Italian Botanist 20: 149–165 (2025)
doi: 10.3897/italianbotanist.20.170749
https://italianbotanist.pensoft.net

RESEARCH ARTICLE

Italian Botanist
Published by Società Botanica Italiana

Palaeofloristic record of *Linnaea borealis* during the Mid-Holocene in the Friuli Venezia Giulia plain

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Academic editor: Lorenzo Peruzzi | Received 1 September 2025 | Accepted 6 October 2025 | Published 17 December 2025

Citation: Zappa J, Mercuri AM, Astuti G, Lastrucci L, Torri P, Florenzano A (2025) Palaeofloristic record of *Linnaea borealis* during the Mid-Holocene in the Friuli Venezia Giulia plain. Italian Botanist 20: 149–165. https://doi.org/10.3897/italianbotanist.20.170749

Abstract

Glacial relicts represent invaluable biogeographical markers of past climatic oscillations, offering unique insights into the historical dynamics of species distribution and the long-term persistence of biodiversity within refugia. *Linnaea borealis*, common name Twinflower, is a small suffruticose plant that grows in coniferous and mixed forests at altitudes ranging from 1,200 to 2,100 m a.s.l. This species is considered a relict of the Ice Age, as its current distribution is limited to circumboreal zones of the Northern Hemisphere. In Italy, currently it was observed living only in the mountains in four northern alpine regions (Valle d'Aosta, Piemonte, Lombardia, Trentino–Alto Adige), while fossil pollen records were found in a wetland area in the plain in Friuli Venezia Giulia. The finding in plains (30 m a.s.l.) in strata dated 6,000 years ago, i.e. significantly later than the post-glacial retreat, opens up the question of whether its disappearance did not occur in post-glacial times in the Palù basin, which possibly may have functioned as a refuge area for this species in the lowlands. This study focuses on the first evidence of past presence of this species, previously unrecorded and currently not present, in the Palù basin. Thanks to detailed palynological analyses, pollen grains of *L. borealis* were identified in Mid-Holocene pollen samples taken from a core drilled in this area. This approach underscores the importance of combining floristic and palynological data to reconstruct the biogeography and ecology of species. In the case of *L. borealis*, such analyses offer new perspectives on the past presence of this species in areas where it is no longer found today, shedding light on how climate changes during the Holocene may have influenced its distribution.

Keywords

Biodiversity, Biogeography, Caprifoliaceae, Palynology, Palù basin

Introduction

Reconstructing the historical distribution of plant species is one of the central challenges in biogeography and historical ecology. Understanding where and how species were distributed in the past is not only crucial for interpreting current ecological patterns, but also for anticipating future responses to climate and anthropogenic changes (Willis and Birks 2006; Svenning et al. 2008).

Modern floristic data alone may be insufficient to accurately reconstruct a species' biogeographical history, as they provide only a static snapshot of current occurrences (Birks 2003). In this context, palynology (the study of fossil pollen) offers a vital temporal perspective on past plant communities, enabling researchers to integrate the spatial resolution of floristic records with a stratified temporal dimension (Moore et al. 1991; Bennett and Willis 2001). This integrated approach provides insights into long-term vegetation dynamics and allows for a more robust reconstruction of past species distributions and habitat shifts.

Climatic relicts are the surviving remnants of formerly widespread populations that became isolated due to climatic shifts and consequential habitat degradation (Hampe and Jump 2011). These populations have survived in small refugia, where local environmental conditions have remained suitable, even when surrounding habitats became inhospitable (Woolbright et al. 2014). Among such areas, the Alpine region has historically served as a major refuge, shaping the distribution and diversity of European flora and fauna through repeated glacial–interglacial cycles (e.g., Europe: Taberlet et al. 1998; Eastern Alps: Tribsch and Schönswetter 2003).

In fact, along the Pleistocene (2.58 million–11,700 years Before Present), glacial advances fragmented the vegetation cover of the Alps, forcing many plant species into extra- or intra-glacial refugia. Some of these taxa, now called glacial relicts, still persist in isolated alpine microhabitats characterised by cool, stable conditions reminiscent of the late Quaternary environments in which they once thrived. Their current disjunct distributions provide biogeographical evidence of glacial expansions and postglacial retreats across the Alpine area (Holderegger and Thiel-Egenter 2009).

Numerous arctic-alpine and boreal-alpine species have been studied as classic glacial relicts, mostly with molecular and plant fossil identifications, including the following examples in the Alps and across Europe: *Nuphar pumila* (Timm) DC. (Bétrisey et al. 2020), *Saxifraga oppositifolia* L. (Winkler et al. 2012), *Eritrichium nanum* (L.) Schrad. ex Gaudin subsp. *nanum* (Stehlik et al. 2002) and the *Ranunculus alpestris* group (Paun et al. 2008), *Carex aquatilis* Wahlenb. (Andreas 1951), *Dryas octopetala* L. (Tralau 1963; Birks 2008), *Betula nana* L. (Birks 2008). These taxa are among the most valuable bioindicators of stable microclimatic conditions and play essential roles in biodiversity conservation, palaeoecological reconstruction and studies on plant resilience to climate change.

In this study, we focus on *Linnaea borealis* L., a circumboreal species currently surviving as a relict in several valleys of the Italian Alps (Valle d'Aosta, Piemonte, Lombardia, and Trentino-Alto Adige), where it inhabits subalpine acidophilous conifer forests with a mossy understorey. These Alpine populations are predominantly clonal, relying on vegetative reproduction, which reflects both a limited capacity for dispersal and a high degree of in situ persistence (Wieberg et al. 2016). A recent work from Pipenbaher et al. (2025), assessed the genetic variability of *L. borealis* by comparing small, isolated eastern-southeastern Alpine populations with core boreal populations, confirming their status as glacial relicts surviving in specific microhabitats and underscoring the need for targeted conservation strategies.

According to Christenhusz (2013) and Thiem and Buk-Berge (2017), *L. borealis* is a plant belonging to the Caprifoliaceae family; it is an evergreen, stoloniferous dwarf shrub. Its common name, Twinflower, comes from the distinctive inflorescence which is composed by two bell-shaped flowers growing on a forked stem of letter Y shape. Its blooming time is between June and August, in Europe most commonly in June – beginning of July. Blossoms persist for about 7 days, and after pollination the flower develops into a fruit in the form of a small, dry, one-seeded capsule, which lasts 36 days. It spreads by zoochory (seeds are dispersed by birds or by small animals, thanks also to small hair bracts that can attach to animal's fur). Its reproductive strategy is predominantly clonal, with low production of viable seeds and the pollen dispersion is insect-pollinated.

Linnaea borealis is a circumboreal species with ecological adaptations typical of cold, humid bioclimatic zones (see also Suppl. material 1). It exhibits a marked preference for shaded or semi-shaded environments characterised by low light intensity and stable microclimatic conditions. This species typically occurs in subalpine coniferous forests (composed mainly by *Picea abies* (L.) H.Karst. and *Abies alba* Mill. with other *Pinus* species), where it forms part of a moss-rich understorey (with *Rhododendron* spp. and *Vaccinium myrtillus* L. underwood). It thrives on podzolic soils that are acidic, moist, and rich in organic matter, particularly humic forest litter in cold and cool climates. These edaphic and microclimatic requirements reflect its status as a climate-sensitive relict, dependent on long-term ecological stability and minimally disturbed forest structures (Wróblewska 2013; Pignatti et al. 2018; Pipenbaher et al. 2025). As a shallow-rooted species, it is particularly susceptible to drought; in dry years, its populations tend to decline, whereas they increase markedly during wetter years (Piękoś-Mirkowa and Mirek 2003). In the past century, numerous localities where this species was once widespread and abundant (such as in Wolin National Park and the Wisełka region in Poland) have been well documented (Piotrowska 1966). Many of these populations are now gradually disappearing, primarily due to changes in habitat conditions. In addition to increasing drought, a major contributing factor is the alteration of forest structure and understory composition caused by anthropogenic destruction of locations (as observed in Serpelice region in Poland; Ciosek et al. 2015) or for the negative effects of the densification of shrub layers of some competitive species such as *Vaccinium myrtillus* and *V. vitis-idaea* L. (as observed in Grala-Dąbrowizna region in Poland; Ciosek et al. 2015). Although *L. borealis* is shade-tolerant, excessive canopy closure and shrub expansion may reduce microhabitat heterogeneity, air circulation, and soil moisture availability, ultimately making conditions unsuitable for its persistence (Niva 2003; Niva et al. 2006; Scobie and Wilcock 2009). These reductions in *L. borealis* communities are largely the result of natural succession in the shrub layer and also of some forest management practices (as reported by staff of Wolin National Park, see Thiem and Buk-Berge 2017).

Due to its strict ecological and climatic requirements, according to Bartolucci et al. (2018, 2024) in Italy the species is confined to just four northern regions (Valle d'Aosta, Piemonte, Lombardia, and Trentino-Alto Adige) where it is restricted to a few mountain sites at high elevations (from approximately 1,200 to 2,100 m a.s.l.). In particular:

(i) in Valle d'Aosta, the species is listed as nearly threatened by Bovio (2016);

(ii) in Piemonte, the presence of the species is a recent finding, while the species was previously considered at locally extinct (Selvaggi et al. 2023, 2024);

(iii) in Lombardia, it is considered at a moderate extinction risk, and a new locality in the Orobic Alps was also discovered (Cretti and Bona 2015);

(iv) in Trentino-Alto Adige, and particularly within the Adamello Brenta Natural Park, it is also considered at a moderate extinction risk (Festi and Prosser 2008) and occurs both inside and outside the park boundaries.

Lastly, an interesting aspect of this species concerns the ethnobotanical knowledge. *L. borealis* has a long history of use in folk medicine across Europe and North America, where it was employed to treat ailments such as shingles, rashes, rheumatism, and menstrual pain, often through decoctions, ointments, or smoke inhalation (Brondegaard 1959; Alm 2006). In Nordic countries, the plant was commonly boiled in water or milk and consumed on an empty stomach, while in North America, indigenous tribes used it for cramps, fevers, and headaches (Moerman 1998). Today, *L. borealis* is also used in cosmetics for skin care, prompting renewed interest in its phytochemical properties and ongoing pharmaceutical research, particularly in Poland.

Pollen attributed to *Linnaea borealis* was found in the PaluOFF1 core (Friuli Venezia Giulia) between 9,800 and 6,300 cal yr BP (calibrated years Before Present), despite the species current absence and lack of the species in previous records in the area (Pini 2004; Zappa et al. 2023). The comparison between fossil and herbarium pollen, using standard morphological criteria and statistical analysis, confirmed the local presence of the species (Zappa et al. 2025). The aim of this paper is, therefore, to discuss the significance of the presence of *L. borealis* in the Palù basin during the Mid-Holocene. Pollen from a terrestrial core provides this new information on the species spatial and temporal distribution. By integrating current sources, the research intends to propose the Palù basin as a potential refugium for this species during the Holocene.

Materials and methods

Study area

The Palù basin (30 m a.s.l.), located in the Pordenone province between Caneva and Polcenigo (Friuli Venezia Giulia), is a ca. 100-hectare wetland formed during the late glacial period due to fluvial and tectonic dynamics that led to the creation of a lake, later evolving into a complex system of peatlands, ponds, and rivers (Bassetti and Cavulli 2002; Peresani and Ravazzi 2002). Its sedimentary sequence, spanning from the Last Glacial Maximum (15,000–11,000 yr BP) to the present, preserves a detailed archive of environmental and climatic changes in the southern Friulian Prealps, making it a site of high palaeoecological (Pini 2004; Zappa et al. 2023) and archaeological significance (Micheli et al. 2023). In fact, thanks to the large amount of archaeological evidence, Palù di Livenza was inscribed in 2011 on the World Heritage List of UNESCO – "Prehistoric pile-dwellings around the Alps". Excavation campaigns, including a Neolithic village, were carried out between 2013 and 2021 under the coordination of Roberto Micheli (Superintendence of Friuli Venezia Giulia) (Micheli et al. 2022).

Currently, the land within the Palù basin is primarily used for agricultural purposes, although several natural areas remain preserved (Fig. 1). The surrounding lowland landscape includes vineyards, arable land, pastures, urban settlements, and mineral extraction sites. In contrast, higher elevations (such as the Cansiglio Massif) are predominantly covered by coniferous and mixed forests.

The Palù basin holds significant naturalistic value and has been designated as a Biotope since 2018, owing to its remarkable environmental, faunal, and vegetational features, many of which are globally threatened. As a spring-fed wetland, the site plays a crucial role as a reproductive habitat for numerous amphibian species (many of which are nationally or even internationally protected) and are particularly vulnerable to human disturbance.

The dominant vegetation at Palù di Livenza belongs to the 147b series (Blasi 2010), which comprises three intertwined phytosociological sigmeta: *Buglossoido-Ostryo carpinifoliae*, *Mercuriali ovatae-Ostryo carpinifoliae*, and *Ostryo carpinifoliae-Fraxino orni*. Each is characterized by specific assemblages of deciduous trees, shrubs,

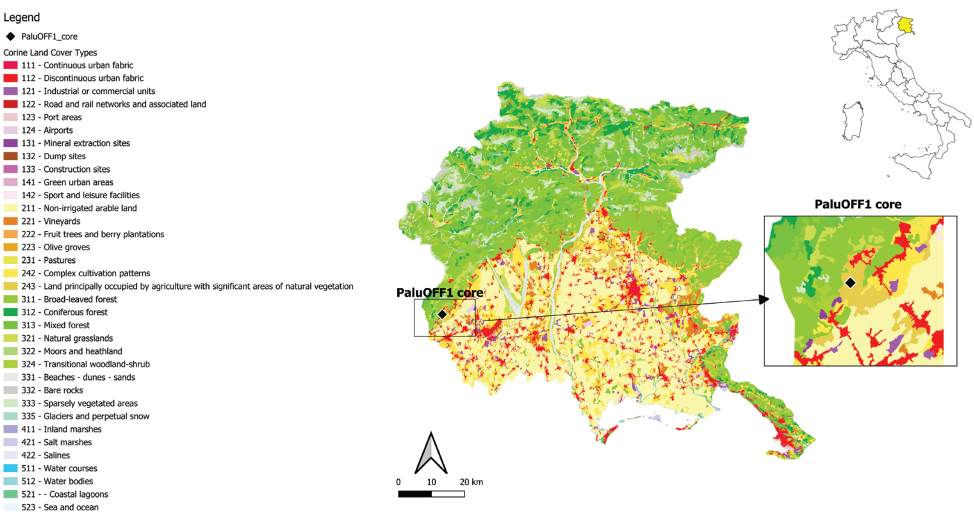

Figure 1. Corine Land Cover based map showing the main land cover types in Friuli Venezia Giulia with focus on the surrounding of the Palù di Livenza basin and the PaluOFF1 coring site. The map was carried out using QGIS version 3.42 (QGIS Development Team 2009) with the Corine Land Cover 2018 vector data (100 m resolution) as base layer (EAA 2019).

and herbaceous species typical of the pre-Alpine region. The area also includes high-value spring habitats, forming part of the broader "spring belt" of the Po Plain, where groundwater emerges due to the presence of fine, impermeable sediments (Zappa et al. 2008). These springs sustain a rich mosaic of wetland habitats, including alkaline fens dominated by *Cladium mariscus* (L.) Pohl and other hygrophilous species, which form distinct vegetational zones around ponds and lakes.

Palynological studies on past pollen record

In February 2022, a sediment core (PaluOFF1) was manually cored approximately 300 meters from the Palù di Livenza Neolithic site. The coring reached a depth of around 6 m, ending upon contact with the "Argille Azzurre" formation, indicative of the Last Glacial Maximum. Excluding the potentially reworked top layer (55 cm), the 555 cm core was sub-sampled and 54 palynological samples were collected every 10 cm under sterile conditions at the Laboratory of Palynology and Palaeobotany in Modena. Then, 21 samples were selected for pollen analysis based on the sedimentological characteristics of the core. Palynological samples were processed using standard protocols (Florenzano et al. 2012) aimed at removing inorganic matter and concentrating palynomorphs, with *Lycopodium* tablets added to estimate pollen concentrations. The treatment included deflocculation, acid treatments, acetolysis and heavy liquid separation. Pollen analyses were performed at high magnification (1000×) under optical microscope using keys, atlases and the reference collection of the laboratory (e.g., Reille 1992; Beug 2015).

For the purpose of this research only general and selected results will be reported. In particular, the useful sums reported and discussed together with the *L. borealis* record are: coniferous forests, upland forests, mixed oakwood (the widely spread type of forest present in the basin) and sums of plants related to hygrophilous environment (both trees and herbs).

Linnaea borealis distribution

We reconstructed the historical and current distribution of *L. borealis* using peer-reviewed literature on floristic data (occurrence of species, records of species in the environment or in Herbaria), the results of which are reported in Table 1. Furthermore, research on the GBIF platform (Global Biodiversity Information Facility) was carried

Table 1. Table providing a summary of the Italian regions where the species occurs, the main reported localities of its populations, the principal vegetation associations and the key bibliographic sources.

Region	Occurrence	Vegetation associations	Main references
Valle d'Aosta	Val di Rhêmes, Valgrisenche	Shaded slopes with conifers	Conti et al. (2005), Bovio (2016)
Piemonte	Val d'Ossola (e.g., Valle Devero)	Acidic, undisturbed areas	Selvaggi et al. (2024)
Lombardia	Upper Valtellina, Stelvio National Park, Orobic Alps	Coniferous woods at 1,600–2,000 m	Conti et al. (2005), Cretti and Bona (2015)
Trentino-Alto Adige	Renon Plateau, Van Ridanna, Val di Fumo, Val di Tovel	Rare; in acidophilous beech and spruce woods	Bartolucci et al. (2018); Festi and Prosser (2008)

out aimed at identifying places where *L. borealis* was recorded on the field (in Italy) or is present in herbaria collection (with Italian provenance even if stored in European herbaria). The georeferenced data obtained were then used to create a map using R (R Core Team (2025)) and related packages (Wickham 2016; Pebesma 2018; Chamberlain et al. 2025; Giraud 2025; Massicotte and South 2025).

Results

The PaluOFF1 pollen record

Palynological analyses carried out on PaluOFF1 samples gave robust results. The preservation state of pollen grains in sediment was quite always good, allowing the identification at the lowest possible taxonomic level of pollen grains (Fig. 2). In general, a rich biodiversity was found, with a floristic list which includes 117 taxa, 31 AP–Arboreal Plants and 86 NAP–Non-Arboreal Plants (see also Suppl. material 2). To

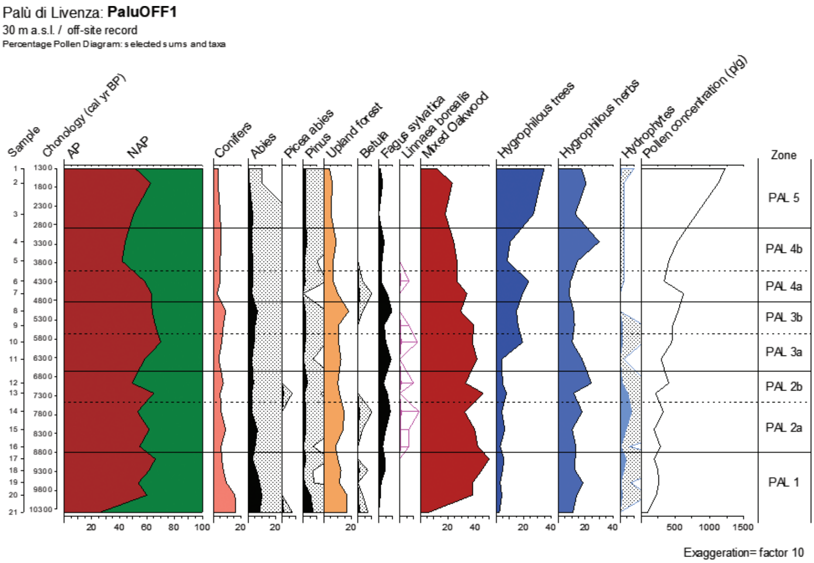

Figure 2. PaluOFF1 off-site core synthetic pollen diagram. AP includes arboreal pollen (trees, shrubs and lianas), NAP the non-arboreal pollen (herbs). Conifers curves includes *Abies*, *Picea abies*, *Pinus* that are also displayed separately, upland forest are the sum of all conifers + *Fagus sylvatica* and *Betula* (displayed also separately), Mixed oakwood includes *Acer campestre* type, *Carpinus betulus*, *Corylus avellana*, deciduous *Quercus*, *Ostrya carpinifolia/Carpinus orientalis* type, *Fraxinus excelsior* type, *Tilia platyphyllos* type, *Tilia cordata* type and *Ulmus*; hygrophilous trees (*Alnus*, *Populus*, *Salix*), hygrophilous herbs (Cyperaceae, *Lilium martagon*, *Lythrum*, cf. *Pancratium*, *Paris* type, *Phragmites australis*, *Scilla* type, *Scirpus* type, *Sparganium emersum* type, *Typha latifolia* type) and hydrophytes (*Butomus umbellatus*, *Myriophyllum*, *Potamogeton*, *Sparganium erectum* type) are also reported. Depth bars represent 10× exaggeration. Chronology is based on the age-depth model performed on two radiocarbon dates (cal yr BP) and published in Zappa et al. (2025). The diagram was drawn with Tilia software (Grimm 2004). This site is included in the BRAIN database (ID: NFV28; Mercuri et al. 2024).

highlight the main features of the vegetation cover along the core, selected sums of taxa were used, focusing on key groups such as mixed oakwood (the predominant forest type at the site) and hygrophilous trees and herbs, as illustrated in Fig. 2. The pollen record spans 10,000 yrs of vegetation history and was subdivided into five pollen zones through cluster analysis, which grouped samples exhibiting similar vegetation assemblages. *Linnaea borealis* appears consistently across several pollen zones, from its first occurrence in PAL2a to its last in PAL4a, corresponding to a chronological interval between 8,600 and 4,200 cal yr BP.

Linnaea borealis specimen in Italy from floristic surveys and herbarium data

To obtain an overview of the species' records in Italy, the starting point was the collection of reports and bibliographic references found on Acta Plantarum (https://www.actaplantarum.org/) and related entries. A summary of the species presence on the field is reported in Table 1. Subsequently, a search on the GBIF (https://www.gbif.org/) allowed for the identification of occurrences of *Linnaea* in Italy (both herbarium specimens and field observations from the scientific community). The research gave 119 records, 94 of which are georeferenced and could be included in the elaborated map (Fig. 3). One record was excluded as it was determined to be erroneous. This analysis provided evidence of the current and limited distribution of the species within the national territory.

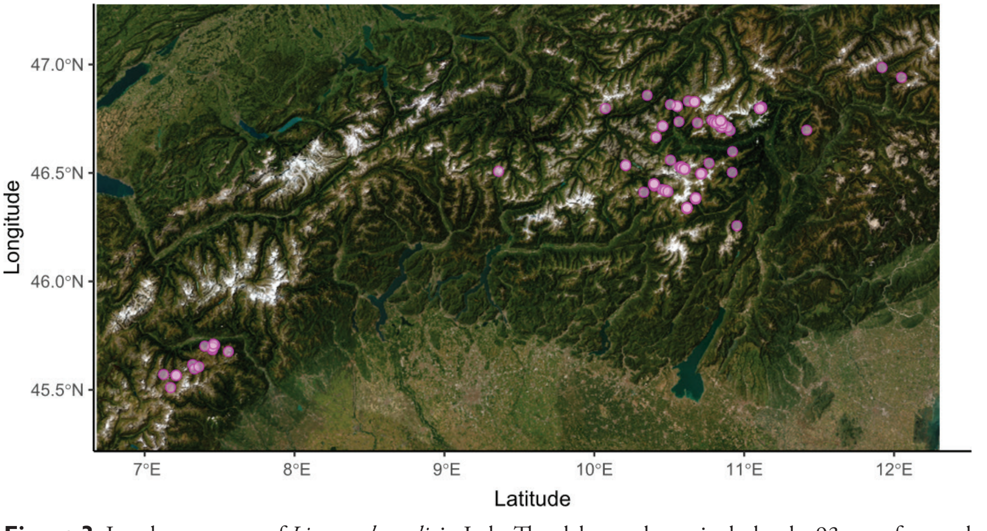

Figure 3. Local occurrence of *Linnaea borealis* in Italy. The elaborated map includes the 93 georeferenced records found. 81 records derive from observations in the field and 12 derive from historical records documented in herbaria. Data were retrieved from GBIF.org (https://doi.org/10.15468/dl.exupdc, accessed on 03rd October 2025) and were elaborated using R. Occurrence points are displayed with transparency, allowing those underneath to remain visible when they are close in space. As points overlap due to their proximity, their shades intensify.

Discussion

Present day distribution of *Linnaea borealis* in Italy

The integrated approach that merged research on bibliographic sources with quantitative georeferenced occurrence data on the GBIF platform allowed for the construction of a picture of the species' national presence. The results confirmed that *Linnaea borealis* is currently confined to a few, geographically scattered locations, primarily in montane and subalpine environments in northern Italy. Both herbarium specimens and modern field records corroborate a distribution pattern that is highly fragmented and restricted in extent. This limited geographic range emphasise the rarity of *L. borealis* within the Italian flora. Its patchy distribution raises important questions about the environmental variables and factors that have allowed the species to persist in certain microhabitats while disappearing from others. Unlike in Poland, where several studies have shown that the decline of *Linnaea* communities is linked also to anthropogenic pressures on the landscape (Ciosek et al. 2015), no specific research is currently available in Italy to investigate the causes of this decline in areas where the species has persisted as a glacial relict. The current distribution of this species in Italy appears to be influenced by only ecological and climatic factors. This highlights the need for further investigation into the roles of microclimatic stability and specific habitat characteristics in the areas where the species persisted or persists. In this context, reconstructing its past occurrence and analysing the composition of the plant communities that coexisted with it are essential steps toward understanding the environmental conditions that have supported its long-term survival in some areas.

Palaeoecological context and role of the Palù basin as a refugium area

The presence of *Linnaea borealis* in palaeoecological records from Friuli Venezia Giulia offers a unique opportunity to investigate climatic and ecological dynamics in the Mid-Holocene landscape. As a cold-adapted species, *L. borealis* serves as a valuable indicator of past climatic conditions in the study site. In Italy, several glacial refugia have been identified through palaeoecological and palynological evidence, including the Euganean Hills where the persistence of *Castanea sativa* Mill. during the Last Glacial Maximum has been documented (Krebs et al. 2004). According to Holderegger and Thiel-Egenter (2009), glacial refugia can be broadly categorised into three types: high-altitude nunataks, peripheral refugia, and lowland refugia. Among these, lowland refugia are considered particularly important for the survival of cold-adapted mountain taxa, as they may have offered microclimatic stability and ecological continuity during periods of glacial advance and subsequent climatic oscillations. The Palù basin, due to its geomorphological setting and hydrological features, may represent one such lowland refugium during a central Mid-Holocene phase.

The PaluOFF1 pollen sequence provides compelling insights into the local vegetation dynamics and ecological stability of the Palù di Livenza basin during the Holocene. The data reveal a densely forested landscape, characterised by mixed deciduous and coniferous forests. Dominant taxa include elements of temperate broadleaf forests such as deciduous *Quercus*, alongside upland and montane elements like *Fagus sylvatica* L., *Betula*, *Abies alba*, *Picea abies*, and *Pinus*. This composition is consistent with the ecological preferences of *L. borealis*, which is typically associated with cool, humid, and semi-shaded forest environments composed of mixed and conifer species. Furthermore, wetland habitats were spread within the basin (Zappa et al. 2023; Zappa 2025), which may have contributed to maintaining local microrefugia for cold-adapted species.

The earliest occurrence of *L. borealis* pollen (PAL2a; Fig. 2) is associated with a significant expansion of hygrophilous taxa. This includes both arboreal elements such as *Alnus*, *Populus*, and *Salix*, and a suite of herbaceous wetland plants including Cyperaceae, *Lythrum*, *Sparganium emersum* type, and *Butomus umbellatus* L. Notably, this phase also corresponds to a marked increase in coniferous (*Abies*, *Picea abies*, *Pinus*) and other upland forest taxa (*Fagus sylvatica*, *Betula*), reinforcing the idea that the surrounding landscape retained montane characteristics despite ongoing climatic shifts at the broader regional scale. In fact, palaeoclimatic reconstructions for central and southern Europe during the Early and Mid-Holocene suggest persistent climatic variability and regionally asynchronous responses (Magny et al. 2012; Vannière et al. 2013). Nevertheless, between ca. 8,600 and 4,200 cal yr BP, the pollen assemblage of the PaluOFF1 record reflects phases of relatively cool and moist conditions in the Palù basin, which created favourable ecological niches for the persistence of glacial relicts such as *L. borealis*. During possibly Early and Mid-Holocene, these conditions allowed *L. borealis* to survive in the area, although today it is mostly confined to higher-altitude habitats. As a classical boreal-montane relict species, highly sensitive to climatic fluctuations, *L. borealis* is typically associated with stable, mesic microclimates, often in ecotonal areas where lowland and upland vegetation overlap. Thus, the survival of *L. borealis* beyond the Last Glacial Maximum in a lowland area suggests that the Palù basin maintained a set of environmental conditions sufficiently cool and humid to allow the continued presence of this species.

Comparing the results from PaluOFF1 with previous palynological analyses of sequences sampled on-site (Pini 2004; Zappa et al. 2023), it is evident that no *L. borealis* pollen was detected, despite the possibility that the plant may have had practical uses for Neolithic populations. In fact, while the ethnobotanical record in Europe remains fragmentary, the repeated references to *L. borealis* in folk medicine suggest that its properties were widely recognised and valued, especially in northern and central Europe. In light of these medicinal uses, the identification of *L. borealis* in post glacial and Early Holocene palaeoecological records from the Friuli Venezia Giulia region may carry not only ecological and biogeographical significance, but also cultural and symbolic implications. Although no macroremains or direct archaeobotanical evidence of *L. borealis* have been recovered from Neolithic archaeological contexts at the site, this absence cannot be taken as conclusive proof of non-use. Unlike species whose uses are concentrated in easily identifiable parts (e.g., seeds, fruits), *L. borealis* is typically employed in its entirety, which may limit the likelihood of its preservation and recovery (as seed, fruit or pollen) in archaeological sediments. Nonetheless, its presence within the local vegetation mosaic (as found in PaluOFF1), especially in plant associations that align with its ecological preferences, strongly implies that it formed part of the landscape during periods of human occupation, potentially contributing to the ecosystem structure even in the absence of direct human exploitation. The early Neolithic period in Friuli Venezia Giulia was marked by increased sedentism, experimentation with cultivated and wild plant resources, and a growing knowledge of the local environment. In this context, the survival of this potentially valuable relict species in ecologically stable microhabitats may have offered an accessible resource for herbal remedies or ritual practices.

Addressing the local significance of the PaluOFF1 pollen record

A central issue concerns the origin of *L. borealis* pollen and whether it could have reached the site via long-distance dispersal from higher elevations. Several lines of evidence argue strongly against this possibility. Firstly, *L. borealis* is an entomophilous species, with heavy, sticky pollen adapted for insect pollination rather than wind transport significantly limiting the long-distance air dispersion. Secondly, the taphonomic characteristics and overall condition of the record are excellent, with no signs of disturbance or degradation. The depositional context appears regular, consistent and undisturbed, strongly suggesting that the material has not undergone any reworking. Thirdly, the record shows not a single isolated occurrence but a continuous and repeated signal of *L. borealis* pollen across the sequence. This temporal persistence suggests the growth of a local population within the basin, rather than sporadic inputs from more distant montane regions. Finally, the good preservation state of pollen in the record is a further indicator of its local provenance. Pollen grains when transported over long distances, often show signs of folding or degradation due to the mechanical stress of transport. In this case, however, their excellent preservation represents additional evidence of local deposition. Therefore, the presence of *L. borealis* in the PaluOFF1 record is interpreted as evidence for an autochthonous population, maintained by the basin's relatively stable microclimatic conditions.

Conclusions

The integrated analysis of past and current data highlights the rarity of *L. borealis* in Italy, underlining the importance of past occurrence investigations to better understand the ecological and historical factors behind its present-day rarity and distribution. The presence of *L. borealis* in the Palù basin between 8,600 and 4,200 cal yr BP, confirmed by morphological pollen analysis, should have been due to anthropogenic or natural conditions. Pollen of *L. borealis* has been found within plant communities that correspond well to its typical ecological habitat. The Palù basin, due to its spring-fed and relatively stable environmental conditions, may have acted as a lowland refugium, preserving suitable microhabitats for the persistence of this species even during periods of broader ecological and climatic changes elsewhere. Regarding possible uses of this plant by local Neolithic communities, no clear evidence of specific uses by Neolithic populations has been found as it was not directly recovered from the archaeological site but only off-site. However, this absence may be due to the fact that the entire plant is typically used for remedies rather than just the floral parts, which may reduce its finding in the archaeobotanical record inside the archaeological site.

Acknowledgements

An acknowledgement is owed to Roberto Micheli of Superintendence of Archaeology, Fine Arts, and Landscape of Friuli Venezia Giulia for the availability and the research conducted at Palù di Livenza and to prof. Alessandro Fontana and Giacomo Vinci of the University of Padua for their collaboration on palaeoenvironmental research in the Palù basin. We would also like to thank Lorenzo Braga for his support during the data extraction from GBIF and the subsequent creation of figure 3 in R. We acknowledge the three reviewers – Donatella Magri, Gabriele Galasso and Lorenzo Peruzzi – for their constructive comments. This research started as part of a PhD project co-financed in the frame of the National Operational Programme Research and Innovation 2014-2020 (CCI 2014IT16M2OP005), ESF REACT-EU resources, Action IV.4 "PhDs and research contracts on innovation topics" and Action IV.5 "PhDs on Green topics".

This research was funded by the project "National Biodiversity Future Center–NBFC", implemented under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4–Call for tender No. 3138 of December 16, 2021, rectified by Decree n.3175 of December 18, 2021 of Italian Ministry of University and Research funded by the European Union – Next Generation EU. Project code CN_00000033, Concession Decree No. 1034 of June 17, 2022, adopted by the Italian Ministry of University and Research, CUP E93C22001090001.

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Supplementary material 1

Summary of the ecological requirements of *Linnaea borealis* in its typical environment in the Northern Hemisphere where it is commonly distributed nowadays

Authors: Jessica Zappa, Anna Maria Mercuri, Giovanni Astuti, Lorenzo Lastrucci, Paola Torri, Assunta Florenzano

Data type: docx

Explanation note: Data summarised from Pignatti et al. (2018); POWO (2025); Thiem and Buk-Berge (2017).

Link: https://doi.org/10.3897/italianbotanist.20.170749.suppl1

Supplementary material 2

Table reporting all 117 taxa found in the 21 samples of PaluOFF1 pollen sequence divided between Arboreal Plants (AP) and Non-Arboreal Plants (NAP)

Authors: Jessica Zappa, Anna Maria Mercuri, Giovanni Astuti, Lorenzo Lastrucci, Paola Torri, Assunta Florenzano

Data type: docx

Link: https://doi.org/10.3897/italianbotanist.20.170749.suppl2