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Integrative analysis of stem-reduction patterns in the inflorescences of *Carlina acaulis* subsp. *caulescens* from the Appennino Abruzzese Mountains (Central Italy)

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Carlina species are well-known multi-use plants with edible, decorative, and officinal applications. In Italy, *Carlina acaulis* L., 1753 shows wide variability in stem length, ranging from acaulescent (non-stemmed) individuals to plants with a very prominent stem. This variability was often attributable to two subspecies presence, respectively: subsp. *acaulis* and subsp. *caulescens* (Lam.) Schübl. & G.Martens, 1834. According to recent references, subsp. *acaulis* is now to be considered restricted to north and central-northern Italy (including north Apennine), while subsp. *caulescens* can be found throughout the Italian peninsula, from north to south. However, specimens with strongly reduced stems can be observed even more at the south, like in our study area. In such cases, this variability is now to be considered as a simple intrasubspecific variability within subsp. *caulescens*. The apparent discrepancy between reduced and prominent stems within the same subspecies, although it could be a key morphological character for subspecies determination, is intriguing and warrants further investigation. How could this variability be influenced by genetic factors (i.e., internal subspecies variability, possible hybridization with similar taxa, or genetic remnants of subsp. *acaulis*) or by environmental stressors/conditions? In our study, we examined the characteristics of *C. a.* subsp. *caulescens* alongside closely related taxa, looking for potential drivers of stem size by integrating botanical, environmental and ecological observations, morphological and chemical traits study, as well as genetic and soil microbiota analyses. These findings provide a multidisciplinary perspective on the factors shaping morphological and chemical diversity in *Carlina* taxa, offering new insights into their phenotype variation.

KEYWORDS
***Carlina acanthifolia*, *Carlina acaulis*, decaulescence, environmental stressors, morphotype traits, novel taxa, stem variation**

1 Introduction

1.1 Background

The genus *Carlina* L. 1753 includes about 30 species distributed mainly across the Northern Hemisphere, typically occurring in temperate habitats; a dozen of which are part of the Italian flora (Bartolucci et al., 2018). The species of this genus are well-known multi-purpose plants with edible, decorative, and officinal uses.

Coste (1937) still regarded as an important reference in France, treated both *Carlina acaulis* and *Carlina acanthifolia* as biennial species. In another major reference Flora (e.g., Verloove et al., 2024) and in earlier editions authored by different botanists since 1973, only *Carlina acaulis* is mentioned, described as a biennial hemicryptophyte. Devesa Alcaraz et al. (2014) listed both *Carlina acaulis* and *Carilina acathifolia* as perennial herbs. Other authors (e.g., Tutin et al., 1976) describes the studied species as long-lived monocarpous perennial, which blooms and fruits only once in its lifetime and dies after it produces seeds. In others standard Floras this is not clearly specified, or they are generically listed as hemicryptophyte (e.g., Pignatti, 1982). This different interpretation lead sometimes to misinterpretations regarding the longevity and the ability to produce one or more flowering events over the course of its life cycle for both species; anyway, *C. acaulis* and *C. acanthifolia* can show a huge variability. For that reason, several taxa were described over time (e.g., Coste, 1937) that are now considered as synonyms (POWO, 2026a).

In Europe, according to the aforementioned Floras, it can be stated, in a very broad and simplified way, that *Carlina acaulis* is more widely distributed in northern and cooler regions, whereas *Carlina acanthifolia* is more common in southern and warmer areas; therefore, the latter distribution area is relatively overall "shifted southwards". Both the *Carlina* species could occur from sea level up to hills and even in man-made reliefs. Lambinon et al. (1992) mentioned the presence of *Carlina acaulis* in Atlantic costal dunes and on spoil tips, sometimes known as Terrils, Terracones or Terrikones (see Cianfaglione, 2024); towards the south both are often found progressively at higher elevations. We used these Floras to understand and apply the ecological and morphological traits relevant to our study plant specimens.

In Italy, *C. acaulis* is common from the north to the central peninsula, while in the south it becomes progressively rarer and mostly found in higher elevations, reaching its southernmost limit around the Pollino Mountains area, while is not present on the islands; while *Carlina acanthifolia*, although also a common species, has a similar distribution even if shifted south, with the two species showing full overlap between the northern and central Apennines (Pignatti, 1982; Bartolucci et al., 2018 and addenda). Unlike other European and Mediterranean countries where these species may be rare or locally rare, in Italy both species must be regarded as unequivocally common following Pignatti et al., 2018; Bartolucci et al., 2018 and addenda).

Both species (*Carlina* acaulis and *C. acanthifolia*) shows a wide variability. *Carlina acaulis*, in particular exhibits a wide variability in stem length, ranging from specimens with clearly developed stems (Figure 1), to those with a completely acaulescent individuals (Figure 2). Pignatti (1982) defined *Carlina acaulis* as usually acaulescent, with the flower head at the center of the basal leaf rosette, but long-stemmed individuals with a leafy stem up to 30 cm tall can also be found. He added that these tall forms appear within the same populations under particular ecological conditions, such as plants that develop late at the end of the vegetation season, after mowing, or in specific conditions such as at the edges of woodlands. Pignatti also reported that these two forms were in the past referred to as var. *alpina* Jacq., and var. *caulescens* DC., but he did not consider stem length to be a hereditary trait, generically citing the genetic knowledge available at the time, and therefore he did not recognise any taxonomic significance for both these described forms. For this species binary form variation, with almost no intermediate forms, he cited Goethe who considered this species an example of plant metamorphosis, recognizing the primary importance of form as a harmonious whole and anticipating ideas that are only now being discussed. This variability has successively been interpreted as the result of the presence of two subspecies of *Carlina acaulis* L. 1753, namely: the nominal subspecies (subsp. *acaulis*; and the stemmed subspecies [subsp. *caulescens* (Lam.) Schübl. & G.Martens 1834].

In Flora Europaea (Tutin et al., 1976), the authors mention also subsp. *simplex* that usually has a stem of 15–60 cm, simple or branched, with up to 6 capitula, rarely short or absent. *Carlina acaulis* subsp. *simplex* (Waldst. & Kit.) Nyman according to the most important online databases, in older literature appears as *Carlina acaulis* subsp. *simplex* (Waldst. & Kit.) Schübl. & G. Martens; *Carlina acaulis* f. *simplex* (Waldst. & Kit.) Nyman; *Carlina simplex* Waldst. & Kit.; *Carlina subacaulis* var. *simplex* (Waldst. & Kit.) DC.; *Carlina subacaulis* DC; or more generically as *Carlina acaulis* var. *simplex*.

This botanical entity actually is anyway not recognised as valid by principal global online databases (e.g., Euro+Med, 2026 and POWO, 2026a, b), treated as a synonym and included within the broader concept of *Carlina acaulis* L.

The atypical decaulescent form we found shares several traits in common with a *Carlina* taxon described for the Balkans [*Carlina acaulis* f. *nana* (Degen) Meusel & Werner], like the stemless form,

FIGURE 1
An example of *Carlina acaulis* subsp. *caulescens* f. *typica* from the study area, with long developed stems. In the image, the typus specimen of the newly proposed form (f. *typica*) is shown.

FIGURE 2
Examples of *Carlina acaulis* subsp. *caulescens* f. *decaulescens* specimens studied in the same area, representing the non-stemmed phenotype. It should be noted that the morphological characters of this decaulescent form tend to converge towards those of *C. acaulis* subsp. *acaulis*. In the top right, the typus specimen of the newly proposed form (f. *decaulescens*) is shown.

occurring sporadically in similar locations of the main species, though more rarely (von Degen, 1938; Meusel and Werner, 1962). However, this taxon has generally been considered over time as heterotypic synonyms of *Carlina aggregata* Willd., 1803 and of *Carlina acaulis* (cf. CWG, 2025; WFO, 2026; POWO, 2026a, b - databases online).

According to recent literature, the subsp. *acaulis* is now considered restricted to northern Italy (including the northern Apennines), whereas subsp. *caulescens* occurs throughout the peninsula, from north to south (Conti et al., 2005; Bartolucci et al., 2018 et add. nec. seqq.). Nevertheless, as we observed, specimens with markedly reduced stems can still be found further south, for example in central Italy, as it is for our study area. In such cases, this condition is now regarded simply as intraspecific variability within *Carlina acaulis* subsp. *caulescens*. This raises a very interesting question regarding this species, since *Carlina acaulis* is, by definition, a stemless species, as indicated by its name; however, there is a subspecies that develops a well-formed, sometimes even tall stem, as the subspecies name (*Carlina acaulis* subsp. *caulescens*) suggests. Interestingly, within this subspecies, it is possible to find again stemless individuals, thus expressing a trait (the stem length) that is highly unexpectable and contradictory.

This variability is intriguing and fascinating. This oxymoronic taxonomic condition and unusual stem-length specimens' expression stimulate curiosity, calling for further research. For these reasons, we focused our study on *Carlina acaulis* subsp. *caulescens* living in the study area, where specimens can be found with a strongly reduced, if not completely absent, stem.

To understand the possible patterns underlying stem reduction in the *Carlina acaulis* subsp *caulescens*, we first conducted a botanical survey and a morphological trait assessment, completed by molecular analyses to investigate the phytochemical (chemical traits) and genetic characteristics that are typical of the taxon under study. This allowed us, on the one hand, to evaluate the functional traits of this taxon and to determine whether these features may result from possible genetic introgressions from neighbouring taxa, which share botanical and ecological similitudes, and distribution (Pignatti, 1982). In addition, we analysed closely related taxa in the same way to assess whether the stem-reduction phenomenon could be attributable to genetic influence from nearby species.

Afterwards, we also planned environmental and ecological observations to evaluate whether the traits related to the stem reduction might instead be determined by environmental conditions or stress factors.

This also allowed us to study and describe the morphological, chemical, genetic, and ecological characteristics of several *Carlina* taxa, which are poorly studied or even entirely unstudied when considering the Italian accessions.

1.2 Research hypotheses

- Are *Carlina acaulis* subsp. *caulescens* individuals without a stem, or with a very reduced stem, typical *Carlina acaulis* subsp. *acaulis*, or are they morphologically atypical *Carlina acaulis* subsp. *caulescens*?
- Do they fall within the normal interspecific variability, or are they the result of probable hybrids with other acaulescent *Carlina* taxa with which they coexist, from which they may have inherited the reduced stem trait?
- The expression of the decaulescent phenotype could be associated with possible teratological phenomena, or malformations caused by environmental stressors, or pests?
- Could they represent taxa introduced by humans, or are they naturally occurring beyond their currently presumed range?

2 Materials and methods

In order to answer those questions, a first botanical analysis was carried out in the study area to identify the specimens belonging to taxa that are the focus of our research, as well as other taxa used for comparison tests. The second phase of our study consisted of a morphological trait assessment aimed at identifying and selecting

samples of *Carlina acaulis* subsp. *caulescens* showing a markedly reduced stem development.

For each specimen, we recorded environmental characteristics (geographical position, slope or flat terrain) and possible stressors (trampling, grazing pressure, drought-related stress, or the presence of pathogens or other herbivores). For each taxa, after the flowering (September), we collected only specimens that appeared healthy and in good condition, and that did not show any malformations such as stunting plants or plants showing teratological traits (e.g., poorly developed flowers or leaves; curbed or weak stems, leaves and flowers; fasciation), nor individuals damaged by trampling, predators, or pathogens with visible etiological signs (e.g., rot, fungal or bacterial decay, desiccation, spots, folding, or pronounced bite marks), or by severe drought. This selection was made in order to focus the study not only on potential environmental stressors, but also on other possible influencing factors. The aerial parts were consequently collected and analysed to assess genetic and phytochemical traits. A genetic survey was carried out on one inflorescence per sample. The corresponding adjacent soil per sample was collected around each plant both to analyse and to characterise the microbiota, resulting in three independent plant–soil units. Each taxa was treated as a distinct biological unit, allowing a comparative assessment of plant-associated and soil microbial communities. The studied taxa belongs to the subfamily Carduoideae, tribe Cardueae, and subtribe Carlininae (APG, 2016). It should be noted that genetic species diversity and phylogenetic relationships within this subtribe remain largely unresolved, or simply deductive, with only a few preliminary or hypothetical reconstructions based on few studies currently available (Kadereit and Jeffrey, 2007; Funk et al., 2009; Barres et al., 2013; Pignatti et al., 2018; Herrando-Moraira et al., 2019) and no specific data were found from Italian peninsula accessions. This study aims to explore the stem development phenotypic variability in greater depth in order to better understand the possible origin of the variation in caulescence patterns in *Carlina acaulis* subsp. *caulescens*, which may be more unpredictable than currently assumed. Moreover, it seeks to clarify the extent to which this trait is driven by genotype rather than by environmental conditions, specific stressors, or by hybridisation with other similar taxa like *Carlina acanthifolia*. This investigation may also contribute to a clearer and more detailed understanding of the phylogeny, taxonomy, phytochemical, botanical and genetics of this genus.

2.1 Study area

The study area is located in the northern portion of the "Altopiano di Campo Felice" plain, between 1540 and 1560 m a.s.l.; reference GPS point coordinates: 42°14'00.6"N - 13°25'08.9"E. The study site is highly representative of the conditions of the central Apennines. In addition, the area is relatively homogeneous from both a topographical and ecological perspective, which helps avoid significant environmental factors differences that could influence the phenotype, particularly the expression of the stem. The northern portion of the "Altopiano di Campo Felice" plain was selected as the sampling site because it is relatively drier than the southern part and is representative of the environmental conditions required for the optimal development of the *Carlina* taxa under study. These taxa are typically xerothermic and heliophilous plants that usually grow in meadows and pastures (Pignatti, 1982) on soils developed on sedimentary calcareous substrates, within warm and dry xerothermic secondary grassland formations (cf. Tela Botanica, 2026 - database online) that are favoured under human disturbance, particularly farming. Within the study area, *Carlina acaulis* and *Carlina acanthifolia* were also recorded in roadside habitats, including verges and ditches along major asphalt roads. As in many areas of the Apennines, the landscape of the study area has been strongly shaped by human activities, over time. These anthropogenic pressures have transformed the landscape, vegetation, and related ecosystems into secondary (semi-natural, or artificial) systems of anthropogenic origin (Figure 3). Deforestation and grazing have historically been the most significant human impacts in the area, as is typical for Italy, Europe in general, and therefore also the Apennine mountains (e.g., Cianfaglione, 2014; Pratesi, 2019; Cianfaglione et al., 2025). As we observed on site and through aerial and ground photographs, as well as by reviewing general sources, including daily news and local chronicles (e.g., Flora di Lucoli, 2012; Mountain Wilderness, 2024), human pressure has progressively increased in recent years due to the gradual intensification of motorised equipment use, the expansion of tourism, recreational, and sporting activities, as well as urban, agro-pastoral, commercial, and infrastructure development. The presence of these anthropogenic pressures was considered important in our study, as it is both representative of Apennine Mountains environments and allows for the assessment of potential influences from stressors associated with stem development patterns.

2.2 Study design and sample collection (exploratory analysis)

We monitored the study area for five years, looking for the presence of the studied taxa, their distribution, and their possible dynamics, while analysing the classical environmental stressors

FIGURE 3
A detail of the landscape of the study area, with the forest remnants on the mountains, belonging to the *Fagus sylvatica* vegetation belt, and the plain below with secondary grasslands created by human activity. Here grow *Carlina acaulis* subsp. *caulescens*, in both the *typica* and *decaulescens* forms; together with *Carlina acanthifolia* subsp. *acanthifolia*.

related to water, temperature, pests, and human impact that could affect them.

After that, we selected healthy and well-developed samples so as not to alter the possible relationship between genetic and environmental factors, in order to understand how both could be correlated with the phenotypic expression of stem length, nor to risk affecting the chemical composition and morphological characteristics of the plant's traits, which could have influenced our results or comparison capacity.

For the samples to be analysed in the laboratory, see the specific treatments in the dedicated sections below. In any case, two situations must be distinguished for these analyses: for the genetic analysis, the amount of material required was minimal and had to come from a single genetic individual (one plant per studied taxon), which was morphologically and taxonomically characterised with maximum care. For each taxon, single plants were identified, and DNA was extracted from three samples per plant (triplicates), taken from the flowers, leaves, and small root fragments. Each sample analysis needed max 500 mg of plants dry material. 500 mg of soil surrounding the roots of these same individuals was also collected to extract the environmental DNA of the micro-organisms present in the rhizosphere. For the phytochemical analysis, a larger amount of material was needed (i.e., 107 g of dry aerial parts). For this purpose, additional leaves were collected only from selected plants growing within a few meters of each other, for a total of 4–5 individuals per analysed taxon considered for the phytochemical study. This was done to avoid environmental differences influencing the results and to minimise harvesting disturbance. Our sampling strategy was designed to follow both the principle of caution and the goal of minimizing the impact on local plant specimens, local populations, and the ecosystem of the study area. All procedures are further detailed in the dedicated sections below.

2.2.1 Ecological and botanical analysis

A first step was to search for, identify, and record the presence of individuals with absent or highly reduced stems within populations of typical *Carlina acaulis* subsp. *caulescens* with well-developed stems. Subsequently, an initial assessment was carried out to exclude specimens with poorly developed or absent stems that could be attributed to clearly identifiable causes, such as direct trampling, water stress, fire, pathogen attacks, dieback, malformations (e.g., curvature, teratologies due to fasciation), or insect damages or damaged flowers. This was done in order to limit factors that could make it more difficult to assess the extent to which genetic factors, as opposed to environmental ones, may influence the expression of this phenotype. Among the ten samples per taxon that passed the selection, we chose the best-developed individuals, namely those that, based on the measurements of morphological traits, matched the average morphological characteristics accepted for *Carlina acaulis* subsp. *caulescens* (e.g., Pawłowski and Jasiewicz, 1972; Tutin et al., 1976; Pignatti, 1982; Pignatti et al., 2018). For each selected specimen, a healthy, undamaged sample of the aerial parts, from the collar to the flowers, was collected for chemical and genetic analyses. The samples harvested for further phytochemical and genetic analyses were collected during the summer, after the flowering, on 15 September 2023. For each taxon, three biological replicates (n = 3) consisting of healthy aerial parts were collected. Samples were initially air-dried in a shaded and well-ventilated area at room temperature, ensuring that the temperature did not exceed 40 °C, and were subsequently stored at −20 °C until molecular and phytochemical analyses.

Since this species is monocarpic and because non-caulescent specimens of *Carlina acaulis* subsp. *caulescens* are relatively rare in nature, we intentionally limited destructive sampling in order to minimise potential impacts on the local population. For this reason, three biological replicates per taxon (n = 3) were collected and analysed, each processed in triplicate for molecular and microbiological analyses.

To facilitate sample identification throughout the analyses, the investigated taxa were coded as follows: *Carlina acaulis* subsp. *acaulis* = 1B; *Carlina acanthifolia* subsp. *acanthifolia* = 2B; and *Carlina acaulis* subsp. *caulescens* = 3B; as can also be seen in the diagrams shown later in Figures 4 and 5.To evaluate whether the non-caulescent specimens observed in the study area could be attributable to *Carlina acaulis* subsp. *acaulis* rather than to atypical forms of subsp. *caulescens*, comparative analyses were performed between the collected specimens and *Carlina acaulis* subsp. *acaulis* (1B). In addition, to explore the possibility of hybridization with related taxa, this study was designed as an exploratory and hypothesis-generating comparative analysis involving *Carlina acaulis* subsp. *caulescens* (3B) and *Carlina acanthifolia* subsp. *acanthifolia* (2B).

The second hypothesis is supported by the presence of probable interspecific hybrid forms (e.g., Meusel and Kästner, 1994). Following Devesa Alcaraz et al. (2014) almost two botanical entities should be considered as hybrid between *Carlina acanthifolia* and *Carlina acaulis*, mentioned as: *Carlina* × *vayredae* Gaut., in: "Cat. Fl. Pyrénées-Orientales: 244, 1897" and *Carlina* × *clemenceaui* Sennen, in: "Bull. Soc. Bot. France 74: 380, 1927" reported from

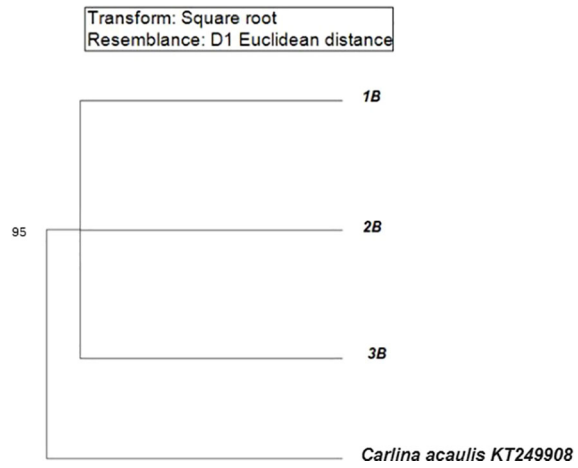

FIGURE 4
Hierarchical clustering (dendrogram) of ITS amplicon sequences (150 bp) obtained from *Carlina* inflorescence samples (1B, 2B, 3B) and the reference sequence *Carlina acaulis* (accession number KT249908.1). Clustering was performed on square-root-transformed data using Euclidean distance (D1). All samples clustered together with the reference sequence at approximately 95% similarity, indicating high genetic similarity and no relevant differentiation at the species level.

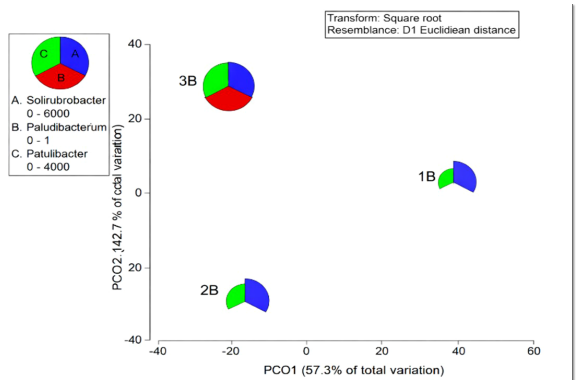

FIGURE 5
Principal coordinates analysis (PCoA) of soil bacterial communities associated with the three *Carlina* species samples (1B, 2B, and 3B). The analysis was performed on square-root-transformed data using Euclidean distance (D1). The first two axes explain 57.3% (PCO1) and 42.7% (PCO2) of the total variation, respectively. Pie charts represent the relative contribution of the main bacterial genera driving sample positioning in the ordination space, including *Solirubrobacter*, *Paludibacterium* and *Patulibacter*.

FIGURE 6
An example showing that it is not rare to find even all the three taxa we studied growing very close to each other in the study area. In this picture we can observe *Carlina acaulis* subsp. *caulescens* f. *decaulescens*; *Carlina acaulis* subsp. *caulescens* f. *typica*; and *Carlina acanthifolia* subsp. *acanthifolia*; together with their respective floral remains during spring.

northeastern Spain, of which "the herbarium material was not possible to be consulted".

Frère Sennen (Granier-Blanc É. M.) in note 707 (see Sennen, 1927) provides a very interesting detailed account of the variability of *Carlina acaulis* in the Ceretania (Cerdanya or Cerdagne) region in the Eastern Pyrenees Mountains, recognizing the coexistence of two distinct morphological forms: a non-stemmed and a stemmed one. The non-stemmed form is described as occurring with the stem very appressed to the ground, generally monocapitate, and bearing a relatively medium or small capitulum (calathidium). In contrast, the stemmed form typically appears in dense clonal-like clusters, sometimes producing up to ten tall stems arising from a single rootstock, and bearing conspicuously large capitula markedly different from those of the non-stemmed form. Sennen also reports the presence of numerous intermediate morphotypes, which he interpreted as potential hybrids between the two forms. Additionally, he mentions several specimens that he regarded as putative hybrids between those tree *Carlina* morphotypes, and with *Carlina acanthifolia* (mentioned as *C. cynara*).

Since both species (*Carlina acaulis* and *Carlina acanthifolia*) frequently occupy the same habitats (see Figure 3) and are often in close contact (Figure 6), as in our study area, they could interact causing occasional hybridisation events. In this context, the high variability observed in *Carlina acanthifolia* could also play a decisive role in shaping the variability of hybrid forms found in nature, given its relative variability observed in the study area (Figure 7), but also as known in the cited literature. Furthermore, being a stemless species with a flower head close to the ground, it could potentially contribute genetic traits related to stem reduction in possible hybridisations. *Carlina acaulis* shares several morphological similarities with *Carlina acanthifolia*, to the point that the latter taxon was in the past also referred to as *Carlina acaulis* (*sensu* Lamarck, 1783). The relative morphological and systematics closeness between the two species, together with the fact that both species often share the same habitats and can be found flowering at the same time, during summer, as it is in our study area (end of July to August), may lead to the hypothesis that a hybridisation should be possible in some conditions.

Finally, three soil samples were collected, each in duplicate, to analyse the associated microbial communities and explore possible relationships between soil biodiversity, environmental conditions, and stem-length phenotypic variability.

Our fieldwork permitted interactions with people frequenting the study area, enabling informal conversations with them. These interactions were random and opportunistically used as non-structured sources of information and were treated as anonymised interviews in accordance with ISE - International Society of Ethnobiology (2008) and AAA - American Anthropological Association (2012) ethical guidelines. We selected individuals regularly visiting or working in the area, resulting in 50 conversations conducted with local shepherds, farmers, and various types of tourists (e.g., hikers, trekkers, runners, and vacationers). Additionally, we carried out 10 intentional meetings using a snowball sampling approach with elderly residents (70–100 years old) from the nearby Lucoli and Rocca di Cambio municipalities, seeking further details on local traditional knowledge. No distinctions were made regarding gender, social status, or educational level, as this was considered unnecessary for our aims and the study does not constitute a detailed ethnobotanical investigation. Instead, our objective was to determine whether differences existed in the

FIGURE 7
Two of the studied *Carlina acanthifolia* subsp. *acanthifolia* specimens from the study area showing a relative variability. This taxon is also acaulescent, resembling in some way both *Carlina acaulis* subsp. *acaulis* and *C. acaulis* subsp *caulscens* f. *decaulescens*.

perception, knowledge, and uses of the taxa encountered in the study area, and to assess the potential risks or benefits related to human impact. In all cases, anonymity was ensured in compliance with the ethical guidelines mentioned above.

2.3 Phytochemical analysis

2.3.1 Chemicals

The following chemicals were used for the phytochemical analysis: 96% ethanol and distilled water for the extraction; dichloromethane and methanol in mixture at different concentrations for the column chromatography separation on silica gel (40–63 µm); 2N sulphuric acid for the developments of TLCs; deuterated chloroform and methanol for the identification of the metabolites via NMR spectroscopy.

All the solvents and silica gel were purchased from Sigma Aldrich whereas TLCs were purchased from Fluka Analytical.

2.3.2 Instruments

Proton NMR spectra were acquired at 298° K on a Bruker Avance II 400 MHz instrument (Billerica, Massachusetts, USA) and recorded with 32 transients, a spectral width of 9013.7 Hz (corresponding to 15 ppm) and a data set points matrix of 64K for an acquisition time of 7.3 s. The recycle delay was set at 6.55 s. Chemical shifts were referenced to TMS (s, 0 ppm) for spectra in $CDCl_3$ while the signal peak relative to CD_2HOD (m5, 3.31 ppm) was used as reference for spectra in CD_3OD.

2.3.3 Extraction

107 g of *Carlina acaulis* subsp. *caulescens* aerial parts were extracted by maceration in 2 L of a solution composed of 96% ethanol and distilled water in ratio 9:1 *v/v*. The maceration lasted 28 days for a more exhaustive extraction of the plant material. The resulting solution was then filtrated and concentrated by means of Rotavapor at reduced pressure at the temperature of 55 °C. During this step, the solution pH was checked on litmus paper to verify that it was not too acid (pH < 5.5) or too basic (pH > 8.5) and prevent unwanted secondary reactions like the hydrolysis of compounds. The solution pH was about 7.5. In the end, a dark green extract weighing 19 g was obtained.

2.3.4 Separation and identification

4 g of this extract was separated by means of column chromatography using silica gel (107 g, ratio about 1:30 *w/w*) as stationary phase and mixtures of dichloromethane and methanol at different concentration ratios. The beginning ratio was 98:2 *v/v* (150 mL) but this was modified during the chromatographic run, in order to let the elution of more polar compounds, to 97:3 *v/v* (100 mL), 95:5 *v/v* (150 mL), 8:2 *v/v* (200 mL), 7:3 *v/v* (150 mL), 6:4 *v/v* (100 mL).

During this separation step, all the compounds were identified by comparing their spectroscopic and data with those reported in the literature: α-amyrin (1), β-amyrin (2) (Tajuddeen et al., 2022) and lupeol 3-acetate (3) (Abdullah et al., 2022) in mixture with triglycerides from the assembly of fractions 71–93 for the total weight of 60.6 mg; the same previous mixture was also found in the assembly of fractions 94–112 for the total weight of 68.9 mg; lupeol (4) (Ni et al., 2019) in mixture with triglycerides from the assembly of fractions 113–120 for the total weight of 33.6 mg and from the assembly of fractions 121–126 for the total weight of 15.5 mg; apigenin (5) (Chang et al., 2017) in mixture with fatty acids from the assembly of fractions 127–130 for the total weight of 31.5 mg; chlorogenic acid (6) (Hu et al., 2019) in mixture with saccharides from the assembly of fractions 139–162 for the total weight of 156.3 mg.

2.4 Genetic plants and related soil bacterial community analysis

2.4.1 DNA extraction

Total genomic DNA was extracted separately from soil and inflorescence samples using protocols optimised for environmental and plant-derived matrices. Total genomic DNA was extracted from soil and inflorescence samples using matrix-specific commercial kits, according to the manufacturers' instructions. DNA from soil samples was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, USA), which is optimised for complex environmental matrices. DNA from inflorescence samples was extracted using the GenElute™ Genomic DNA Miniprep Kit (Sigma-Aldrich, USA), specifically designed for plant tissues and plant-associated microorganisms. Extracted DNA was quantified and assessed for quality prior to downstream molecular analyses. DNA concentration and quality were assessed prior to downstream molecular analyses.

2.4.2 Amplicon sequencing

Plant species identification was performed by amplification and sequencing of a short fragment (~150 bp) of the Internal Transcribed Spacer (ITS) region, a widely used marker for plant taxonomic identification. While the limited length of the amplified fragment does not provide the same discriminatory power as longer ITS regions, it was nonetheless useful for supporting taxonomic attribution in the analysed samples and for comparison with reference sequences available in public databases (Hajibabaei et al., 2006; Taberlet et al., 2012). For *Carlina acaulis* subsp. *caulescens*, taxonomic assignment was evaluated against the

GenBank reference sequence KT249908.1. Molecular analysis therefore provided supportive evidence for the identification of the *Carlina* taxa analysed.

2.4.3 Soil bacterial community analysis (16S rRNA)

Soil bacterial communities were characterised through 16S rRNA gene amplicon sequencing in order to investigate the composition and structure of the bacterial communities associated with the sampled soils (Giampaoli et al., 2014). Bacterial DNA extracted from soil samples was used for amplification of the 16S rRNA gene using universal bacterial primers targeting the V1–V2 hypervariable regions. Amplicon libraries were prepared according to the Illumina 16S Metagenomic Sequencing Library Preparation protocol, including adapter ligation, indexing, purification, and normalization steps. Sequencing was performed on an Illumina iSeq100 platform using paired-end sequencing chemistry. Taxonomic assignment was performed at the genus level in order to evaluate similarities and differences among bacterial communities associated with the analysed *Carlina* taxa.

2.4.4 Bioinformatic and statistical analyses

Raw sequencing reads were processed using a standard bioinformatic pipeline including quality filtering, trimming, normalization, and taxonomic assignment. Low-quality reads and potential sequencing artefacts were removed prior to downstream analyses. Taxonomic classification was performed against a reference bacterial database, and downstream community analyses were specifically conducted at the genus level in order to identify the dominant and most discriminant bacterial genera associated with the analysed samples. Relative abundance tables based on genus-level assignments were used for all ecological and multivariate analyses. Relative abundance data were square-root transformed before multivariate analyses in order to reduce the influence of highly abundant taxa. A Euclidean distance (D1) resemblance matrix was calculated to evaluate similarities and dissimilarities among samples. Community differences were assessed through Analysis of Similarities (ANOSIM), while Similarity Percentage Analysis (SIMPER) was used to identify the bacterial genera contributing most to the observed dissimilarities between groups. Patterns of bacterial community composition at the genus level were visualised using Principal Coordinates Analysis (PCoA).

3 Results, discussion and perspectives

The phytochemical analysis performed on the aerial parts of *Carlina acaulis* subsp. *caulescens* evidenced the presence of six secondary metabolites namely α-amyrin (1), β-amyrin (2), lupeol 3-acetate (3), lupeol (4), apigenin (5) and chlorogenic acid (6) (Figure 8).

Hierarchical clustering based on Euclidean distance of square-root-transformed ITS sequence data showed that all inflorescence samples clustered together with the reference sequence *Carlina acaulis* (accession number KT249908) at approximately 95% similarity (Figure 4).

This level of similarity indicates limited genetic divergence among samples and is consistent with intraspecific or subspecific variation, with no evidence supporting species-level novelty. The 16S rRNA amplicon sequencing analysis revealed the presence of complex bacterial communities in the soils associated with the three *Carlina* taxa. At the genus level, the overall community structure was broadly similar across samples, while differences in the relative abundance of specific taxa were observed. Pairwise comparison of soil bacterial communities showed moderate levels of dissimilarity among the samples associated with the different *Carlina* taxa.

The average dissimilarity values were:

- 14.2% between *Carlina acaulis* subsp. *acaulis* and *Carlina acanthifolia* subsp. *acanthifolia*,
- 14.2% between *Carlina acaulis* subsp. *acaulis* and *Carlina acaulis* subsp. *caulescens*,
- 12.95% between *Carlina acanthifolia* subsp. *acanthifolia*, and *Carlina acaulis* subsp. *caulescens*.

These values indicate a largely shared bacterial background, with detectable taxon-associated differences in community composition.

Principal Coordinates Analysis (PCoA) highlighted a separation of soil samples along the first two axes. The first principal coordinate (PCO1) explained 57.3% of the total variance, while the second coordinate (PCO2) accounted for 42.7%, together explaining more than 80% of the overall variability. ANOSIM analysis indicated limited but structured differences among soil bacterial communities associated with the three *Carlina* taxa (global $R = 0.553$, $p > 0.05$), in agreement with the moderate dissimilarity values observed in pairwise comparisons. SIMPER analysis identified several bacterial genera as major contributors to the observed dissimilarities among soil samples. In particular, variations in the relative abundance of *Solirubrobacter, Patulibacter, Aridibacter, Pseudonocardia, Chitinimonas*, and *Gaiella* accounted for a substantial proportion of the differences between groups. Among these, *Solirubrobacter* and *Patulibacter* consistently contributed to dissimilarity across pairwise comparisons, suggesting a potential association between these taxa and the different *Carlina* species and subspecies (Figure 5).

This study provides an exploratory molecular and ecological characterization of three *Carlina* taxa by integrating plant DNA barcoding with soil bacterial community analysis. ITS-based identification supported the taxonomic assignment of the analysed specimens to *Carlina acaulis* subsp. *acaulis*, *Carlina acanthifolia* subsp. *acanthifolia*, and *Carlina acaulis* subsp. *caulescens*, confirming their attribution to already described taxa within the genus *Carlina* and excluding species-level novelty.

Hierarchical clustering of short ITS fragments (~150 bp) revealed a high degree of similarity between the analysed samples and reference sequences, with no clear separation among taxa. The observed similarity (approximately 95%) falls within the range commonly associated with intraspecific or subspecific variation in plant ITS barcoding studies, supporting taxonomic consistency

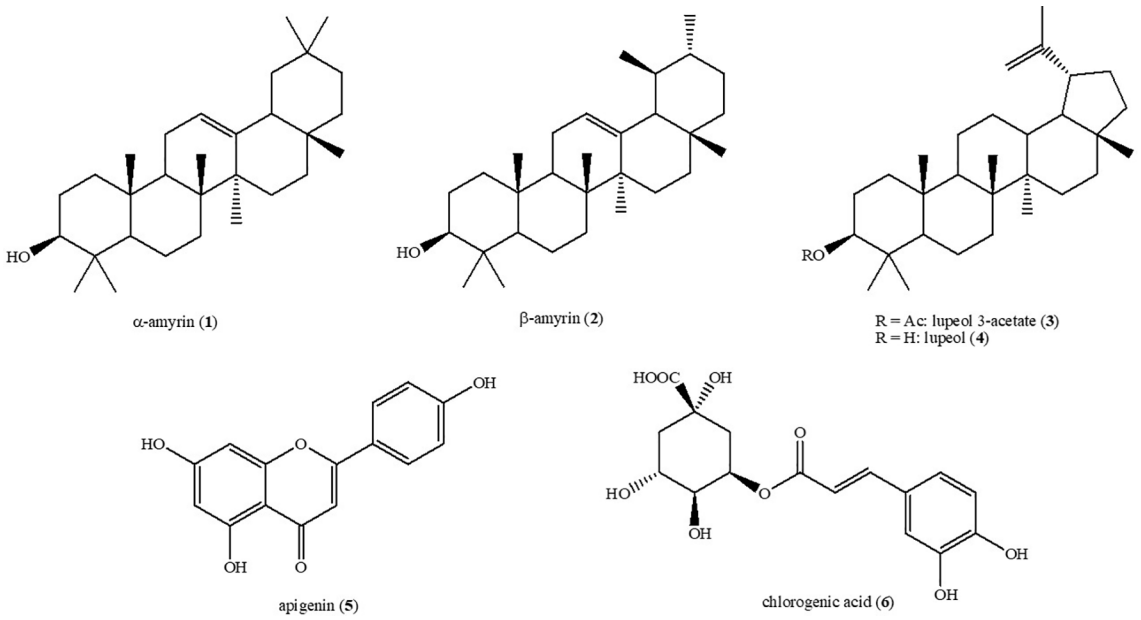

FIGURE 8
Chemical structures of the identified compounds in the aerial parts of *C. acaulis* subsp. *caulescens*..

rather than genetic divergence (Chase et al., 2007; Hajibabaei et al., 2006). Although longer ITS regions may provide increased phylogenetic resolution, short ITS fragments have been shown to retain sufficient discriminatory power for reliable species identification, particularly in exploratory frameworks and when DNA quality or amplification efficiency is limiting.

Soil bacterial community analysis revealed broadly similar community structures across samples, as indicated by moderate average dissimilarity values (approximately 13–14%) and by multivariate ordination. These findings suggest the presence of a shared bacterial background among soils associated with different *Carlina* taxa, with limited but structured variation. Such levels of dissimilarity are consistent with those commonly reported in natural, low-disturbance soils, where community composition is shaped by environmental gradients rather than strong host-driven effects (Lauber et al., 2009).

SIMPER analysis identified a small number of bacterial genera, most notably *Solirubrobacter*, *Patulibacter*, and *Paludibacterium*, as the primary contributors to the observed differences among soil samples. These genera are widely reported in oligotrophic, semi-arid, and natural soil environments and are often associated with the turnover of organic matter and adaptation to nutrient-poor conditions (Davis et al., 2011; Ludwig et al., 2015). Their occurrence is therefore ecologically coherent with the typical habitats of *Carlina* species, which are known to thrive in calcareous, nutrient-poor, and environmentally harsh habitats (dry, hot, and stressed by human activities, mainly as pasture and trampling).

The presence of these bacterial taxa should not be interpreted as evidence of plant-specific microbial associations. Rather, the observed patterns likely reflect the influence of shared soil properties and habitat conditions. Environmental filtering is recognised as a major driver of soil microbial community composition, often outweighing host plant identity in natural ecosystems (Berg and Smalla, 2009; Fierer, 2017). The lack of distinct clustering among samples further supports the interpretation that differences in bacterial communities are moderate and environmentally driven.

Overall, this study highlights the value of combining plant DNA barcoding with soil microbial community analysis to support taxonomic identification and ecological interpretation. Future studies incorporating larger sample sizes, multiple genetic markers, and detailed environmental metadata will be necessary to further explore plant–soil–microbiota interactions within the genus *Carlina* and to disentangle the relative roles of habitat and host identity.

The integration of morphological, genetic, phytochemical, and soil microbiota analyses suggests that the occurrence of stem-reduced individuals within the investigated populations is most consistent with intrasubspecific morphological variability within *Carlina acaulis* subsp. *caulescens*.

Genetic barcoding did not reveal differentiation from reference sequences, while the phytochemical profile corresponds to metabolite patterns widely reported across the genus *Carlina*. Soil bacterial communities were largely shared among samples and did not show taxon-specific signatures, suggesting that local microbial composition is unlikely to represent a primary driver of the observed stem phenotype morphological variation.

Several limitations of this exploratory study should be acknowledged. First, the limited number of samples reduces statistical power and prevents robust ecological or taxonomic generalizations; therefore, the observed patterns should be considered preliminary and mainly hypothesis-generating. Second, plant identification was based on a short fragment of the ITS region (~150 bp). Although widely used as a DNA barcode, such a short sequence provides lower discriminatory power compared to longer regions and may limit the detection of fine-scale intra-specific variation. The inclu-

sion of additional markers or longer barcode regions would likely improve phylogenetic resolution.

Third, soil microbiota analysis was performed on bulk soil collected adjacent to the plants rather than on rhizosphere or root-associated compartments, which are more directly shaped by plant–microbe interactions. In addition, the use of a single bacterial marker (16S rRNA) constrains taxonomic resolution and does not allow functional characterization of microbial communities.

Finally, the phytochemical analysis focused on the identification of major metabolites without quantitative comparison among taxa or comprehensive metabolomic profiling; therefore, subtle chemical differences potentially associated with morphological variability cannot be excluded.

Overall, while the adopted methodological approach is appropriate for a hypothesis-generating framework, further studies including larger sample sizes, additional genetic markers, rhizosphere-targeted microbiome analyses, and more comprehensive phytochemical investigations are needed to better elucidate the ecological and evolutionary factors underlying variability within *Carlina acaulis* subsp. *caulescens*.

The compounds identified in the species belong to three different classes of natural compounds i.e., triterpenoids (1-4), flavonoids (5) and caffeoyl-quinic acids (6).

Their presence is reported for the first time in this morphotype of *Carlina acaulis* subsp. *caulescens* as well as in an Italian population of the species itself. In fact, the previous studies on *Carlina acaulis* subsp. *caulescens* have regarded populations collected in Poland (Strzemski et al., 2016, 2017).

Conversely, their occurrence is well-documented within the *Carlina* genus and particularly in the species *Carlina acanthifolia*, *and Carlina acaulis*, where β-amyrin (2), lupeol 3-acetate (3), lupeol (4) and chlorogenic acid (6) have been identified together (Dombris and Raynaud, 1981; Jaiswal et al., 2011; Strzemski et al., 2016, 2017; Saralieva et al., 2023; Petkova et al., 2024; Sowa et al., 2023a, b, c).

Instead, α-amyrin (1) has been evidenced only in three of the species excluding *Carlina acanthifolia* (Strzemski et al., 2016; Sarlieva et al., 2023; Petkova et al., 2024) and apigenin (5) only in two excluding *Carlina acanthifolia* and *Carlina acaulis* (Đorđević et al., 2012), not representing a significant parameter of chemophenetic discordance, yet because of their isolation in the nominal species.

Right for this situation in general, it was not possible to verify if this morphotype derives from a plant hybridization process, at least under the phytochemical standpoint. Further data from future studies in this context may help clarify the direction to be taken. Unfortunately, revisions and changes in scientific names have gradually increased the difficulty of managing and using genetic and phytochemical data assigned to taxa whose names or taxonomic status have changed. A particularly striking case concerns *Carlina utzka* (*sensu* Pignatti, 1982), which is no longer considered a species, but rather a subspecies of *Carlina acanthifolia* [*Carlina acanthifolia* All. subsp. *utzka* (Hacq.) Meusel and Kästner, 1994], with a critical conservation status taxon (Bilz et al., 2011; Melnyk, 2011; Sowa et al., 2020). Taxon that is now considered typical only for Balkans and central Europe, doubtful for Italy, to be considered as probably extinct (Bartolucci et al., 2018) or never occurred in Italy, except if as a possible entity that need to be confirmed in north-eastern Italy (Acta Plantarum, 2026).

Also *Carlina acaulis* has been interpreted in various ways over time, just as *Carlina acanthifolia*. Over time, some different ideas and interpretations have followed about this species, to the present day, and there is certainly still much to do, to clarify, and to discover.

The multifactorial study carried out supports the hypothesis that, in the study area, the non-caulescent individuals of *Carlina acaulis* should be attributed to atypical specimens of subsp. *caulescens* rather than to typical specimens of *Carlina acaulis* subsp. *acaulis*. This also addresses the question of whether these could represent specimens of *Carlina acaulis* subsp. *acaulis* introduced accidentally or overlooked in previous floristic surveys conducted in Italy, while not being able to exclude the possible presence of rare occurrences in the region.

Following the cited literature, as plant traits, we focused also on floral characters and reproductive dynamics rather than exclusively on vegetative ones. According to Devesa Alcaraz et al. (2014); PLADIAS (2026) and CCDB (2026a, b) online databases, the chromosome numbers of the two *Carlina* species under study are compatible with possible hybridization events (2n=20). However, the available literature provides no sufficiently detailed or objective evidence in this regard, as it is largely based on indirect assumptions or deductions, as observed in the reviewed literature. Floral morphology, anthesis, spatial proximity, and the coexistence of the taxa in the same habitat with overlapping distribution areas are also compatible with possible hybridization phenomena, although this is not supported by the results of our analysis; nevertheless, a rare or occasional occurrence cannot be excluded. For these reasons, we preferred to refer to them more generically as intermediate forms rather than hybrids.

Genetic, ecological, and chemophenetic data do not support sufficient differences to justify the separation of typical and atypical specimens into distinct taxa; however, they can be easily distinguished based on morphological traits related to stem development, since we did not find plants that were more malformed, more affected, or more stressed than the typical forms. Atypical forms, despite their rarity, tend to be relatively more easily found disturbed, damaged, or crushed. However, these events occur late in development stage, even after flowering, and therefore do not justify the lack of stem elongation.

Based on the observation of ecological and environmental characteristics, as well as stressors, we can state that only a few rare specimens showed damaged or poorly developed plant, while the majority appeared healthy and exhibited well-developed flowers, both in the typical and decaulescent forms. Therefore, the reduced stem length observed in the atypical form cannot be attributed exclusively to malformations, pathological conditions, or environmental stressors that might limit or influence the development. Tall (typical long-stemmed) and decaulescent (stemless) forms occurred within the same populations under a range of ecological conditions. The typical long-stemmed habit was not specifically associated with plants developing late in the growing season, following mowing, nor under specific conditions such as forest edges.

The available bibliographic sources mentioning acaulescent and caulescent forms within *Carlina acaulis* are unclear as to whether they refer to what were later treated as two distinct subspecies (subsp. *acaulis* and subsp. *caulescens*), or to different variants within one or the other subspecies, or whether they conflate those interpretations. Furthermore, subsequent nomenclatural and systematic revisions have made the interpretation even more complex and often less consistent. In light of current taxonomic and systematic position clarifications, our analysis has shown that within subsp. *caulescens* there are also acaulescent forms that cannot be attributed to the nominal subspecies (subsp. *acaulis*) representing distinct intrasubspecific variation. Accordingly, and in order to clarify these issues, we propose the recognition of new forms.

Taking into account the various stemless *Carlina* phenotypes reported in the literature, which have been treated as invalid, unclear, or synonymous, and in light of our study results, we suggest treating the two forms observed in our study area as follows:

- *Carlina acaulis* subsp. *caulescens* f. *decaulescens* Cianfaglione, forma nova hoc loco. *Characteribus caulibus valde reductis vel absentibus. Inflorescentia unica, in centro rosulae basalis foliorum posita.* See Figure 2 for the typus and its visual description, in particular the second specimen positioned in the upper left part of the image.
- *Carlina acaulis* subsp. *caulescens* f. *typica* Cianfaglione, forma nova hoc loco. *Characteribus caulibus fere vel plene evolutis. Inflorescentiis pluribus, singulis in apice sui cuiusque caulis dispositis; caulibus ex rosula basali foliorum caulinorum orientibus*. See Figure 1 for the typus and its visual description.

In each form, the internal floral morphology, root morphology, and leaf morphology represent the typical life traits of the average nominal species, as indicated in the cited Floras. In the first proposed form, the stem is absent or nearly so, with a single inflorescence appressed to the ground and located in the center of the basal leaf rosette. In the second proposed form, the stem is typically well developed and bears cauline leaves. Several inflorescences are present per plant; each inflorescence is borne at the apex of its own stem, with stems arising from the same basal rosette.

From our results, the ecological, phytochemical, and genetic differences were not sufficiently evident to justify a higher-level separation of the typical (f. *typica*; with normal stems) and atypical (*f. decaulescens;* non-stemmed) forms into distinct higher-ranking taxa. The two forms remain distinguishable only through the observation of morphological traits associated with the length of the flowering stem.

In order to better clarify, catalogue, and interpret plant diversity, we propose a third possible form that can be inferred based on past descriptions, taxonomic proposals, and various interpretations of specimens that are attributable to the *Carlina acaulis* subsp. *caulescens* group. This third form groups plants that cannot be clearly assigned to either of the two previously proposed forms, as they develop stems to an intermediate degree, falling between the dwarf-stem and subcaulescent morphotypes.

- We propose this taxon as *Carlina acaulis* subsp. *caulescens* f. *intermedia* Cianfaglione, forma nova hoc loco. *Haec forma morphologice characteres intermedios inter duas formas antea propositas exhibet.*

Our results represent a contribution to the knowledge of the botanical, ecological, chemical, and genetic characteristics and related traits of the genus *Carlina*, a relatively little-known and poorly documented genus, especially when considering Italian accessions, for which no data were available in the literature until now.

Further studies are needed to investigate the reasons of the stem reduction in the study subspecies and to determine whether other stem-reduced and stemless phenotypes previously proposed falling into *Carlina acaulis* subsp. *caulesecens* may belong to the form we propose, as well as to assess how closely or distantly related they are between them and others related taxa.

Microbiota analyses revealed high biodiversity in all soil samples. On one hand, this indicates that the soil ecological system is healthy and rich of biodiversity. However, comparisons between samples collected under the caulescent and non-caulescent plants consistently show a healthy soil ecological system in all cases. Despite this, the results do not provide evidence to distinguish environmental conditions or soil microorganism diversity that could account for specific factors or stressors contributing to the phenomenon of decaulescence in populations of *Carlina acaulis* subsp. *caulescens*.

This work contributes to raising awareness that the taxonomic status, phenotypic traits, and ecological assessment of the studied *Carlina* species still require further investigation. It also highlights the need to reconsider and emphasise the use of lower–rank taxa such as varieties and forms (cf. Cianfaglione and Pedrotti, 2019), which over time have increasingly been misused, synonymised with higher-ranking taxa, or neglected, thereby complicating the cataloguing and understanding of biological diversity, phenotypic variation, conservation and management strategies, and the classification of interspecific and intergeneric diversity within the genus *Carlina*.

About biological form, life cycle and behaviour of both *Carlina* species (*C. acaulis* and *C. acanthifolia*), an apparent inconsistency emerges when considering the reference Floras cited. Attempting to resolve this in a logically consistent manner, we assumed that these two species should be regarded as monocarpic hemicryptophytes, that can live for two or more years while producing only a single fruiting event during their life cycle. Conversely, based on observations conducted during the monitoring period in our study area, we surprisingly observed that at least one third of the flowering individuals of both species were able to regenerate leaves from the basal portion of the plants that had flowered in the previous year. We could confidently identify these as reiterative leaves rather than new seedlings, since the new sprouts were clearly oriented from the main hypogeal axis and did not show the typical morphological characteristics of new seedlings, being already arranged in leaf rosettes like in each adult sample and lacking cotyledons, as shown in Figure 9 and Figure 10, respectively for *C. acanthifolia* (first image) and *C. acaulis* (second image). The aerial portion of the monitored samples was always dead in spring, often completely detached, degraded or rotten. The hypogenous part was always

consistently found to be gnawed, frequently visited by rodents or colonised by insects like beetles or ants. Plants undergoing vegetative regrowth appeared as though the new shoots originated laterally from the main hypogenous portion, creating a sort of secondary ramification, generally arising in the underground portion, 3–5 cm below the soil surface. Individuals lacking the ability to reiterate appeared to have generally been subjected to predation and consequently a decay. At the beginning of the following growing season, the plants frequently showed signs of decay and detachment of the last year epigeous portion as a clear evidence of predation o of trampling. This condition resembles what is known for cultivated artichokes, which are often found died or rotten, in gardens, detached from the root after being gnawed by voles during winter. In our study area, both *Carlina* species have shown the ability to survive for several years (at least four) after their first flowering event, and to undergo multiple flowering cycles (one per year) for the same functional genetic individual. This capacity appears to be limited more by herbivory damage and decay agents than by any intrinsic, self-regulated end-of-cycle process. The ability to reiterate seems to depend on the mode of predation, particularly on whether the damage affects the collar region and the upper hypogeal tissues, which can allow resprouting and prevent

FIGURE 9
The reiteration of *Carlina acanthifolia* subsp. *acanthifolia* after flowering, during the early vegetative season. In the picture we can observe the spring appearance, with the above-ground remains from the previous year as well as from the year before, clearly showing the ability to reiterate and flower multiple times in successive years. The situation in this photo is particularly unusual because the above-ground parts were not separated from the rest of the plant, having been spared for several consecutive years by rodents and other herbivore attacks, remaining attached to the underground portion.

FIGURE 10
The reiteration of *Carlina acaulis* subsp. *caulescens* after flowering, during the early vegetative season.

plant decay. Injuries and trampling occurring after flowering or during the winter up to the following growing season may instead favour the decay of the plants. We can hypothesise that the apparent discrepancies between our observations on both *Carlina* species and the apparently contrasting information reported in the cited Floras may be linked to population-level variability or to differences in the intensity and type of predation, as well as to winter damage associated with varying environmental conditions, local natural stressors, and human impacts. All these factors may act directly or indirectly by promoting the decay. This would warrant future *ad hoc* monitoring at the individual plant level, for several years, in order to better elucidate the underlying causes and dynamics, and to assess any potential differences, if present, between the typical and decaulescent forms of *Carlina acaulis* subsp. *caulescens*.

Environmental analyses, including genetic and soil microbiota analyses, did not reveal any affinity of the current forms of *Carlina acaulis* subsp. *caulescens* with possible hybrids involving *Carlina acanthifolia*, which has similar ecological and morphological characteristics and often shares the same habitat. Nor did they show affinity with the subspecies *acaulis* of *Carlina acaulis*. This therefore also rules out the presence of *Carlina acaulis* subsp. *acaulis* at the study site, and consequently excludes the possibility of an anthropogenic introduction of this taxon. The *Carlina acaulis* non-stemmed specimens that we studied may be considered as decaulescent forms of *Carlina acaulis* subsp. *caulescens*. The forms described in the literature with developed stems, although clearly shorter than usual, should consequently be considered intermediate forms. All three forms: the typical, the intermediate,

and the decaulescent one, should therefore be regarded as three different forms, representing a mere expressions of intrasubspecific phenotypic variability. Although *Carlina acaulis* subsp. *caulescens* is a common taxon in Italy and particularly for the study area, it can be argued that the intermediate forms emerging from the cited literature, and especially the decaulescent one should be regarded as rare, deserving of greater scientific attention, warranting further monitoring and investigation. This applies also from a conservation perspective and even from a cultural standpoint.

Our fieldwork activities also allowed us to meet people who frequent these areas allowing us to gather information on the taxa under study as well as on local perceptions, uses, and recollections. Although this activity does not constitute a comprehensive or detailed ethnobotanical investigation, these conversations provided valuable information on the taxa under study and offered insights into local perceptions, knowledge, uses, and memories. This helped us better understand potential conservation threats and identify which taxa may be more vulnerable to anthropogenic impacts. This also supports the need to assign and recognise clear names to distinguish and refer to the different taxa we analysed and proposed, as the different morphological forms did not appear to be perceived in the same way, nor to face the same levels of risk according to interview outcomes. These interviews highlighted specific risks faced by members of the *Carlina* genus, particularly the decaulescent form *Carlina acaulis* subsp. *caulescens* f. *decaulescens*, since it is the rarer taxa, and the most subject in relation to both harvesting and damaging activities. The studied *Carlina* species and associated grasslands have historically been favoured by human pressure, both past and present; nevertheless, non-stemmed forms are more threatened, as they are frequently collected for traditional uses, new agri and food trends, and for decoration purposes, and are more easily damaged by trampling or machinery, likely due to their lower visibility. Human impact on the study area is evidenced by local press sources, as well as by analyses of photographs, aerial imagery, and field observations. Traces of human, livestock, and mechanical trampling are frequent, and damaged specimens were often recorded as a consequence of such disturbances, as observed during our fieldwork.

The expression of the decaulescence biological phenomenon, it cannot be directly associated with possible teratological phenomena or malformations caused by environmental stressors or pests, since we observed in the field that these specimens were not influenced by temperature or drought conditions, since we found each year healthy specimens from both forms (typical and decaulescent), often vegetating very close each other. The analysed environmental factors would logically justify the presence of smaller or dwarf individuals, as well as different chemical profiles or other reduced morphological and reproductive traits. However, in our case, all the characteristics of the decaulescent form fall fully within the average range of the subspecies, according to the cited literature, except for the stem length morphology. Field analyses enabled us to select healthy specimens, excluding plants exhibiting clear malformations (i.e., fasciation, curling, swelling), or showing signs of pathogens, herbivore attacks, or trampling, from the genetic and chemical analyses. This approach was intended to minimise environmental bias and consequently to better assess the extent to which these traits may instead be associated with genetic factors. Soil biota analyses revealed a high microbial biodiversity associated with the rhizosphere; however, they did not provide sufficient resolution to detect patterns that could explain the observed variation in stem expression. No clear microbial or edaphic differences emerged that could account for the pronounced stem development phenotypic divergence. These results indicate the need for further deeper investigations in a broader spatial sampling design.

4 Conclusions

The knowledge of *Carlina acaulis* as well *as Carlina acanthifolia* is very complex, and this is reflected in the considerable changes that these two botanical entities have undergone over time in their taxonomic interpretation. This, in turn, reflects in one hand the lack of knowledge and in the other hand it underlines the many contradictions or still unresolved issues, despite the significant progress in the understanding and study of the Carlineae. Many aspects remain to be clarified, and our study contributes to this objective. This makes our study locally significant while also offering insights of broader relevance for understanding this genus diversity on a wider global geographical scale.

Our investigation, combined with recent advances from literature, has made it possible to clarify many biogeographic, taxonomic and systematic positions, allowing previous interpretations to be disambiguated through the proposal of new taxa related to *Carlina acaulis* subsp. *caulescens* forms, as presented in our study. Otherwise, our results, when compared with the bibliographic analysis of the cited literature, indicate that after more than a century of hypotheses and investigations, and despite significant scientific progress, as well as the development of modern techniques and knowledge, the questions regarding traits and phenotypic variability in both studied *Carlina* species, as well as the complex stem expression in *Carlina acaulis*, remain open and intriguing issues, leaving much still to be explored in future research.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AB: Methodology, Writing – review & editing. CF: Conceptualization, Methodology, Writing – review & editing. FV: Formal analysis, Methodology, Writing – original draft. DDV: Formal analysis, Writing – review & editing. RS: Resources, Visualization, Writing – review & editing. LS: Formal analysis, Methodology, Writing – review & editing. VRS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. KC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

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References

AAA - American Anthropological Association (2012). Statement on ethics: Principles of professional responsibility. Available online at: https://ethics.americananthro.org/category/statement/ (Accessed March 23, 2026).

Abdullah, S. A., Jamil, S., and Zainuddin, Z. (2022). Chemical constituents from the twigs of Malaysian Syzygium griffithii. *Chem. Nat. Compd.* 58, 548–549. doi: 10.1007/s10600-022-03731-9

Acta Plantarum (2026). Flora d'Italia. Scheda taxon. *Carlina acanthifolia* All. subsp. utzka (Hacq.) Meusel & Kästner. Available online at: https://www.actaplantarum.org/flora/flora_info.php?id=1768 (Accessed March 23, 2026).

APG - The Angiosperm Phylogeny Group (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot. J. Linn. Soc* 181, 1–20. doi: 10.1111/boj.12385

Barres, L., Sanmartín, I., Anderson, C. L., Susanna, A., Buerki, S., Galbany-Casals, M., et al. (2013). Reconstructing the evolution and biogeographic history of tribe Cardueae (Compositae). *Am. J. Bot.* 100, 867–882. doi: 10.3732/ajb.1200058

Bartolucci, F., Peruzzi, L., Galasso, G., Albano, A., Alessandrini, A., Ardenghi, N. M. G., et al. (2018). An updated checklist of the vascular flora native to Italy. *Plant Biosyst.* 152, 179–303. doi: 10.1080/11263504.2017.1419996

Berg, G., and Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol. Ecol.* 68, 1–13. doi: 10.1111/j.1574-6941.2009.00654.x

Bilz, M., Kell, S. P., Maxted, N., and Lansdown, R. V. (2011). *European Red List of Vascular Plants* (Luxembourg: Publications Office of the European Union).

CCDB (2026a). Chromosome Counts Database (CCDB, version 1.66.6). Available online at: https://ccdb.tau.ac.il/Angiosperms/Compositae/Carlina/Carlina%20acanthifolia%20All./ (Accessed March 23, 2026).

CCDB (2026b). *Chromosome Counts Database (CCDB, version 1.66.6)*. Available online at: https://ccdb.tau.ac.il/Angiosperms/Compositae/Carlina/Carlina%20acaulis%20L./. (Accessed March 24, 2026).

Chang, Y., Zhang, P., Zhang, X., Chen, J., Rausch, W.-D., Gula, A., et al. (2017). Cytotoxic activities of flavonoids from the traditional Mongolian medicinal herb Clematis aethusifolia Turcz. *Nat. Prod. Res.* 31, 1223–1227. doi: 10.1080/14786419.2016.1230112

Chase, M. W., Cowan, R. S., Hollingsworth, P. M., Van Den Berg, C., Madriñán, S., Petersen, G., et al. (2007). A proposal for a standardised protocol to barcode all land plants. *Taxon* 56, 295–299. doi: 10.1002/tax.562004

Cianfaglione, K. (2014). "*L'importanza degli alberi e del bosco. Cultura, scienza e coscienza del territorio (2 vols.)*," Trento, Italy: Temi Editore, 980.

Cianfaglione, K. (2024). An editorial to introduce the new journal wild: issues, approaches, ideas and proposals. *Wild* 1, 30–38. doi: 10.3390/wild1010003

Cianfaglione, K., Bottacci, A., Landi, S., Schirone, B., Pedrotti, F., and Di Bonito, M. (2025). "Conifers reforestations: An environmental problem or a possible resource?," in *Rethinking Networks in Times of Transition: Ecological, Energy and Social Issues*. Eds. L. Dobigny and B. Robyns (London, UK and Hoboken: ISTE & Wiley), 37–85. doi: 10.1002/9781394427550.ch3

Cianfaglione, K., and Pedrotti, F. (2019). The Red Beech at the "Montagna di Torricchio", Marche Region, Central Italy. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 47, 860–866. doi: 10.15835/nbha47311583

Conti, F., Abbate, G., Alessandrini, A., and Blasi, C. (2005). *An Annotated Checklist of the Italian Vascular Flora* (Roma: Palombi Editore), 71.

Coste, H. (1937). *Flore Descriptive Et Illustrée De La France, De La Corse Et Des Contrées Limitrophes: Tome 2 : 358-366. Second Tirage* (Paris: Librairie des Sciences et des Arts).

CWG – Compositae Working Group (2025). Global Compositae Database. Carlina aggregata f. nana Degen. Available online at: https://www.compositae.org/gcd/aphia.php?p=taxdetails&id=1240360 (Accessed March 23, 2026).

Davis, K. E. R., Sangwan, P., and Janssen, P. H. (2011). Acidobacteria, Rubrobacteridae and Solirubrobacterales are abundant in Australian soils. *Environ. Microbiol.* 13, 2011–2025. doi: 10.1111/j.1462-2920.2011.02459.x

Devesa Alcaraz, J. A., Quintanar Sánchez, A., and García García, M.Á. (2014). *Flora Iberica. Plantas Vasculares De La Península Ibérica E Islas Baleares (Vol. XVI(I): Compositae [Partim])*. Eds. J. A. Devesa Alcaraz, A. Quintanar Sánchez and M.Á. García García (Madrid, Spain: Real Jardín Botánico, CSIC). Available online at: http://www.floraiberica.es/floraiberica/texto/pdfs/16_159_001_Carlina.pdf (Accessed March 23, 2026).

Dombris, M. A., and Raynaud, J. (1981). The flavonoid heterosides from *Carlina corymbosa* leafy twigs. *Plantes Médicinales Phytothérapie* 15, 21–24.

Đorđević, S., Tadić, V., Petrović, S., Kukić-Marković, J., Dobrić, S., Milenković, M., et al. (2012). Bioactivity assays on *Carlina acaulis* and *C. acanthifolia* root and herb extracts. *Dig. J. Nanomater. Biostruct.* 7, 1213–1222.

Euro+Med. (2026). *Database Online:* Carlina acaulis *subsp. simplex*. Available online at: https://europlusmed.org/cdm_dataportal/taxon/058e0163-e611-4e8a-9e57-ab67a5cb3b49/synonymy?highlight=d727367c-cfed-4914-b28f-7f8f655f7cf4#d727367c-cfed-4914-b28f-7f8f655f7cf4 (Accessed March 23, 2026).

Fierer, N. (2017). Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* 15, 579–590. doi: 10.1038/nrmicro.2017.87

Flora di Lucoli (2012). La ricerca floristica nel territorio di Lucoli nel 2010 e nel 2011. Le ultime dalla natura, marzo 2012. Available online at: https://www.lucoli.it/floradilucoli/News.html (Accessed March 23, 2026).

Funk, V. A., Susanna, A., Steussy, T. F., and Bayer, R. J. (2009). *Systematics, Evolution, and Biogeography of Compositae* (Vienna: International Association for Plant Taxonomy (IAPT), 965.

Giampaoli, S., Berti, A., Di Maggio, R. M., Pilli, E., Valentini, A., Valeriani, F., et al. (2014). The environmental biological signature: NGS profiling for forensic comparison of soils. *Forensic Sci. Int.* 240, 41–47. doi: 10.1016/j.forsciint.2014.02.028

Hajibabaei, M., Smith, M. A., Janzen, D. H., Rodriguez, J. J., Whitfield, J. B., and Hebert, P. D. N. (2006). A minimalist barcode can identify a specimen whose DNA is degraded. *Mol. Ecol. Notes* 6, 959–964. doi: 10.1111/j.1471-8286.2006.01470.x

Herrando-Moraira, S., Calleja, J. A., Galbany-Casals, M., Garcia-Jacas, N., Liu, J.-Q., López-Alvarado, J., et al. (2019). Nuclear and plastid DNA phylogeny of the tribe Cardueae (Compositae) with Hyb-Seq data: A new subtribal classification and a temporal framework for the origin of the tribe and the subtribes. *Mol. Phylogenet. Evol.* 137, 313–332. doi: 10.1016/j.ympev.2019.05.001

Hu, W., Zhou, J., Shen, T., and Wang, X. (2019). Target-guided isolation of three main antioxidants from *Mahonia bealei* (Fort.) Carr. leaves using HSCCC. *Molecules* 24, 1907. doi: 10.3390/molecules24101907

ISE - International Society of Ethnobiology (2008). Code of Ethics. Version 2008. Available online. Available online at: https://www.ethnobiology.net/what-we-do/core-programs/ise-ethics-program/code-of-ethics/ (Accessed March 23, 2026).

Jaiswal, R., Deshpande, S., and Kuhnert, N. (2011). Profiling the chlorogenic acids of *Rudbeckia hirta*, Helianthus tuberosus, *Carlina acaulis* and *Symphyotrichum novae-angliae* leaves by LC-MS^n. *Phytochem. Anal.* 22, 432–441. doi: 10.1002/pca.1299

Kadereit, J. W., and Jeffrey, C. (2007). *The Families and Genera of Vascular Plants, Volume Viii, Flowering Plants. Eudicots* (Berlin, Heidelberg: Springer). doi: 10.1007/978-3-540-31051-8

Lamarck, J.-B. (1783). *Encyclopédie Méthodique. Botanique* (Paris: chez Panckoucke librairie, Hôtel de Thou; and Liège, chez Plomteux, Imprimeurs d'Etat), 623.

Lambinon, J., de Langhe, J.-E., Delvosalle, L., and Duvigneaud, J. (1992). *Nouvelle Flore De La Belgique, Du Grand-Duché De Luxembourg, Du Nord De La France Et Des Régions Voisines (Ptéridophytes Et Spermatophytes); 4^e Éd* (Meise, Belgium: Edition du Jardin botanique de Meise), 1092.

Lauber, C. L., Hamady, M., Knight, R., and Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure. *Appl. Environ. Microbiol.* 75, 5111–5120. doi: 10.1128/AEM.00335-09

Ludwig, W., Euzéby, J., Schumann, P., Busse, H.-J., Trujillo, M. E., Kämpfer, P., et al. (2015). "Road map of the phylum Actinobacteria," in *Bergey's Manual of Systematic Bacteriology*. Eds. M. Goodfellow, P. Kämpfer, H.-J. Busse, M. E. Trujillo, K. Suzuki and W. Ludwig (Hoboken, NJ, USA: John Wiley & Sons, Inc., Bergey's Manual Trust), 1–37. doi: 10.1002/9781118960608.bm00029

Melnyk, V. (2011). *Carlina onopordifolia*. The IUCN Red List of Threatened Species 2011: e.T161848A5503822. doi: 10.2305/IUCN.UK.2011-1.RLTS.T161848A5503822.en (Accessed March 23, 2026).

Meusel, H., and Kästner, A. (1994). *Lebensgeschichte Der Gold- Und Silber-Disteln. Monographie Der Mediterran-Mitteleuropäischen Compositen-Gattung Carlina. Band Ii: Artenvielfalt Und Stammesgeschichte Der Gattung* (Wien–New York: Springer Verlag), 697. doi: 10.1007/978-3-7091-3069-8

Meusel, H., and Werner, K. (1962). Über die Gliederung von *Carlina acaulis* L. und *Carlina vulgaris* L. *Wissenschaftliche Z. der Universität Halle Math.-Nat. Reihe* 11, 279–292.

Mountain Wilderness (2024). Abruzzo, non c'è tregua per i Piani di Pezza: un'area protetta su cui sorgerà uno stadio per lo sci. Il Fatto Quotidiano. 7 Marzo. Ambiente; Opinioni dal Blog. Online. Available online at: https://www.ilfattoquotidiano.it/2024/03/07/abruzzo-non-ce-tregua-per-i-piani-di-pezza-unarea-protetta-su-cui-sorgera-uno-stadio-per-lo-sci/7468842/ (Accessed March 23, 2026).

Ni, L., Qiu, Y.-T., Shi, Y., Chen, J., and Xu, H.-Y. (2019). Chemical constituents of the roots of *Ormosia hosiei*. *Chem. Nat. Compd.* 55, 972–974. doi: 10.1007/s10600-019-02866-6

Pawłowski, B., and Jasiewicz, A. (1972). *Flora Polska. Rośliny Naczyniowe Polski I Ziem Ościennych. Tom 13. Dwuliścienne, Zrosłopłatkowe. Część Iv*. Eds. B. Pawłowski and A. Jasiewicz (Warszawa–Kraków: Państwowe Wydawnictwo Naukowe), 94–100.

Petkova, N., Ivanov, I., Saralieva, E., Georgieva, D., Nikolova, K., Eftimov, T., et al. (2024). Bioactive compounds of *Carlina acanthifolia* All. roots obtained by fractional extraction and their 3D fluorescence spectra. *Opt. Quantum Electron.* 56, 1284. doi: 10.1007/s11082-024-07044-9

Pignatti, S. (1982). *Flora D'Italia (Vol. 3)* (Bologna: Edagricole), 217.

Pignatti, S., Guarino, R., and La Rosa, M. (2018). *Flora D'Italia. Second Rev. & Augmented Edition. Vol. 3: Asteraceae* (Bologna: Edagricole).

PLADIAS (2026). Database of the Czech flora and vegetation. Available online at: https://pladias.cz/en/taxon/data/Carlina%20acaulis%20subsp.%20acaulis? (Accessed March 23, 2026).

POWO (2026a). Plants of the World Online. *Carlina acaulis* L. Available online at: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:189213-1#synonyms (Accessed March 23, 2026).

POWO (2026b). Plants of the World Online. Carlina aggregata f. nana. Available online at: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:77181590-1 (Accessed March 23, 2026).

Pratesi, F. (2019). *Storia Della Natura D'italia* (Soveria Mannelli (CZ): Rubbettino Editore), 1–236.

Sarlieva, E. N., Petkova, N. T., Ivanov, I. G., Aneva, I. Y., Georgiev, V. G., and Nikolova, K. T. (2023). Polyphenolic compounds, triterpenes, carlina oxide, antioxidant activity and carbohydrate profile of different vegetal parts of *Carlina vulgaris* L. *Carlina acanthifolia* All. and *Carlina corymbosa* L. *Trop. J. Natural Product Res.* 7, 4242–4248. doi: 10.26538/tjnpr/v7i10.18

Sennen, F. (1927). Nombreuses localités de plantes nouvelles pour la Cerdagne, observées durant les vacances des années 1915 à 1926 (1). *Bull. la Société Botanique France* 5e série, tome III, 74, 355–410.

Sowa, S., Kulik, M., Koroluk, A., Toporowska, J., Marek, E., Szewczyk, W., et al. (2020). Genetic structure of *Carlina acanthifolia* subsp. utzka populations on the north-western margins of the species range. *Global Ecol. Conserv.* 24, e01225. doi: 10.1016/j.gecco.2020.e01225

Sowa, I., Mołdoch, J., Dresler, S., Kubrak, T., Soluch, A., Szczepanek, D., et al. (2023b). Phytochemical profiling, antioxidant activity, and protective effect against H_2O_2-induced oxidative stress of *Carlina vulgaris* extract. *Molecules* 28, 5422. doi: 10.3390/molecules28145422

Sowa, I., Mołdoch, J., Paduch, R., Strzemski, M., Szkutnik, J., Tyszczuk-Rotko, K., et al. (2023a). Polyphenolic composition of *Carlina acaulis* L. extract and cytotoxic potential against colorectal adenocarcinoma and cervical cancer cells. *Molecules* 28, 6148. doi: 10.3390/molecules28166148

Sowa, I., Paduch, R., Mołdoch, J., Szczepanek, D., Szkutnik, J., Sowa, P., et al. (2023c). Antioxidant and cytotoxic potential of *Carlina vulgaris* extract and bioactivity-guided isolation of cytotoxic components. *Antioxidants* 12, 1704. doi: 10.3390/antiox12091704

Strzemski, M., Wójciak-Kosior, M., Sowa, I., Rutkowska, E., Szwerc, W., Kocjan, R., et al. (2016). Carlina species as a new source of bioactive pentacyclic triterpenes. *Ind. Crops Prod.* 94, 498–504. doi: 10.1016/j.indcrop.2016.09.025

Strzemski, M., Wojnicki, K., Sowa, I., Wojas-Krawczyk, K., Krawczyk, P., Kocjan, R., et al. (2017). *In vitro* antiproliferative activity of extracts of *Carlina acaulis* subsp. caulescens and *Carlina acanthifolia* subsp. *utzka*. *Front. Pharmacol.* 8, 371. doi: 10.3389/fphar.2017.00371

Taberlet, P., Coissac, E., Hajibabaei, M., and Rieseberg, L. H. (2012). Environmental DNA. *Mol. Ecol.* 21, 1789–1793. doi: 10.1111/j.1365-294x.2012.05542.x

Tajuddeen, N., Swart, T., Hoppe, H. C., and van Heerden, F. R. (2022). Phytochemical, antiplasmodial, and cytotoxic investigation of *Euclea natalensis* A.DC. subsp. *natalensis* leaves. *Chem. Biodiversity* 19, e202200150. doi: 10.1002/cbdv.202200150

Tela Botanica (2026). *Carlina acaulis* f. Nana (Degen) Meusel & Werner. Available online at: https://www.tela-botanica.org/bdtfx-nn-77800-synthese (Accessed March 23, 2026).

Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., et al. (1976). *Flora Europaea* (Cambridge: Cambridge University Press) 4, I–505.

Verloove, F., Van Rossum, F., Devriese, H., Matysiak, J.-P., Ronse, A., Van De Beek, A., et al. (2024). *Nouvelle Flore De La Belgique, Du Grand-Duché De Luxembourg, Du Nord De La France Et Des Régions Voisines (Ptéridophytes Et Spermatophytes). Septième Édition* (Meise, Belgium: Edition du Jardin botanique de Meise), 1–1000, ISBN: .

von Degen, Á. (1938). *Flora Velebitica, III Band* (Budapest: Verlag der Ungarischen Akademie der Wissenschaften), 1–597.

WFO Plant list (2026). *Carlina Aggregata* f. Nana Degen. Available online at: https://www.worldfloraonline.org/taxon/wfo-0000125016 (Accessed March 23, 2026).