Barbara Valle (Department of Life Sciences, Università degli Studi di Siena, Siena, Italy; NBFC, National Biodiversity Future Center, Palermo, Italy
Coleoptera, families	Daniele Debiasi (Research and Museum Collections Office, Climate and Ecology Unit, MUSE— Museo delle Scienze, Trento, Italy)
Coleoptera, Apionidae	Enzo Colonnelli (Indipendent taxonomist, Roma, Italy)
Coleoptera, Buprestidae	Daniele Debiasi (Research and Museum Collections Office, Climate and Ecology Unit, MUSE— Museo delle Scienze, Trento, Italy)
Coleoptera, Byturidae	Daniele Debiasi (Research and Museum Collections Office, Climate and Ecology Unit, MUSE— Museo delle Scienze, Trento, Italy)
Coleoptera, Cantharidae	Gianfranco Liberti (Indipendent taxonomist, Milano, Italy)
Coleoptera, Cerambycidae	Daniele Debiasi (Research and Museum Collections Office, Climate and Ecology Unit, MUSE— Museo delle Scienze, Trento, Italy)
Coleoptera, Chrysomelidae	Giulia Magoga (Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, University of Milano, Milano, Italy)
Coleoptera, Coccinellidae	Daniele Debiasi (Research and Museum Collections Office, Climate and Ecology Unit, MUSE— Museo delle Scienze, Trento, Italy)
Coleoptera, Curculionidae	Enzo Colonnelli (Indipendent taxonomist, Roma, Italy)
Coleoptera, Dasytidae	Gianfranco Liberti (Indipendent taxonomist, Milano, Italy)
Coleoptera, Elateridae	Daniele Debiasi (Research and Museum Collections Office, Climate and Ecology Unit, MUSE— Museo delle Scienze, Trento, Italy)
Coleoptera, Malachidae	Gianfranco Liberti (Indipendent taxonomist, Milano, Italy)
Coleoptera, Mordellidae	Enrico Ruzzier (Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Legnaro, Italy)
Coleoptera, Oedemeridae	Daniele Debiasi (Research and Museum Collections Office, Climate and Ecology Unit, MUSE— Museo delle Scienze, Trento, Italy)
Coleoptera, Scarabeidae	Daniele Debiasi (Research and Museum Collections Office, Climate and Ecology Unit, MUSE— Museo delle Scienze, Trento, Italy)
Coleoptera, Scraptiidae	Enrico Ruzzier (Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Legnaro, Italy)
Coleoptera, Staphylinidae	Adriano Zanetti (Civic Museum of Natural History, Lungadige Porta Vittoria, Verona, Italy)
Coleoptera, Tenebrionidae	Daniele Debiasi (Research and Museum Collections Office, Climate and Ecology Unit, MUSE— Museo delle Scienze, Trento, Italy)
Dermaptera	Marco Bonelli
Diptera, families	Daniele Sommaggio (Department of Life Sciences, University of Modena and Reggio Emilia, Modena and Reggio Emilia, Italy)
Diptera, Anthomyiidae	Verner Michelsen (Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark)
Diptera, Chloropidae	Paula Riccardi (Center for Integrative Biodiversity Discovery, Museum für Naturkunde, Leibniz-Institute for Evolution and Biodiversity Science, Berlin, Germany)
Diptera, Faniidae	Andrzej Grzywacz (Nicolaus Copernicus University in Toruń, Faculty of Biological and Veterinary Sciences, Department of Ecology and Biogeography, Toruń, Poland)
Diptera, Muscidae	Daniele Avesani (Zoology Section, Civic Museum of Natural History of Verona, Verona, Italy)
Diptera, Phoridae	Ronald Henry Lambert Disney (Department of Zoology, University of Cambridge, Cambridge, United Kingdom)
Diptera, Syrphidae	Daniele Sommaggio (Department of Life Sciences, University of Modena and Reggio Emilia, Modena and Reggio Emilia, Italy)
Hymenoptera, wasp families (2021)	Filippo Di Giovanni (Department of Agriculture Food and Environment, University of Pisa, Pisa, Italy)

Hymenoptera, wasp families (2022)	Vladimir Žikić (Department of Biology and Ecology, University of Niš, Niš, Serbia)
Hymenoptera, Apoidea	Andree Cappellari (Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padua, Padua, Italy) Maurizio Mei (Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Roma, Roma, Italy)
Hymenoptera, Braconidae	Vladimir Žikić (Department of Biology and Ecology, University of Niš, Niš, Serbia); Željko Tomanović (Department of Invertebrate Zoology and Entomology, University of Belgrade, Belgrade, Serbia)
Hymenoptera, Formicidae	Francesco Pietra (Department of Biosciences, University on Milano, Milano, Italy) Cristina Castracani (Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy) Enrico Schifani (Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy)
Hymenoptera, Ichneumonidae	Filippo Di Giovanni (Department of Agriculture Food and Environment, University of Pisa, Pisa, Italy)
Hymenoptera, Thentrediinae	Daniele Sommaggio (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy)
Lepidoptera	Melania Massaro (Zoology of Invertebrate Section, Civic Museum of Natural Science Enrico Caffi, Bergamo, Italy)
Orthoptera	Marco Bonelli
Thysanoptera	Gregorio Vono (Department of Agriculture, Mediterranea University of Reggio Calabria, Reggio Calabria, Italy)

Supplementary material S2. Number of specimens collected in 2021 and 2022 for each land management type within each altitudinal sector on the valley.

		Low valley			Mid valley		High valley	
Year	**Order**	**Apple orchards**	**Pastures**	**Hay meadows**	**Pastures**	**Hay meadows**	**Pastures**	**Hay meadows**
2021	Diptera	105	47	110	138	164	26	57
2021	Hymenoptera	49	80	149	24	17	12	1
2021	Coleoptera	10	60	23	4	14	15	89
2021	Thysanoptera	729	127	513	13	42	97	0
2021	Hemiptera	12	25	17	7	19	0	3
2021	Lepidoptera	3	4	2	3	2	1	0
2021	Orthoptera	0	0	3	0	0	0	0
2021	Dermaptera	2	0	0	0	0	0	0
2021	Neuroptera	0	0	0	0	0	0	0
2021	Collembola	2	1	0	1	1	0	1
2021	Aracnidae	1	3	2	0	1	0	0
2021	Acari	0	0	0	1	0	2	1
2022	Diptera	22	23	72	84	66	46	36
2022	Hymenoptera	12	35	50	15	5	6	2
2022	Coleoptera	2	23	8	5	6	26	36
2022	Thysanoptera	301	92	485	7	57	62	0
2022	Hemiptera	8	26	28	8	13	6	6
2022	Lepidoptera	0	0	1	0	0	0	0
2022	Orthoptera	0	1	0	4	0	1	0
2022	Dermaptera	0	0	0	0	0	0	0
2022	Neuroptera	2	0	0	0	0	0	0
2022	Collembola	0	0	0	0	2	0	0
2022	Ragni	1	3	1	0	1	2	0
2022	Acari	0	0	2	0	2	0	1

Supplementary Material S3. Relative abundance of most represented arthropod orders sampled during field work of 2021 and 2022.



Supplementary Material S4. Families relative abundance of the most represented orders (Coleoptera, Diptera, and Hymenoptera) collected during the fieldwork. "ND"= family not identified.

Coleoptera	Low valley			Mid valley		High valley	
Family	Apple Orchards	Pastures	Hay meadows	Pastures	Hay meadows	Pastures	Grasslands
Apionidae	-	2.38	29.03	-	-	-	-
Buprestidae	-	8.33	16.13	-	-	-	-
Byturidae	-	5.95	-	-	-	-	-
Cantharidae	7.69	-	12.90	-	-	2.44	0.79
Cerambycidae	-	-	-	-	4.76	-	-
Chrysomelidae	-	2.38	6.45	33.33	33.33	-	0.79
Cryptophagidae	-	-	3.23	-	-	-	-
Coccinellidae	-	-	3.23	-	4.76	-	-
Curculionidae	15.38	-	6.45	11.11	-	-	-
Dasytidae	-	20.24	-	22.22	-	97.56	96.06
Elateridae	-	-	3.23	-	9.52	-	-
Meloidae	7.69	-	-	-	-	-	-
Mordellidae	-	-	3.23	-	-	-	-
Nitidulidae	23.08	54.76	6.45	-	-	-	-
Oedemeridae	7.69	1.19	3.23	-	-	-	-
Scarabeidae	38.46	3.57	-	22.22	14.29	-	-
Scraptiidae	-	1.19	3.23	-	-	-	-
Staphylinidae	-	-	-	-	9.52	-	2.36
Tenebrionidae	-	-	3.23	-	-	-	-
ND	-	-	-	11.11	23.81	-	-
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Diptera	Low valley			Mid valley		High valley	
Family	Apple Orchards	Pastures	Hay meadows	Pastures	Hay meadows	Pastures	Grasslands
Agromyzidae	2.84	1.16	-	-	0.44	-	1.03
Anthomyiidae	2.84	6.98	6.84	16.80	5.26	8.33	9.28
Atelestidae	-	-	-	0.40	-	-	-
Bibionidae	-	-	-	1.20	2.63	-	-
Cecidomyiidae	2.84	-	0.53	0.40	-	1.39	1.03
Ceratopogonidae	-	1.16	-	-	-	-	-
Chamaemyiidae	-	-	-	0.40	-	-	1.03
Chloropidae	31.91	22.09	10.53	8.80	5.70	1.39	4.12
Conopidae	-	-	-	0.40	-	-	-
Dolichopodidae	-	1.16	-	0.80	0.88	1.39	2.06
Drosophilidae	2.84	4.65	0.53	-	-	-	-
Empididae	-	1.16	-	0.80	0.88	-	-
Ephydridae	-	-	1.05	-	-	-	-
Fanniidae	-	-	-	-	0.44	-	-
Hybotidae	2.13	1.16	0.53	0.40	0.88	-	1.03
Lauxanidae	-	-	-	-	0.44	-	-
Lonchopteridae	-	-	-	-	0.44	-	-
Milichidae	-	-	-	-	0.44	-	-
Muscidae	14.89	38.37	29.47	49.20	40.35	65.28	44.33
Phoridae	4.96	1.16	-	4.00	3.51	-	6.19

Polleniidae	-	-	-	-	0.44	-	-
Psilidae	-	-	-	0.40	0.44	-	-
Sarcophagidae	-	1.16	-	0.40	-	-	-
Scathophagidae	-	-	-	-	0.88	-	-
Sciaridae	3.55	2.33	2.63	1.60	6.58	1.39	8.25
Sepsidae	-	2.33	0.53	-	0.44	-	-
Simuliidae	7.09	-	0.53	-	-	-	-
Sphaeroceridae	-	-	0.53	-	-	-	-
Syrphidae	21.99	13.95	42.63	12.80	28.07	20.83	21.65
Tachinidae	1.42	1.16	1.58	0.80	0.44	-	-
Tephritidae	-	-	1.58	-	0.44	-	-
ND	0.71	-	0.53	0.40	-	-	-
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Hymenoptera	Low valley			Mid valley		High valley	
Family	Apple Orchards	Pastures	Hay meadows	Pastures	Hay meadows	Pastures	Grasslands
Andrenidae	1.54	0.83	4.48	2.44	-	-	-
Apidae	23.08	9.17	15.92	12.20	27.27	-	-
Braconidae	4.62	0.83	1.99	4.88	-	5.26	-
Chalcicoidea	-	0.83	-	-	-	5.26	-
Chalcidoidea	1.54	-	-	-	-	-	-
Colletidae	1.54	0.83	-	-	-	-	-
Cynipidae	-	-	0.50	-	-	-	-
Diapriidae	1.54	0.83	-	-	-	5.26	-
Eulophidae	-	-	-	-	9.09	5.26	-
Eulphidae	-	-	0.50	-	-	5.26	-
Figitidae	-	1.67	-	-	-	-	25.00
Formicidae	36.92	46.67	67.16	51.22	27.27	42.11	-
Halictidae	6.15	21.67	4.98	7.32	-	5.26	5-
Ichneumonidae	-	-	-	-	4.55	-	25.00
Ichneumonidae	1.54	1.67	-	-	-	-	-
Megachilidae	-	9.17	1.49	-	4.55	-	-
Mymaridae	-	0.83	0.50	-	9.09	-	-
Platygastridae	3.08	1.67	-	9.76	-	-	-
Pteromalidae	3.08	0.83	-	-	-	-	-
Scelionidae	9.23	1.67	1.00	4.88	4.55	-	-
Tenthredinidae	-	0.83	1.00	2.44	4.55	-	-
Torymidae	-	-	-	-	-	5.26	-
ND	6.15	-	0.50	4.88	9.09	21.05	-
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Supplementary Material S5. Abundance of specimens identified at least at genus level collected for each land use and altitudinal sector within Martello Valley in 2021 and 2022. BME: low valley apple orchards; BPA: low valley pastures; BPR: low valley hay meadows; MPA: mid valley pastures; MPR: mid valley hay meadows; VPA: high valley pastures; VPR: high valley grasslands.

Year	Order	Family	Genus	Species	Author	BME	BPA	BPR	MPA	MPR	VPA	VPR
2021	Coleoptera	Buprestidae	*Anthaxia (Anthaxia)*	*Anthaxia (Anthaxia) podolica*	Mannerheim, 1837	0	2	0	0	0	0	0
2021	Coleoptera	Buprestidae	*Anthaxia (Melanthaxia)*	*Anthaxia (Melanthaxia) helvetica*	(Stierlin, 1868)	0	2	0	0	0	0	0
2021	Coleoptera	Buprestidae	*Anthaxia (Melanthaxia)*	*Anthaxia (Melanthaxia) quadripunctata*	(Linnaeus, 1758)	0	1	3	0	0	1	0
2021	Coleoptera	Byturidae	*Byturus*	*Byturus ochraceus*	(Scriba, 1791)	0	1	0	0	0	0	0
2021	Coleoptera	Cantharidae	*Cantharis*	*Cantharis livida*	Linnaeus, 1758	1	0	1	0	0	0	0
2021	Coleoptera	Cantharidae	*Malthodes*	*Malthodes* cfr. *fuscus*		0	0	1	0	0	0	0
2021	Coleoptera	Cantharidae	*Rhagonycha (Rhagonycha)*	*Rhagonycha (Rhagonycha) fulva*	(Scopoli, 1763)	0	0	2	0	0	0	0
2021	Coleoptera	Cerambycidae	*Brachyta*	*Brachyta interrogationis*	(Linnaeus, 1758)	0	1	0	0	0	0	0
2021	Coleoptera	Chrysomelidae	*Cryptocephalus*	*Cryptocephalus transiens*	Franz, 1949	0	1	1	0	0	0	0
2021	Coleoptera	Chrysomelidae	*Cryptocephalus*	*Cryptocephalus violaceus*	Laicharting, 1781	0	0	0	2	0	0	0
2021	Coleoptera	Chrysomelidae	*Gastrophysa (Gastrophysa)*	*Gastrophysa (Gastrophysa) viridula*	(De Geer, 1775)	0	0	0	0	2	0	0
2021	Coleoptera	Chrysomelidae	*Neocrepidodera*	*Neocrepidodera peirolerii*	(Kutschera, 1860)	0	0	0	0	5	0	0
2021	Coleoptera	Coccinellidae	*cfr. Ceratomegilla*	cfr. *Ceratomegilla notata*		0	0	0	0	1	0	0
2021	Coleoptera	Coccinellidae	*Coccinella (Coccinella)*	*Coccinella (Coccinella) septempunctata*	(Linnaeus, 1758)	0	0	1	0	0	0	0
2021	Coleoptera	Dasytidae	*Clanoptilus*	*Clanoptilus affinis* subsp. *subconcolor*	(Pic, 1911)	0	1	0	0	0	0	0
2021	Coleoptera	Dasytidae	*Danacea*	*Danacea nigritarsis* subsp. *alpina*	Pic, 1894	0	10	0	0	0	0	0
2021	Coleoptera	Dasytidae	*Dasytes*	*Dasytes alpigradus*	Kiesenwetter, 1863	0	0	0	1	0	15	90
2021	Coleoptera	Elateridae	*Agrypnus*	*Agrypnus murinus*	(Linnaeus, 1758)	0	0	1	0	0	0	0
2021	Coleoptera	Elateridae	*Ctenicera*	*Ctenicera cuprea*	(Fabricius, 1775)	0	0	0	0	2	0	0
2021	Coleoptera	Nitidulidae	*Meligethes*		Stephens, 1830	1	40	1	0	0	0	0
2021	Coleoptera	Oedemeridae	*Oedemera*	*Oedemera* gr. *virescens*		0	0	1	0	0	0	0
2021	Coleoptera	Oedemeridae	*Oedemera (Oedemera)*	*Oedemera (Oedemera) podagrariae*	(Linnaeus, 1767)	1	0	0	0	0	0	0
2021	Coleoptera	Scarabeidae	*Phyllopertha*	*Phyllopertha horticola*	Linnaeus, 1758	5	0	0	1	2	0	0
2021	Coleoptera	Scarabeidae	*Trichius*	*Trichius fasciatus*	Linnaeus, 1758	0	2	0	0	0	0	0
2021	Coleoptera	Staphylinidae	*Eusphalerum*			0	0	0	0	0	0	1
2021	Coleoptera	Tenebrionidae	*Cteniopus*	*Cteniopus sulphureus*	(Linnaeus, 1758)	0	0	1	0	0	0	0
2022	Coleoptera	Buprestidae	*Anthaxia (Melanthaxia)*	*Anthaxia (Melanthaxia) quadripunctata*	(Linnaeus, 1758)	0	2	1	0	0	0	0
2022	Coleoptera	Byturidae	*Byturus*	*Byturus ochraceus*	(Scriba, 1791)	0	4	0	0	0	0	0
2022	Coleoptera	Cantharidae	*Cantharis*	*Cantharis fibulata*	Märkel, 1852	0	0	0	0	0	0	1
2022	Coleoptera	Cantharidae	*Malthodes*	*Malthodes trifurcatus*	Kiesenwetter, 1852	0	0	0	0	0	1	0
2022	Coleoptera	Chrysomelidae	*Cryptocephalus*			0	1	0	1	0	0	1
2022	Coleoptera	Cryptophagidae	cfr. *Atomaria*			0	0	1	0	0	0	0

2022	Coleoptera	Dasytidae	*Clanoptilus*	*Clanoptilus affinis* subsp. *subconcolor*	(Pic, 1911)	0	5	0	0	0	0	0
2022	Coleoptera	Dasytidae	*Danacea*	*Danacea nigritarsis* subsp. *alpina*	Pic, 1894	0	1	0	0	0	0	0
2022	Coleoptera	Dasytidae	*Dasytes*	*Dasytes alpigradus*	Kiesenwetter, 1863	0	0	0	1	0	25	31
2022	Coleoptera	Nitidulidae	cfr. *Meligethes*			0	1	0	0	0	0	0
2022	Coleoptera	Nitidulidae	cfr. *Meligethes*			0	3	1	0	0	0	0
2022	Coleoptera	Oedemeridae	*Oedemera (Oedemera)*	*Oedemera (Oedemera) flavipes*	(Fabricius, 1792)	0	1	0	0	0	0	0
2022	Coleoptera	Scarabeidae	*Oxythyrea*	*Oxythyrea funesta*	(Poda, 1761)	0	1	0	0	0	0	0
2022	Coleoptera	Scarabeidae	*Phyllopertha*	*Phyllopertha horticola*	Linnaeus, 1758	0	0	0	1	1	0	0

Year	Order	Family	Genus	Species	Author	BME	BPA	BPR	MPA	MPR	VPA	VPR
2021	Diptera	Fanniidae	*Fannia*	cf. *Fannia novalis*		0	0	0	0	1	0	0
2021	Diptera	Muscidae	*Coenosia*			2	0	1	17	1	17	24
2021	Diptera	Muscidae	*Coenosia*	*Coenosia ambigua*	Séguy, 1923	0	0	0	0	0	1	0
2021	Diptera	Muscidae	*Coenosia*	*Coenosia obscuricula*	(Rondani, 1871)	0	0	1	2	0	16	24
2021	Diptera	Muscidae	*Coenosia*	*Coenosia pedella*	(Fallén, 1825)	0	0	0	14	0	0	0
2021	Diptera	Muscidae	*Coenosia*	*Coenosia styriaca*	Hennig, 1961	0	0	0	0	1	0	0
2021	Diptera	Muscidae	*Coenosia*	*Coenosia verralli*	Collin, 1953	2	0	0	0	0	0	0
2021	Diptera	Muscidae	*Drymeia*			0	1	0	5	5	2	0
2021	Diptera	Muscidae	*Drymeia*	*Drymeia alpicola*	(Rondani, 1871)	0	0	0	2	1	1	0
2021	Diptera	Muscidae	*Drymeia*	*Drymeia brumalis*	(Rondani, 1866)	0	1	0	0	3	0	0
2021	Diptera	Muscidae	*Drymeia*	*Drymeia hamata*	(Fallén, 1823)	0	0	0	3	0	1	0
2021	Diptera	Muscidae	*Helina*	*Helina annosa*	(Zetterstedt, 1838)	0	0	0	1	1	0	0
2021	Diptera	Muscidae	*Helina*	*Helina obtusipennis*	(Fallén, 1823)	0	0	0	0	0	0	1
2021	Diptera	Muscidae	*Helina*	*Helina quadrum*	(Fabricius, 1805)	0	0	1	0	0	0	0
2021	Diptera	Muscidae	*Helina*	*Helina reversio*	(Harris, 1780)	0	0	0	0	2	0	0
2021	Diptera	Muscidae	*Hydrotaea*			0	0	1	0	1	0	0
2021	Diptera	Muscidae	*Hydrotaea*	*Hydrotaea albipuncta*	(Zetterstedt, 1845)	0	0	0	0	1	0	0
2021	Diptera	Muscidae	*Phaonia*	*Phaonia alpicola*	(Zetterstedt, 1845)	0	0	0	0	0	0	1
2021	Diptera	Muscidae	*Phaonia*	*Phaonia angelicae*	(Scopoli, 1763)	6	0	0	0	0	0	0
2021	Diptera	Muscidae	*Phaonia*	*Phaonia consobrina*	(Zetterstedt, 1838)	0	0	0	0	0	1	0
2021	Diptera	Muscidae	*Phaonia*	*Phaonia hybrida*	(Schnabl, 1888)	0	0	0	6	5	0	0
2021	Diptera	Muscidae	*Phaonia*	*Phaonia meigeni*	Pont, 1986	0	0	0	0	1	0	0
2021	Diptera	Muscidae	*Phaonia*	*Phaonia serva*	(Meigen, 1826)	0	0	0	2	6	0	0
2021	Diptera	Muscidae	*Thricops*			12	30	47	71	54	2	5
2021	Diptera	Muscidae	*Thricops*	*Thricops cunctans*	(Meigen, 1826)	12	28	33	18	32	0	3
2021	Diptera	Muscidae	*Thricops*	*Thricops innocuus*	(Zetterstedt, 1838)	0	1	9	8	5	0	0

2021	Diptera	Muscidae	*Thricops*	*Thricops longipes*	(Zetterstedt, 1845)	0	0	0	1	3	0	0
2021	Diptera	Muscidae	*Thricops*	*Thricops nigritellus*	(Zetterstedt, 1838)	0	0	0	19	14	2	0
2021	Diptera	Muscidae	*Thricops*	*Thricops rostratus*	(Meade, 1882)	0	0	0	0	0	0	2
2021	Diptera	Muscidae	*Thricops*	*Thricops semicinereus*	(Wiedemann, 1817)	0	1	2	24	0	0	0
2021	Diptera	Psilidae	*Chamaepsila*	*Chamaepsila atra*	(Meigen, 1826)	0	0	0	0	1	0	0
2021	Diptera	Stratiomyidae	*Pachygaster*	*Pachygaster atra*	Panzer, 1787	0	0	1	0	0	0	0
2021	Diptera	Stratiomyidae	*Sargus*			0	0	1	0	0	0	0
2021	Diptera	Syrphidae	*Cheilosia*	*Cheilosia himantopa*	(Panzer 1798)	1	0	0	0	0	0	0
2021	Diptera	Syrphidae	*Cheilosia*	*Cheilosia proxima*	(Zetterstedt, 1843)	0	0	0	0	1	0	0
2021	Diptera	Syrphidae	*Cheilosia*	*Cheilosia vernalis*	(Fallen, 1817)	0	0	0	0	1	0	0
2021	Diptera	Syrphidae	*Cheilosia (Cheilosia)*	*Cheilosia (Cheilosia) melanura*	(Becker, 1894)	0	0	0	0	1	0	0
2021	Diptera	Syrphidae	*Cheilosia (Cheilosia)*	*Cheilosia (Cheilosia) rhynchops*	Egger, 1860	0	0	0	0	2	0	0
2021	Diptera	Syrphidae	*Cheilosia (Cheilosia)*	*Cheilosia flavipes*	(Panzer, 1798)	0	1	0	0	0	0	0
2021	Diptera	Syrphidae	*Cheilosia (Eucatosyrphus)*	*Cheilosia (Eucatosyrphus) longula*	(Zetterstedt, 1838)	0	0	0	0	2	0	0
2021	Diptera	Syrphidae	*Cheilosia (Taeniocheilosia)*	*Cheilosia (Taeniocheilosia) nigripes*	(Meigen, 1822)	1	1	0	0	0	0	0
2021	Diptera	Syrphidae	*Cheilosia (Taeniocheilosia)*	*Cheilosia (Taeniocheilosia) pedemontana*	Rodani, 1857	0	0	0	0	1	0	0
2021	Diptera	Syrphidae	*Cheilosia (Taeniocheilosia)*	*Cheilosia (Taeniocheilosia) vangaveri*	Timon-David, 1937	0	0	0	0	0	0	1
2021	Diptera	Syrphidae	*Chrysotoxum*	*Chrysotoxum bicinctum*		0	0	3	1	0	0	0
2021	Diptera	Syrphidae	*Chrysotoxum*	*Chrysotoxum fasciatum*	(Müller, 1764)	0	0	0	1	0	0	0
2021	Diptera	Syrphidae	*Chrysotoxum*	*Chrysotoxum festivum*	(Linnaeus, 1758)	0	0	2	0	0	0	0
2021	Diptera	Syrphidae	*Dasysrphus*	*Dasysrphus pinastri*	(De Geer, 1776)	0	0	0	0	1	0	0
2021	Diptera	Syrphidae	*Episyrphus*	*Episyrphus balteatus*	(De Geer, 1776)	6	0	2	0	0	0	0
2021	Diptera	Syrphidae	*Eristalis*	*Eristalis interrupta*	(Poda, 1761)	0	2	0	0	0	0	0
2021	Diptera	Syrphidae	*Eristalis*	*Eristalis rupium*	Fabricius, 1805	0	0	0	0	0	1	0
2021	Diptera	Syrphidae	*Eristalis*	*Eristalis tenax*	(Linneus., 1758)	6	0	6	0	0	0	0
2021	Diptera	Syrphidae	*Eupeodes*	*Eupeodes corollae*	(Fabricius, 1794)	2	0	0	1	2	0	0
2021	Diptera	Syrphidae	*Melanostoma*	*Melanostoma mellinum*	(Linnaeus, 1758)	1	1	1	6	0	0	0
2021	Diptera	Syrphidae	*Melanostoma*	*Melanostoma mellinum*	Maschio	1	1	1	6	0	0	0
2021	Diptera	Syrphidae	*Merodon*	*Merodon cinereus group*		0	0	1	0	0	0	0
2021	Diptera	Syrphidae	*Neocnemodon*	*Neocnemodon* cfr. *larusi/pubescens*		0	0	0	0	2	0	0
2021	Diptera	Syrphidae	*Orthonevra*	*Orthonevra tristis*	(Loew, 1871)	0	0	0	0	1	0	0
2021	Diptera	Syrphidae	*Parasyrphus*	*Parasyrphus annulatus*	(Zetterstedt, 1838)	1	0	0	0	13	0	1
2021	Diptera	Syrphidae	*Parasyrphus*	*Parasyrphus lineolus*	(Zetterstedt, 1843)	0	0	0	0	1	0	0
2021	Diptera	Syrphidae	*Pipiza*	*Pipiza notata*	Meigen, 1822	0	0	0	0	1	0	0
2021	Diptera	Syrphidae	*Platycheirus*	*Platycheirus albimanus*	(Fabricius, 1781)	1	0	0	0	0	0	0
2021	Diptera	Syrphidae	*Sphaerophoria*			2	0	8	2	4	0	0
2021	Diptera	Syrphidae	*Sphaerophoria*	*Sphaerophoria interrupta*	(Fabricius, 1805)	0	0	2	2	3	0	0

2021	Diptera	Syrphidae	*Sphaerophoria*	*Sphaerophoria scripta*	(Linnaeus, 1758)	2	0	4	0	1	0	0
2021	Diptera	Syrphidae	*Syritta*	*Syritta pipiens*	(Linnaeus, 1758)	1	0	8	0	0	0	0
2021	Diptera	Syrphidae	*Syrphocheilosia*	*Syrphocheilosia claviventris*	(Strobls, 1910)	0	0	0	0	1	0	5
2021	Diptera	Syrphidae	*Xylota*	*Xylota caeruleiventris*	Zetterstedt, 1838	0	0	0	0	1	0	0
2021	Diptera	Syrphidae	*Xylota*	*Xylota jakutorum*	Bagachanova, 1980	1	0	0	0	2	0	0
2022	Diptera	Conopidae	*Zodion*	*Zodion cinereum*	(Fabricius, 1794)	0	0	0	1	0	0	0
2022	Diptera	Psilidae	*Chamaepsila*	*Chamaepsila nigra*	(Fallén, 1820)	0	0	0	1	0	0	0
2022	Diptera	Scathophagidae	*Scathophaga*			0	0	0	0	2	0	0
2022	Diptera	Syrphidae	*Cheilosia*	*Cheilosia pagana*	(Meigen, 1822)	0	0	1	0	0	0	0
2022	Diptera	Syrphidae	*Chrysotoxum*	*Chrysotoxum bicinctum*	(Linnaeus, 1758)	0	0	0	1	0	0	0
2022	Diptera	Syrphidae	*Chrysotoxum*	*Chrysotoxum fasciatum*	(Müller, 1764)	0	0	1	0	0	0	0
2022	Diptera	Syrphidae	*Episyrphus*	*Episyrphus balteatus*	(De Geer, 1776)	1	0	6	1	0	0	0
2022	Diptera	Syrphidae	*Episyrphus*	*Epysyrphus balteatus*	(De Geer, 1776)	0	0	0	0	1	0	0
2022	Diptera	Syrphidae	*Eristalis*	*Eristalis similis*	(Fallen, 1817)	0	0	0	0	0	1	0
2022	Diptera	Syrphidae	*Eristalis*	*Eristalis tenax*	(Linneus., 1758)	0	1	16	1	0	1	0
2022	Diptera	Syrphidae	*Eupeodes*	*Eupeodes corollae*	(Fabricius, 1794)	0	3	0	0	0	0	2
2022	Diptera	Syrphidae	*Eupeodes*	*Eupeodes lapponicus*	(Zetterstedt, 1838)	1	0	6	2	3	3	10
2022	Diptera	Syrphidae	*Eupeodes*	*Eupeodes luniger*	(Meigen, 1822)	0	0	0	2	0	1	0
2022	Diptera	Syrphidae	*Eupeodes*	*Eupeodes nitens*	(Zetterstedt, 1843)	0	0	0	1	0	0	0
2022	Diptera	Syrphidae	*Melanostoma*	*Melanostoma mellinum*	(Linnaeus, 1758)	0	0	1	0	0	0	0
2022	Diptera	Syrphidae	*Meliscaeva*	*Meliscaeva auricollis*	(Meigen, 1822)	0	0	2	0	0	0	0
2022	Diptera	Syrphidae	*Parasyrphus*	*Parasyrphus annulatus*	(Zetterstedt, 1838)	0	0	2	0	1	0	0
2022	Diptera	Syrphidae	*Parasyrphus*	*Parasyrphus lineolus*	(Zetterstedt, 1843)	0	0	0	0	1	0	0
2022	Diptera	Syrphidae	*Parasyrphus*	*Parasyrphus vittiger*	(Zetterstedt, 1843)	0	0	1	1	1	0	0
2022	Diptera	Syrphidae	*Pipiza*	*Pipiza notata*	Meigen, 1822	0	0	0	1	1	0	0
2022	Diptera	Syrphidae	*Platycheirus*	*Platycheirus albimanus*	(Fabricius, 1781)	0	0	0	1	0	0	0
2022	Diptera	Syrphidae	*Scaeva*			2	1	1	3	15	8	2
2022	Diptera	Syrphidae	*Scaeva*	*Scaeva pyrastri*	(Linnaeus, 1758)	0	0	0	0	0	1	0
2022	Diptera	Syrphidae	*Scaeva*	*Scaeva selenitica*	(Meigen, 1822)	1	1	1	3	15	7	2
2022	Diptera	Syrphidae	*Sphaerophoria*	*Sphaerophoria interrupta*	(Fabricius, 1805)	0	2	1	1	0	0	0
2022	Diptera	Syrphidae	*Sphaerophoria*	*Sphaerophoria scripta*	(Linnaeus, 1758)	0	0	7	0	3	0	0
2022	Diptera	Syrphidae	*Syrphus*	*Syrphus ribesii*	(Linnaeus, 1758)	0	0	0	1	1	0	0
2022	Diptera	Syrphidae	*Syrphus*	*Syrphus torvus*	Osten Sacken, 1875	0	0	2	4	0	0	0
2022	Diptera	Syrphidae	*Syrphus*	*Syrphus vitripennis*	Meigen, 1822	0	0	5	2	0	0	0

Year	Order	Family	Genus	Species	Author	BME	BPA	BPR	MPA	MPR	VPA	VPR
2021	Hymenoptera	Andrenidae	*Andrena*	*Andrena fulvago*	(Christ, 1791)	1	0	0	0	0	0	0
2021	Hymenoptera	Andrenidae	*Andrena*	*Andrena haemorrhoa*	(Fabricius, 1781)	0	0	1	0	0	0	0
2021	Hymenoptera	Andrenidae	*Andrena*	*Andrena thoracica*	(Fabricius, 1775)	0	1	0	0	0	0	0
2021	Hymenoptera	Andrenidae	*Panurgus*	*Panurgus banksianus*	(Kirby, 1802)	0	0	6	1	0	0	0
2021	Hymenoptera	Apidae	*Apis*	*Apis mellifera*	Linnaeus, 1758	12	1	12	0	6	0	0
2021	Hymenoptera	Apidae	*Bombus*	*Bombus lapidarius*	(Linnaeus, 1758)	1	5	0	0	0	0	0
2021	Hymenoptera	Apidae	*Bombus*	*Bombus monticola*	Smith, 1849	0	0	0	1	0	0	0
2021	Hymenoptera	Apidae	*Bombus*	*Bombus pascuorum*	(Scopoli, 1763)	0	0	1	0	0	0	0
2021	Hymenoptera	Apidae	*Bombus*	*Bombus pyrenaeus*	Pérez, 1879	0	0	0	4	0	0	0
2021	Hymenoptera	Apidae	*Bombus*	*Bombus sylvarum*	(Linnaeus, 1761)	0	0	2	0	0	0	0
2021	Hymenoptera	Apidae	*Bombus*	*Bombus terrestris/lucorum*		0	0	1	0	0	0	0
2021	Hymenoptera	Braconidae	*Alysiinae*			1	0	1	0	0	0	0
2021	Hymenoptera	Braconidae	*Aphidius*			0	1	0	0	0	0	0
2021	Hymenoptera	Braconidae	*Asobara*			2	0	0	1	0	0	0
2021	Hymenoptera	Braconidae	*Aspilota*			0	0	0	0	0	1	0
2021	Hymenoptera	Braconidae	*Coelinidea*			0	0	1	0	0	0	0
2021	Hymenoptera	Braconidae	*Dacnusa/Chorebus*			0	0	1	0	0	0	0
2021	Hymenoptera	Formicidae	*Camponotus*	*Camponotus ligniperda*	(Latreille, 1802)	0	1	0	0	0	0	0
2021	Hymenoptera	Formicidae	*Formica*	*Formica clara*	Forel, 1886	0	4	0	0	0	0	0
2021	Hymenoptera	Formicidae	*Formica*	*Formica exsecta*	Nylander, 1846	0	0	0	4	0	0	0
2021	Hymenoptera	Formicidae	*Formica*	*Formica fusca*	Linnaeus, 1758	0	0	4	0	0	0	0
2021	Hymenoptera	Formicidae	*Formica*	*Formica lemani*	Bondroit, 1917	0	1	0	11	6	5	0
2021	Hymenoptera	Formicidae	*Formica*	*Formica rufibarbis*	Fabricius, 1793	0	0	1	0	0	0	0
2021	Hymenoptera	Formicidae	*Formica*	*Formica sanguinea*	Latreille, 1798	0	3	0	0	0	0	0
2021	Hymenoptera	Formicidae	*Lasius*	*Lasius emarginatus*	(Olivier, 1792)	2	0	0	0	0	0	0
2021	Hymenoptera	Formicidae	*Lasius*	*Lasius niger*	(Linnaeus, 1758)	12	0	96	0	0	0	0
2021	Hymenoptera	Formicidae	*Lasius*	*Lasius psammophilus*	Seifert, 1992	2	5	0	0	0	0	0
2021	Hymenoptera	Formicidae	*Myrmica*	*Myrmica rubra*	(Linnaeus, 1758)	0	24	9	0	0	0	0
2021	Hymenoptera	Formicidae	*Tapinoma*	*Tapinoma subboreale*	Seifert, 2012	0	1	0	0	0	0	0
2021	Hymenoptera	Formicidae	*Temnothorax*	*Temnothorax unifasciatus*	(Latreille, 1798)	0	1	0	0	0	0	0
2021	Hymenoptera	Formicidae	*Tetramorium*	*Tetramorium immigrans*	Santschi, 1927	0	4	0	0	0	0	0
2021	Hymenoptera	Formicidae	*Tetramorium*	*Tetramorium indocile*	Santschi, 1927	0	1	0	0	0	0	0
2021	Hymenoptera	Halictidae	*Dufourea*	*Dufourea alpina*	Morawitz, 1865	0	0	0	0	0	1	0
2021	Hymenoptera	Halictidae	*Dufourea*	*Dufourea dentiventris*	(Nylander, 1848)	1	0	0	0	0	0	0
2021	Hymenoptera	Halictidae	*Halictus*	*Halictus simplex*	Blüthgen, 1923	0	2	0	0	0	0	0
2021	Hymenoptera	Halictidae	*Halictus*	*Halictus subauratus*	(Rossi, 1792)	0	1	0	0	0	0	0

2021	Hymenoptera	Halictidae	*Halictus*	*Halictus tumulorum*	(Linnaeus, 1758)	0	1	0	0	0	0	0
2021	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum albipes*	(Fabricius, 1781)	0	1	0	0	0	0	0
2021	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum calceatum*	(Scopoli, 1763)	2	0	0	0	0	0	0
2021	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum* cf. *fratellum*		0	0	0	0	0	0	2
2021	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum cupromicans group*		1	0	1	2	0	0	0
2021	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum laticeps*	(Schenck, 1870)	0	1	0	0	0	0	0
2021	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum leucozonium*	(Schrank, 1781)	0	2	0	0	0	0	0
2021	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum morio*	(Fabricius, 1793)	0	2	2	0	0	0	0
2021	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum zonulum*	(Smith, 1848)	0	0	2	0	0	0	0
2021	Hymenoptera	Ichneumonidae	*Diadegma*			0	2	0	0	0	0	0
2021	Hymenoptera	Ichneumonidae	*Stenomacrus*			1	0	0	0	0	0	0
2021	Hymenoptera	Megachilidae	*Chelostoma*	*Chelostoma campanularum*	Kirby, 1802)	0	1	0	0	0	0	0
2021	Hymenoptera	Megachilidae	*Chelostoma*	*Chelostoma florisomne*	(Linnaeus, 1758)	0	2	0	0	1	0	0
2021	Hymenoptera	Megachilidae	*Heriades*	*Heriades truncorum*	(Linnaeus, 1758)	0	0	3	0	0	0	0
2021	Hymenoptera	Megachilidae	*Hoplitis*	*Hoplitis leucomelana*	(Kirby, 1802)	0	1	0	0	0	0	0
2021	Hymenoptera	Megachilidae	*Megachile*	*Megachile lagopoda*	(Linnaeus, 1761)	0	1	0	0	0	0	0
2021	Hymenoptera	Megachilidae	*Osmia*	*Osmia gallarum*	Spinola, 1808	0	6	0	0	0	0	0
2021	Hymenoptera	Tenthredinidae	*Dolerus*	*Dolerus nitens*	Zaddach, 1859	0	0	0	0	1	0	0
2021	Hymenoptera	Tenthredinidae	*Tenthredo*	*Tenthredo arcuata*	Forster, 1771	0	1	2	0	0	0	0
2021	Hymenoptera	Tenthredinidae	*Tenthredo*	*Tenthredo mioceras*	(Enslin, 1912)	0	0	0	1	0	0	0
2022	Hymenoptera	Andrenidae	*Panurgus*	*Panurgus banksianus*	(Kirby, 1802)	0	0	2	0	0	0	0
2022	Hymenoptera	Apidae	*Apis*	*Apis mellifera*	Linnaeus, 1758	1	1	14	0	0	0	0
2022	Hymenoptera	Apidae	*Bombus*	*Bombus humilis*	Illiger, 1806	0	2	0	0	0	0	0
2022	Hymenoptera	Apidae	*Bombus*	*Bombus lapidarius*	Linnaeus, 1758)	0	1	1	0	0	0	0
2022	Hymenoptera	Apidae	*Bombus*	*Bombus lucorum/terrestris*		1	0	0	0	0	0	0
2022	Hymenoptera	Apidae	*Bombus*	*Bombus pascuorum*	(Scopoli, 1763)	0	1	1	0	0	0	0
2022	Hymenoptera	Braconidae	*Chorebus*			0	0	1	0	0	0	0
2022	Hymenoptera	Braconidae	*Synaldis*			0	0	0	1	0	0	0
2022	Hymenoptera	Colletidae	*Hylaeus*	*Hylaeus annulatus*	(Linnaeus, 1758)	1	0	0	0	0	0	0
2022	Hymenoptera	Colletidae	*Hylaeus*	*Hylaeus confusus*	Nylander, 1852	0	1	0	0	0	0	0
2022	Hymenoptera	Formicidae	*Formica*	*Formica fusca*	Linnaeus, 1758	0	0	0	1	0	0	0
2022	Hymenoptera	Formicidae	*Formica*	*Formica lemani*	Bondroit, 1917	0	0	0	4	0	2	0
2022	Hymenoptera	Formicidae	*Formica*	*Formica rufibarbis*	Fabricius, 1793	0	1	0	0	0	0	0
2022	Hymenoptera	Formicidae	*Lasius*			0	0	1	0	0	0	0
2022	Hymenoptera	Formicidae	*Lasius*	*Lasius alienus*	(Foerster, 1850)	0	2	0	0	0	0	0
2022	Hymenoptera	Formicidae	*Lasius*	*Lasius niger*	(Linnaeus, 1758)	7	0	15	0	0	0	0
2022	Hymenoptera	Formicidae	*Myrmica*	*Myrmica rubra*	(Linnaeus, 1758)	0	8	4	0	0	0	0

2022	Hymenoptera	Formicidae	*Temnothorax sp.*			0	0	0	0	0	1	0
2022	Hymenoptera	Halictidae	*Halictus*			0	1	0	0	0	0	0
2022	Hymenoptera	Halictidae	*Halictus*	*Halictus confusus/tumulorum*		0	0	1	0	0	0	0
2022	Hymenoptera	Halictidae	*Halictus*	*Halictus simplex*	Blüthgen, 1923	0	4	0	0	0	0	0
2022	Hymenoptera	Halictidae	*Halictus*	*Halictus simplex-group*		0	3	0	0	0	0	0
2022	Hymenoptera	Halictidae	*Halictus*	*Halictus subauratus*	(Rossi, 1792)	0	1	0	0	0	0	0
2022	Hymenoptera	Halictidae	*Lasioglossum*			0	0	1	0	0	0	0
2022	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum albipes*	(Fabricius, 1781)	0	0	1	0	0	0	0
2022	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum calceatum*	(Scopoli, 1763)	0	0	1	0	0	0	0
2022	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum leucozonium*	(Schrank, 1781)	0	1	0	0	0	0	0
2022	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum lissonotum*	(Noskiewicz, 1926)	0	1	0	0	0	0	0
2022	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum morio*	(Fabricius, 1793)	0	4	1	0	0	0	0
2022	Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum nitidulum*	(Fabricius, 1804)	0	1	0	1	0	0	0
2022	Hymenoptera	Platygastridae	*Inostemma*			0	0	0	2	0	0	0

Supplementary Material S6. Summary of Baesyan generalised linear model to assess the abundance of arthropod taxa in relation of different parameters. Columns for effects and estimated parameters are: "Estimate": posterior mean estimate; "Est. Err.": standard error of the posterior mean; "l-95%": lower limit of the 95% Bayesian credible interval; "u-95%": upper limit of the 95% Bayesian credible interval; "Rhat": Gelman-Rubin convergence statistic; "Bulk_ESS": bulk effective sample size for the estimate; "Tail ESS": the tail effective sample size of the estimate, and "pd": Probability of Direction meaning the probability that a parameter is strictly positive or negative.

Family: MV (negbinomial)
Links: mu = log; shape = identity
mu = log; shape = identity
mu = log; shape = identity
mu = log; shape = identity
mu = log; shape = identity
mu = log; shape = identity
mu = log; shape = identity
Formula: Coleoptera ~ management + apple_orchard + hay_meadow + bot_family + n_flowers + altitude + day + (1 | sito) + (1 | y)
Formicidae ~ management + apple_orchard + hay_meadow + bot_family + n_flowers + altitude + day + (1 | sito) + (1 | y)
Hym.wasps ~ management + apple_orchard + hay_meadow + bot_family + n_flowers + altitude + day + (1 | sito) + (1 | y)
Hemiptera ~ management + apple_orchard + hay_meadow + bot_family + n_flowers + altitude + day + (1 | sito) + (1 | y)
Nematocera ~ management + apple_orchard + hay_meadow + bot_family + n_flowers + altitude + day + (1 | sito) + (1 | y)
Brachycera ~ management + apple_orchard + hay_meadow + bot_family + n_flowers + altitude + day + (1 | sito) + (1 | y)
Syrphidae ~ management + apple_orchard + hay_meadow + bot_family + n_flowers + altitude + day + (1 | sito) + (1 | y)
Wild.Apoidea ~ management + apple_orchard + hay_meadow + bot_family + n_flowers + altitude + day + (1 | sito) + (1 | y)
Data: data (Number of observations: 287)
Draws: 3 chains, each with iter = 2000; warmup = 1000; thin = 1;
total post-warmup draws = 3000
Group-Level Effects:
~site (Number of levels: 14)

	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
sd(coleotteritot_Intercept)	0.67	0.35	0.1	1.49	1	680.00	582
sd(formicidaetot_Intercept)	1.22	0.5	0.52	2.42	1.01	1070.00	1378
sd(vespetot_Intercept)	0.38	0.28	0.01	1.09	1.01	816.00	960
sd(emitteritot_Intercept)	0.35	0.2	0.03	0.82	1.01	887.00	958
sd(nematoceritot_Intercept)	1.22	0.56	0.38	2.55	1	1007.00	1650
sd(brachiceritot_Intercept)	0.32	0.15	0.04	0.67	1	441.00	303
sd(sirfiditot_Intercept)	0.28	0.19	0.01	0.72	1	775.00	1324
sd(apselvtot_Intercept)	0.34	0.28	0.01	1.05	1	1109.00	1790

~year (Number of levels: 2)

	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
sd(coleotteritot_Intercept)	0.93	1.23	0.01	4.19	1	1138.00	1578
sd(formicidaetot_Intercept)	1.19	1.25	0.04	4.57	1	1222.00	1455
sd(vespetot_Intercept)	0.87	1.16	0.02	4.03	1	1146.00	1595
sd(emitteritot_Intercept)	1.25	1.27	0.13	4.86	1	1138.00	1220
sd(nematoceritot_Intercept)	1.38	1.2	0.11	4.62	1	1466.00	1389
sd(brachiceritot_Intercept)	0.96	1.04	0.05	3.8	1	1116.00	1562
sd(sirfiditot_Intercept)	1.83	1.35	0.44	5.45	1	2003.00	2312
sd(apselvtot_Intercept)	1.24	1.27	0.08	4.75	1	1466.00	1172

Population-Level Effects:

		Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS	pd
Coleoptera	Intercept	-0.52	0.92	-2.79	0.97	1.00	1718	1137	77.50%
Formicidae	Intercept	-2.48	1.05	-4.72	-0.56	1.00	2130	1772	98.90%
Hym.Wasps	Intercept	-1.82	0.76	-3.78	-0.39	1.00	2102	1164	98.27%
Hemiptera	Intercept	-0.93	0.97	-3.31	0.86	1.00	2056	1827	89.93%
Nematocera	Intercept	-1.92	1.1	-4.26	0.24	1.00	2358	1733	96.37%
Brachycera	Intercept	1.08	0.79	-0.79	2.66	1.00	1755	1325	93.53%
Syrphidae	Intercept	-0.22	1.2	-2.94	2.08	1.00	1843	1726	56.00%

Wild.Hym.Apoidea	Intercept	-1.32	1	-3.57	0.63	1.00	1850	1689	93.40%
	Parameter	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	**Rhat**	**Bulk_ESS**	**Tail_ESS**	**pd**
Coleoptera	Management	-0.67	0.25	-1.16	-0.19	1.00	1426	1483	99.30%
Coleoptera	Apple Orchard	-0.37	0.28	-0.94	0.18	1.00	2070	1832	92.47%
Coleoptera	Hay Meadows	-0.25	0.32	-0.85	0.42	1.00	1968	1671	80.77%
Coleoptera	Fabaceae	-1.03	0.35	-1.71	-0.34	1.00	3677	1814	99.87%
Coleoptera	Ranunculaceae-Rosaceae	-0.73	0.31	-1.33	-0.14	1.01	5110	1802	99.33%
Coleoptera	N Flowers	0.06	0.02	0.02	0.1	1.00	4190	2165	100%
Coleoptera	Altitude	-0.42	0.36	-1.14	0.26	1.00	1552	1219	90.90%
Coleoptera	Day	-0.69	0.16	-1.02	-0.38	1.00	5161	2278	100%
Formicidae	Management	0.36	0.53	-0.65	1.51	1.00	1840	1512	76.70%
Formicidae	Apple Orchard	-0.23	0.43	-1.13	0.61	1.00	1744	1628	72.73%
Formicidae	Hay Meadows	-0.46	0.51	-1.5	0.5	1.00	1879	1740	83.60%
Formicidae	Fabaceae	0.06	0.41	-0.73	0.84	1.00	3420	2356	56.87%
Formicidae	Ranunculaceae-Rosaceae	-0.65	0.44	-1.53	0.18	1.00	4533	2524	93.70%
Formicidae	N Flowers	0.06	0.02	0.01	0.11	1.00	3440	2103	99.07%
Formicidae	Altitude	-1.08	0.61	-2.41	0.05	1.00	1462	1360	97.17%
Formicidae	Day	-0.77	0.18	-1.14	-0.43	1.00	5579	2332	100%
Hym.Wasps	Management	0.24	0.22	-0.18	0.68	1.00	2482	1744	88.67%
Hym.Wasps	Apple Orchard	0.23	0.19	-0.16	0.61	1.01	2372	1785	90.27%
Hym.Wasps	Hay Meadows	-0.03	0.25	-0.53	0.45	1.01	2669	1745	55.17%
Hym.Wasps	Fabaceae	-0.02	0.33	-0.69	0.62	1.00	4194	2611	52.53%
Hym.Wasps	Ranunculaceae-Rosaceae	-0.4	0.38	-1.18	0.32	1.00	4767	2294	85.97%
Hym.Wasps	N Flowers	0.04	0.02	0	0.07	1.00	3826	2451	96.83%
Hym.Wasps	Altitude	-0.18	0.28	-0.76	0.32	1.00	2547	1809	75.90%
Hym.Wasps	Day	0.14	0.14	-0.14	0.43	1.00	5559	2670	83.53%
Hemiptera	Management	-0.04	0.15	-0.35	0.26	1.00	2430	1950	62.40%
Hemiptera	Apple Orchard	-0.22	0.15	-0.53	0.08	1.00	2422	1655	93.03%
Hemiptera	Hay Meadows	0.13	0.17	-0.21	0.49	1.00	2159	1530	80.40%
Hemiptera	Fabaceae	0.32	0.23	-0.13	0.75	1.00	3529	2270	92.43%
Hemiptera	Ranunculaceae-Rosaceae	-0.39	0.26	-0.91	0.12	1.00	5270	2335	93.83%
Hemiptera	N Flowers	0.01	0.01	-0.01	0.04	1.00	4177	2096	84.73%
Hemiptera	Altitude	-0.42	0.2	-0.82	-0.05	1.00	2122	1644	98.40%
Hemiptera	Day	-0.04	0.09	-0.22	0.14	1.00	6508	1931	68.63%
Nematocera	Management	0.17	0.44	-0.69	1.11	1.00	1965	1908	65.93%
Nematocera	Apple Orchard	0.45	0.44	-0.43	1.35	1.00	1826	1710	86.27%
Nematocera	Hay Meadows	0.78	0.56	-0.26	1.97	1.00	1887	1457	93.57%
Nematocera	Fabaceae	-0.08	0.43	-0.9	0.78	1.00	3407	2352	57.03%
Nematocera	Ranunculaceae-Rosaceae	-0.38	0.47	-1.28	0.56	1.00	3999	2412	78.37%
Nematocera	N Flowers	0.02	0.02	-0.02	0.07	1.00	3686	2410	82.63%
Nematocera	Altitude	0.18	0.64	-1.19	1.41	1.00	1808	1776	64.07%
Nematocera	Day	-0.14	0.18	-0.51	0.19	1.00	5223	1742	78.90%
Brachycera	Management	0.19	0.12	-0.06	0.42	1.00	1649	1433	93.93%
Brachycera	Apple Orchard	0.17	0.13	-0.1	0.42	1.00	1956	1563	90.60%
Brachycera	Hay Meadows	0.17	0.15	-0.12	0.47	1.00	2207	2009	89.50%
Brachycera	Fabaceae	-1.11	0.17	-1.44	-0.77	1.00	4599	2376	100%
Brachycera	Ranunculaceae-Rosaceae	-0.35	0.16	-0.67	-0.03	1.00	5426	2213	98.23%
Brachycera	N Flowers	0.04	0.01	0.02	0.06	1.00	3983	2736	100%
Brachycera	Altitude	0.27	0.18	-0.09	0.62	1.00	1894	1668	93.33%
Brachycera	Day	0.15	0.08	0	0.3	1.00	5635	2355	97.33%
Syrphidae	Management	0.23	0.14	-0.06	0.49	1.00	2232	1543	95.07%
Syrphidae	Apple Orchard	0.28	0.15	-0.03	0.58	1.00	2658	1879	96.40%
Syrphidae	Hay Meadows	0.58	0.17	0.24	0.92	1.00	2835	2041	99.63%
Syrphidae	Fabaceae	-1.96	0.29	-2.56	-1.41	1.00	4357	2483	100%
Syrphidae	Ranunculaceae-Rosaceae	-0.1	0.23	-0.54	0.35	1.00	4816	2437	67.87%
Syrphidae	N Flowers	0.04	0.02	0.01	0.07	1.00	4293	2522	99.67%
Syrphidae	Altitude	0.14	0.2	-0.27	0.52	1.00	2191	1682	77.50%
Syrphidae	Day	0.23	0.11	0.03	0.44	1.00	4853	2271	98.90%
Wild.Hym.Apoidea	Management	-0.14	0.27	-0.66	0.4	1.00	2931	1830	72.50%
Wild.Hym.Apoidea	Apple Orchard	-0.39	0.17	-0.75	-0.05	1.00	2977	2275	98.23%
Wild.Hym.Apoidea	Hay Meadows	-0.46	0.23	-0.95	-0.05	1.00	2532	2274	98.10%

Wild.Hym.Apoidea	Fabaceae	-0.9	0.34	-1.56	-0.25	1.00	4000	2511	99.60%
Wild.Hym.Apoidea	Ranunculaceae-Rosaceae	-0.82	0.35	-1.51	-0.15	1.00	5260	2251	99.07%
Wild.Hym.Apoidea	N Flowers	-0.01	0.02	-0.05	0.04	1.00	3777	2233	59.33%
Wild.Hym.Apoidea	Altitude	-1.42	0.27	-2.02	-0.91	1.00	2582	1833	100%
Wild.Hym.Apoidea	Day	0.17	0.13	-0.08	0.42	1.00	5107	1889	90.47%

Family Specific Parameters:

	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
Shape_Coleoptera	0.48	0.09	0.33	0.68	1.00	4263	2218
Shape_Formicidae	0.42	0.09	0.27	0.62	1.00	4167	2477
Shape_Hym. Wasps	1.96	7.45	0.45	6.67	1.00	2478	1331
Shape_Hemiptera	2.16	1.28	0.99	5.01	1.00	4102	1453
Shape_Nematocera	0.57	0.32	0.23	1.37	1.00	3537	2019
Shape_Brachycera	1.42	0.2	1.06	1.85	1.00	5571	2170
Shape_Syrphidae	0.93	0.19	0.63	1.37	1.00	4905	2268
Shape_Wild Hym. Apoidea	1.14	0.56	0.5	2.5	1.00	3803	1873

Draws were sampled using sampling (NUTS). For each parameter, Bulk_ESS and Tail_ESS are effective sample size measures, and Rhat is the potential scale reduction factor on split chains (at convergence, Rhat = 1).

Supplementary Material S7. Summary of Baesyan generalised linear model to assess the diversity of Diptera Syrphidae and Hymenoptera Apoidea communities in relation of different parameters. Columns for effects and estimated parameters are: "Estimate": posterior mean estimate; "Est. Err.": standard error of the posterior mean; "l-95%": lower limit of the 95% Bayesian credible interval; "u-95%": upper limit of the 95% Bayesian credible interval; "Rhat": Gelman-Rubin convergence statistic; "Bulk_ESS": bulk effective sample size for the estimate; "Tail ESS": the tail effective sample size of the estimate, and "pd": Probability of Direction meaning the probability that a parameter is strictly positive or negative.

Family: MV (lognormal, lognormal)
Links: mu = identity; sigma = identity
mu = identity; sigma = identity
Formula: Shannon_Apoidea ~ Management + Apple_orchard + Hay_meadows + Bot_Family+ N_Flowers + Altitude + Day + (1 | site) + (1 | year)
Shannon_Syrphidae ~ Management + Apple_orchard + Hay_meadows + Bot_Family+ N_Flowers + Altitude + Day + (1 | site) + (1 | year)
Data: data_agg_div (Number of observations: 287)
Draws: 3 chains, each with iter = 2000; warmup = 1000; thin = 1;
total post-warmup draws = 3000
Group-Level Effects:
~site (Number of levels: 14)

	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
sd(ShannonAd_Intercept)	0.05	0.02	0.01	0.1	1	698	703
sd(ShannonSd_Intercept)	0.03	0.02	0	0.09	1	953	743

~year (Number of levels: 2)

	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
sd(ShannonApoidea_Intercept)	0.47	0.77	0	2.72	1	482	1170
sd(ShannonSyrphidae_Intercept)	0.73	1.04	0.03	3.64	1.01	597	1209

Population-Level Effects:

		Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS	pd
Shannon Apoidea	(Intercept)	0.08	0.42	-0.83	1.13	1.01	598	742	76.30%
Shannon Syrphidae	(Intercept)	0.14	0.65	-1.35	1.54	1.00	973	750	74.83%
	Parameter	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS	pd
Shannon Apoidea	Management	-0.04	0.02	-0.07	0.00	1.00	1386	1521	97.50%
Shannon Apoidea	Apple Orchard	-0.05	0.02	-0.09	-0.01	1.00	1570	1790	98.83%
Shannon Apoidea	Hay Meadows	-0.04	0.02	-0.08	0.01	1.00	1479	1587	95.23%
Shannon Apoidea	Fabaceae	-0.05	0.03	-0.10	0.01	1.00	3634	2427	95.60%
Shannon Apoidea	Ranunculaceae-Rosaceae	-0.04	0.03	-0.10	0.01	1.00	3398	2347	94.83%
Shannon Apoidea	N Flowers	0.00	0.00	0.00	0.00	1.00	3345	2607	59.23%
Shannon Apoidea	Altitude	-0.11	0.03	-0.16	-0.05	1.00	1250	1455	100.00%
Shannon Apoidea	Day	0.02	0.01	0.00	0.04	1.00	4375	2192	94.70%
Shannon Syrphidae	Management	0.04	0.02	-0.01	0.08	1.00	1824	1956	95.50%
Shannon Syrphidae	Apple Orchard	0.00	0.03	-0.05	0.05	1.00	1876	1554	53.73%
Shannon Syrphidae	Hay Meadows	0.10	0.03	0.04	0.15	1.00	2396	1749	99.77%
Shannon Syrphidae	Fabaceae	-0.20	0.05	-0.30	-0.11	1.00	3054	2363	100.00%
Shannon Syrphidae	Ranunculaceae-Rosaceae	0.04	0.05	-0.06	0.14	1.00	3414	2251	77.67%
Shannon Syrphidae	N Flowers	0.01	0.00	0.00	0.01	1.00	4057	2357	95.97%
Shannon Syrphidae	Altitude	0.01	0.03	-0.05	0.07	1.00	1476	1873	63.43%
Shannon Syrphidae	Day	0.02	0.02	-0.03	0.06	1.00	4177	2053	77.47%

Family Specific Parameters:

	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
sigma_Shannon Apoidea	0.18	0.01	0.16	0.19	1	2721	2162
sigma_Shannon Syrphidae	0.33	0.01	0.3	0.36	1	4130	2077

Draws were sampled using sampling (NUTS). For each parameter, Bulk_ESS and Tail_ESS are effective sample size measures, and Rhat is the potential scale reduction factor on split chains (at convergence, Rhat = 1).

Chapter 3.2

ITS2 metabarcoding quantitative reliability in assessing the composition of pollen carried by insects

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ITS2 metabarcoding quantitative reliability in assessing the composition of pollen carried by insects

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Abstract

To unravel complex plant-pollinator networks, it is essential to be able to obtain qualitative and quantitative information about the composition of insect-carried pollen. This information can be gathered by light microscopy identification of pollen grains, but this process can be time-consuming and requires trained specialists. In this context, ITS2 metabarcoding can be a powerful tool, providing a high taxonomic resolution in identifying taxa represented in a pollen sample. However, a possible critical point of this approach may be its quantitative reliability in estimating the relative abundance of pollen taxa, especially when ITS2 is used as a single-marker. The aim of this work was to test the qualitative and quantitative reliability of ITS2 metabarcoding approach in assessing the composition of pollen carried by insects. Pollen samples consisting of a limited number of grains were retrieved from different bee families collected in different environments. The NGS reads obtained by ITS2 metabarcoding were compared, at family level, with the pollen grain counts obtained by light microscopy. Moreover, the proportion of NGS reads belonging to plants in flower occurring in the sampling area was evaluated at genus level. The results highlighted a good correlation between the relative abundances obtained with metabarcoding and microscopy, and a good accuracy of metabarcoding in providing quantitative results independently of bee taxon and pollen taxa composition. Moreveor, this study indicates that ITS2 metabarcoding can also be applied when the available amount of pollen is a constraint, a crucial point in the perspective of an actual evaluation of pollination networks.

Keywords: bees; plant-pollinator networks; palynology; pollination; single-marker metabarcoding

1. Introduction

The complex interactions between plants and insect pollinators play a vital role in the sustenance of terrestrial ecosystems and the maintenance of their biodiversity (Garibaldi et al., 2011; Potts et al., 2016; Ollerton, 2017). In the last decade, many studies focused on these interactions (e.g., Geslin et al., 2013; Jędrzejewska-Szmek and Zych, 2013; Olito and Fox, 2015; Lefebvre et al., 2018; Walton et al., 2020). However, most of these studies are based on flower-visitation networks, under the assumption that all the flower visiting species are true pollinators or, at least, that the frequency of visits by a taxon is indicative of its role in pollination (Cuartas-Hernández and Medel, 2015; Traveset et al., 2016; De Manincor et al., 2020). As a matter of fact, a flower visitor can be defined pollinator only when it transfers viable pollen from one flower to the receptive stigma of a conspecific one (Zhao et al., 2019). Moreover, the frequency of visits by a taxon is not always correlated to its impact on plant pollination (King et al., 2013). In this context, networks based on the pollen actually carried by flower visitors may offer more comprehensive insights into the roles of different insect taxa in pollination and their visitation history (De Manincor et al., 2020). Moreover, pollen-based networks should provide both qualitative and quantitative information about the composition of the pollen carried by flower visitors (e.g., Devoto et al., 2011; Banza et al., 2015; Pornon et al., 2016; Lang et al., 2018; Bell et al., 2019; Macgregor et al., 2019; Bleha et al., 2021; Bonelli et al., 2020). This information would allow to evaluate which pollinator species are fundamental for the success of plant reproduction and to identify which plant species could serve as important trophic resources for flower-visiting species (De Manincor et al., 2020).

Pollen grains are commonly identified through traditional microscopy methods, mainly based on morphological characters such as shape and exine ornamentation (Li et al., 2004; Del Pozo-Banos et al., 2015; Kailas et al., 2017; Pospiech et al., 2019). These features are observable using light microscope (Feás et al., 2010; Umber et al., 2022), scanning electron microscopy (Li and Flenley, 1999; Jones and Bryant, 2014; Bahadur et al., 2018), and transmission electron microscopy (Grote et al., 2000; Soares et al., 2021). Morphological identification of pollen is widely employed in different research areas, such as aerobiology (Mercuri, 2015), archaeology (Yang et al., 2018), paleobotany

(Dunker et al., 2021), forensic science (Mildenhall et al., 2006), melissopalynology (Von Der Ohe et al., 2004), and plant-pollinator interactions (e.g., Gaona et al., 2019; Bonelli et al., 2020; Bleha et al., 2021; Weinman et al., 2023). However, identification and quantification of large amounts of pollen material by microscopy are time-consuming processes that require specialized training (Bell et al., 2017). Moreover, for many taxa, microscopy proved to be effective and reliable for the identification of pollen only at the family level, while the taxonomic assignment at genus level often proven challenging or even impossible in many cases (Lindbladh et al., 2002; Galimberti et al., 2014; Hawkins et al., 2015; Kraaijeveld et al., 2015).

An alternative to this approach is represented by the DNA metabarcoding. DNA barcode and high-throughput sequencing enable comprehensive identification of unknown taxa within a mixed pollen sample by comparing the obtained DNA sequences with a references database (Evans and Kitson, 2020; Leidenfrost et al., 2020). This approach can be particularly useful when several pollen samples must be analysed.

The most used DNA barcodes for pollen analysis include plastid genome regions such as ribulose-1,5-biphosphate carboxylase (*rbcl*) and *trnL-trnF* intergenic spacer (*trnL*), or nuclear genome regions as ribosomal Internal Transcribed Spacer 1 (ITS1) and Internal Transcribed Spacer 2 (ITS2) (Lowe et al., 2022; Omelchenko et al., 2022). Among these, *rbcl* is rarely used alone but is often combined with other barcode loci to increase species discrimination (Hollingsworth et al., 2011; Tommasi et al., 2021; Lowe et al., 2022). ITS1 has lower amplification efficiency and taxonomic resolution compared to ITS2 (Gous et al., 2019; Omelchenko et al., 2022). *trnL* and ITS2 markers are commonly used individually or together in a multi-locus analysis (Bontšutšnaja et al., 2021; Carneiro de Melo Moura et al., 2022; Polling et al., 2022). Many authors agree that *trnL* exhibits poor taxonomic resolution, often failing to go beyond the family level (Kraaijeveld et al., 2015; Leontidou et al., 2018; Richardson et al., 2019; Baksay et al., 2020). In contrast, ITS2 demonstrates high resolution in identifying plants, at least at genus level, if adequate reference databases are available (Keller et al., 2015; Richardson et al., 2015a; Richardson et al., 2015b; Gous et al., 2019; Richardson et al., 2019; Stallman et al., 2019; Milla et al., 2022). In view of these considerations, ITS2 seems to be a good marker for pollen analysis when genus-level resolution is required.

However, a possible limitation of ITS2 metabarcoding may be its quantitative reliability, as the effectiveness of this marker in estimating the relative abundance of pollen taxa in mixed samples is still debated, especially when used as a single-marker (Kraaijeveld et al., 2015; Smart et al., 2017; Stallman et al., 2019; Polling et al., 2022).

Considering the advantages of single-marker analysis over multi-marker analysis (i.e., economic efficiency and time-saving), understanding the quantitative reliability of ITS2 alone in assessing the relative frequency of plant taxa in pollen mixtures is an important aspect to be elucidated. To the best of our knowledge only few studies tested the quantitative efficiency of ITS2 metabarcoding for pollen retrieved from insects, obtaining mixed results (Keller et al., 2015; Richardson et al., 2015a; Richardson et al., 2015b; Bänsch et al., 2020; Wizenberg et al., 2023). Moreover, most of these studies considered only pollen from the honeybee (*Apis mellifera* Linnaeus, 1758), collected by using pollen traps installed at the entrance of the hives. Bänsch and colleagues (2020) also considered *Bombus terrestris* (Linnaeus, 1758), another social bee species, for which, however, only the pollen of two plant taxa was identified. All these studies were performed in agricultural landscapes, while there is a lack of studies in natural and seminatural environments.

The aim of the present study was to evaluate the reliability of ITS2 in assessing the presence and relative frequency of plant taxa in the pollen carried by various social and solitary bee species (Hymenoptera: Apoidea: Anthophila) collected across diverse Alpine environments characterized by different land use (apple orchard, pasture, hay meadow, alpine grassland) and altitudes (from about 1000 to 2700 m a.s.l.). This heterogeneous context guaranteed a significant variability in the taxonomic composition of pollen loads. Taking into consideration that the available amount of pollen can be limiting factor for some insect species and environmental contexts, ITS2 metabarcoding was performed on pollen samples consisting of a limited number of grains, much lower than in previous works.

In detail, (i) the taxonomic composition and relative abundance of pollen loads obtained with ITS2 metabarcoding and light microscopy were compared at family level, and (ii) the proportion of NGS reads belonging to plants in flower actually occurring in the sampling area was evaluated at genus level.

2. Material and methods

2.1 Sampling area

The sampling area is located in the eastern European Alps within the largest Alpine protected area in Italy: Stelvio National Park (figure 1.a). The bee sampling was performed in the Martello Valley (Bolzano, South Tyrol, Italy) (figure 1.b) within seven environment types that differ for the combination of land use (apple orchard, pasture, hay meadow and alpine grassland) and elevation (low, mid, and high valley) (table 1). For each environment type two sampling sites were selected (figure1.c, Table S1). The size of each sampling site was of approximately 1,500 m^2.

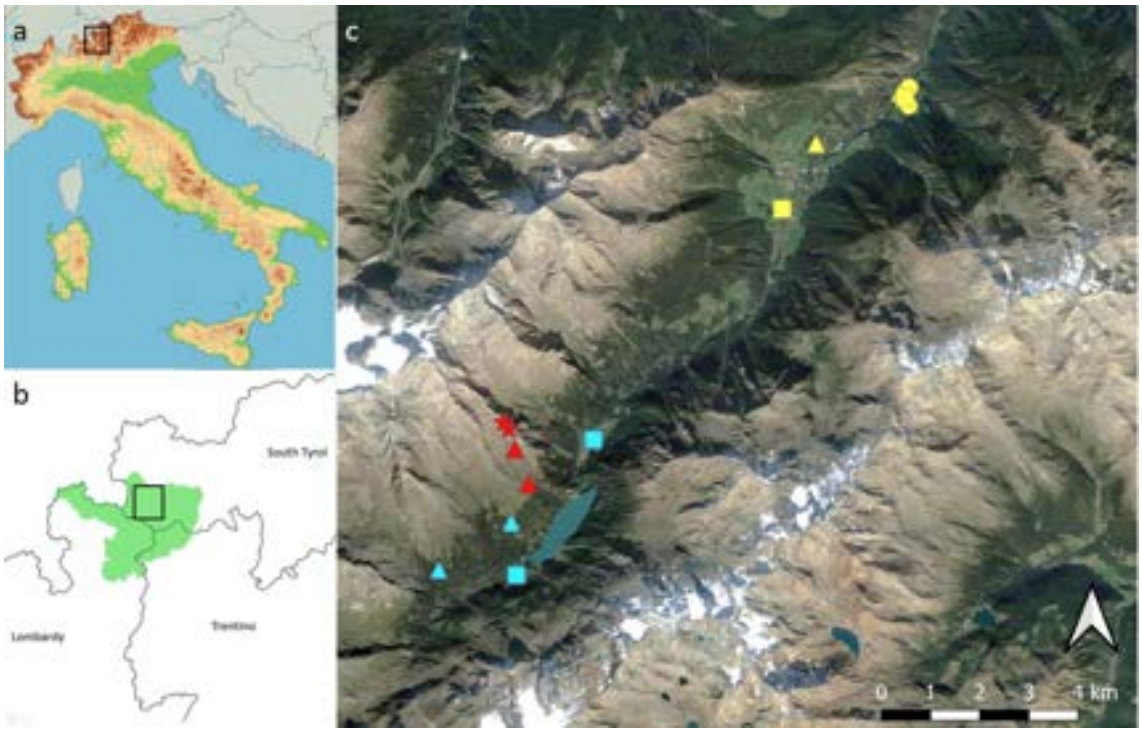

Figure 1. a. Stelvio National Park in the European Alps; **b.** Martello valley within the Stelvio National Park (green area); **c.** Selected sampling sites along the Martello valley: six sites, characterized by three different land uses, were located in the low valley (yellow), four sites, characterized by two different land uses, were located in the mid valley (blue), four sites, characterized by two different land uses, were located in the high valley (red). Dots: apple orchards; triangles: pastures; squares: hay meadows; stars: alpine grasslands.

Table 1. The seven selected environment types for the bee sampling performed in the Martello valley.

		Altitudinal sector		
		Low valley	Mid valley	High valley
		900 – 1600 m asl	1601 – 2300 m asl	2301 – 3000 m asl
Land use	Apple orchard	X		
	Pasture	X	X	X
	Hay meadow	X	X	
	Alpine grassland			X

2.2 Bee sampling and in-field blooming plant checklist

The bee sampling was conducted in the framework of a broader project on pollinators in Alpine environments (IMPOLLINET). In detail, bees (Hymenoptera: Apoidea: Anthophila) were collected during six sampling sessions conducted between late June and late August 2021. Each sampling site was surveyed once during each session. Bees were collected by free observation sampling (Bonelli et al., 2022); specifically, four operators freely walked within each site for 15 minutes, collecting all the observed bees in the area. Each specimen was then stored separately in collection tubes with 70% ethanol and preserved at 4 °C.

After every sampling session, a checklist of plants in flower was compiled by two botanists for each site. This checklist included all the entomophilous plant species in flower observed within and in the proximity of each site, covering an area of approximately 2,000 m^2 (Table S2). The resulting checklist was helpful for morphological identification of pollen material, and it was fundamental in verifying the genus-level results obtained with metabarcoding.

2.3 Pollen collection and quantification

Each bee sample was vortexed for at least 30 seconds to remove all the pollen from the insect pollen-carrying structures and body (Bonelli et al., 2020). Then, each bee specimen was observed under the stereomicroscope to ensure the successful removal of all pollen. If necessary, the aforementioned process was repeated. Therefore, each bee specimen was transferred into a new collection tube and identified by expert taxonomists at the species level. The collection tubes containing the pollen were maintained at 65 °C to facilitate ethanol evaporation until the suspension volume was less than 30 µl.

Pollen grains from bees belonging to the same family and collected in the same type of environment (i.e., same land use and altitudinal sector), were merged into a single sample as reported in table 2. Since *Apis mellifera* is a managed species, specimens belonging to this species were kept separated from other Apidae.

Table 2. Specimens collected during the six sampling sessions. "Total specimen": number of bees from which pollen was retrieved or each group; "Pollen sample code": Progressive number of resulting collection tubes from merged pollen material.

Land use	Sector	Collection date	Species	Family	Total specimens	Pollen sample code
Apple orchard	Low valley	30-VII-2021	*Andrena* cf. *fucata*	Andrenidae	1	1
		05-VII-2021	*Apis mellifera* Linnaeus, 1758	Apidae	1	2
		05-VII-2021	*Hylaeus confusus* Nylander, 1852	Colletidae	1	3
		05-VII-2021	*Lasioglossum morio* (Fabricius, 1793)	Halictidae	1	4
Pasture	Low valley	29-VII-2021	*Andrena hattorfiana* (Fabricius, 1775)	Andrenidae	1	5
Hay meadow	Low valley	28-VI-2021	*Bombus pascuorum* (Scopoli, 1763)	Apidae	1	6
		28-VI-2021	*Lasioglossum zonulum* (Smith, 1848)	Halictidae	1	7
Pasture	Mid valley	29-VI-2021	*Andrena rogenhoferi* Morawitz, 1872	Andrenidae	2	8
		29-VI-2021	*Andrena fulvago* (Christ, 1791)	Andrenidae		
		29-VI-2021	*Apis mellifera* Linnaeus, 1758	Apidae	3	9
		03-VII-2021	*Apis mellifera* Linnaeus, 1758	Apidae		
		30-VII-2021	*Apis mellifera* Linnaeus, 1758	Apidae		
		30-VII-2021	*Bombus pyrenaeus Perez, 1879*	Apidae	3	10
		30-VII-2021	*Bombus monticola* Smith, 1849	Apidae		
		30-VII-2021	*Bombus lapidarius* (Linnaeus, 1758)	Apidae		
Hay meadow	Mid valley	08-VII-2021	*Apis mellifera* Linnaeus, 1758	Apidae	1	11
		28-VII-2021	*Bombus mesomelas* Gerstaecker, 1869	Apidae	5	12
		08-VII-2021	*Bombus ruderarius* (Muller, 1776)	Apidae		
		08-VII-2021	*Bombus hortorum* (Linnaeus, 1761)	Apidae		
		08-VII-2021	*Bombus pyrenaeus* Perez, 1879	Apidae		
		08-VII-2021	*Bombus ruderarius* (Muller, 1776)	Apidae		
Pasture	High valley	29-VII-2021	*Bombus mendax* Gerstaecker, 1869	Apidae	4	13
		29-VII-2021	*Bombus pyrenaeus* Perez, 1879	Apidae		
		29-VII-2021	*Bombus terrestris/lucorum*	Apidae		
		29-VII-2021	*Bombus ruderarius* (Muller, 1776)	Apidae		
		03-VII-2021	*Dufourea alpina* Morawitz, 1865	Halictidae	2	14
		03-VII-2021	*Dufourea alpina* Morawitz, 1865	Halictidae		
Alpine grassland	High valley	26-VIII-2021	*Bombus pyrenaeus* Perez, 1879	Apidae	1	15

To estimate the number of pollen grains in each pollen sample, the volume of the suspension was measured, and three samples (1 µl each) were withdrawn and put on slides to count pollen grains by light microscopy. The mean number of pollen grains contained in 1 µl of suspension was used to estimate the total amount of pollen grains in the initial suspension.

Despite some studies have reported that at least 10,000 pollen grain are required for both optimal DNA extraction and quantification (Swenson and Gemeinholzer, 2021;

Omelchenko et al., 2022), our preliminary experiments (unpublished data) showed that 200-500 pollen grains were enough to obtain high-quality DNA, successful amplification of the ITS2 gene, and accurate taxonomic identification. Therefore, for each pollen sample, two subsamples consisting in a suspension containing approximately 500 pollen grains were prepared: one was used for microscopy analysis and one for DNA extraction and ITS2 metabarcoding analysis.

2.4 Light microscope analysis

Each subsample prepared for light microscopy was subjected to acetolysis according to Erdtman (1960). After the last centrifugation step, the resulting pellet was resuspended in a mixture of 100 µl glycerol and distilled water in a 1:1 ratio (v:v). Microscope slides were mounted and sealed with wax for the subsequent pollen identification and counting. This process was carried out using a Leica DM – RD microscope equipped with ruler and a Leica MC170 HD camera (Leica Camera AG, Wetzlar, DE). Pollen was identified to the lowest possible taxonomic level following published pollen guides (Moore et al., 1991; Buchner and Weber, 2000) and the image database "PalDat" (Buchner and Weber, 2000). Any morphotypes that could not be unambiguously identified at species level were grouped together at the genus or family level.

2.5 ITS2 metabarcoding and bioinformatic analysis

Each subsample prepared for metabarcoding analysis was placed at 56 °C to facilitate the complete evaporation of ethanol. Then, genomic DNA was extracted from pollen samples using a modified version of the CTAB (Cetyl Trimethylammonium Bromide) protocol (Doyle and Doyle, 1987) reported in Bonelli et al. (2019). Briefly, 50 µl of extraction buffer (1.4 M NaCl; 20 mM EDTA; 100 mM Tris pH 8.0; 2% CTAB) and 5 µl of 20 mg/ml proteinase K were added to each sample, then sporopollenin were mechanically disrupted by using pre-sterilized pestles and samples were left in a water bath at 56 °C overnight. One volume of chloroform was added to samples which were then centrifuged at 11,500 × g for 5 min at 4 °C. Supernatant was collected and, DNA was precipitated by two successive washes in ethanol, each one followed by a centrifugation at 1,300 × *g* at 4 °C for 15 and 5 min, respectively. Lastly, the pellet

containing the DNA was resuspended in 30 µl of molecular biology grade water. The amount of DNA was determined by Qubit (Invitrogen™ Qubit™ 4, Carlsbad, CA, USA).

After DNA extraction, two libraries were prepared from each sample to obtain two technical replicates (replicate A and replicate B). The ITS2 region was amplified using the pair of standard DNA barcoding primers reported by Bänsch et al. (2020): ITS2-S2F forward: 5' – ATGCGATACTTGGTGTGAAT – 3' (Chen et al., 2010), ITS4-R reverse: 5' – TCCTCCGCTTATTGATATGC – 3' (White et al., 1990) with Illumina overhang sequences appended. These primers generate ITS2 amplicons with an expected length of ~340 bp. The parameters of the thermocycler (Applied Biosystems ProFlex PCR System, ThermoFisher Scientific, Waltham, MA, USA) were set as follow: initial denaturation at 94 °C for 3 min, 32 cycles of 94 °C denaturation for 20 s, annealing at 56 °C for 20 s, and extension at 72 °C for 40 s, and a final extension step at 72 °C for 5 min. ITS2 amplification products were checked on 1% agarose gel.

After the first PCR, the following steps of the Next Generation Sequencing (NGS) pipeline were performed by Genomix4life S.R.L (Baronissi, Italy). The ITS2 amplicons were purified, and a second PCR step was applied to integrate relevant flow-cell binding domains and unique indices (Nextera XT Index kit; Illumina, San Diego, CA, USA). The obtained libraries were then sequenced on the MiSeq platform (Illumina) in a 2 x 250 bp paired-end mode. The raw sequence underwent quality control analysis using FastQC (Andrews et al., 2010).

Bioinformatic analysis to determine the taxonomic composition of the pollen samples was performed using QIIME2 v.2022.2 (Bolyen et al., 2019). To obtain Amplicon Sequence Variants (ASVs) the raw reads were processed with the dada2 algorithm (Callahan et al., 2016) implemented in QIIME2 (trunc-len-f = 230, trunc-len-r = 230, max-ee-f = 2, max-ee-r =2). A naïve Bayes classifier (Bokulich et al., 2018) was trained on the PLANiTS reference database (Banchi et al., 2020) and then used to obtain the taxonomic assignment for each ASV (confidence threshold = 0.8). The concordance of the two technical replicates, both in terms of identified ASVs and taxonomic composition, was assessed using the QIIME2 plugin q2-quality-control. For the comparison with light microscopy data, the datasets from the two technical replicates

were collapsed at the genus and family levels and then merged by averaging the abundance of each taxon in the two replicates.

2.6 Analysis to compare family-level pollen composition obtained by DNA metabarcoding and light microscopy

A comparison of pollen composition, both in terms of presence-absence and relative abundances, has been carried out for each sample using the R software v.4.2.2 (R Core Team, 2022). The comparison between light microscopy and metabarcoding was performed at the family level, since it was the lowest rank for which taxonomic identification obtained with light microscopy was available for all pollen grains. Pollen loads diversity and composition was compared using different indices: Shannon's entropy (Shannon and Weaver, 1949) and Pielou's evenness (Pielou, 1966) were computed to estimate alpha diversity, while beta-diversity dissimilarities were estimated using Jaccard (Jaccard, 1908) and Bray-Curtis indices (Sorensen, 1948). The Wilcoxon signed-rank test (Wilcoxon, 1992) was used to detect statistically significant differences in the alpha diversity estimates, while the Mantel test (Mantel, 1967) was used to test for differences in the dissimilarity matrices. Linear regression and the Pearson correlation coefficient (Pearson, 1895) were applied to measure the linear correlation between the relative abundances obtained with the two different methods (light microscopy and metabarcoding).

2.7 Analysis to compare genus-level pollen composition obtained by DNA metabarcoding with the checklist of plants in flower

To evaluate the reliability of the method, the relative abundance of plant genera detected by DNA metabarcoding was compared with the list of plant genera in flower observed in field. It was not possible to operate at species level, since several of the species identified by DNA metabarcoding are not present within the South Tyrolean wild flora (referred to Bartolucci et al., 2018) (e.g., *Geum aleppicum*, *Euphrasia cuneata*, *Trifolium uniflorum, Sambucus canadensis*, *Cucurbita ficifolia*). To mitigate the potential for contamination, all genera detected through metabarcoding with a relative abundance below 0.5% were filtered out. Moreover, the optimal habitat (Pignatti et al.,

2017–2019) of those genera identified with molecular method but not observed in blooming during the in-field observations was checked to evaluate the metabarcoding identification reliability. In particular, the different types of vegetation cover within a radius of 500 meters from the center of each plot were identified using the Land Use Map (Ufficio Pianificazione territoriale e cartografia – GeoCatalogo (http://www.buergernetz.bz.it)) on QGis software (version 3.30.1). Those genera whose ideal habitat was present among the identified types of vegetation cover were considered as potentially present. The genera identified with metabarcoding were divided into three groups: "detected" (if the genus was present in the in-field observation); "undetected potentially present" (if the genus was not present in the in-field observation but its optimal habitat was identified within a radius of 500 meters); "undetected not present" (if the genus was not present in the in-field observation and its optimal habitat was not present within a radius of 500 meters). Finally, for each pollen sample, the relative abundance of NGS reads for "detected", "undetected potentially present", and "undetected not present" genera was calculated to evaluate the identification reliability of the method.

3. Results

3.1 Overview of the results obtained from palynological analysis conducted by light microscopy and metabarcoding

A total of 8,573 pollen grains were counted across fifteen samples using light microscopy (average per sample: 571; min: 104; max: 1,557); 99.78% of the grains were identified to family level, 35.70% to genus level, and 1.96% to species level, for a total of 23 plant families, 19 genera, and 9 species detected (Table S3).

A total of 878,976 paired-end reads (average per sample 29,299; min 10,261; max: 49,636) were obtained from the sequencing. A total of 721,564 sequences (average per sample 24,052; min 8,168; max: 41,965) corresponding to 727 ASVs (amplicon sequence variants) were obtained. 98.7% of the reads were assigned at least to the taxonomic level of family (90.9% of the ASVs), 95.4% to the genus level (85.3% of the ASVs), and 30.0% to the species level (50.0% of the ASVs) for an amount of 33 families, 84 genera, and 74 species detected.

For each pollen sample, sequences obtained for technical replicates were very similar (average of shared sequences per sample = 95.3%, SEM = 1.26%) and they provided consistent results in terms of the relative abundance of taxa, regardless of the taxonomic level chosen (Fig. S1).

3.2 Quantitative comparison of pollen composition obtained by microscopy and metabarcoding analysis

Overall, DNA metabarcoding analysis detected more plant families, genera, and species than microscopy. While nineteen families were identified by both methods, the molecular approach detected fourteen additional families, and microscopy identified four unique families (Table S4). Notably, six of the families detected only by metabarcoding (Amaryllidaceae, Cistaceae, Cucurbitaceae, Cupressaceae, Cyperaceae, and Rutaceae) had very low abundances (less than 50 reads each) which may suggest that their presence is not accurately representative of the sample and could be due to artefactual factors (e.g., contamination of the pollen loads with non-pollen DNA). Additionally, each of the four families identified only by microscopy (Gentianaceae, Orchidaceae, Onagraceae, and Pinaceae) was represented by few pollen grains (less than 20). Thus, the inability of metabarcoding in detecting these families may be due to the insufficient amount of DNA or to a suboptimal amplification.

Table 3. Alpha diversity estimates for morphological and molecular pollen identification.

	Morphology (light microscope)			Molecular (*ITS2* metabarcoding)		
Pollen sample code	N. of families	Shannon	Pielou	N. of families	Shannon	Pielou
1	2	0.08	0.08	7	0.46	0.16
2	2	0.08	0.08	6	0.07	0.03
3	6	1.38	0.54	6	0.20	0.08
4	1	0	0	9	0.13	0.04
5	8	1.16	0.39	22	2.68	0.60
6	6	1.31	0.51	8	1.24	0.41
7	10	2.16	0.65	15	2.17	0.56
8	2	0.08	0.08	11	0.29	0.08
9	3	1.06	0.67	7	1.03	0.37
10	9	1.71	0.54	11	1.01	0.29
11	1	0	0	13	0.60	0.16
12	4	1.48	0.74	10	1.30	0.39
13	4	0.41	0.20	12	1.16	0.32
14	3	0.16	0.10	13	0.92	0.25
15	2	0.14	0.14	11	0.70	0.20

The alpha diversity estimates obtained with the two methods for the same sample differed (table 3). However. the results of the Wilcoxon signed-rank test showed significant differences only in the number of plant families detected (W = 1.5, p-value = 0.0002), while no significant difference was recorded for both the Shannon's entropy (W = 40, p-value = 0.28) and the Pielou's evenness (W = 52, p-value = 0.68). The Mantel test applied to dissimilarity matrices for the two methods (microscopy and metabarcoding) showed a significant correlation when using the Bray-Curtis index (rho = 0.65, p-value = 0.001) while no significant correlation could be identified with the Jaccard index (rho = 0.17, p-value = 0.16), probably due to the presence of low abundance families uniquely identified by a single method.

From a quantitative point of view, the plant family relative abundance obtained by microscopy analysis was positively correlated with the estimated relative abundance obtained by ITS2 metabarcoding (linear regression: R^2 = 0.74, p-value < 2.2×10^{-16}; Pearson: coefficient = 0.86, p-value < 2.2×10^{-16}) (figure 2).

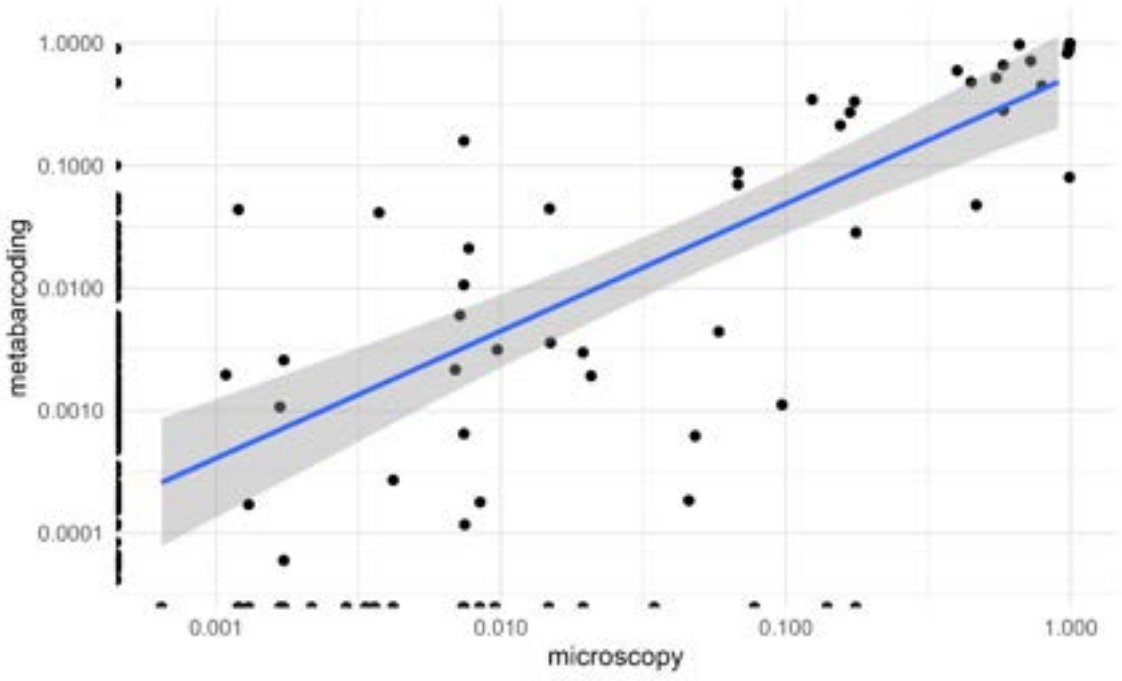

Figure 2. Linear regression representing the correlation between plant family relative abundance obtained with microscopy and metabarcoding.

More in detail, plant family relative abundance obtained by light microscopy and DNA metabarcoding was similar in almost all pollen samples independently from bee taxon, land use and altitude (figure 3), with pollen families showing discrepancies of no more

than 25% of relative abundance between the two methods for each sample, except for Halictidae (hay meadows located in low valley) and *Apis* (hay meadows located in mid valley).

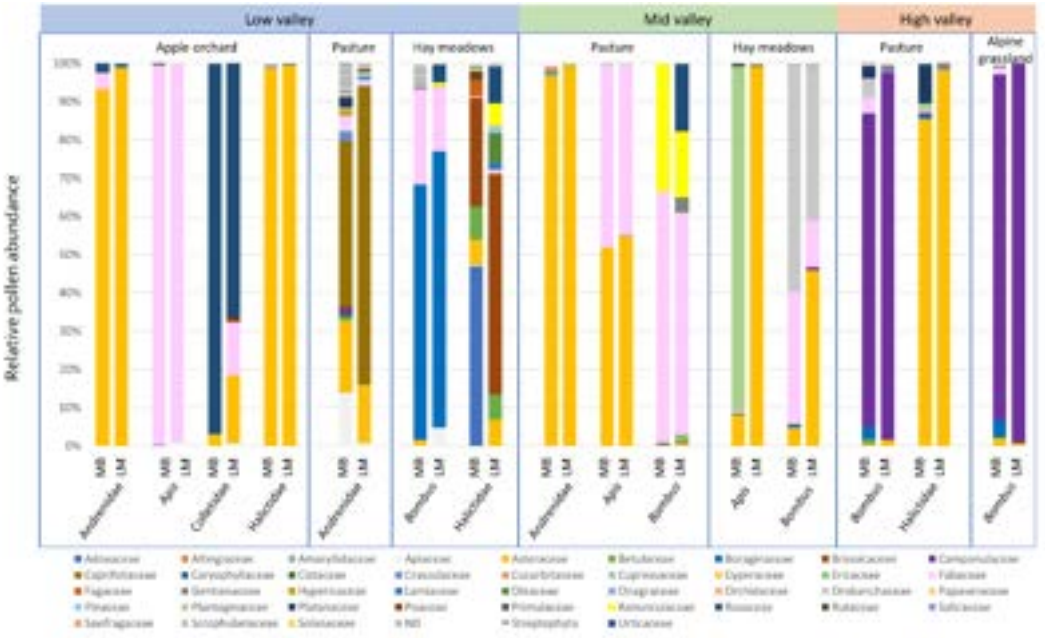

Figure 3. Relative abundance of pollen families obtained by light microscopy (LM) and *ITS2* Metabarcoding (MB) for each bee taxon sampled in the seven environments in which sampling was conducted. Relative abundance of each pollen family identified by metabarcoding is the mean of NGS reads obtained for the replicate A and the replicate B. Relative abundance of each pollen family identified by microscopy is the result of pollen count.

3.3 Comparison of pollen composition obtained by metabarcoding with the checklist of plants in flower

The comparison of metabarcoding data with the list of plants observed in the field showed a good correspondence for each pollen sample (figure 4). The majority of NGS reads observed in each sample were attributed to flowering plant genera recorded within the studied environment, with an average abundance of 93.6% falling into the category of "detected" genera. Additionally, on average, 3.6% of the reads belong to genera not recorded during fieldwork but potentially present due to suitable habitats in the vicinity ("undetected potentially present"). Conversely, an average of 1.7% of the reads belong to genera not detected during field activities and not expected to be present in the environment ("undetected not present"). Furthermore, approximately 1% of reads in each sample were excluded due to being assigned to genera with a relative abundance lower than 0.5%. The pollen sample that showed the greatest discrepancy

was that from *Bombus* collected in low valley hay meadow environment. In this sample, pollen belonging to *Symphytum* represented more than 50% of the pollen grains, while this genus was not observed blooming during in-field observations, but its habitat was nearby. Moreover, for this pollen sample a similar relative abundance of *Symphytum* was detected with light microscopy (Table S3), thus excluding an error of DNA metabarcoding in the taxonomic assignment.

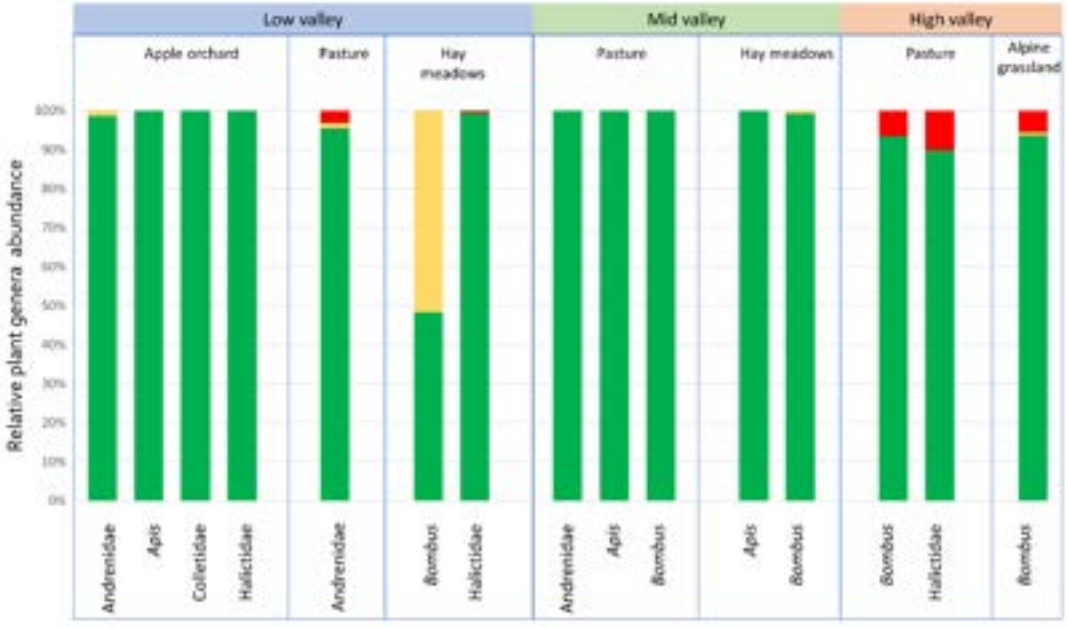

Figure 4. Comparison between genera identified by metabarcoding and in-field observation (percentages refer to identified genera NGS reads abundance). In-field detected genera (green), in-field undetected but potentially present genera (yellow), and in-field undetected and not present genera (red).

4. Discussion

This work aimed to evaluate the accuracy of ITS2 metabarcoding in providing quantitative insights into the composition of pollen loads carried by bee taxa collected in different environmental contexts.

To evaluate the effectiveness of this method, the family-level pollen load composition obtained through ITS2 reads has been compared with that obtained counting pollen grains identified by light microscopy. Similar analyses have been conducted in few previous works yielding mixed results. While previous studies have generally agreed on the qualitative superiority of metabarcoding due to its higher sensitivity and deeper taxonomic resolution (Keller et al., 2015; Richardson et al., 2015a; Richardson et al., 2015b; Wizenberg et al., 2023), the quantitative concordance of the two methods is still

debated. Some studies have shown no correlation between the relative abundances obtained with the two methods (Richardson et al., 2015a; Richardson et al., 2015b) while others indicated good correlations (Keller et al., 2015; Wizenberg et al., 2023). In this study an evident correlation (Pearson coefficient = 0.79) between the results obtained with ITS2 metabarcoding and light microscopy was found. Also considering the single samples, the relative abundances of plant families recorded with the two methods were very similar in more than 85% of the samples (13 out 15). As stated by Wizenberg et al. (2023), a multi-locus analysis might increase the accuracy of a quantitative characterization. However, this also implies an increase in the analysis costs (Wizenberg et al., 2023), that may not be justifiable if a sufficiently detailed picture of pollen composition can be obtained using a single marker. Therefore, an accurate cost-benefit analysis should be conducted to choose the most appropriate approach, considering the desired outcomes.

Given the quantitative reliability showed by DNA metabarcoding in estimating the relative abundance of plant families in pollen mixtures, it would have been interesting to evaluate whether similar results can be obtained also at the genus level. However, the low taxonomic accuracy achievable with light microscopy due to the natural morphological variability of pollen grains (Galimberti et al., 2014; Hawkins et al., 2015), the small subtle features that diversify closely related taxa (Richardson et al., 2015a; Zavada and Hackley, 2022) as well as the incomplete reference databases for mountain plants (Buchner and Weber, 2000; Bosch et al., 2009), made a direct evaluation not possible. Nonetheless, the comparison of plant genera identified by metabarcoding with in-field plants in flower showed that also the genus-level accuracy of ITS2 taxonomic assignment ITS2 is quite high, as all the detected plant genera are present in the flora of the sampled area. Moreover, the proportion of NGS reads belonging to plant genera shared with plants in flower occurring in each environment was high too, with the highest discrepancies not exceeding 10%. A specialized ITS2 database for plant species presents within the selected sampling area would further enhance the accuracy of genus-level assignment as well as to enable a more precise identification of pollen to species level (Banchi et al., 2020).

Another noteworthy finding of this study is that ITS2 metabarcoding is applicable even in contexts where the limited amount of available pollen represents a constraint. As stated before, the reliability of ITS2 metabarcoding in providing quantitative information about the composition of the pollen load carried by bees was tested by few previous studies (Keller et al., 2015; Richardson et al., 2015a; Richardson et al., 2015b; Bänsch et al., 2020; Wizenberg et al., 2023). However, in these works the DNA was extracted from a quantity of pollen ranging from 15 mg to 10 g, where each gram can be estimated to contain from about 500,000 to 90 million grains (Pitsios et al., 2006; Anderson et al., 2014). We instead used samples consisting of approximately 500 pollen grains (weighting approximately between 0.0055 mg and 1 mg). This is a crucial point to consider in the perspective of an actual evaluation of pollination networks, as dealing with this quantity of grains can be a common situation in pollen-based networks (Alarcón, 2010; Devoto et al., 2011; Orford et al., 2015; Urbanowicz et al., 2017). These findings suggest that ITS2 metabarcoding may be employed also for the analysis of pollen carried not only by bees, but also by other flower-visiting taxa (e.g., Diptera, Coleoptera, Lepidoptera) not having specialized structures for the accumulation of pollen grains and thus often carrying less pollen than bees (Larson et al., 2018). Despite this, also these flower visiting insects can play a fundamental role in plants pollination in different environmental contexts (Abrol, 2012; Orford et al., 2015; Wardhaugh, 2015; Lefebvre et al., 2018; McNabe and Cobb, 2021; Requier et al., 2023).

In conclusion, this study offers novel insights into the applicability of ITS2 metabarcoding techniques for characterizing the family-level composition of low quantities of pollen carried by insects, not only qualitatively but also in terms of taxa relative abundance. results could be useful to support researchers in choosing the most appropriate method to construct reliable quantitative pollen-based networks in different ecological contexts.

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Declarations

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Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Supplementary Material

Table S1. Identification data of selected sampling sites within the sampling area.

Sampling site ID	Sector	Elevation (m asl)	Management	Longitude (E) (UTM WGS84 – 32T)	Latitude (N) (UTM WGS84 – 32T)
BME1	Low valley	978	Apple orchard	639100	5160373
BPA1	Low valley	1000	Pasture	639102	5160268
BPR1	Low valley	1007	Hay meadow	639009	5160164
BME2	Low valley	1019	Apple orchard	639089	5160005
BPA2	Low valley	1313	Pasture	637207	5159233
BPR2	Low valley	1335	Hay meadow	636515	5157903
MPR1	Mid valley	1713	Hay meadow	632679	5153213
MPR2	Mid valley	1895	Hay meadow	631113	5150456
MPA2	Mid valley	2281	Pasture	631006	5151523
MPA1	Mid valley	2246	Pasture	629530	5150562
VPA1	High valley	2343	Pasture	631355	5152310
VPA2	High valley	2524	Pasture	631080	5153022
VPR1	High valley	2611	Alpine grassland	630958	5153413
VPR2	High valley	2683	Alpine grassland	630851	5153557

Table S2. Checklist of all the entomophilous plant species observed in flower during the in-field surveys within a radius of 30 meters for each land management type.

		Low valley			Mid valley		High valley	
Species	Author	Apple orchard	Pasture	Hay meadow	Pasture	Hay meadow	Pasture	High altitude grasslands
Adoxaceae								
Sambucus nigra	L.	X		X				
Apiaceae								
Aegopodium podagraria	L.	X				X		
Carum carvi	L.			X				
Daucus carota	L.	X	X					
Heracleum sphondylium	L.			X				
Imperatoria ostruthium	L.				X	X		
Mutellina purpurea	(Poir.) Reduron, Charpin et Pimenov				X	X		X
Oreoselinum nigrum	Delarbre		X					
Pimpinella major	(L.) Huds.			X		X		
Pimpinella saxifraga	L.		X					
Asteraceae								
Achillea millefolium	L.	X	X	X	X	X		
Achillea moschata	Wulfen				X		X	X
Achillea tomentosa	L.		X					
Adenostyles alliariae	(Gouan) A. Kern.					X		
Aconitum napellus	L. em. Skalicky				X	X	X	
Antennaria dioica	(L.) Gaertn.				X		X	X
Arnica montana	L.						X	
Aster alpinus	L				X		X	X
Bellis perennis	L.					X		
Carduus defloratus	L.		X			X		
Carduus nutans	L.		X					
Carlina acaulis	L.				X	X		
Centaurea stoebe	L.		X					
Cirsium heterophyllum	(L.) Hill					X		
Crepis aurea	(L.) Cass.				X	X		
Crepis biennis	L.					X		
Erigeron alpinus	L.				X			X
Erigeron annuus	(L.) Desf.			X				
Erigeron uniflorus	L.				X		X	X
Hieracium murorum	L.		X					
Homogyne alpina	(L.) Cass.						X	X
Jacobaea abrotanifolia	(L.) Moench				X		X	
Jacobaea carniolica	(Willd.) Schrank						X	X
Lactuca perennis	L.		X					

Leontodon hispidus	L.		X	X	X		X		
Leucanthemopsis alpina	(L.) Heywood						X	X	
Leucanthemum vulgare	Lam.			X		X			
Matricaria chamomilla	L.	X		X		X			
Pilosella aurantiaca	(L.) F.W. Schultz et Sch. Bip.					X			
Pilosella glacialis	(Reyn. ex Lachen.) F.W. Schultz et Sch. Bip				X				
Pilosella officinarum	Vaill.				X	X	X	X	
Pilosella piloselloides	(Vill.) Sojak		X						
Scorzoneroides helvetica	(Merat emend. Widder) Holub						X	X	
Solidago virgaurea	L.				X		X	X	
Taraxacum officinale	Weber	X	X	X		X			
Tolpis staticifolia	(All.) Sch. Bip.						X		
Tussilago farfara	L.					X			
Berberidaceae									
Berberis vulgaris	L.		X						
Boraginaceae									
Echium vulgare	L.	X	X	X					
Myosotis alpestris	F.W. Schmidt					X			
Myosotis laxa	Lehm.				X	X			
Brassicaceae									
Arabis glabra	(L.) Bernh.		X						
Biscutella laevigata	L.		X		X	X			
Capsella bursa-pastoris	(L.) Medik.	X		X		X			
Cardamine amara	L.					X			
Cardamine resedifolia	L.					X			
Erysimum odoratum	Ehrh.		X						
Erysimum rhaeticum	(Schleich. ex Hornem.) DC.		X						
Rorippa sylvestris	(L.) Besser	X		X					
Sinapis arvensis	L.			X					
Campanulaceae									
Campanula barbata	L.					X	X		
Campanula persicifolia	L.			X					
Campanula rotundifolia	L.		X						
Campanula scheuchzeri	Vill.				X	X	X	X	
Campanula trachelium	L.		X						
Phyteuma betonicifolium	Vill.				X	X			
Phyteuma hemisphaericum	L.						X	X	
Soldanella alpicola	F.K. Mey.						X	X	
Caryophyllaceae									
Cerastium arvense	L.				X				
Cerastium fontanum	Baumg.					X			
Cerastium holosteoides	Fr.			X					
Dianthus sylvestris	Wulfen		X						
Minuartia verna	(L.) Hiern						X	X	
Silene acaulis	(L.) Jacq.						X	X	
Silene alba	(Mill.) E.H.L. Krause	X		X		X			
Silene dioica	(L.) Clairv.		X	X		X			
Silene nutans	L.		X		X		X		
Silene vulgaris	(Moench) Garcke	X		X					
Stellaria media	(L.) Vill.	X							
Gypsophila vaccaria	(L.) Sm.					X			
Chenopodiaceae									
Blitum bonus-henricus	(L.) Rchb.			X					
Cistaceae									
Helianthemum nummularium	(L.) Mill.		X		X				
Hieracium glanduliferum	Hoppe							X	
Convolvulaceae									
Calystegia sepium	(L.) R. Br.	X							
Crassulaceae									
Sedum rupestre	L.		X		X				
Sedum sexangulare	L.		X						
Sempervivum arachnoideum	L.		X		X				
Sempervivum montanum	L.				X		X		
Dipsacaceae									

Knautia drymeia	Heuff.					X		
Scabiosa columbaria	L.		X					
Ericaceae								
Arctostaphylos uva-ursi	(L.) Spreng.				X		X	
Erica carnea	L.						X	
Kalmia procumbens	(L.) Gift, Kron et P.F.						X	X
Rhododendron ferrugineum	L.						X	X
Vaccinium myrtillus	L.						X	
Euphorbiaceae								
Euphorbia seguieriana	Neck.		X					
Euphrasia alpina	Lam.		X		X	X	X	
Euphrasia minima	Jacq. e1 DC.				X		X	X
Fabaceae								
Anthyllis alpicola	Brugger		X			X		
Astragalus alpinus	L.					X		
Astragalus onobrychis	L.		X					
Hedysarum hedysaroides	(L.) Schinz et Thell.					X		
Lathyrus sylvestris	L.		X					
Lotus corniculatus	L.	X	X	X	X	X	X	X
Medicago lupulina	L.		X	X		X		
Medicago sativa	L.			X				
Melilotus indicus	(L.) All.	X						
Trifolium alpinum	L.				X		X	
Trifolium badium	Schreb.				X	X		
Trifolium montanum	L.		X					
Trifolium pratense	L.	X	X	X	X	X		
Trifolium repens	L.	X	X	X	X	X		
Vicia cracca	L.	X		X		X		
Vicia sepium	L.	X		X		X		
Gentianaceae								
Gentiana acaulis	L.					X	X	X
Gentiana brachyphylla	Vill.					X		
Gentiana nivalis	L.						X	X
Gentiana verna	L.						X	X
Geraniaceae								
Geranium robertianum	L.	X						
Geranium sylvaticum	L.					X		
Hypericaceae								
Hypericum perfoliatum	L.		X					
Juncaceae								
Juncus jacquinii	L.							X
Lamiaceae								
Ajuga pyramidalis	L.					X	X	
Ajuga reptans	L.					X		
Atocion rupestre	(L.) Raf.					X		
Bartsia alpina	L.					X		
Clinopodium acinos	(L.) Kuntze		X					
Clinopodium alpinum	(L.) Kuntze	X	X					
Glechoma hederacea	L.	X	X	X		X		
Lamium album	L.	X	X	X		X		
Prunella grandiflora	(L.) Scholler		X					
Prunella vulgaris	L.	X	X	X				
Salvia pratensis	L.		X			X		
Thymus praecox	Opiz		X		X		X	
Lentibulariaceae								
Pinguicula leptoceras	Rchb.					X		
Liliaceae								
Gagea serotina	(L.) Ker Gawl.							X
Onagraceae								
Epilobium angustifolium	L.					X		
Epilobium montanum	L.	X						
Orchidaceae								
Coeloglossum viride	(L.) Hartm.						X	
Nigritella nigra	(L.) Rchb.				X		X	
Orobancaceae								
Pedicularis kerneri	Dalla Torre						X	X
Rhinanthus alectorolophus	(Scop.) Pollich					X		
Papaveraceae								
Chelidonium majus	L.		X					
Plantaginaceae								

Plantago lanceolata	L.	X	X	X				
Plantago media	L.		X			X		
Poligonaceae								
Bistorta officinalis	Delarbre	X				X		X
Polygalaceae								
Polygala vulgaris	L.	X	X		X	X		
Polygonaceae								
Polygonum aviculare	L.	X						
Rumex acetosa	L.			X				
Rumex acetosella	L.					X		
Rumex alpinus	L.					X		
Primulaceae								
Androsace obtusifolia	All.						X	X
Primula farinosa	L.					X		
Primula glutinosa	Wulfen							X
Ranunculaceae								
Pulsatilla alpina	(L.) Delarbre						X	
Ranunculus acris	L.	X	X	X	X	X	X	
Ranunculus bulbosus	L.		X					
Ranunculus kuepferi	Greuter et Burdet							X
Ranunculus montanus	Willd.						X	X
Thalictrum aquilegiifolium	L.					X		
Trollius europaeus	L.					X		
Rosaceae								
Alchemilla vulgaris	L. s.s.				X	X		
Fragaria vesca	L.		X	X				
Geum montanum	L.						X	X
Geum rivale	L.					X		
Geum urbanum	L.	X						
Potentilla argentea	L.		X					
Potentilla aurea	L.				X	X	X	X
Potentilla grandiflora	L.				X		X	
Potentilla neumanniana	Rchb.		X					
Potentilla recta	L.					X		
Rosa canina	L.		X					
Rubiaceae								
Galium anisophyllon	Vill.				X	X	X	
Galium mollugo	L.			X				
Galium pumilum	Murray		X					
Santalaceae								
Thesium alpinum	L.				X			
Saxifragaceae								
Saxifraga aizoides	L.					X		
Saxifraga bryoides	L.							X
Scrophulariaceae								
Veronica arvensis	L.			X				
Veronica bellidioides	L.				X		X	X
Veronica chamaedrys	L.		X	X	X	X		
Veronica serpyllifolia	L.		X	X	X	X		
Solanaceae								
Solanum dulcamara	L.		X					
Thymelaeaceae								
Daphne striata	Tratt.						X	
Urticaceae								
Urtica dioica	L.	X	X	X		X		

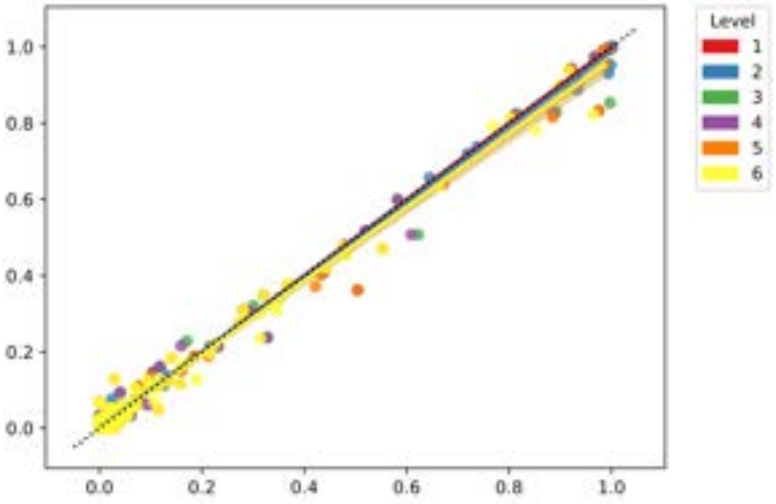
Table S3. Morphological identification at lowest possible taxonomic level and quantification of pollen grains collected from sampled bees.

		Low Valley							Mid Valley					High Valley		
		Aplle orchard				Pasture	Hay meadows		Pasture			Hay meadows		Pasture		High grassland
Code	\	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Family	**Taxon**	Andrenidae	Apidae (Apis)	Colletidae	Halictidae	Andrenidae	Apidae (Bombus)	Halictidae	Andrenidae	Apidae (Apis)	Apidae (Bombus)	Apidae (Apis)	Apidae (Bombus)	Apidae (Bombus)	Halictidae	Apidae (Bombus)
Apiaceae	Apiaceae sp.1	0	3	1	0	1	0	0	0	0	0	0	0	0	0	0
Apiaceae	Apiaceae sp.2	0	0	0	0	0	40	0	0	0	0	0	0	0	0	0
Asteraceae	Asteroidea	50	0	4	3	20	0	7	4	4	3	0	24	2	2	11
Asteraceae	Cichorioidea	776	0	20	919	1	0	0	589	840	1	346	25	2	233	1
Betulaceae	*Alnus*	1	0	0	0	0	0	0	1	2	0	0	0	0	0	0
Betulaceae	Betulaceae	0	0	0	1	0	0	7	0	0	0	0	0	0	0	0
Boraginaceae	*Symphytum* sp.	0	0	0	0	0	510	0	0	0	0	0	0	0	0	0
Boraginaceae	*Echium vulgare*	0	0	0	0	0	93	0	0	0	0	0	0	0	0	0
Boraginaceae	*Eritrichium* cfr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brassicaceace	*Rorippa sylvestris*	0	0	0	0	0	0	60	0	0	0	0	0	0	0	0
Campanulaceae	*Phyteuma* sp.	0	0	0	0	0	0	0	0	0	1	1	1	261	0	1544
Cariophyllaceae	*Silene acaulis*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Caprifoliaceae	Caprifoliaceae	0	0	0	0	107	0	0	0	0	0	0	0	0	0	0
Ericaceae	*Rhododendron ferrugineum*/ *Vaccinium myrtillus*	0	0	0	0	0	1	0	1	2	12	1	0	0	0	1
Fabaceae	*Vicia* sp.	1	0	15	0	0	63	0	0	0	0	0	0	0	0	0
Fabaceae	Fabaceae	0	0	0	0	2	77	1	0	688	336	0	13	1	0	0
Fabaceae	*Lotus* cfr.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fabaceae	*Trifolium* sp.	0	352	4	0	0	0	0	0	0	0	0	0	0	0	0
Gentianaceae	*Gentiana* sp.	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0
Cupressaceae	*Juniperus communis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lamiaceae	*Thymus* sp.	0	0	0	0	1	0	2	0	0	0	0	0	0	1	0
Lamiaceae	*Teucrium* cfr.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Oleacea	*Fraxinus* sp.	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0
Onagraceae	*Epilobium montano*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Orchideaceae	*Nigritella nigra*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Orobancaceae	*Euphrasia* sp./ *Rhinanthus* sp.	0	0	0	0	0	0	0	0	0	0	0	42	0	0	0
Pinaceae	*Picea abies*	0	0	0	0	1	3	2	0	2	1	0	0	0	0	0
Plantaginaceae	*Plantago* sp.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Poaceae	Poaceae	1	0	2	0	1	0	0	0	0	1	0	0	0	0	0
Primulaceae	*Androsace* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Primulaceae	*Soldanella pusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Ranunculaceae	Ranunculaceae	0	0	0	0	0	7	6	1	0	0	0	0	0	0	0
Ranuncolaceae	*Aconitum napellus*	0	0	0	0	0	0	0	1	0	101	0	0	0	0	0
Rosaceae	Rosaceae	6	0	90	0	0	38	10	0	0	102	0	0	2	0	0
Rosaceae	*Geum urbanum*	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Rosaceae	*Potentilla* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salicaceae	*Salix* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Saxifragacea	Saxifragacea	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
ND	ND	2	0	1	0	2	4	1	1	2	1	2	0	0	3	0
Total		838	355	137	925	137	836	104	598	1540	580	351	105	268	242	1557

Table S4. Qualitative and quantitative results of morphological and molecular analysis. "NGS reads" column reports the mean of reads between the two technical replicates for each sample.

	Morphology (LM)		Molecular (ITS2 metabarcoding)	
Plant family	Pollen grains	Mean rel. abund.	NGS reads	Mean rel. abund.
Apiales;Apiaceae	45	0.00472640895	3377.0	0.009999910494
Asparagales;Amaryllidaceae	0	0.00000000000	3.5	0.000012618410
Asparagales;Orchidaceae	1	0.00027548209	0.0	0.000000000000
Asterales;Asteraceae	3887	0.42472850826	110912.5	0.313794907602
Asterales;Campanulaceae	1808	0.13197521050	44530.5	0.115226152821
Boraginales;Boraginaceae	603	0.04808612440	24289.0	0.052420598393
Brassicales;Brassicaceae	60	0.03846153846	2853.0	0.019239285353
Caryophyllales;Caryophyllaceae	1	0.00027548209	12.0	0.000035847620
Cucurbitales;Cucurbitaceae	0	0.00000000000	26.5	0.000128586489
Cupressales;Cupressaceae	0	0.00000000000	31.5	0.000093903803
Dipsacales;Adoxaceae	0	0.00000000000	4463.5	0.031229144977
Dipsacales;Caprifoliaceae	107	0.05206812652	9356.5	0.027489606746
Ericales;Ericaceae	18	0.00188986888	48.0	0.000127670610
Ericales;Primulaceae	1	0.00027548209	261.5	0.001012179311
Fabales;Fabaceae	1553	0.16511408286	90059.0	0.193036505555
Fagales;Betulaceae	12	0.00483686886	1473.0	0.007806292908
Fagales;Fagaceae	0	0.00000000000	450.0	0.002959024775
Gentianales;Gentianaceae	20	0.00229885057	0.0	0.000000000000
Lamiales;Lamiaceae	5	0.00215909391	29.5	0.000199954923
Lamiales;Oleaceae	8	0.00512820513	1.5	0.000003629105
Lamiales;Orobanchaceae	42	0.02666666667	15833.5	0.043013550948
Lamiales;Plantaginaceae	1	0.00048661800	8821.0	0.062046122042
Lamiales;Scrophulariaceae	0	0.00000000000	111.0	0.000326120488
Malpighiales;Hypericaceae	0	0.00000000000	297.5	0.000874061669
Malpighiales;Salicaceae	0	0.00000000000	75.5	0.000369902800
Malvales;Cistaceae	0	0.00000000000	31.5	0.000106356040
Myrtales;Onagraceae	1	0.00007955449	0.0	0.000000000000
Pinales;Pinaceae	9	0.00220942635	0.0	0.000000000000
Poales;Cyperaceae	0	0.00000000000	21.0	0.000102878267
Poales;Poaceae	5	0.00165435104	249.5	0.001424766620
Proteales;Platanaceae	0	0.00000000000	3347.5	0.010959657110
Ranunculales;Ranunculaceae	116	0.01623652840	8429.5	0.022624081183
Rosales;Rosaceae	250	0.06607930136	25161.5	0.066541459395
Rosales;Urticaceae	0	0.00000000000	561.0	0.001416835134
Sapindales;Rutaceae	0	0.00000000000	48.0	0.000209245018
Saxifragales;Crassulaceae	0	0.00000000000	624.5	0.001830312199
Saxifragales;Saxifragaceae	1	0.00018993352	213.0	0.000719169410
Undetermined/Unassigned	19	0.00409828657	11.5	0.000084041290
Undetermined Streptophyta	0	0.00000000000	4767.0	0.012535620491

Figure S1. Qualitative and quantitative comparison of the two technical replicates. x-axis = relative abundance of taxa in the replicate A; y-axis = relative abundance of taxa in the replicate B. Colours correspond to different taxonomic levels (1=kingdom, 2=phylum, 3=class, 4=order, 5=family, 6=genus).



Chapter 4

Ph.D. side projects

Chapter 4.1

Campanula bergomensis (Campanulaceae), a new plant species from Bergamo Prealps (Northern Italy)

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Campanula bergomensis (Campanulaceae), a new species from Bergamo Prealps (Northern Italy)

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Abstract

A new species of *Campanula* (Campanulaceae), *C. bergomensis sp. nov.*, is described from the Bergamo Prealps (Orobic Prealps, Lombardy, Northern Italy), based on both morphological and molecular evidence. The new species was considered in the past as an isolated population of *C. cespitosa*, which presents an eastern Alpine distribution. *C. bergomensis* is morphologically well distinguishable from *C. cespitosa* on the number of flowers in the racemose inflorescence, the corolla shape, and the whitish-yellow pollen surface with many spinulae. Genetically, the presence of an insertion of 81-bp in the *trnL-F* sequences is very characteristic. Further studies are needed to better define the phylogenetic relationship among the three closely related species, *C. bergomensis*, *C. cespitosa* and *C. cochlearifolia*. *C. bergomensis* inhabits dolomitic debris cones at low elevations. The species is range-restricted and is severely threatened by human activities. Therefore, it is urgent to adopt protection and conservation measures for the new species.

Key words: Biodiversity, *Campanula cespitosa*, Endemism, Genetics, Morphology

Introduction

The Bergamo Prealps (Lombardy, Northern Italy), located in the southwestern portion of the Eastern Alps, are known as a biodiversity hotspot, due to the very high number of narrow endemic species (Merxmüller, 1954; Fenaroli, 1973; Aeschimann *et al.*, 2011, Martini *et al.*, 2012), the highest in the Alps (Aeschimann *et al.*, 2004). This high rate of endemism is caused by the complex geomorphological and climatic history of this Alpine sector (Cesati, 1848; Giacomini, 1943; Kunz & Reichstein, 1959; Pitschmann & Reisigl, 1959). The proximity of this area to the margins of the Quaternary ice sheet allowed the formation and maintenance of refugia (Tribsch & Schönswetter, 2003). Currently, its central position forms a bridge between the western and eastern Alpine floras, under a favourable climatic condition characterized by mild winters and high summer precipitation (Martini *et al.*, 2012).

Among the Alpine genera, *Campanula* L. has the highest number of endemic species (Aeschimann *et al*., 2004). In the Bergamo Prealps this is underlined by several endemic *Campanula* taxa: *C. raineri* Perp., *C. elatinoides* Moretti, *C. carnica* Schiede ex Mert. & W.D.J.Koch *subsp. puberula* Podlech, (Martini *et al*., 2012), *C. martinii* F.Fen., Pistarino, Peruzzi & Cellin. (Fenaroli *et al*., 2013). Another endemic is *C. cespitosa* Scop., an eastern Alpine species with two distinct ranges, north and south of the Alpine chain (Pawlowski, 1970): from the Viennese to the Steyr Alps in the north, and from Slovenia to the river Adige in the south. Its presence in the Bergamo Prealps was first observed by Rota (1843), Bergamaschi (1853), Chenevard (1915), and later confirmed by Hess *et al*. (1967), Ravazzi (2007), and Martini *et al*. (2012) (Fig. 1). Surprisingly, despite all these confirmed records, the presence of this species in the Bergamo Prealps is reported only in local floras (Martini *et al*., 2012), but it is missing from national or international floras. In fact, it is not reported in the Bergamo Prealps in Aeschimann *et al*. (2004), Pignatti (1982; 2017–2019) and in the most recent updated checklist of the Italian flora (Bartolucci *et al*., 2018). Considering the high degree of endemism of *Campanula* in the Alps and the poorly known occurrence of *C. cespitosa* in the Bergamo Prealps, in addition to the significant distance between these populations from the rest of its distribution range, we decided to further study this taxon in Lombardy. Based on multiple phytogeographic, morphological and genetic evidence, we concluded that the populations from the Bergamo Prealps represent a species new to science, which we propose to name *Campanula bergomensis*.

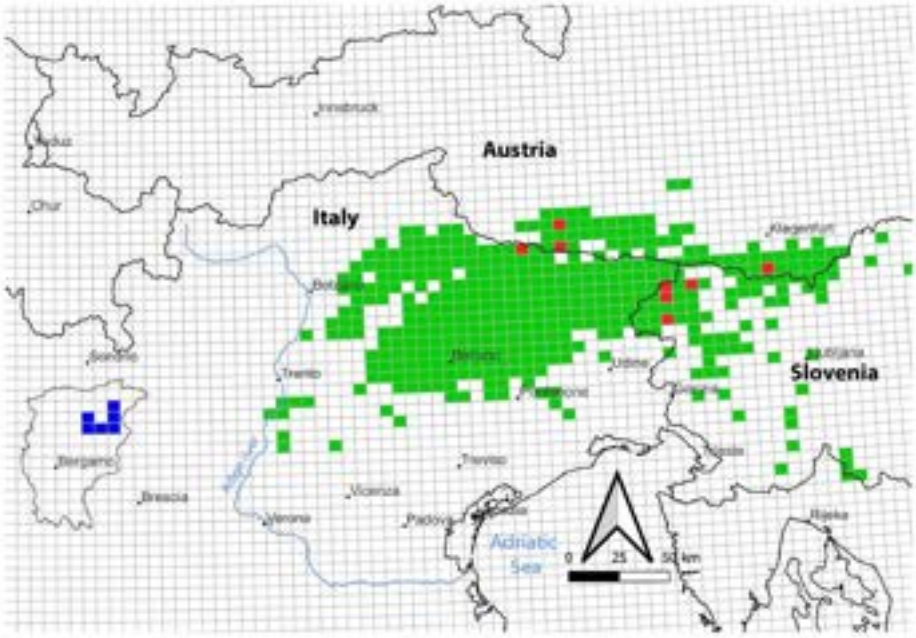

FIGURE 1. Distribution of *Campanula bergomensis* (blu squares) and *C. cespitosa* (green and red squares). Red squares indicate the grids where *C. cespitosa* samples for genetic analysis were collected. Cartographic grid is taken from Ehrendorfer and Hamann (1965). Bibliographic data of *C. cespitosa* are: Andreatta *et al*. 2020 (Veneto, Italy), Martini *et al*. 2023 (Friuli-Venezia Giulia, Italy), Wilhalm *et al*. 2014 (Alto Adige, Italy), Jogan *et al*. 2001 (Slovenia), Hartl *et al*. 1992 and Stradinger *et al*. 2009 (Austria).

Materials and methods

The morphological analysis of *Campanula bergomensis* was performed on samples collected in three valleys (Table 1); from each valley, 40–50 individuals (130 in total) were collected. The morphological comparison with the closely related species *C. cespitosa* was performed using 10–20 individuals of this species from three different sites (50 in

total) (Table 1). For leaf trait measurements, individuals were collected and preserved in moistened blotting paper until traits were measured.

For the pollen analysis and comparison, at least 3 flowers in full anthesis from each population sampled of *C. bergomensis* and *C. cespitosa* were collected and dried (Table 1). For *C. cespitosa*, the pollen from an herbarium specimen was also included (Table 1).

For the genetic analysis, *C. bergomensis* samples were collected in summer 2022. In order to perform detailed genetic comparison with the closely related *C. cespitosa*, the latter was sampled in three different areas within its distribution range (see Fig. 1). Samples of *C. cochlearifolia*, a species closely related to *C. cespitosa*, were also collected (Table 1). For genetic analysis, five specimens of each investigated species were sampled (four *C. cochlearifolia*) (Table 1). From each specimen 5 to 15 young leaves were collected. Specimens were preserved in plastic bags hermetically closed and filled with silica gel to reduce moisture and dehydrate the leaves. Bags were then preserved under cool and dry conditions.

TABLE 1. Sampling dates for morphological and genetic analysis. "-" indicates missing data. * samples from Val Resia (UD) are from *Herbarium* F. Martini of Museo Friulano di Storia Naturale Udine (Italy) (*leg.* F. Martini).

Species	Country, region	Site	Elevation (m a.s.l)	Sampling date for plant morphology	Sampling date for pollen morphology	Sampling date for genetics	Sample IDs for genetics
C. bergomensis	Italy, Lombardy	Songavazzo (BG), Valle di Fruec	920–930	20 Jul. 2019	20 Jul. 2019	-	-
	Italy, Lombardy	Oneta (BG), Val dei Dadi	770–850	21 Jul. 2019	21 Jul. 2019	8 Aug. 2022	BG1–2
	Italy, Lombardy	Cusone (BG), Valle Cabrosa	515–745	25 Aug. 2019	25 Aug. 2019	8 Aug. 2022	BG3
	Italy, Lombardy	Oneta (BG), Valle di Tede	710	-	-	8 Aug. 2022	BG3–4
C. cespitosa	Italy, Veneto	Cortina d'Ampezzo (BL), Passo Tre Croci	1210	30 Jul. 2019	30 Jul. 2019	-	-
	Italy, Friuli-Venezia Giulia	Resia (UD), Valle del Rio Bierman	600	-	Herbarium *	-	-
	Italy, Friuli-Venezia Giulia	Sauris (UD)	1240	1 Aug. 2020	-	-	-
	Italy, Veneto	Sappada-Prevenzano (BL), Parve river springs	1085–1890	-	-	8 Aug. 2022	CePl–5
	Italy, Trentino	Primiero San Martino (TN), Val Canali	1410–1580	2 Aug. 2020	-	-	-
	Slovenia, Gorizia Region	Žaga- Kobarid, Robič - Stara selo; Predel-Borve:, Log pod Mangartom; Kranjska Gora	240–1320	-	-	18 Aug. 2022	Ce11–5
	Austria, Carinthia	Ferlach, Loibl Pass, Golbass (Oberdorn); Kotschach-Mauthen, Plöcken Pass	700–980	-	-	19 Aug. 2022	CeA1–5
C. cochlearifolia	Italy, Lombardy	Bormio (SO), Passo dello Stelvio	2200	-	-	11 Jul. 2023	CeV2–5

Seeds from specimens from Val dei Dadi were collected in the field on 14th September 2019 in order to cultivate the new species *ex situ* in "Città Studi" Botanical Garden (University of Milan). 450 seeds were sown in the tropical greenhouse on 26 January 2021, although only 14 seedlings germinated and, on 3 October 2023, only two plants had survived, which are currently cultivated outdoors.

Following the method proposed by Pierce *et al.* (2013), the CSR (competitive-stress tolerant-ruderal) strategy was calculated for the new species using three leaf parameters: leaf dry matter content (LDMC), specific leaf area (SLA), and leaf area (LA). In particular, 130 cauline and 30 basal leaves were measured from samples from Val Dei Dadi, Val di Frucc and Val Cabrosa (Table 1).

Light Microscopy (LM) and Scanning Electron Microscopy (SEM) observations were conducted using pollen grains acetolyzed according to Bonelli *et al.* (2020). The measurements of acetolyzed pollen, mounted in glycerine jelly, were taken in LM with a 100x immersion objective lens on a Leica DM-RD microscope equipped with a Leica

MC170 HD camera (Leica Camera AG, Wetzlar, DE). For pollen grain shape, P (polar axis), E (equatorial diameter), and P/E ratio, 50 pollen grains in equatorial view were measured and analyzed for each population (Eustacchio *et al*., 2023). The measurements were performed with ImageJ software (Schneider *et al*., 2012). For SEM observations, acetolyzed and air-dried pollen was sputtered with a 25-nm layer of gold in argon plasma (AGAR Automatic Sputter Coater Mod. B7341, Scientific Limited, Stansted, UK) equipped with a quartz crystal thickness monitor. At least 30 pollen grains in equatorial and polar view were observed under the electron microscope (Model Leo-1430, Zeiss Inc., Thornwood, NY, USA). Palynological terminology follows Rodondi *et al*. (2004), Punt *et al*. (2007) and Hesse *et al*. (2009).

DNA was extracted within two months from sample collection. Genomic DNA was extracted and purified using DNeasy Plant Pro Kit (QIAGEN©, Germantown, MD, USA) starting from 10 mg of dry leaves. For the genetic analysis two plastidal markers were PCR-amplified and sequenced. For the PCR amplification of *matK* and *trnL-F* genes primers from Crowl *et al*. (2014) were used. Amplifications of plastidal genes were performed with the following PCR cycles: an initial denaturation step at 96 °C for 90 s, followed by 34 cycles at 95 °C for 30 s, 50 °C for 60 s, and 72 °C for 90 s, and a final extension step of 20 min at 72 °C. Amplification products were checked on a 1.5% agarose gel and sequenced by an external sequencing service provider (Eurofins Genomics, Italy), using the same two primers as used for amplification.

Prior to phylogenetic analyses, sequence electropherograms were visually inspected using Benchling (Benchling, 2023) to detect mis-calls. Besides the sequences from *C. bergomensis* and *C. cespitosa* generated by us, we added all taxa with publicly available *matK* and *trnL* sequences included in the *3* clade (the one with *C. cespitosa*) from the phylogenetic tree in Crowl *et al*. (2014). To further complement the phylogenetic reconstruction, *C. hawkinsiana* and *C. lasiocarpa* were added at the time the analysis was conducted; they were within the first four species with the highest total alignment score after a nucleotide blast against *Campanula* (taxid:40568; organism field) using BG5 *trnL-F* sequence as a query. *Jasione montana* was used as outgroup. Alignments were performed using Muscle included in AliView v1.28 (Larsson, 2014). After manual trimming of upstream and downstream unaligned portions, multialignments built from *matk* and *trnL-F* sequences were manually merged to build a concatenated plastidial dataset. Phylogenetic analyses were run using MEGA v11.0.13 (Tamura *et al*., 2021). The best substitution model was estimated using the appropriate MEGA function. A maximum likelihood (ML) tree was constructed with the following parameters: General Time Reversible model (gamma distributed with invariant sites), four discrete gamma categories, use all sites, 5000 bootstrap replications (all other options were left to default parameters). Single-nucleotide polymorphic sites (SNPs) in the multialignment were counted using the SNP-sites v2.5.1 tool (Page *et al*., 2016). Small insertions/deletions (indels) were counted by visual inspection of the alignments. For both variant types, a variant was counted if occurring at least in one species of the multialignment. GenBank accession numbers of sequences used in this study are reported in Table S1a in supplementary material.

Results

The analysis of morphological traits indicates a clear separation between *C. bergomensis* and the closely related species *C. cespitosa* (see section on taxonomic relationships).

New sequences and trace files generated in this work have been uploaded to GenBank (Table S1b in supplementary material). The alignment of *trnL-F* sequences from all species considered revealed the presence of an 81bp (base pair) long tandem repeat in the *C. bergomensis* accessions. A total of 194 SNPs and 3 indels were identified comparing the *matK* sequence of all species. For *trnL-F*, SNP number was lower (96), but a higher number of indels was detected (17). The genetic variants identified in these sequences allowed a phylogenetic reconstruction (ML tree) which provides a robust support for considering the three related groups *C. bergomensis* (99 bootstrap value; Fig. 2, purple vertical line), *C. cespitosa* (95 bootstrap value; Fig. 2, blue vertical line) and *C. cochleariifolia* (96 bootstrap value; Fig. 2, green vertical line) as distinct species. On the other hand, although *C. cespitosa* and *C. cochleariifolia* species are clearly separated, their relationship remains unresolved due to the low bootstrap value (40) of their ancestral node. Importantly, the significant bootstrap value supporting the *C. bergomensis* group (99) suggests its separation from the other two related entities. The congruence among the morphological, genetic and geographic evidence supports the recognition of *C. bergomensis* as a species new to science.

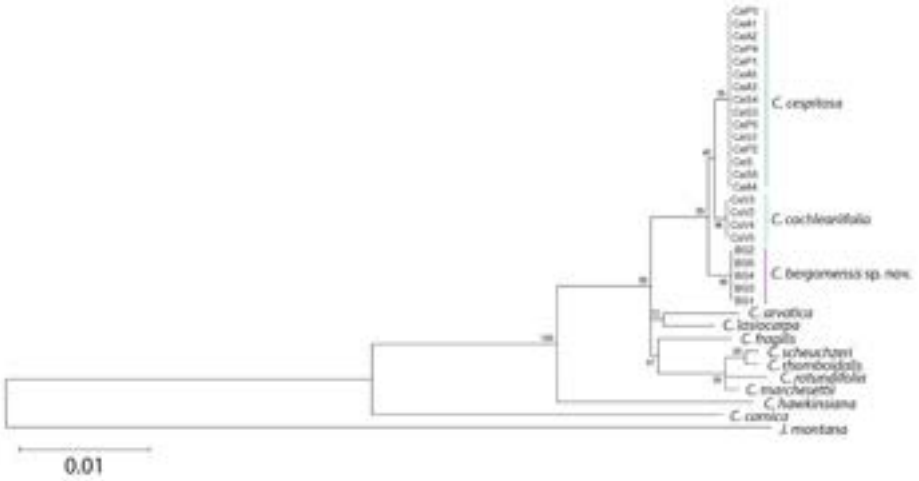

FIGURE 2. ML phylogenetic reconstruction of relevant *Campanula* species. Species selection is explained in the methods section. Bootstrap values are shown on each node. *Jasione montana* is selected as the outgroup.

Taxonomy

***Campanula bergomensis* F. Mangili & L. Mangili *sp. nov.* (Figs. 3–8)**

Description:—Perennial herb, (4–) 10–20 (–30) cm tall, with ascending-erect, glabrous or sparsely hairy, square-cross-section stems, with strong, horizontal creeping rhizomes from which a rosette of leaves develops (Fig. 3A–C; Fig. 4). Roots robust, branched and elongated underground. Basal leaves forming rosettes, with 10–20 mm long petioles, lamina oval-rhomboid 9–12 x 6–7 mm, glabrous, margin weakly toothed, sometimes sparsely hairy, often witheread during summer anthesis (Fig. 3A–D; Fig. 4); stem leaves numerous, sessile, linear-lanceolate, acute, (7–) 15–21 (–30) mm, glabrous, margin entire, on lower portion of stem close together, then spaced and decreasing upwards (Fig. 3A, Fig. 4). Inflorescence a raceme with 2–4 flowers, supported by long peduncles (Fig. 3A; Fig. 4). Calyx glabrous, with laciniae 2–3 mm slightly larger than the tube and appressed to the corolla (Fig. 3E, Fig. 4). Buds and flowers pendulous; corolla (9–) 11–12 (–14) mm, hemispherical-campanulate, not constricted in upper part, light blue (Fig. 3E–F, Fig. 4); trifid stigma not protruding. Fresh pollen color whitish, becoming yellow when drying out; mature pollen grains suboblate (P/E= 0.87), released as free monads of medium size (30 µm); grains radially symmetric, isopolar, triporate and zonoporate (Fig. 5); tectum discontinuous with muri (thickness around 0.20 µm) and lumina; spinulae present, shorter than 3 µm; sometimes granules present; ornamentation rugulate-microreticulate and echinate (Fig. 5). Capsule pendulous, ovoid, dehiscent. Seeds about 0.9 mm long (Fig. 3G; Fig. 4).

Type:—ITALY, Val Borlezza BG, Clusone, Ponte della Selva, Val Cabeosna, sentiero di fondovalle, 625 m, ghiaione ricco di calcare, 15.VIII.2007, *Leg. Perico*, 18 Apr. 2023 (holotype BER!; isotypes in BER!; paratypes ibidem 15.VIII.2007, *leg. Perico*), (Fig. 6).

Synonyms:—*Campanula cespitosa sensu* Martini *et al.* (2012), non Scop., Fl. Carniol., ed. 2, 1: 143 (1771); *Campanula rotundifolia pro parte sensu* auct. fl. bergomensis, non L., Sp. Pl.: 163 (1753).

Phenology:—Flowering from mid-July to mid-August.

Habitat and ecology:—*Campanula bergomensis* grows on dolomitic debris cones, ledges of rough grassland, generally on poorly developed and well-drained soils (Fig. 3B). Its elevational distribution ranges from 450 to 1,250 m a.s.l. The area of occurrence is characterized by an annual average temperature of 11 °C in Clusone (data from Ufficio Idrografico del Po, 1958–1982[a]) and rainy climate (1500 ml/y in Clusone; Ceriani & Carelli, 1990). The species is particularly associated with wide and flat debris cones, an unusual geomorphological feature in the Lombardian

Prealps. *C. bergomensis* can be found on the edge of *Pinus mugo* Turra vegetation (with *Amelanchier ovalis* Medik., *Sesleria coerulea* (L.) Ard., *Calamagrostis varia* (Schrad.) Host, *Hieracium* sp., *Dryas octopetala* L., *Globularia cordifolia* L.), where the slopes are sparsely vegetated. *C. bergomensis* shows a S-R strategy (leaf traits and CSR graph are reported in supplementary material in Table S2 and Fig. S1, respectively).

Distribution range:—*Campanula bergomensis* occurs in a few areas of the Orobic Prealps, within the borders of Bergamo Province (Lombardy, Italy). (Fig. 1, 7).

FIGURE 3. Morphological features of *C. bergomensis* A: plant habitus (scale bar 5 cm). B: the plant in its habitat. C: winter habitus. D: leaf shapes (scale bar 1 cm). E, F: flower shape and fresh pollen color (scale bars 1 cm). G: seeds on millimetre paper to show shape and size.

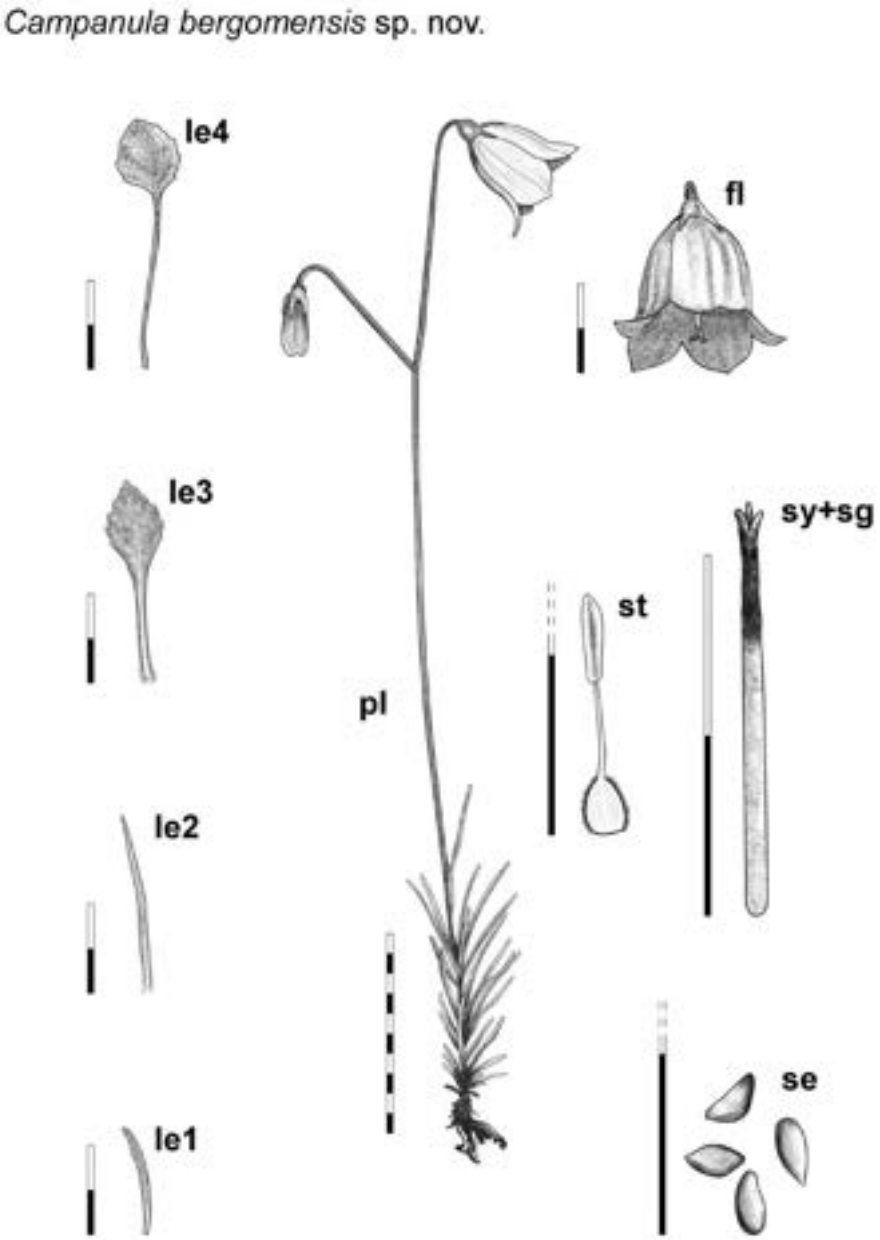

FIGURE 4. Illustration of *C. bergomensis* (bars subdivisions 5 mm). "le"= leaf, "pl"=plant, "fl"=flower, "st"=stamen", "sy"= style", "sg"= stigma, "se"=seeds".

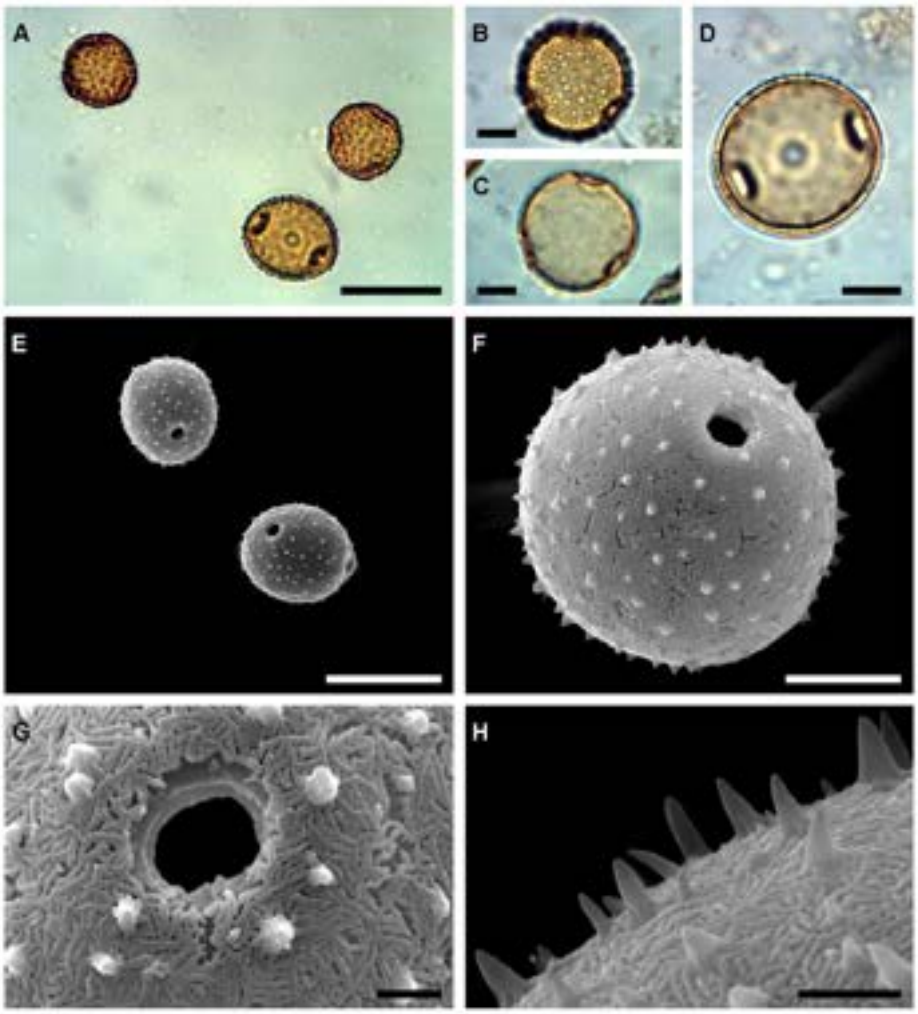

FIGURE 5. Pollen morphology of *C. bargomensis*. **A–D:** LM micrographs; **E–H:** SEM micrographs. A: Pollen grains as free monads showing the pores and the echinate ornamentation (scale bar = 50 μm). B: Pollen grain in polar view, the focal plane is on the echinate pole (scale bar = 10 μm). C: Pollen grain in polar view, the focal plane is on the equator showing the equatorial position of the pores (scale bar = 10 μm). D: Pollen grain in equatorial view, showing the suboblate shape (scale bar = 10 μm). E: Pollen grains as free monads showing echinate ornamentation (scale bar = 30 μm). F: Pollen grain showing the rugulate-microreticulate and echinate ornamentation (scale bar = 10 μm). G: The rugulate-microreticulate ornamentation stops near the pore margin (scale bar = 2 μm). H: The echinate ornamentation coexists with a rugulate-microreticulate pattern (scale bar = 2 μm).

FIGURE 6. Holotype of *C. bergomensis* sp. nov.

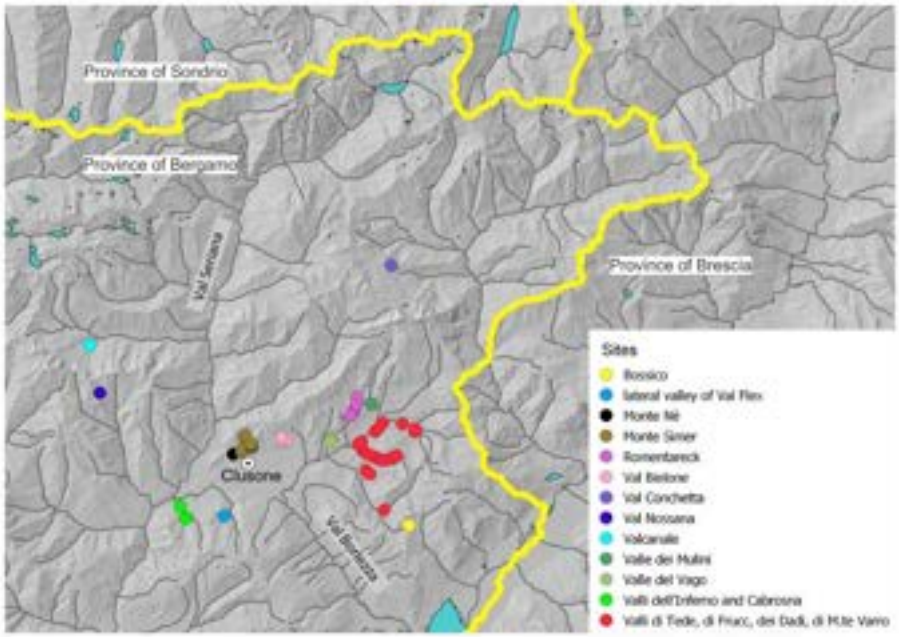

FIGURE 7. Distribution of *Campanula bergomensis*.

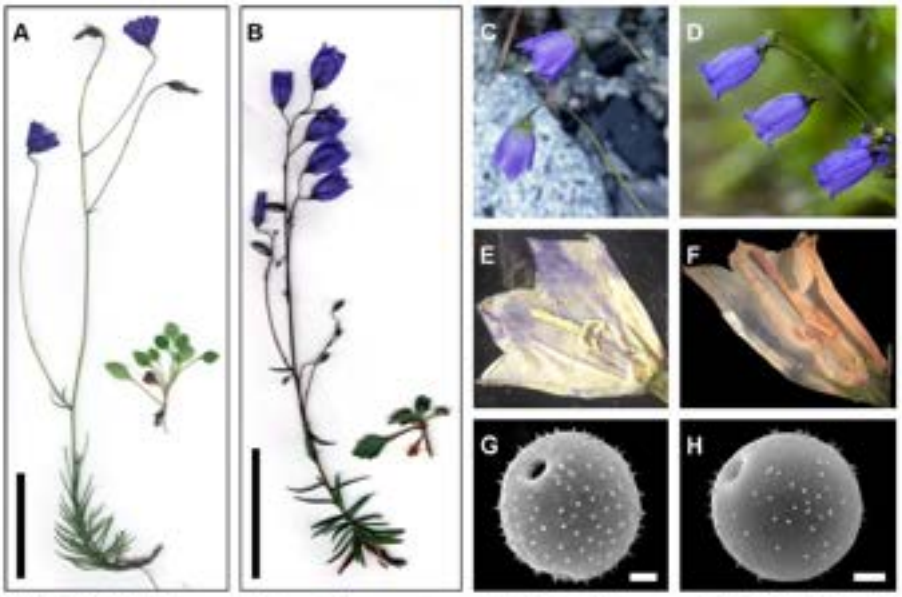

FIGURE 8. Morphological comparison between *C. bergomensis sp. nov.* (A, C, E, G) and *C. cespitosa* (B, D, F, H). A, B: plants habitus (scale bars 5 cm). C, D: flower shape. E, F: dried pollen color. G, H: pollen ornamentation observed with SEM (scale bars 5 μm).

Etymology:—The epithet refers to the Province of Bergamo (called "Bergomum" in Roman antiquity), where all known populations of the new species are found.

Taxonomic remarks:—Morphological and genetic evidence support *Campanula bergomensis* as a species new to science. In particular, it is morphologically distinguished from *C. cespitosa* for having 1) the racemose inflorescence with less flowers compared to *C. cespitosa* (2–3 and 6, on average, respectively; Fig. 8 and Fig. S2); 2) the shape of the corolla (not constricted at the apex, like in *C. cespitosa)*; and 3) the whitish-yellow pollen surface with many spinulae, instead of pink and with few spinulae like in *C. cespitosa* (Fig. 8). Genetically, *C. bergomensis* is well-distinguished from, though close to both *C. cespitosa* and *C. cochleariifolia* (Fig. 2), which have been consistently recovered as sister species (Mansion *et al.* 2012; Crowl *et al.* 2014, 2016). Although morphology would suggest that *C. bergomensis* is closer to *C. cespitosa* than to *C. cochleariifolia*, which is macroscopically very different, our genetic data are not so clear in confirming the phylogenetic relationships among these three closely related species and further investigations are needed, possibly including the comparison of more genes.

Discussion

Biogeographic considerations

Among the three closely related species, *C. cochleariifolia*, has the widest distribution range, being a South-European orophyte, which includes that of *Campanula bergomensis* and *C. cespitosa*. The co-occurrence of *C. cochleariifolia* and *C. bergomensis* was documented in phytosociological relevés from Val dei Dadi (Ravazzi, 2007). In contrast, *C. bergomensis* and *C. cespitosa* have clearly separated distribution ranges, the first being present only in a few sites of the Bergamo Prealps (Central-Southern Alps), the second occurring in the Eastern Alps, at least 100 km apart (the westernmost record is from Monte Finonchio—Folgaria, TN; Prosser *et al.*, 2019). The two disjunct, though closely related species *C. bergomensis* and *C. cespitosa* may have originated in isolation resulted from the Pleistocene Ice Ages, a well-known driver of such distributional pattern in the Alps, particularly important in the unglaciated peripheral areas (Merxmüller, 1954, Schönswetter *et al.*, 2003). However, a pre-glacial origin has been suggested for some endemic taxa occurring in the same area as a result of the late Tertiary dismantling of a once continuous southern Prealpine karstic platform characterized by limestone/dolomitic substrate (Bini, 1978, Ravazzi, 2007). The occurrence of *C. bergomensis* on peculiar carbonate landforms, such as flat and wide debris cones, uncommon in the Central Prealps but distinctive in the Eastern Alps, may support that hypothesis. In addition, Crowl *et al.* (2016) dated the split between *C. cespitosa* and *C. cochleariifolia* between 0.3 and 4.6 million years: this range, although wide, would support this hypothesis.

Threats

Some populations of *Campanula bergomensis* grow within a protected area of the Natura 2000 Network, Habitat Directive. In particular, populations of Val Bielone, Romentareck, Val dei Mulini (Colle di Passeraia), Monte Simer are included in the SAC (Special Area of Conservation) "Val Sedornia, Valzurio, e Pizzo della Presolana" (IT2060005); Val Nossana population is included in SAC "Val Nossana—Cima di Grem" (IT2060009). The main threat to *C. bergomensis* is currently the high frequency of motorcycles on and off the trails, and also off-trail, in all the valleys where the largest populations of the new species subsist (Val di Tede, Val di Monte Varro, Val Righenzolo, Val dei Dadi). The frequent passage of mountain bikes and motorcycles severely disrupts the stability of the debris conoids where *C. bergomensis* grows. Less critical is the episodic hydro-geological thread due to sudden floods caused by heavy precipitation events (Ravazzi *et al.*, 2007). The IUCN conservation status of the new species is under evaluation.

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Supplementary Material

Table S1: a) GenBank accession numbers of online sequences used for genetic comparison; b) GenBank accession numbers of sequences produced for this study.

a)

Species name	matK Genebank accession number	TrnL-F Genebank accession number
Jasione montana	EU713247.1	EF088782.1
Campanula carnica	LT706651.1	KJ512670.1
Campanula hawkinsiana	LT706717.1	EF213146.1
Campanula scheuchzeri	MZ204490.1	EF088762.1
Campanula rhomboidalis	LT706738.1	KJ512689.1
Camoanula rotundifolia	LT706721.1	MH509299
Campanula marchesettii	KJ512581.1	KJ512686.1
Campanula fragilis	EU713321.1	FJ426580.1
Campanula arvatica	EU713344.1	JF747591.1
Campanula lasiocarpa	JN571947.1	AB219602.1

b)

Sample IDs	species name	TrnL-F Genebank accession number	matK Genebank accession number
BG5	*C. bergomensis* sp. nov.	OR670347	OR641997
BG2		OR670348	OR641996
BG4		OR670349	OR641998
BG3		OR670350	OR641999
BG1		OR670351	OR642000
CeP2	*C. cespitosa*	OR670352	OR641984
CeA1		OR670353	OR641995
CeP3		OR670354	OR641994
CeS4		OR670355	OR641988
CeP1		OR670356	OR641991
CeA2		OR670357	OR641993
CeA5		OR670358	OR641990
CeP5		OR670359	OR641986
CeS1		OR670360	OR641985
CeS2		OR670361	OR641983
CeS3		OR670362	OR641987
CeS5		OR670363	OR641981
CeP4		OR670364	OR641992
CeA3		OR670365	OR641989
CeA4		OR670366	OR641982
CoV4	*C. cochleariifolia*	OR670367	OR642003
CoV3		OR670368	OR642001
CoV5		OR670369	OR642004
CoV2		OR670370	OR642002

Table S2: leaf traits of *Campanula bergomensis*.

Site	Leaf Fresh Weight (mg)	Leaf Dry Weight (mg)	Leaf Area (mm^2)	Site	Leaf Fresh Weight (mg)	Leaf Dry Weight (mg)	Leaf Area (mm^2)
Val di Frucc	2.3	-	13.724	Val dei Dadi	2	-	8.637
Val di Frucc	2.1	0.6	14.527	Val dei Dadi	1.8	-	9.246
Val di Frucc	2.2	-	16.572	Val dei Dadi	7.4	2	33.015
Val di Frucc	2.1	0.9	12.452	Val dei Dadi	2.9	0.9	13.55
Val di Frucc	1.2	-	9.19	Val dei Dadi	3.2	0.5	14.364
Val di Frucc	1.6	-	11.531	Val dei Dadi	1.9	0.7	8.997
Val di Frucc	2.9	1.1	19.463	Val dei Dadi	2.5	1	13.137
Val di Frucc	3.1	1.2	16.756	Val dei Dadi	3.2	-	15.926
Val di Frucc	2	0.5	12.679	Val dei Dadi	4.5	1.3	17.422
Val di Frucc	1.7	-	13.51	Val dei Dadi	2.8	-	10.933
Val di Frucc	3.9	0.8	18.588	Val dei Dadi	3.7	1	13.729
Val di Frucc	2.8	1.1	15.49	Val dei Dadi	1.6	0.6	9.248
Val di Frucc	1.4	0.7	8.044	Val dei Dadi	3.6	0.9	16.877
Val di Frucc	2.4	0.8	14.805	Val dei Dadi	0.9	-	6.509
Val di Frucc	2.7	1.1	20.138	Val dei Dadi	1.9	-	10.16
Val di Frucc	3.3	0.9	16.089	Val Cabrosna	1	0.4	8.515
Val di Frucc	3.5	1	15.106	Val Cabrosna	1.3	-	8.7
Val di Frucc	3.5	1.1	21.009	Val Cabrosna	1.8	0.6	10.503
Val di Frucc	1.8	0.9	12.16	Val Cabrosna	2.5	-	14.781
Val di Frucc	4.2	1	28.818	Val Cabrosna	2	-	14.457
Val di Frucc	3.2	0.6	21.007	Val Cabrosna	2.3	-	11.239
Val di Frucc	4.2	1.8	21.197	Val Cabrosna	1.5	-	11.834
Val di Frucc	4.3	1	25.609	Val Cabrosna	1.5	-	9.454
Val di Frucc	2.9	0.9	17.949	Val Cabrosna	1.9	0.5	11.741
Val di Frucc	3.9	0.1	29.507	Val Cabrosna	2	-	12.223
Val di Frucc	3.8	0.8	25.453	Val Cabrosna	1.9	0.6	11.956
Val di Frucc	3.3	1.3	20.473	Val Cabrosna	2.3	-	13.315
Val di Frucc	3	1	23.45	Val Cabrosna	2.5	0.8	15.832
Val di Frucc	2.6	0.9	15.752	Val Cabrosna	2.3	0.5	14.916
Val di Frucc	7	2.2	43.578	Val Cabrosna	2.1	-	9.135
Val di Frucc	2	0.8	11.415	Val Cabrosna	2.5	0.6	14.532
Val di Frucc	2.8	1.1	13.639	Val Cabrosna	2.6	0.5	14.28
Val di Frucc	1.7	-	10.396	Val Cabrosna	1.5	0.4	11.655
Val di Frucc	2.9	0.6	14.203	Val Cabrosna	1.8	-	11.088
Val di Frucc	4.5	1.3	22.408	Val Cabrosna	1.6	0.4	8.279
Val di Frucc	2.8	0.9	13.767	Val Cabrosna	5	1.5	24.359
Val di Frucc	2.6	0.4	14.448	Val Cabrosna	2.8	-	15.27
Val di Frucc	1.4	-	9.227	Val Cabrosna	3.5	0.9	18.705
Val di Frucc	3.2	0.6	16.293	Val Cabrosna	4	-	20.1
Val di Frucc	3.3	0.7	17.27	Val Cabrosna	4.4	0.8	22.67
Val dei Dadi	5	1	23.852	Val Cabrosna	1.8	-	11.141
Val dei Dadi	12.3	3.2	45.93	Val Cabrosna	3.3	1	16.802
Val dei Dadi	6.4	1.7	23.36	Val Cabrosna	2.5	-	14.693
Val dei Dadi	5.6	1.4	24.082	Val Cabrosna	3.5	0.9	17.429
Val dei Dadi	6	1.6	25.574	Val Cabrosna	4.6	1.2	22.264
Val dei Dadi	3.6	1.1	17.701	Val Cabrosna	4.2	1.3	18.556
Val dei Dadi	3	0.5	16.385	Val Cabrosna	3.9	1.5	16.344
Val dei Dadi	7.6	2.1	33.429	Val Cabrosna	1.6	-	8.631
Val dei Dadi	9.1	2.2	37.041	Val Cabrosna	2.3	-	8.743
Val dei Dadi	5.5	1.3	27.04	Val Cabrosna	2.4	0.7	12.087
Val dei Dadi	3	0.8	15.192	Val Cabrosna	1.8	-	6.707
Val dei Dadi	2.8	-	14.775	Val Cabrosna	2.2	0.3	10.575
Val dei Dadi	3.2	-	13.878	Val Cabrosna	2.8	0.7	16.784
Val dei Dadi	3.4	0.5	16.817	Val Cabrosna	2.7	0.8	11.137
Val dei Dadi	2.3	0.6	17.277	Val Cabrosna	1.9	-	12.416
Val dei Dadi	1.6	-	9.773	Val Cabrosna	3.1	0.9	17.755
Val dei Dadi	3.7	-	20.484	Val Cabrosna	4.5	1.2	23.431
Val dei Dadi	2.9	0.7	12.856	Val Cabrosna	3.1	0.5	17.516
Val dei Dadi	3	0.6	17.735	Val Cabrosna	2.6	0.8	15.438
Val dei Dadi	4.3	0.7	24.469	Val Cabrosna	4.9	1.1	27.436
Val dei Dadi	2	0.6	8.365	Val Cabrosna	2.3	0.4	13.96
Val dei Dadi	1.7	0.7	9.667	Val Cabrosna	2.9	-	12.777
Val dei Dadi	3.6	1.2	15.964	Val Cabrosna	0.8	-	5.344
Val dei Dadi	2.7	-	15.041	Val Cabrosna	5	1.6	29.725
Val dei Dadi	3.1	1	13.503	Val Cabrosna	7.8	1.6	35.865

Figure S1: graph of *Campanula bergomensis* CSR strategy.

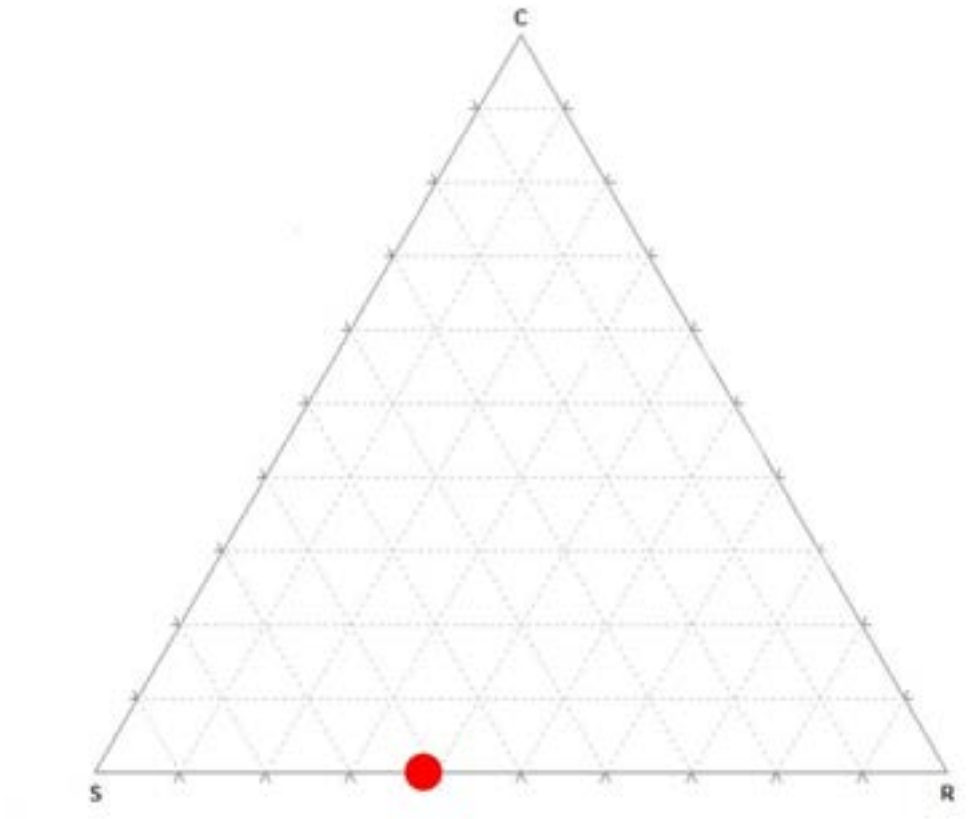
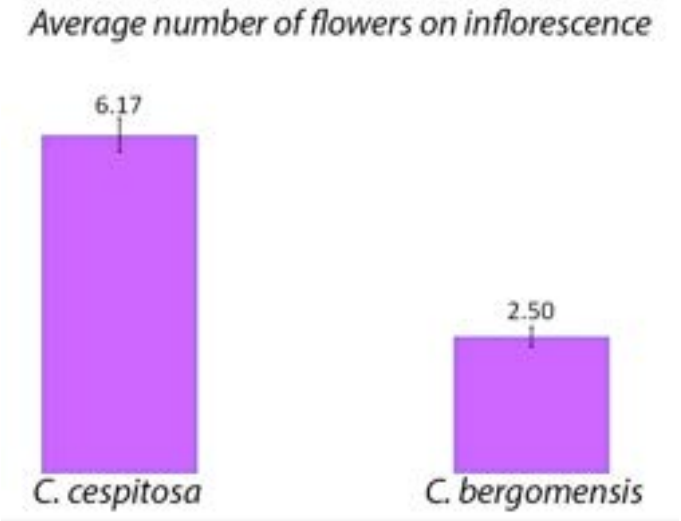


Figure S2: average number of flowers in each inflorescence in *C. cespitosa* and *C. bergomensis*, with the standard error (individuals considered: 322 for *C. cespitosa,* 125 for *C. bergomensis*).



Chapter 4.2

Orenebria tresignore (Coleoptera: Carabidae: Nebriinae), a new species from Orobic Alps (Northern Italy).

Work in preparation

Oreonebria tresignore (Coleoptera: Carabidae: Nebriinae), a new species from Orobic Alps (Northern Italy)

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Overview of the work

1. Study case

In 2014, the ground beetle *Oreonebria soror* subsp. *tresignore* (Coleoptera: Carabidae) was described by Szallies & Huber (2014) from a population found surrounding a snowfield on Pizzo Tresignori (Orobic Alps, Southern Central Italian Alps). These authors describe the morphology of this new entity comparing it with the Southern Eastern Alpine sub-species *Oreonebria soror* subsp. *soror* (Daniel, 1903) and the closely related Northern Central Alpine species *Oreonebria angustata* (Dejean, 1830) among which "*soror*" and "*tresignore*" were previously considered sub-species. The results highlighted how these three entities differ from each other mostly for the shape of their male genitalia, and for the different chaetology on their vertex, clypeus, and elytrae.

The ecology and distribution of *Orenebria soror* subsp. *tresignore* were subsequently investigated by Gobbi et al. (2018). The data collected over three years in an extended area of the Orobic Alps, highlighted that this subspecies is not related only to the Pizzo Tresignori, but it is distributed among most of the Orobic mountain sector, never overlapping with *O. soror* subsp. *soror* distribution range. Indeed, the currently known distribution range of *O. soror* subsp. *soror* comprise the easternmost portions of the Southern Alpine chain, within the Southern Rhaetian Alps (Ledoux & Roux, 2005; Grottolo et al., 2016) (Figure1).

Oreonebria soror subsp. *tresignore*, *O. soror* subsp. *soror* and *O. angustata* share a quite similar microthermal preferences, as they are all cold-adapted species, because living at high altitude, near snowfields, or surrounding permanent snow or glaciers (Tenan et al., 2016; Gobbi et al., 2018). However, *Orenebria soror* subsp. *tresignore* showed to be more closely related to glacial and periglacial environments than the other two entities. Indeed, this species was observed only within the first 50 cm from the edge of glaciers and glaciret, while beyond this threshold disappear (Gobbi et al., 2018); thus, it can be defined as a cold stenotherm species (or cryophilic species). Conversely, *O. soror* subsp. *soror*, as well as *O. angustata*, show a lower sensitivity to the presence of ice as they can be found also far from the glacier in more mature habitats (Tenan et al., 2016).

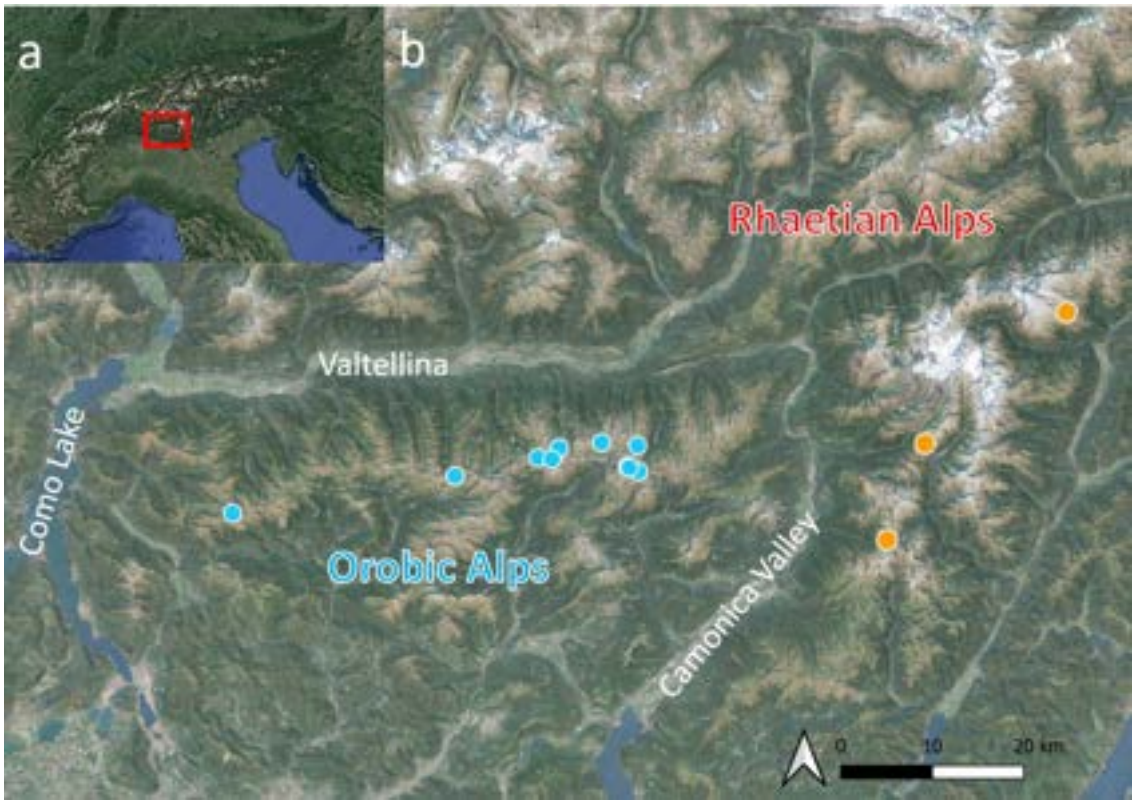

Figure1_a. Location of the Southern Alps within the European Alps; **b.** Distribution of *Oreonebria soror* subsp. *tresignore* (blue dots) and *Oreonebria soror* subsp. *soror* (orange dots) within the Southern European Alps.

Given the clear different morphology of male genitalia (Szallies & Huber 2014), the different distribution range which characterize *O. soror* subsp. *tresignore* and *O. soror* subsp. *soror*, the differences in thermal requirement and the high level of endemism that characterize Orobic Alps (Graf et al., 2014), we hypothesised that these two entities are two different species.

To test this hypothesis, we compared genetically *Oreonebria soror* subsp. soror and *Oreonebria soror* subsp. *tresignore*.

2. Materials and methods

DNA extraction and amplification

Oreonebria soror subsp. *soror* and *O. soror* subsp. *tresignore* samples were collected among 2018 and 2020 (Table1) and stored in ethanol 100% at -20 °C. DNA was extracted from the bodies, excluding the head, the elytra, and the legs, following the CTAB protocol (Doyle & Doyle, 1987) described in Bonelli et al. (2019). Then, the DNA was purified using Monarch Genomic DNA Purification Kit (New England BioLabs,

Massachusetts, MA, USA). In according with markers used for phylogenetic analysis conducted by Kavanaugh et al. (2021) on carabid beetle of supertribe Nebriitae, for this study three mitochondrial (*Cytochrome A oxidase,* "COIa"; *Cytochrome B oxidase*, "COIb", and 16S) were amplified using primers reported in table2 and PCR protocols reported in table3. Amplification products were checked on a 1.5% agarose gel and sequenced by a commercial sequence service provider (Eurofins Genomics, Italy).

Table 1. Samples collection data; Sample code= 'TRE': *Oreonebria soror* subsp. *tresignore*; 'SOR': *Oreonebria soror* subsp. *soror*.

Year	Sample	Province; Region	Locality	Coordinate (UTM WGS84-32T)	Altitude (m asl)	Sample code
2018	*O. s. tresignore*	Bergamo; Lombardy	Trobio Glacier	N 5101002.64 E 584080.44	2522	TRE 1
2019	*O. s. tresignore*	Bergamo; Lombardy	Pizzo Tre Signori	N 5095890.00 E 540943.00	2240	TRE 2
2019	*O. s. tresignore*	Bergamo; Lombardy	Publino Pass	N 5099960.00 E 565245.00	2260	TRE 3
2018	*O. s. tresignore*	Bergamo; Lombardy	Malgina Glaciret	N 5103591.86 E 581266.02	2575	TRE 4
2019	*O. s. tresignore*	Bergamo	Vedretta del Lupo	N 5103033.00 E 576650.00	2500	TRE 5
2018	*O. s. soror*	Brescia; Lombardy	Forcel Rosso Pass	N 5103533.94 E 616586.32	2580	SOR 1
2018	*O. s. soror*	Brescia; Lombardy	Forcel Rosso Pass	N 5103533.94 E 616586.32	2580	SOR 2
2018	*O. s. soror*	Brescia; Lombardy	Forcel Rosso Pass	N 5103533.94 E 616586.32	2580	SOR 3
2018	*O. s. soror*	Brescia; Lombardy	Listino Pass	N 5092976.52 E 612452.33	2650	SOR 4
2018	*O. s. soror*	Brescia; Lombardy	Listino Pass	N 5092976.52 E 612452.33	2650	SOR 5
2020	*O. s. soror*	Trento; Trentino-Alto Adige	Amola Rock Glacier	N 5117881.00 E 632056.00	2434	SOR 6
2020	*O. s. soror*	Trento; Trentino-Alto Adige	Amola Rock Glacier	N 5117881.00 E 632056.00	2434	SOR 7
2020	*O. s. soror*	Trento; Trentino-Alto Adige	Amola Rock Glacier	N 5117881.00 E 632056.00	2434	SOR 8
2020	*O. s. soror*	Trento; Trentino-Alto Adige	Amola Rock Glacier	N 5117881.00 E 632056.00	2434	SOR 9
2020	*O. s. soror*	Trento; Trentino-Alto Adige	Amola Rock Glacier	N 5117881.00 E 632056.00	2434	SOR 10

Table 2. Markers used and respective primers sequences.

DNA	Region	Primer Name	Direction	Sequence	Literature
Mitochondrial	COIa	LCO1490 F	Forward	GGTCAACAAATCATAAAGATATTGG	Folmer et al 1994
Mitochondrial	COIa	HCO2198 R	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al 1994
Mitochondrial	COIb	Jerry F	Forward	CAACATTTATTTTGATTTTTTGG	Simon et al., 1994
Mitochondrial	COIb	Pat R	Reverse	TCCAATGCACTAATCTGCCATATTA	Simon et al., 1994
Mitochondrial	16S	16sbR F	Forward	CCGGTTTGAACTCAGATCATG	Polak et al. 2016
Mitochondrial	16S	16SaR aka LR-N-13398 R	Reverse	CGCCTGTTTAACAAAAACAT	Polak et al. 2016

Table3. PCR conditions for each region amplified in according with primers used. "D" indicates the denaturation phase; "Cycles" indicates the number of cycles; for the touchdown reactions, the number of cycles in each of the three cycling rounds is given; "Cycles temp" indicates the temperature of each cycle phase; "Cycles time" indicates the duration of each cycle phase; "Ext" indicate temperature and time of the extension phase.

Region	D. Time (min)	D. Temp. (°C)	Cycles	Cycles temp. (°C)	Cycles time (s)	Ext	Literature
COIa	4	90	35	94, 52, 72	240, 30, 45	72 °C; 10 min	Folmer et al., 1994
COIb	3	94	35	94, 52, 72	25; 25; 70	72 °C; 7 min	Maddison et al., 2012
16S	3	94	35	94, 47, 72	30; 30; 45	72 °C; 7 min	Polak et al., 2016

Phylogenetic analysis

For phylogenetic analyses, *Oreonebria angustata* DNA sequences from the work conducted by Kavanaugh et al. (2021), were used as outgroups. Sequence alignments were performed using Benchling (Benchling, 2023). After manual trimming of upstream and downstream unaligned portions, multialignments built from 16S, COIa and COIb sequences were manually merged to build a concatenated mitochondrial dataset. The sequences that represented the same haplotype in different samples were collapsed using FaBox tool (Villesen, 2007) with the "DNA to haplotype collapser and converter" function. Phylogenetic analyses were run using MEGA v10.2.6 (Tamura et al., 2021). The best substitution model was estimated using the appropriate MEGA function. A maximum likelihood (ML) tree was constructed with the following parameters for the mitochondrial dataset: Tamura 3-parameter model (gamma distributed with invariant sites), four discrete gamma categories, pairwise deletion, 1000 bootstrap replications (all other options were left to default parameters).

3. Overview of the main results and discussion

As hypothesised by morphological analysis, phylogenetic reconstruction provides a robust support in identifying *Oreonebria tresignore* and *O. soror* as different species from the ancestor *O. angustata*. Moreover, phylogeny also provides a robust support in keeping *Oreonebria tresignore* (97 boostrap value) and *Oreonebria soror* (99 boostrap value) separated from each other (Figure2).

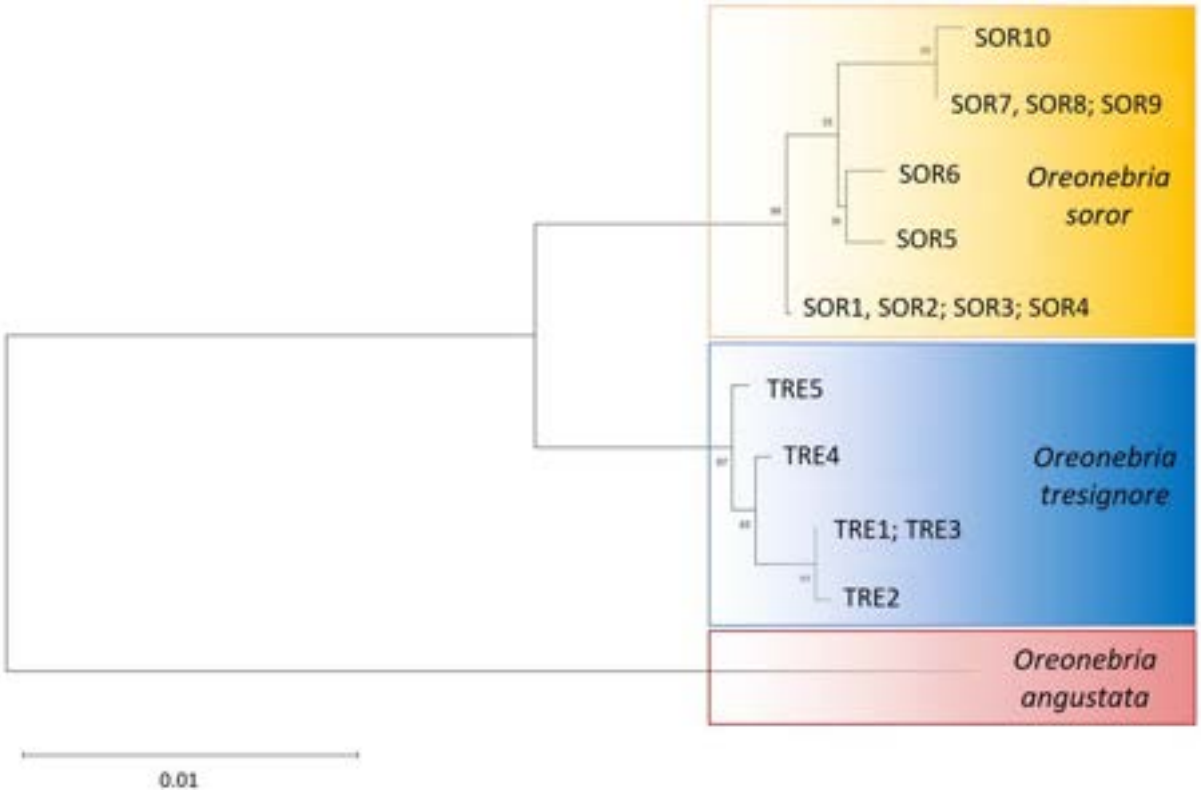

Figure 2. ML Phylogenetic reconstruction of *Oreonebria tresignore*, *O. soror and O. angustata* based on mitochondrial markers. *Oreonebria angustata* is selected as the outgroup.

As previously observed by Szallies & Huber, (2014), both *O. soror* and *O. tresignore* are closely related taxa of *O. angustata*. This last species presents numerous populations widely distributed in the inner part of the Wester-Central European Alps of Switzerland and nearly Italy (Rhaetian Alps) (Ledoux & Roux, 2005). The speciation of *O. angustata* into two new species, could be related to glacial and interglacial cycles occurred during the Pleistocene period. Indeed, during the glacier extension phases, *O. angustata* could have followed the glaciers advancement from the inner Alps toward South, reaching Orobic Alps and Southern Rhaetian Alps. With the glacier retreatment, Orobic and Rhaetian population of *O. angustata* could be remained isolated from the northernmost populations by the presence of a wide valley as Valtellina, leading to a first species diversification (*O. soror*). Subsequently, the further glaciers retreat in the Southern Alps at the end of the Pleistocene, may have caused the isolation of the population within the Orobian Alps from those of Rhaetian Alps given the presence of the Val Camonica Valley between the two Alpine sectors, ending the gene flow that had probably persisted until that moment between *O. soror* population. From this moment, *O. soror*

population located within Orobian Alps could have been diversified genetically leading to the origin of *O. tresignore* species, now clearly genetically separated by *O. soror*.
From a perspective of the conservation status of the species, the risk of extinction for *O. tresignore* is considered high (Gobbi et al., 2018). Indeed, glaciers and glacirets surface on Orobic Alps are very limited and probably will disappear within few decades. Given the particular ecology of *O. tresignore*, populations of this species are supposed to migrate following the glaciers forehead. Due to its inability to fly due to its brachyptery and its cold microclimate dependency, *O. tresignore* is supposed to be unable to migrate along long distances to find other suitable habitats.

This work will include additional output as:

- Phylogenetic reconstruction of *Oreonebria tresignore* and *O*. *soror* based on two nuclear markers (wingless and topoisomerase genes).
- Phylogenetic reconstruction of *Oreonebria* genus (*O. tresignore*, *O. soror*, and *Oreonebria* species reported in Kavanaugh et al. (2021) "Phylogeny of the supertribe Nebriitae (Coleoptera, Carabidae) based on analyses of DNA sequence data".

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Chapter 5

Conclusions

Conclusions

This Ph.D. project provided new insights about plant and flower-visiting arthropod communities that characterize different Alpine environments. Moreover, this project allowed the development of different methodological approaches suitable for a comprehensive study of flower-arthropod interactions including the composition of biotic communities, the behaviour of arthropods on flowers, and the composition of pollen transported by flower visitors.

The characterization of flower-visiting arthropod communities in two different mountain and seasonal contexts allowed for a better understanding of how their composition is influenced by different environmental parameters.

Firstly, it was highlighted how Alpine plant species that flower very early in the season, such as *Androsace brevis*, establish important relationships with numerous arthropod taxa (paragraphs 2.3; 2.4). In this alpine context, the only factor that influences the snow melt period and the composition of the arthropod community is represented by meteorological conditions (especially temperature), as there are only very limited human activities (*e.g.,* mountain hut and trekkers). The occurrence of different meteorological conditions led the composition of flower visiting arthropod community (and potential pollinators) to change over years. Given this variability in community composition, it is difficult to predict the effects of ongoing climate warming on plant-arthropod interactions. However, the well-known longevity of many alpine plant species could allow them to adapt to changing flower-visiting arthropod communities, and potentially ensure reproductive success. A long-term study will be essential in gaining a better understanding of these complex plant and arthropod interactions and how they evolve in response to increasing temperatures.

Analyses conducted in a broader context (Stelvio National Park paragraph 3.1), throughout the entire vegetative season, showed that inter-annual meteorological conditions do not affect the distributional pattern of the observed arthropod taxa on flowers, either along the altitudinal gradient or in the different land management considered. This result emphasized how flower-visiting arthropod communities are more stable in an environment that is not strongly influenced by weather conditions. Excluding the effects of altitude, the abundance of the most common flower visitors (wild bees, syrphids, non-syrphid flies, beetles, and hymenopteran wasps) is highly influenced by the type of land use and the corresponding level of mechanization. This reveal that changes in land use can lead to substantial modification in biotic communities, particularly in the abundances of Hymenoptera Apoidea, Coleoptera, Hymenoptera Formicide, Hymenoptera wasps, and Diptera Brachycera.

Moreover, this study showed how the biodiversity of wild bees decrease in hay meadows and apple orchards, allowing us to hypothesis how the repeated passage of heavy vehicles on the soil (such as tractors and mechanized mowers), could make nesting activities challenging, or even impossible, for many wild bee species. On the other hand, the increase in the biodiversity of syrphids in highly human-treated environments could be related to the high presence of hemipters, whose larvae many species of syrphids feed on. This study highlighted the need to preserve the biodiversity of mountain ecosystems through sustainable and compatible land management programs.

It will be fundamental, soon, to also identify the plant-pollination network within each environment considered for the study conducted within the Stelvio National Park. Plant-pollination networks will be fundamental in identifying plant–pollinator associations, in order to understand the ecological role of each component of these biotic communities in the survival of mountain ecosystems.

Another important result concerns the importance of neglected taxa such as non-syrphid flies, non-bee, and non-ant hymenopterans (paragraphs **2.4**) as floral visitors, especially during the early season. These neglected taxa, in the absence of the well-known pollinators (such as bees, syrphids and butterflies), could play a fundamental role in the reproductive success of entomophilous plants. The presence of numerous neglected taxa also highlighted the scarcity of taxonomists who are experts in identifying and describing the arthropod fauna of alpine areas. This remark underscores how taxonomic aspects (as noted in the section on braconids **2.5**, and Ph.D. side projects **4.1**; **4.2**) should not be underestimated but, rather, they are still fundamental in the study of biodiversity and biotic relationships, since there are still many gaps in knowledge about the arthropod biodiversity of Alpine contexts.

Important results were also obtained from the methodological studies conducted during the Ph.D. project to achieve comprehensive results for this research. In particular, manual sampling and video observations (paragraph **2.2**) were found to be useful in providing a comprehensive view of the flower-visiting fauna. These methods enable us to: i) obtain detailed results on the specific composition of the flower-visiting arthropod community; ii) obtain qualitative and quantitative results on botanical composition of pollen transported by arthropods which are fundamental for the identification of pollination-networks; iii) observe the largest number of subjects in the least invasive manner possible; iv) identify plant-arthropod interactions that are still very poorly known, and which interactions link arthropods and plants to each other for their own survival. Concerning palynological analysis, the impressive quantitative (and already known qualitative) results obtained by molecular techniques such as ITS2 metabarcoding in palynological analysis (paragraph **3.2**), allowed to lay a good innovative methodological protocol that can provide a valuable support (or even replace) to traditional pollen analysis techniques, in the study of pollen transported by flower-visitors.

Finally, the Ph.D. course allowed to start an active collaboration with the Regional Park of the Orobic Alps and the Stelvio National Park, as well as with the Italian Ministry of the Environment. The involvement of Stelvio National Park Rangers, farmers, and shepherds was also of fundamental importance. These important institutions and valley inhabitants, who are knowledgeable about the work conducted within the environments under their supervision, are crucial for disseminating the emerged results of this study. In addition, the interest of these institutions and people in the research focused on biotic interactions, is fundamental whether the development and implementation of protection and conservation programs for the investigated environments will be necessary to preserve the biotic communities occurring in Alpine areas.

Chapter 6

Ph.D. activities

Ph.D. Courses and tutoring

1) Transferable skills

Academic Year 2020-2021

- Fundamentals of Academic Writing
- Language coaching: interpersonal skills – presentation skills Open Science
- Basic Propaedeutics lesson on IP patents
- Communication on new Media
- Research Assessment

Academic Year 2021-2022

- Language coaching: Interpersonal skills - Interview skills
- Grantsmanship – part 1
- Grantsmanship – part 2
- Self branding
- Workshop for the production of scientific content for new media
- Behind the scenes of a peer reviewed journal
- Research integrity I
- Research integrity II
- Academic writing: Research paper

Academic Year 2022-2023

- Workshop for the preparation of a dissemination/communication plan (preparatory lesson)
- Workshop for the preparation of a dissemination/communication plan (workshop)
- Advanced lecture on using IP to do innovation
- Valorise by creating enterprise
- Open Science: Dati Fair
- The Linkedin profile: tips & Tricks for Ph.D.
- Self branding

2) Ph.D. Courses

Teaching	Sector	Teacher	CFU
Genomics for ecological and evolutionary studies: from DNA sequencing to data analysis	BIO/05, BIO/07, BIO/18	Giulio Formenti	2
Introduction to statistical analysis of ecological environmental data	BIO/07	Roberto Ambrosini	5
Advanced statistical analysis of ecological and environmental data	BIO/07	Roberto Ambrosini	4
The climate crisis – data and impacts	AGR/05, BIO/02, FIS/06	Marco Caccianiga, Maurizio Maugeri, Giorgio Vacchiano	4

3) Thesis supervised

Bechelor thesis:

- "Specific composition of pollen transported by Syrphidae sampled in the Stelvio National Park" (Student: Davide Antenucci – Supervisor: Prof. Marco Caccianiga).
- "Flower-visiting arthropods in alpine environment: video observations on *Androsace brevis* and *Primula hirsute"* (Student: Erica Ceresa – Supervisor: Prof. Marco Caccianiga).
- "Study of plant and arthropod interactions in high-altitude environments through video analysis" (Student: Marta Glisenti – Supervisor: Prof. Marco Caccianiga).
- "Floral visitors in different alpine agro-ecosystems in the Stelvio National Park" (Student: Francesco Pietra – Supervisor: Prof. Morena Casartelli).
- "Study of pollen composition collected by Apoidea and Syrphidae in different agro-ecosystems along an altitudinal gradient within the Stelvio National Park: a molecular approach" (Student: Federica Tonella – Supervisor: Prof. Marco Caccianiga).

Master thesis:

- "High-altitude plant-arthropod interactions: a study about the flower visitors of *Androsace brevis* and *Primula hirsuta* and nocturnal and autumnal visitors of *Androsace brevis"* (Student: Elisa Legoratti – Supervisor: Prof. Marco Caccianiga).
- "Behavioural analysis of flower-visiting arthropods using video observations in mountain environments" (Student: Alberto Chirico – Supervisor: Prof. Morena Casartelli).
- A quantitative analysis of pollen carried by the most common flower-visiting taxa within alpine ecosystems (Student: Francesco Pietra – Supervisor: Prof. Morena Casartelli)

4) Published papers

- Valle, B., Eustacchio, E., Gallo, G.R., Beretta, M., Bonelli, M., Zanzottera, A., Gianfranceschi, L., Federici, G., Mangili, F., Mangili, L., Perico, G., Traini, M., Caccianiga, M. *Campanula bergomensis* (Campanulaceae), a new plant species from Bergamo Prealps (Northern Italy) – Sumbitted to Phytotaxa.
- Tomanović Ž., Žikić V., Pietra F., Eustacchio E., Bonelli M. (2023) - Braconidae (Hymenoptera) in the Central Southern Alps: the first Alpine record of the alien parasitoid *Lysiphlebus testaceipes* (Cresson) and new species for Italy. Redia, 106, 2023: 167-174.
- Eustacchio, E., Bonelli, M., Beretta, M., Monti, I., Gobbi, M., Casartelli, M., & Caccianiga, M. (2023). Pollen and floral morphology of *Androsace brevis* (Hegetschw.) Ces. (Primulaceae), a vulnerable narrow endemic plant of the Southern European Alps. Flora, 301, 152256.
- Bonelli, M., Eustacchio, E., Avesani, D., Michelsen, V., Falaschi, M., Caccianiga, M., Gobbi, M., Casartelli, M. (2022). The early season community of flower-visiting arthropods in a high-altitude alpine environment. Insects, 13, 393.
- Bonelli, M., Melotto, A., Minici, A., Eustacchio, E., Gianfranceschi, L., Gobbi, M., Casartelli, M., Caccianiga, M. (2020). Manual sampling and video observations: an integrated approach to studying flower-visiting arthropods in high-mountain environments. Insects, 11, 881.

5) Paper submitted

- Eustacchio, E., Bonelli, M., Brunetti, M., Montagna, M., Cappellari, A., Gobbi, M., Mei, M., Pedrotti, L., Caccianiga, M., Casartelli, M. *ITS2 metabarcoding quantitative reliability in assessing the composition of pollen carried by insects.* – Submitted to Oecologia.

6) Paper in preparation

- Eustacchio E., Bonelli M., Ficetola F., Pedrotti L., Caccianiga M., Falaschi M., Gobbi M., Casartelli M. *Impact of land management and elevation on composition and structure of alpine lower visiting communities.*
- Bonelli M., Eustacchio E., Losapio G., Ambrosini??, Casartelli M., Gobbi M., Caccianiga M. *Plant-pollinator interactions during early season in Alpine ecosystem: A threeyear study focusing the narrow endemic plant Androsace brevis (Hegetschw.) Ces. (Primulaceae).*
- Bonelli M., Eustacchio, E., Gallo, G.R., Tampucci D., Ornaghi S., Gianfranceschi, L., Valle B., Caccianiga, M., Gobbi M. Oreonebria tresignore *(Coleoptera: Carabidae: Nebriinae), a new species from Orobic Alps (Northern Italy).*

7) Future papers

- Differences in composition of pollen carried by Hymenoptera Apoidea and Diptera Syrpidae: a study case within the Stelvio National Park (Northen Italy).
- Analysis of the behaviour of flower-visiting arthropods considering different elevations and land use types within the Stelvio National Park (Northen Italy).
- How much pollen do flower visitors carry? A quantitative analysis of pollen carried by the most common flower-visiting taxa within alpine ecosystems.

8) Conferences and seminars

- August 28-29, 2023, WorkShop “Naturali Incontri”, Bormio, Stelvio National Park, Italy.
Oral Presentation: A. Chirico; E. Eustacchio; F. Pietra; D. Sciandra; D. Debiasi; L. Pedrotti; M. Bonelli; M. Casartelli; M. Caccianiga; M. Gobbi. “Flower-visiting arthropods behavior: a study case within the Stelvio National Park”.

- August 28-29, 2023, WorkShop “Naturali Incontri”, Bormio, Stelvio National Park, Italy.
Oral Presentation: F. Pietra; M. Bonelli A. Chirico; D. Sciandra; D. Debiasi; L. Pedrotti; E. Eustacchio; M. Casartelli; M. Caccianiga; M. Gobbi. “Study of flower-visiting arthropods communities in different Alpine ecosystems within the Stelvio National Park”.

- February 24, 2023, Talk Grasslands of Eurasian Dry Grassland Group – Online event.
Oral Presentation: E. Eustacchio, M. Bonelli. “Flower-insect interactions in mountain ecosystems”.

- November 16-18, 2022, European Ph.D. Network "Insect Science" – XIII Annual Meeting, Florence, Italy.
Oral Presentation: E. Eustacchio, M. Bonelli, A. Chirico, D. Debiasi, F. Pietra, D. Sciandra, L. Pedrotti, M. Casartelli, M. Gobbi, M. Caccianiga. "Plant-insect interactions in mountain areas: insights for an analysis of pollen loads comparing light microscopy and ITS2 metabarcoding".

- May 4, 2022, Workshop "Studiare e proteggere gli impollinatori nei parchi nazionali delle Alpi", Ministero della Transizione Ecologica.
Oral Presentation: M. Bonelli, E. Eustacchio, L. Pedrotti, M. Casartelli, M. Caccianiga, M. Gobbi. "Impollinet — conoscere e proteggere gli impollinatori: interazioni piante-insetti in prati, pascoli, meleti e praterie del Parco Nazionale dello Stelvio".

- November 17-19, 2021, European Ph.D. Network "Insect Science" – XII Annual Meeting, Florence, Italy
Oral Presentation: E. Eustacchio, M. Bonelli, E. Legoratti, A. Minici, A. Zanzottera, L. Pedrotti, M. Casartelli, M. Gobbi, M. Caccianiga. "Plant-pollinator interactions: a study along spatial-temporal gradient in different land management types within the Stelvio National Park".

- November 17-19, 2021, European Ph.D. Network "Insect Science" – XII Annual Meeting, Florence, Italy.
Oral Presentation: E. Legoratti, E. Eustacchio, A. Minici, M. Gobbi, M. Caccianiga, M. Casartelli., M. Bonelli. "Autumn and night: the neglected side of plant-arthropod interactions"

- March 26-27, 2021, Online Conference "Botanica Sudalpina"
Poster: E. Eustacchio, M. Bonelli, A. Minici, A. Melotto, E. Dinatale, M. Gobbi, L. Gianfranceschi, M. Casartelli, M. Caccianiga. "High mountain plant-pollinator interactions: the case study of the narrow endemic alpine plant *Androsace brevis* (Hegetschw.) Ces. (Primulaceae)".

- March 26-27, 2021, Online Conference "Botanica Sudalpina".
Poster: A. Minici, A. Melotto, M. Bonelli, E. Eustacchio, E. Dinatale, A. Conti, M. Gobbi, L. Gianfranceschi, M. Casartelli, M. Caccianiga. "Video analysis as an innovative approach to investigate plant-arthropod interactions in high mountain environments: The case study of *Androsace brevis* (Hegetschw.) Cesati (Primulaceae)".

- March 26-27, 2021, Online Conference "Botanica Sudalpina".
Oral Presentation: M. Bonelli, E. Eustacchio, A. Minici, E. Dinatale, A. Melotto, F. Mangili, M. Gobbi, M. Beretta, E. Gatti, E. Onelli, L. Gianfranceschi, M. Casartelli, M. Caccianiga. "Living on the edge. Unravelling biology and ecology of the endangered endemic alpine plant *Androsace brevis* (Hegetschw.) Cesati (Primulaceae) by a multidisciplinary approach".

- March 26-27, 2021, Online Conference "Botanica Sudalpina".
Poster: E. Dinatale, M. Bonelli, E. Eustacchio, M. Casartelli, A. Minici, M. Caccianiga, L. Gianfranceschi. "A possible endophytic symbiotic bacterium of endemic species *Androsace brevis* (Primulaceae)".

- March 26-27, 2021, Online Conference "Botanica Sudalpina".
Poster: F. Mangili, M. Graziani, E. Tonini, V. di Michele, E. Eustacchio, M. Bonelli, M. Caccianiga. "Distribuzione, consistenza delle popolazioni e sinecologia di *Androsace brevis* (Hegetschw.) Ces.".

- November 30 – December 4, 2020; European Ph.D. Network "Insect Science" – XI Annual Meeting, Online event.
Oral presentation: E. Eustacchio, M. Bonelli, A. Minici, A. Melotto, E. Dinatale, M. Gobbi, L. Gianfranceschi, M. Casartelli, M. Caccianiga. "Plants and flower-visiting arthropods in mountain ecosystems: the case study of the alpine species *Androsace brevis* (Primulaceae)".

- November 30 – December 4, 2020; European Ph.D. Network "Insect Science" – XI Annual Meeting, Online event.
Oral presentation: A. Minici, M. Bonelli, E. Eustacchio, A. Melotto, E. Legoratti, E. Dinatale, M. Gobbi, M. Casartelli, M. Caccianiga. "Who are my guests? Investigating the arthropod activity on high-altitude flowers by video observations, the example of *Androsace brevis* (Primulaceae)".

- July 28 – 31, 2020: SymBioSe Europe – 23rd Symposium of Biology Students in Europe, Groningen, Netherlands.
Poster: E. Dinatale, M. Bonelli, E. Eustacchio, A. Minici, M. Caccianiga, L. Gianfranceschi. "A possible endophytic symbiont of *Androsace brevis* (Hegetschw.) Cesati (Primulaceae)".

- February 6 – 8, 2020: CYBO – Conference of Young Botanists, Genova, Italy.
Oral Presentation: E. Eustacchio, M. Bonelli A. Minici, A. Melotto, E. Dinatale, M. Gobbi, L. Gianfranceschi, M. Casartelli, M. Caccianiga. "High-mountain plant-pollinators interactions: the case of the narrow endemic alpine plant *Androsace brevis* (Hegetschw.) Ces. (Primulaceae)".

- February 6 – 8, 2020: CYBO – Conference of Young Botanists, Genova, Italy.
Oral Presentation: A. Minici, A. Melotto, M. Bonelli, E. Eustacchio, E. Dinatale, M. Gobbi, L. Gianfranceschi, M. Casartelli, M. Caccianiga. "Videorecording as an innovative method to investigate the behaviour of arthropods on high altitude plants. The case of study of *Androsace brevis* (Hegetschw.) Ces. (Primulaceae)".

- February 6 – 8, 2020: CYBO – Conference of Young Botanists, Genova, Italy.
Oral Presentation: E. Dinatale, M. Bonelli, E. Eustacchio, A. Minici, M. Caccianiga, L. Gianfranceschi. "A possible endophytic symbiont of *Androsace brevis* (Hegetschw.) Cesati (Primulaceae)".

- December 4 – 6, 2019 – European Ph.D. Network "Insect Science" – X Annual Meeting, Genova, Italy.
Oral Presentation: M. Bonelli, E. Eustacchio, A. Minici, A. Melotto, E. Dinatale, M. Gobbi, L. Gianfranceschi, M. Casartelli, M. Caccianiga. "High mountain plant-pollinator interactions: a little known but critical component of fragile ecosystems – The case of the endangered alpine plant *Androsace brevis*"

9) Participation in projects

Project: “Fatal attraction: endangered plant’s lovers”.
Partecipants: 40 participants from 8 countries.
Database: pollinator data for 122 threatened plants in Europe, belonging to 37 plant families

This first database draft has been presented at the Annual Meeting of ConservePlants, Coimbra, August 2023.

The aim is to produce the first complete database by February 2024 and to work on the data paper during March-April 2024.

10) Teaching activities

June 11, 2021, and May 23, 2022
Informative speech during the educational excursion of «Nature conservation and protected areas» course.

Partecipants: 1st years students (cohort 2020) of the Master in «Environmental Management of Mountain Areas (EMMA) LM – 73», University of Bolzano. Teacher: Dr. Gianmaria Bonari

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<< È importante e risaputo che le cose non sempre sono ciò che appaiono.
Per esempio, sul pianeta Terra gli uomini hanno sempre ritenuto di essere
più intelligenti dei delfini. Sostenevano infatti che, mentre loro avevano inventato
un sacco di cose, come la ruota, New York, le guerre, ecc., i delfini non avevano
fatto altro che sguazzare nell'acqua divertendosi.
Al contrario invece, i delfini sapevano da tempo
dell'imminente demolizione della Terra e avevano fatto molti tentativi per avvisare
l'umanità dell'incombente pericolo, ma i loro messaggi erano stati fraintesi e interpretati
come divertenti tentativi di dare calci a palle da football
o di fischiare per avere bocconcini prelibati.
Così alla fine i delfini rinunciarono e se ne andarono dalla Terra coi propri mezzi […].

[…] L'ultimissimo messaggio lanciato dai delfini fu interpretato come
un tentativo estremamente raffinato di fare un doppio salto mortale
all'indietro dentro un cerchio, fischiettando
"The Star-Spangled Banner",
in realtà il messaggio diceva:
"Addio, e grazie per tutto il pesce"**>>**

— Douglas Adams, The Hitchhiker's Guide to the Galaxy