RESEARCH ARTICLE

Different species or altitudinal morphotypes? Testing the taxonomic value of *Dianthus brachycalyx* (Caryophyllaceae)

Jacopo Franzoni,[1] Giovanni Astuti,[2] Fabrizio Bartolucci,[3] Liliana Bernardo,[4] Simone Fior,[5] Gabriele Casazza,[6] Fabio Conti,[3] Duilio Iamonico,[7] Mauro Iberite,[7] Luigi Minuto,[6] Alessia Turini[1] & Lorenzo Peruzzi[1]

1 *PLANTSEED Lab, Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy*
2 *Botanic Garden and Museum, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy*
3 *Floristic Research Centre of the Apennines (School of Bioscience and Veterinary Medicine, University of Camerino – Gran Sasso and Monti della Laga National Park), Via Prov. Km 4.2, 67021 Barisciano (L'Aquila), Italy*
4 *DiBEST, University of Calabria, 87030 Arcavacata di Rende (Cosenza), Italy*
5 *Institute of Integrative Biology, ETH Zürich, Universitätstrasse 16, 8092 Zürich, Switzerland*
6 *DISTAV, University of Genova, Corso Europa 26, 16132 Genova, Italy*
7 *Department of Environmental Biology, Sapienza University of Roma, Piazzale Aldo Moro 5, 00185 Roma, Italy*
Address for correspondence: *Jacopo Franzoni, jacopo.franzoni@phd.unipi.it*

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Abstract Defining species boundaries within plant groups distributed along wide elevational and geographic gradients may lead to unstable taxonomic delimitations using morphological data only. Within the taxonomically challenging genus *Dianthus*, through an integrative approach we tested the taxonomic value of *D. brachycalyx*, a putative species endemic to mountain peaks of the central-southern Apennines, with respect to the widespread central-Mediterranean *D. virgineus*. We measured 30 morphological characters in 452 individuals from 25 populations and obtained 3202 single nucleotide polymorphisms using ddRAD-seq techniques in 394 individuals from 36 populations. For climatic niche comparison, we built a dataset of 348 occurrence points. By comparing morphometric, genetic, and climatic niche data, we showed that *D. brachycalyx* cannot be considered a distinct species. Morphometric separation between the two species is detectable, but high-elevation populations of *D. virgineus* are similar to *D. brachycalyx*. Genetic analyses revealed that the genetic structure of populations of *D. brachycalyx* and *D. virgineus* is mainly shaped by isolation-by-distance, irrespective of their taxonomic attribution. The climatic niches of the two species are overlapping, and the niche differences are just due to different availability of climatic conditions in their ranges. Accordingly, multiple lines of evidence do not support a separation of *D. brachycalyx* from *D. virgineus*, and the former should be considered a heterotypic synonym of the latter.

Keywords Apennines; ddRAD-seq; ecological niche comparison; integrative taxonomy; morphometry; wild carnations

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

The strong ecological pressures in mountain environments are important drivers of morphological differentiation in plants, which may result in intraspecific variation, either caused by local adaptation (Halbritter & al., 2018) or by phenotypic plasticity (Gonzalo-Turpin & Hazard, 2009). Thus, defining species boundaries within plant groups distributed along wide elevational gradients, and heterogeneous environments in general, may lead to unreliable taxonomic delimitations if only morphological data are used.

For instance, the species-rich genus *Dianthus* L. (Fassou & al., 2022) shows great phenotypic responses to the environment in terms of geological substrate, climate, and elevation (Hamzaoğlu & al., 2015; Hardion & al., 2020; Castro & al., 2022; Franzoni & al., 2023; Pålsson & al., 2023). This great responsiveness to different environments, combined with rapid radiation (Valente & al., 2010) and weak reproductive barriers (Carolin, 1957), resulted in morphological patterns that are often difficult to interpret taxonomically. In Europe, this morphological variation has been organized in complexes of closely related taxa that are geographically and/or ecologically isolated but differ only slightly morphologically (Tutin & Walters, 1993). Such a splitting approach led to intricate taxonomies within the genus.

In this context, the *Dianthus virgineus* L. complex (previously erroneously referred as *D. caryophyllus* L. complex or *D. sylvestris* Wulfen complex) is the most emblematic in

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terms of taxonomic complexity (Domina & al., 2021a,b). This complex includes around 30 taxa mainly distributed across the Italian and Balkan peninsulas and major central Mediterranean islands (Marhold, 2011; Meyer, 2011; Tison & de Foucault, 2014; Bartolucci & al., 2024). Although some widespread species are supported by genetic and phenotypic data (Gargano & al., 2023), many other taxa remain underinvestigated. Recent studies on Balkan populations revealed intraspecific variation according to environmental conditions and phylogeographical history, underlining the inconsistency of some of these units (Terlević & al., 2022, 2023). In central-southern peninsular Italy, Sardinia and Sicily, this complex includes 21 taxa, most of which are narrow endemics to montane ranges or isolated peaks (Bacchetta & Brullo, 2000; Bacchetta & al., 2010; Brullo & al., 2015). All these taxa lack karyological discontinuities, are diploid with $2n = 30$ chromosomes and show only a slight geographical genome size variation, not correlated at all with current taxonomy (Franzoni & al., 2024). However, since integrative studies employing multiple lines of evidence are not available, the taxonomic value of all these units is still not clear.

In particular, *Dianthus brachycalyx* A.Huet & É.Huet ex Bacch. & al. is a small-sized carnation endemic to mountain peaks (1500–2500 m a.s.l.) of the central-southern Apennines (Bacchetta & al., 2010; Bartolucci & al., 2024). The distribution range of this species is nested within that of *D. virgineus*, which is widespread across a broad geographical and elevational (0–1800 m a.s.l.) span from southern France to southern Italy (Gargano & al., 2023; Luqman & al., 2023). According to the latest taxonomic revisions of the complex (Bacchetta & al., 2010; Brullo & Guarino, 2017, 2019), *D. brachycalyx* differs morphologically from *D. virgineus* (= *D. longicaulis* Ten.; Domina & al., 2021b) in central-southern Italy by its smaller size, shorter basal and cauline leaves, and less, smaller flowers showing less epicalyx scales. However, the plants growing in the central Apennines are not always easily identified (Conti & Bartolucci, 2015), and in the southern Apennines a morphological continuum between *D. brachycalyx* and *D. virgineus* was observed along an elevational gradient (Rovito & al., 2022), which makes it difficult to clearly diagnose these two taxa. Species delimitation is further limited by a lack of genetic information supporting *D. brachycalyx*.

Here, we employed integrative approaches to taxonomy by comparing morphometric, genetic, and climatic niche data of *Dianthus brachycalyx* and *D. virgineus* to understand whether the former taxon represents a solid and reliable species hypothesis.

■ MATERIALS AND METHODS

Sampling. — We included in the study 35 populations attributed to *Dianthus brachycalyx* (5) and *D. virgineus* (30), including their type localities, by assembling published data and information from newly sampled populations (Fig. 1). Populations were selected to cover the whole distribution range of the two taxa. Morphometric and genetic data of 10 populations from Toscana (central Italy) were retrieved from a previous work (Franzoni & al., 2023). For genetic analyses, 11 populations from Luqman & al. (2023) were included. For each of the 14 newly sampled populations, we collected 10–20 individuals, which were prepared and conserved as herbarium specimens, from which we obtained morphometric data. From a subset of individuals, leaves were collected and quickly silica dried for DNA extraction. The topotypical population of *D. inodorus* (L.) Gaertn., a species that represents a separated genetic lineage from *D. virgineus* (Gargano & al., 2023; Luqman & al., 2023), was sampled and used as an outgroup to build a distance-based tree. Vouchers of the studied materials are deposited in PI or Z+ZT (Appendix 1; suppl. Table S1).

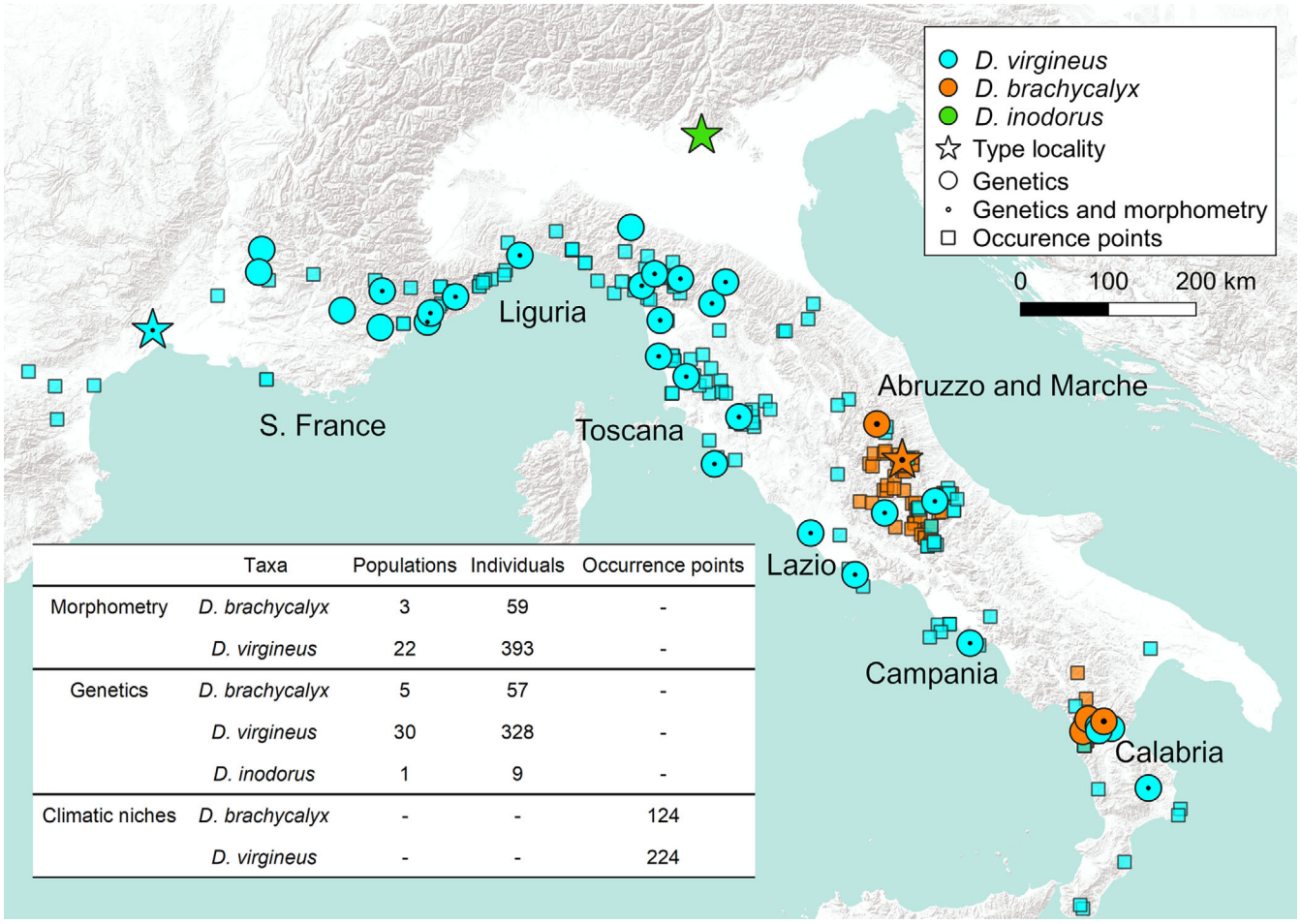
Morphometric analyses. — Morphometric data were obtained from 452 herbarium specimens. We measured 18 numerical and 12 categorical characters concerning stem, leaf, epicalyx, and flower features (Fig. 2, Table 1). All these characters are reported as important to discriminate taxa within the *Dianthus virgineus* complex (Tutin & Walters, 1993; Bacchetta & al., 2010; Brullo & Guarino, 2019; Gargano & al., 2023). An illustration of the measured numerical characters is represented in Fig. 2, whereas the states of the categorical characters are listed in Table 1. In this work, we considered as upper cauline leaves those leaf pairs situated right below the bracts, and as lower cauline leaves those leaf pairs just above the lower internode. We defined as bracts the leaves below the floral pedicel in case of a single-flowered stem (Fig. 2A), or below the first dichotomous branching of the inflorescence in case of a many-flowered stem (Fig. 2B). The floral pedicel of a single-flowered stem was defined as the portion of the stem that supports the flower, inclusive of any scale-like bracteoles not belonging to the epicalyx.

We produced a morphometric dataset including 452 specimens × 30 variables. This matrix was subjected to a series of filtering steps to prepare it for further multivariate analyses. All the analyses were performed in an R environment (R Core Team, 2023) using different packages, as specified case by case. First, we removed all cases containing missing data. Second, we removed all variables exhibiting no significant difference between the two taxa. For all tests, the significance threshold was set at 0.01. For numerical characters, we first assessed if they were normally distributed within taxa and had homogeneous variances between taxa. Deviation from normal distribution was tested using a Shapiro-Wilk test per taxa, with the RVAideMemoire v.0.9-83-7 R package (Herve, 2023). Heteroscedasticity was assessed with the Brown-Forsythe test, implemented in the onewaytests v.3.0 R package (Dag & al., 2018). Differences of non-normal and heteroscedastic characters between taxa were checked with permutation tests, as they do not require any assumption on data distribution, with the rcompanion v.2.4.34 R package (Mangiafico, 2023). Differences of non-normal and homoscedastic characters between taxa were checked with the

Wilcoxon test. Effect size of significant differences in numerical characters was estimated with Cohen's d using the lsr v.0.5.2 R package (Cohen, 1988; Navarro, 2015). Association between taxa and categorical characters was tested with the Fisher exact test, and Cramér's V was used as an effect size estimate (Cramér, 1946; Mangiafico, 2023). We also tested statistical correlation among numerical characters with Spearman's correlation test, to exclude characters showing $r > |0.8|$ from multivariate analyses. Finally, numerical characters were scaled to account for their different units of measure.

Given the mixed nature of our morphometric dataset, to visualize the multivariate morphospace generated by the measured individuals, we opted for a factor analysis of mixed data (FAMD; Pagès, 2004). FAMD is a dimensionality reduction method that can process quantitative and qualitative data, allowing the user to analyze the contribution of both data types in explaining the similarity among studied individuals. The R packages FactoMineR v.2.9 (Lê & al., 2008) and factoextra v.1.0.7 (Kassambara & Mundt, 2020) were employed for this analysis. After exploring the morphospace and the contribution of the characters to it, we tested significant differences of components (called dimensions in FAMD) between taxa with permutation tests. To understand the relation between overall plant morphology and elevation across the study area, we performed a Spearman's correlation test between elevation and principal dimensions and fitted a linear model. All the linear model assumptions were assessed by checking the distribution of residuals. We performed multivariate morphometric analyses on the complete dataset as well as on a reduced dataset that accounted for the different sample size between the two species. This included the three *Dianthus brachycalyx* populations (two from the central Apennines, including type locality, and one from the southern Apennines) with morphometric data and three montane populations of *D. virgineus* (two from the northern Apennines and one from the central Apennines). Specifically, we retained two populations of *D. virgineus* from the northern Apennines and one population from the central Apennines sampled near the type locality of *D. brachycalyx*, but at a lower elevation.

Concerning the morphological description provided in the final taxonomic setting, it contains mean ± standard



	Taxa	Populations	Individuals	Occurrence points
Morphometry	*D. brachycalyx*	3	59	-
	D. virgineus	22	393	-
Genetics	*D. brachycalyx*	5	57	-
	D. virgineus	30	328	-
	D. inodorus	1	9	-
Climatic niches	*D. brachycalyx*	-	-	124
	D. virgineus	-	-	224

Fig. 1. Distribution and number of studied populations and occurrence points of *Dianthus virgineus*, *D. brachycalyx* and *D. inodorus* across southern France and peninsular Italy.

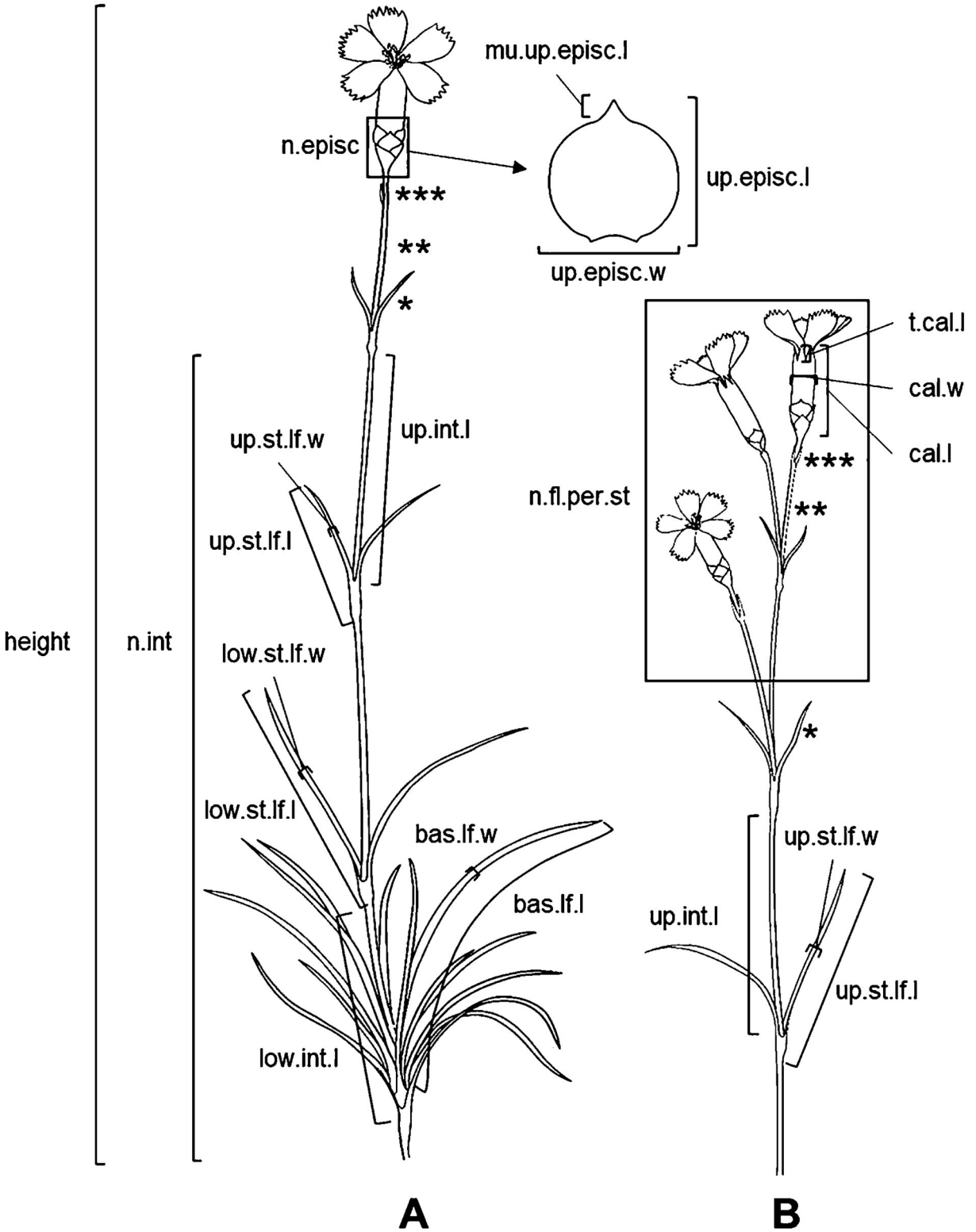


Fig. 2. Typical morphology of individuals belonging to the *Dianthus virgineus* complex. **A**, One-flowered stem; **B**, Many-flowered stem. *, bracts; **, flower pedicels; ***, bracteoles. Numerical characters measured in this study: bas.lf.l, basal leaf length; bas.lf.w, basal leaf width; cal.l, calyx length; cal.w, calyx width; height, plant height; low.int.l, lower internode length; low.st.lf.l, lower stem leaf length; low.st.lf.w, lower stem leaf width; mu.up.episc.l, upper epicalyx scale mucro length; n.episc, number of epicalyx scales; n.fl.per.st, number of flowers per stem; n.int, number of internodes; t.cal.l, calyx teeth length; up.episc.l, upper epicalyx scale length; up.episc.shape, upper epicalyx scale shape; up.episc.w, upper epicalyx scale width; up.st.lf.l, upper stem leaf length; up.st.lf.w, upper stem leaf width; up.int.l, upper internode length. — Illustration by Chiara Di Bella.

deviation values for each numerical character. Variables concerning lower epicalyx scales, corolla, anthers, and ovary features have been measured for this purpose, albeit not included in this study due to a high percentage of missing data.

R scripts for replicating the morphometric analyses are available in suppl. Appendix S1.

Genetic analyses. — DNA extraction followed the protocol employed by Franzoni & al. (2023) relying on the "sbeadex Maxi Plant Kit" (LGC Genomics, Hoddesdon, U.K.) and using a KingFisher Flex Purification System (Thermo Scientific, Waltham, Massachusetts, U.S.A.). Briefly, 10–20 mg of silica-dried leaf samples were finely ground for 4 min using a Retsch-Mill (Retsch, Haan/Düsseldorf, Germany) and metal beads, and incubated with Lysis Buffer PN (350 µl per sample), thioglycerol (3.5 µl per sample), and DCB (10 µl per sample) at 65°C and 1000 rpm for at least 60 min. After centrifugation, 200 µl of the resulting supernatant for each sample was added to a binding solution (400 µl of Binding buffer PN, 9 µl of sbeadex particle suspension). The KingFisher machine was loaded with lysed samples, Wash buffer PN1 (400 µl per sample), Wash buffer PN2 (400 µl per sample), and AMP buffer (100 µl per sample). The concentration of extracted DNA was quantified on a Spark plate reader (Tecan, Männedorf, Switzerland) using

Table 1. List of morphological characters measured in *Dianthus virgineus* and *D. brachycalyx*, character type, unit of measure (for numerical characters) or character states (for categorical characters), and ID.

Variable	Type; unit of measure/character states; ID
Plant height	Continuous; cm; height
Plant habitus	Categorical; hC (caespitose), hS (suffruticose)
Woodystock habitus	Categorical; whC (contracted), whL (loose)
Number of internodes	Integer; n.int
*Lower internode length	Continuous; mm; low.int.l
Upper internode length	Continuous; mm; up.int.l
Basal leaf length	Continuous; mm; bas.lf.l
*Basal leaf width	Continuous; mm; bas.lf.w
Basal leaf shape	Categorical; blC (canaliculate), blF (flat)
Upper stem leaf length	Continuous; mm; up.st.lf.l
Upper stem leaf width	Continuous; mm; up.st.lf.w
*Upper stem leaf margin	Categorical; ulD (denticulate), ulS (smooth)
Lower stem leaf length	Continuous; mm; low.st.lf.l
Lower stem leaf width	Continuous; mm; low.st.lf.w
*Lower stem leaf margin	Categorical; llD (denticulate), llS (smooth)
Number of flowers per stem	Integer; n.fl.per.st
Number of epicalyx scales	Integer; n.episc
Bracteoles on flower pedicel	Categorical; pbY (present), pbN (absent)
*Upper epicalyx scale layout	Categorical: ueaO (overlapping), ueaN (non-overlapping)
Upper epicalyx scale shape	Categorical; uesA (acute), uesO (obtuse)
*Upper epicalyx scale position	Categorical; uepA (appressed), uepD (divaricate)
Upper epicalyx scale length	Continuous; mm; up.episc.l
Upper epicalyx scale mucro length	Continuous; mm; mu.up.episc.l
Upper epicalyx scale width	Continuous; mm; up.episc.w
Calyx length	Continuous; mm; cal.l
Calyx width	Continuous; mm; cal.w
Calyx teeth length	Continuous; mm; t.cal.l
Calyx teeth layout	Categorical: taO (overlapping), taN (non-overlapping)
*Calyx teeth shape	Categorical; tsA (acute), tsO (obtuse)
*Calyx teeth membrane	Categorical; tmV (visible), tmN (not visible)

Asterisk (*) indicates characters filtered out from the multivariate analysis.

the Quantifluor ONE dsDNA kit (Promega, Madison, Wisconsin, U.S.A.). Nine individuals were replicated three times as a positive control.

We genotyped genome-wide single nucleotide polymorphisms (SNPs) with ddRAD-seq (Peterson & al., 2012). Libraries were prepared according to the protocol used by Westergaard & al. (2019). The digestion of 100 ng of high-quality genomic DNA was carried out in a 25 µl reaction volume with 0.4 µl EcoRI-HF (20 U) and 2.5 µl Buffer CutSmart (New England Biolabs, Ipswich, Massachusetts, U.S.A.) for 30 min at 37°C, followed by 0.4 µl Taqα1 (New England Biolabs) for 30 min at 65°C. The double digest was ligated to adaptors in a 30 µl reaction volume using 2 µl P1 Adapter, 2 µl P2 Adapter, 0.8 µl T4 DNA ligase buffer (10×) and 1 µl T4 DNA ligase (400 U/µl). Forty-eight individually barcoded samples were multiplexed in a pooled library. Further, 500–550 bp libraries were selected using the first 0.57× AMPure beads and subsequently 0.12× AMPure beads. This size selection step also removes unligated adapters. The libraries were then washed while attached to 15 µl Dynabeads M-270 Streptavidin beads (Invitrogen, Waltham, Massachusetts, U.S.A.) to select for P2-biotin-labelled adapters. Illumina flowcell annealing sequences, unique double-index primers, multiplexing indices, and sequencing primer annealing regions were added to each library during the PCR amplification performed with a KAPA Hifi Hotstart ready mix (Kapa Biosystems, Wilmington, Massachusetts, U.S.A.) for 8–10 cycles. The libraries were further cleaned using AMPure XP beads and checked for DNA quantity on a Quantus (Promega) using the kit and for optimal fragment sizes on a Tapestation 2200 (Agilent, Santa Clara, California, U.S.A.) using the HS D1000 tape. Sets of two libraries were multiplexed and sequenced in two lanes of 150 bp paired-end reads on an Illumina NovaSeq 6000 at Novogene U.K. (Cambridge, U.K.).

Sequences were demultiplexed using Stacks v.2.41 (Catchen & al., 2013) with default settings. After removing 12 individuals with a low number of reads, the reads from samples were mapped on a reference genome of *Dianthus inodorus* (https://doi.org/10.5061/dryad.x0k6djhng) with BWA-MEM v.0.7.17 (Li, 2013) with default settings, eliminating mapped reads with a mapping quality score below 10 using Sambamba v.0.5.0 (Tarasov & al., 2015). On the resulting BAM files, variants were called with FreeBayes v.1.3.4 (Garrison & Marth, 2012; –max-complex-gap -1 –haplotype-length -1 –min-repeat-entropy 1 -V -F 0.05 –use-best-n-alleles 4), producing a raw VCF file containing 4,569,917 called variants. The raw VCF file was filtered following the dDocent v.2.9.4 pipeline (Puritz & al., 2014), employing VCFtools v.0.1.16 (Danecek & al., 2011) and VCFlib v.1.0.3 (Garrison & al., 2022). Firstly, we kept variants with a quality score higher than 20, minimum mean depth for genotype call of 3, mean depth of 10, minor allele count of 3, minor allele frequency of 5%, and showing less than 50% of missing data. All the individuals showed low percentage of missing data, lower than 50%, so we did not remove any of them. Then we removed sites with more than 5% of missing data. We also filtered sites according to allele balance, mapping quality and their presence on just one read as defined in dDocent. After removing multi allelic SNPs, we filtered out SNPs with allelic frequencies significantly different from the null model of the Hardy-Weinberg equilibrium ($P < 0.01$), according to the test of Wigginton & al. (2005). Finally, with BCFtools v.1.10.2 (Danecek & al., 2021), we pruned the VCF file to keep one SNP for each contig. The mean genotyping error rate of the filtered VCF was calculated with Tiger v.1.0 (Bresadola & al., 2020). The file was transformed to a format compatible for STRUCTURE analyses using PGDSpider v.2.1.1.5 (Lischer & Excoffier, 2012).

To explore the genetic data and to understand the relations among the studied individuals, a distance-based tree was built with SNPRelate v.1.34.1 (Zheng & al., 2012). The resulting dendrogram was customized with the ggtree v.3.8.2 package in R (Yu & al., 2017). We also performed a principal components analyses (PCA) on the genetic datasets by employing the SNPRelate v.1.34.1 R package (Zheng & al., 2012).

We conducted model-based clustering analyses with the software STRUCTURE v.2.3.4 (Pritchard & al., 2000; Falush & al., 2003, 2007; Hubisz & al., 2009). To account for the effect of uneven sample size among populations on the accuracy of the inference of K, we followed the recommendation proposed by Wang (2017). Initially, we performed a STRUCTURE analysis with default priors' parameters (ancestry model with admixture, a relative admixture level [α] equal to 1, without a priori information on sampling localities, and assuming the allelic frequencies correlated among populations). Then, we conducted an analysis with a customized ancestry model, with an initial α value of 0.14 ($\alpha = 1 / K$, using the number of most likely groups obtained in the first analysis, i.e., 7) with a priori information on the sampling localities, and using the uncorrelated frequency model. Each analysis was carried out with 10 replicates for each K from 1 to 10, setting a burn-in period of 10,000 iterations followed by 30,000 iterations of the Markov–Monte Carlo chain. The most likely number of genetically homogeneous clusters (K) was inferred with STRUCTURE HARVESTER v.0.7 (Earl & vonHoldt, 2012), by checking the ΔK statistics (Evanno & al., 2005). Barplots resulting from the analyses were aligned and visualized with the CLUMPAK v.1.1 web server (Kopelman & al., 2015).

To test if the retrieved genetic structure follows an isolation-by-distance pattern, we performed a Mantel test with 9999 iterations, implemented in ade4 v.1.7.22 R package (Dray & Dufour, 2007), between a pairwise F_{ST} matrix, calculated according to Nei's genetic distance (Nei, 1987) with the hierfstat v.0.5.11 R package (Goudet, 2005), and a geographical distance matrix, calculated using the geosphere v.1.5.18 R package (Hijmans, 2016).

PCA and STRUCTURE analyses were also performed on a dataset including a balanced number of samples for the two taxa. The five populations of *Dianthus brachycalyx* were combined with five populations across the range of *D. virgineus*: Morano

(Calabria, southern Italy), Monte Morrone (Abruzzo, central Italy), Libro Aperto (Toscana, central Italy), Andagna (Liguria, northern Italy), and Montferrier sur Lez (Occitanie, southern France; type locality). In this case, the initial α of the STRUCTURE customized analysis was 0.33, as the number of most likely groups obtained in the first analysis was $K = 3$.

Climatic niche comparison. — Niche overlap between *Dianthus brachycalyx* and *D. virgineus* was quantified using the PCA-based method developed by Broennimann & al. (2012). We used 19 bioclimatic variables for the current (2000–2016) time period, at about 1 × 1 km spatial resolution, downloaded from the CHELSA dataset (Karger & al., 2017). To measure niche overlap, we used Schoener's D index (Schoener, 1970), which ranges from 0 (no overlap) to 1 (full overlap). Niche similarity test was employed to check whether the climatic niche occupied by one species with respect to that occupied by the other species is more similar than would be expected at random (Warren & al., 2008). For this test four different background buffers (2, 5, 10, 15 km) were used. The analyses were conducted using the ecospat v.4.0.0 R package (Broennimann & al., 2023).

■ RESULTS

Morphometric analyses. — Eight characters were non-significantly different between taxa and were removed from the morphometric dataset (suppl. Tables S2, S3). After filtering, we obtained a morphological dataset including 424 specimens × 22 characters, 16 numerical and 6 categorical. All retained numerical characters exhibited a correlation coefficient lower than |0.8| (suppl. Table S4).

The first two dimensions of the FAMD analysis on the complete dataset explained 36.10% of the total variation of the dataset (Fig. 3A). The two taxa occupy different portions of the morphospace generated by the first two axes, but with some overlaps, especially between individuals of *Dianthus brachycalyx* and individuals from high-elevation populations of *D. virgineus* (Fig. 3A). The population of *D. brachycalyx* from Calabria (southern Apennines) overlaps with those from Abruzzo and Marche (including the topotypical population, central Apennines), so that no morphological differentiation can be detected. All numerical characters show negative contributions on the first dimension (Fig. 3B), with plant height showing the highest contribution (suppl. Fig. S1A), meaning that plants show an overall reduced size at lower values of dimension 1. Overall, calyx length, lower and upper stem leaf width, number of internodes and epicalyx scales, and upper epicalyx scale width separate the upper-left quadrant from the lower-right quadrant, i.e., *D. brachycalyx* from *D. virgineus* (Fig. 3B). On the other hand, calyx width, upper and lower stem leaf length, upper internode length, basal leaf length, number of stem flowers, and length of calyx teeth explain some of the morphometric variation of *D. virgineus*, mainly covering upper-right and lower-left quadrants (Fig. 3B). Categorical characters have a lower contribution to dimension 1 (Fig. 3C, suppl. Fig. S1A), but the absence of pedicel bracts beneath the flowers and non-overlapping calyx teeth have a high contribution on dimension 2 (suppl. Fig. S1B). Differences of dimensions 1 and 2 between taxa are significant, with the former showing a higher effect size than the latter (suppl. Table S2). Dimension 1 is significantly correlated with the elevation of the studied populations ($r = 0.688$, $P < 0.01$) and a linear model fits well with the data ($R^2 = 0.592$; suppl. Fig. S2). All the linear model assumptions were met. On the contrary, dimension 2 is not significantly correlated with increasing elevation ($r = 0.26$, $P = 0.21$).

The first two dimensions of the FAMD analysis on the balanced dataset explained 35.08% of the total variation (suppl. Fig. S3). In this analysis *Dianthus brachycalyx* is separated from *D. virgineus* (suppl. Fig. S3A), as it shows shorter calyces, calyx teeth, and epicalyx scales (suppl. Fig. S3B). However, concerning qualitative characters, the overlap of the calyx teeth and the presence of pedicel bracteoles do not contribute to the separation as the previous analysis (suppl. Fig. S3C). Dimensions 1 and 2 were significantly different between species ($P < 0.01$) with the former showing an effect size ($d = 1.24$) lower than the latter ($d = 1.57$). However, in this case, neither dimension 1 nor dimension 2 is significantly correlated with increasing elevation ($P > 0.01$, $r = 0.54$; $P > 0.01$, $r = 0.03$, respectively).

Genetic analyses. — We retrieved a VCF file containing 3202 unlinked SNPs characterized across 400 samples, with a mean genotyping error rate of 0.017. In the identity-by-state analysis, all replicates of the same individual formed a monophyletic group with a long branch, thus proving genotypic data reliable (not shown). The distance-based tree (Fig. 4) does not support the current taxonomy. Individuals of *D. brachycalyx* are more similar to individuals originating from geographically close populations of *D. virgineus*, rather than to putatively conspecific individuals from different regions. Overall, a clear geographical signal can be inferred from the dendrogram, with individuals clustered according to their geographical provenance. Moreover, the short branches supporting geographically proximal populations (irrespective of species attribution) point to a low genetic divergence.

The first three axes of the PCA performed on the complete dataset explain 14.47% of the total genetic variation (suppl. Fig. S4A,B) and 20.18% in the analysis performed with the balanced dataset (suppl. Fig. S4C,D). In both analyses, individuals of *Dianthus brachycalyx* do not form a separate group with respect to *D. virgineus*. Populations follow their latitudinal distributions along PC1 with southern populations occupying higher PC values and northern populations showing lower values. PC2 in both analyses (suppl. Fig. S4A,C) and PC3 of the balanced dataset (suppl. Fig. S4D) remark a geographic signal, whereas PC3 of the complete dataset (suppl. Fig. S4B) reveals a slight separation of coastal populations from Lazio (central Italy).

Barplots from STRUCTURE analyses on the complete dataset and the Evanno's test results are reported in Fig. 5 and suppl. Table S5, respectively. The first analysis, performed with

default priors, retrieved $K = 7$ as the most likely number of genetic clusters according to ΔK (suppl. Table S5, Fig. 5B), recognizing two alternative modes with very similar individual assignment to clusters, consistent in all runs (mean similarity scores of 0.989 and 0.991). The second analysis, performed with customized priors, retrieved $K = 2$ as the most likely number of genetic groups (suppl. Table S5, Fig. 5A), with just one mode of individual assignment correlated through all runs (mean similarity score of 0.999). At various K values of both analyses, a geographical signal is apparent, as geographically close populations always belong to the same genetic cluster, irrespective of species attribution. For instance, in the customized analysis, at $K = 2$ (Fig. 5A) populations cluster in two groups, a first one including populations from southern Italy, and a second one including populations from Toscana, Liguria, and southern France, while populations from central-southern Italy exhibit a certain degree of admixture. At higher Ks, genetic structure mirrors the geographical distribution of studied populations at a finer scale, with populations from Toscana, France, Abruzzo, and Lazio grouping in different clusters, and populations from other geographical areas showing admixture among the clusters (not shown). In all the retrieved barplots, populations of *Dianthus brachycalyx* do not form a cluster separate from those of *D. virgineus*. The same results were obtained in the balanced STRUCTURE analysis (suppl. Fig. S5; suppl. Table S4).

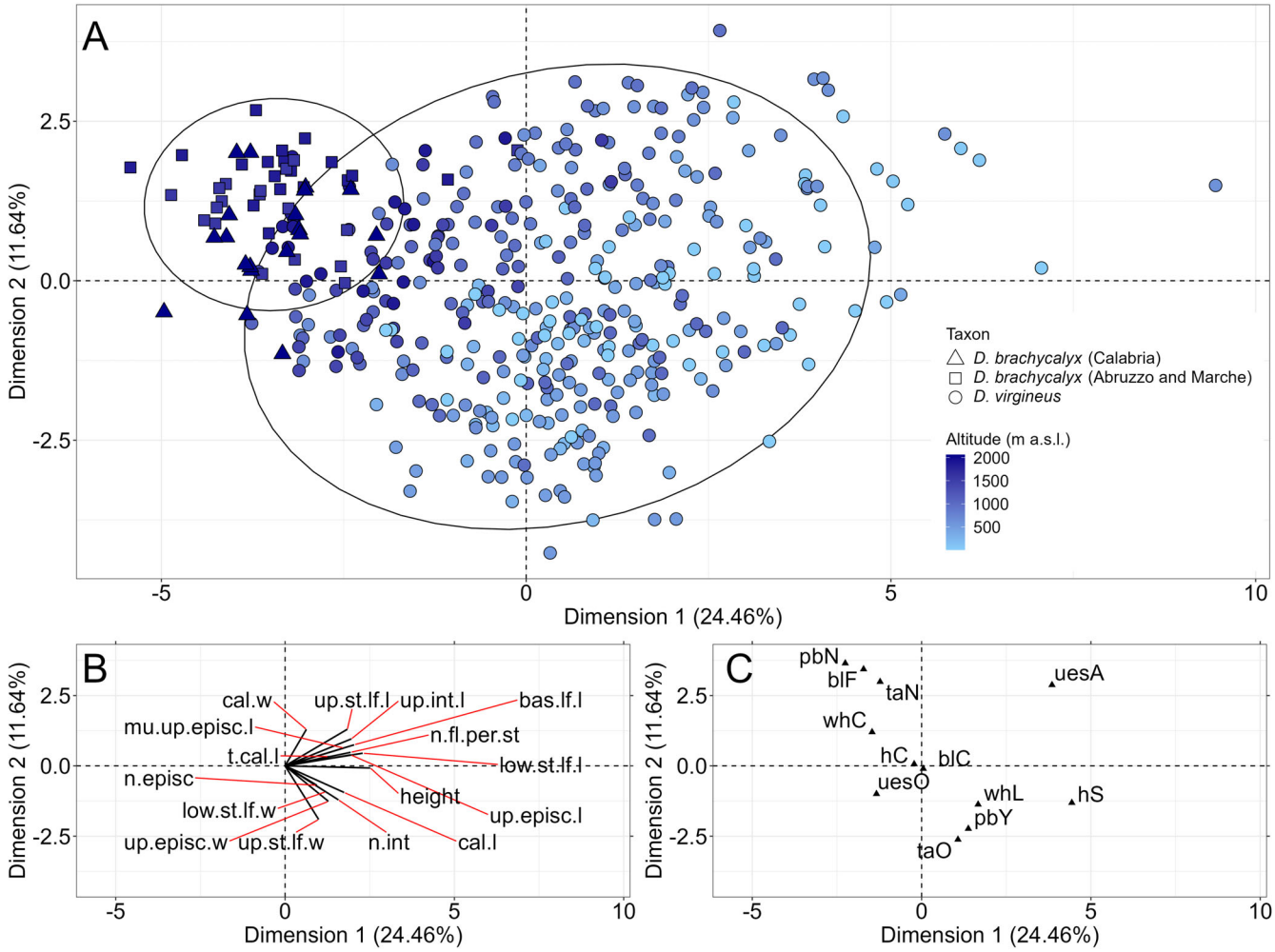


Fig. 3. FAMD results illustrating the relations in the morphospace between *Dianthus brachycalyx* and *D. virgineus* using the complete morphometric dataset. **A**, Scatterplot built with the two dimensions explaining the highest percentage of variation of the data. Points are shaped according to taxa and colored according to altitude of the studied populations. For each taxon, 95% confidence ellipse is plotted. **B**, Biplot showing the relation among the numeric characters on the generated morphospace. bas.lf.l: basal leaf length; cal.l: calyx length; cal.w: calyx width; height: plant height; low.st.lf.l: lower stem leaf length; low.st.lf.w: lower stem leaf width; mu.up.episc.l: upper epicalyx scale mucro length; n.episc: number of epicalyx scales; n.fl.per.st: number of flowers per stem; n.int: number of internodes; t.cal.l: calyx teeth length; up.episc.l: upper epicalyx scale length; up.episc.shape: upper epicalyx scale shape; up.episc.w: upper epicalyx scale width; up.st.lf.l: upper stem leaf length; up.st.lf.w: upper stem leaf width; up.int.l: upper internode length. **C**, Biplot showing the relation among categorical character states on the generated morphospace. Plant habitus: hC (caespitose); hS (suffruticose); woodystock habitus: whC (contracted), whL (loose); basal leaf shape: blC (canaliculate), blF (flat); bracteoles on flower pedicel: pbY (present), pbN (absent); upper epicalyx scale shape: uesA (acute), uesO (obtuse); calyx teeth layout: taO (overlapping), taN (non-overlapping).

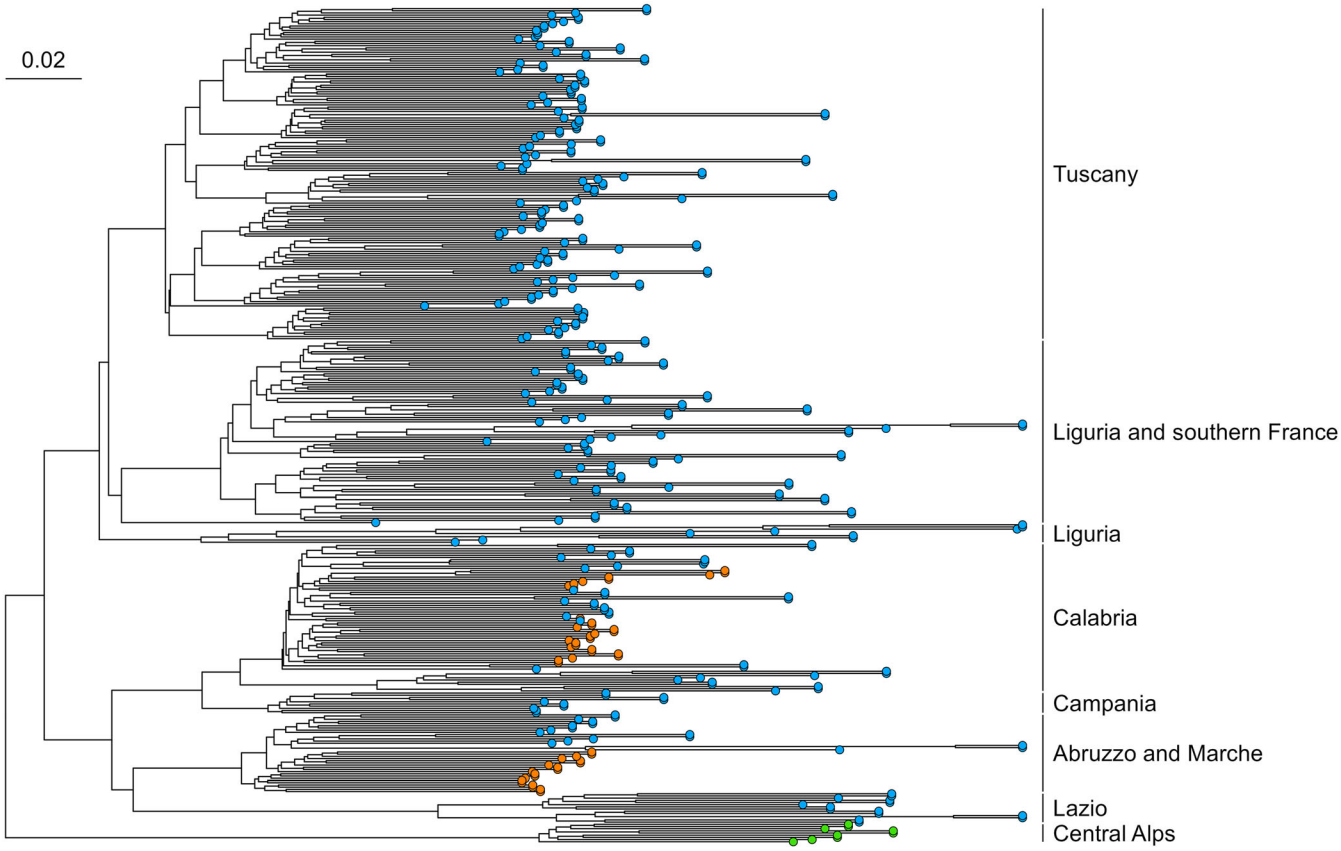


Fig. 4. Distance-based tree crafted with 3202 SNPs characterized in studied individuals (without replicates) of *Dianthus brachycalyx* (orange tips) and *D. virgineus* (blue tips). *Dianthus inodorus* (green tips) was included as outgroup. The scale represents individual dissimilarity.

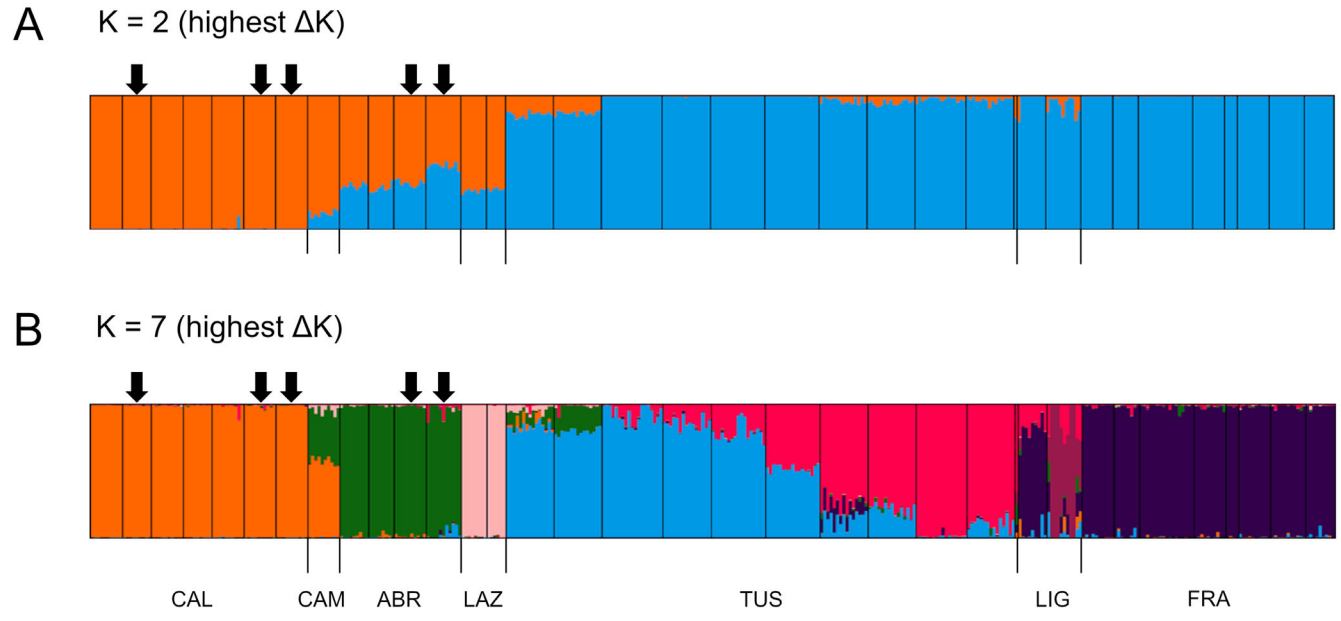


Fig. 5. Bar plots resulting from customized and default STRUCTURE analyses including 389 individuals belonging to 35 populations of *Dianthus brachycalyx* and *D. virgineus*. Populations are ordered according to increasing latitude. Black arrows point at populations of *D. brachycalyx*. Three-letter codes identify geographical regions of the study area: CAL, Calabria; CAM, Campania; ABR, Abruzzo and Marche; LAZ, Lazio; TUS, Toscana; LIG, Liguria; FRA, southern France. **A**, Bar plot of the best *K* according to Evanno's statistics of the customized analysis (*K* = 2, 10/10 modes); **B**, Bar plot of the best *K* according to Evanno's statistics of the default analysis (*K* = 7, 5/10 modes).

The Mantel test between the matrix of the pairwise F_{ST} and the geographical distances among populations was significant ($r = 0.61$, $P < 0.01$). Overall, an isolation-by-distance model fits well with the genetic data (suppl. Fig. S6). However, there are some population pairs showing F_{ST} values higher than expected from geographical distances. These pairs always involve one of the two coastal populations of *D. virgineus* from Lazio (Litorale di Caprolace and Castel Porziano).

Climatic niche comparison. — The climatic niche comparison analysis resulted in a moderate niche overlap between *Dianthus brachycalyx* and *D. virgineus* (Table 2). The similarity tests were non-significant for all the background areas used, meaning that the niches of the two species are similar as expected by chance given the available climatic conditions.

■ DISCUSSION

Our starting hypothesis was that *Dianthus brachycalyx* and *D. virgineus* are distinct species (Bacchetta & al., 2010), supported only when considering them under a strict morphological species concept (Stuessy, 2009). According to our results, the studied populations of *D. brachycalyx* are separated morphologically from *D. virgineus*, exhibiting significantly lower values for the majority of the numerical variables and by a combination of categorical features, like the absence of bracteoles on the flower pedicels and non-overlapping calyx teeth. Nevertheless, there are overlaps between *D. brachycalyx* and montane populations of *D. virgineus*, both showing a generally reduced size of vegetative and reproductive features, possibly explaining previous identification problems (Conti & Bartolucci, 2015) and the morphological continuum (Rovito & al., 2022) observed. This, combined with the covariation of dimension 1 of the FAMD with elevation, questions a taxonomic separation based solely on morphology. Moreover, the studied specimens of *D. brachycalyx* show calyx teeth 4.24 mm (mean value) long and 4 epicalyx scales (just a single specimen has 2), and these values fall within the variation range characterizing *D. virgineus* based on Gargano & al. (2023; calyx teeth (2.9–) 3.6–5.3(–6.5) mm long and 4–6 epicalyx scales).

Table 2. Results of niche similarity tests between *Dianthus brachycalyx* and *D. virgineus*.

Background (km^2)	Niche overlap	*D. virgineus* vs. *D. brachycalyx*	*D. brachycalyx* vs. *D. virgineus*
2 × 2	0.247	ns	ns
5 × 5	0.241	ns	ns
10 × 10	0.240	ns	ns
15 × 15	0.247	ns	ns

Backgrounds were defined by applying 2, 5, 10, 15 km buffer zones around the occurrence points.
ns, non-significant

Dianthus virgineus and *D. brachycalyx* belong to the same genetic lineage (Apennine lineage; Luqman & al., 2023), well distinct from the lineage of *D. inodorus* (Alpine lineage; Luqman & al., 2023). The two Apennine species do not form two distinct genetic clusters. Since we found a positive and significant association between genetic and geographical distances among populations, the clusters identified in the analyses (and their admixtures according to STRUCTURE), result from a continuous genetic variation following spatial distribution of the populations (Bradburd & al., 2018). In other words, across the study area, we detected a genetic cline from southern France to southern Italy, mainly shaped by isolation-by-distance. Populations of *D. brachycalyx* fit in this model as expected from their geographical distribution, but irrespective of their taxonomic attribution. Thus, individuals of *D. brachycalyx* from the central Apennines are genetically closer to individuals of *D. virgineus* from the same geographical area with respect to conspecific populations from the southern Apennines. These results suggest that the taxonomic definition of *D. brachycalyx* actually consists of multiple high-elevation morphotypes. This pattern is documented in other plant species occurring along wide elevational ranges (Trucchi & al., 2017; Halbritter & al., 2018) or heterogeneous environments in general (Roda & al., 2013; Knyazeva & Hantemirova, 2020).

Furthermore, climatic niches of *Dianthus virgineus* and *D. brachycalyx* are overlapping, and niche differences are due to different climatic conditions in their respective ranges.

Our results allow to reject the two-species hypothesis made by Bacchetta & al. (2010), so that only three possible taxonomic scenarios can be formulated for *Dianthus brachycalyx*-*D. virgineus* populations: (1) splitting *D. brachycalyx* in several taxa for each high-elevation morphotype distinct from *D. virgineus*; (2) delimiting two taxa mirroring the two genetic clusters retrieved; (3) lumping *D. brachycalyx* and *D. virgineus* in a single, morphologically variable, species.

The first scenario could be slightly supported on genetic grounds, since populations of *Dianthus brachycalyx* s.str. from central Italy and those from southern Italy belong to different genetic subclusters (Figs. 4, 5). Nonetheless, the very same clusters include also geographically close populations of *D. virgineus*, which are morphologically different from *D. brachycalyx*. Moreover, populations of *D. brachycalyx* s.str. are not distinct from those from Calabria on morphometric grounds (Fig. 3, suppl. Fig. S3).

The second scenario could be supported by genetic data. However, as discussed above, the two clusters would more likely represent two latitudinal extremes of a genetic gradient, and not two independent lineages. Moreover, the populations from the northern and southern clusters cannot be separated in the multivariate morphospace (suppl. Fig. S7).

Finally, the third scenario (one-species hypothesis) is supported by all approaches, since: (a) *Dianthus virgineus* and *D. brachycalyx* belong to a single genetic lineage, structured according to geographical distance, rather than taxonomic attribution of the populations; (b) there are morphological

similarities between *D. brachycalyx* and montane populations of *D. virgineus*; (c) climatic niches of the two species are moderately overlapping; while the two species grow under different climates they don't select different climatic conditions. A one-species hypothesis is further supported by the lack of differences in chromosome number ($2n = 2x = 30$) and genome size between *D. brachycalyx* and *D. virgineus* (Franzoni & al., 2024).

By applying the "Wettstein tesseract", a conceptual tool recently proposed to standardize taxonomic rank decisions (Oberprieler, 2023), the two studied "species" should be included, indeed, within a single species expressing different ecotypes, which are defined as groups diagnosable by ecological and/or morphological features, but showing a sympatric distribution and genetic homogeneity. Also in other studies, the intraspecific morphological variation in *Dianthus* was related to increasing elevation (Castro & al., 2022; Franzoni & al., 2023; Gargano & al., 2023; Pålsson & al., 2023).

■ CONCLUSIONS

Multiple lines of evidence do not allow a separation of *Dianthus brachycalyx* from *D. virgineus*. Thus, we here conclude in formally proposing *Dianthus brachycalyx* A.Huet & É.Huet ex Bacch. & al. as a heterotypic synonym of *Dianthus virgineus* L., i.e., the oldest name available (Bacchetta & al., 2010; Domina & al., 2021b). This work was based on an integrative species-validation approach and provides a solid background to further investigate the *D. virgineus* complex, which contains many taxonomically doubtful species and subspecies distributed in mountain ranges of the central Mediterranean. At the same time, it allows us to generate hypotheses on the evolution and ecology of a widespread central Mediterranean species. For instance, future studies could help to have better insights on the origin and persistence of montane morphotypes along the Apennines.

■ TAXONOMIC SETTING

Dianthus virgineus L., Sp. Pl. 1: 412. 1753 – Lectotype (designated by Domina & al. in Taxon 70: 1098. 2021): Caryophyllus Syl. repens multi florus Bauh., Monspelii sponte, Burser XI: 99 (UPS No. V-174060 [digital image!]).

= *Dianthus brachycalyx* A.Huet & É.Huet ex Bacch., Brullo, Casti & Giusso in Nordic J. Bot. 28: 142. 2010 – Holotype: in elatis ad rupes montis Corno, 1800–2000 m a.s.l., Aprutii, 27 Aug 1856, *Huet du Pavillon 278* (G barcodes G00226658–G00226660 [on three sheets; digital images!]).

Morphological description of Dianthus virgineus as newly circumscribed. – Caespitose perennial herb, 42.38 ± 14.24 cm high with a developed woodystock, with contracted or loosly organized branches, with 2–8 internodes, the lower 42.78 ± 23.06 mm long, the upper 56.13 ± 20.10 mm long. Basal leaves are 81.85 ± 40.32 mm long and 1.02 ± 0.37 mm wide, often canaliculated but sometimes flat. Lower stem leaves are 47.56 ± 24.80 mm long and 0.94 ± 0.36 mm wide, often with a denticulate margin and rarely with a smooth margin. Upper stem leaves are 17.29 ± 8.12 long and 0.94 ± 0.36 mm wide, with a denticulate or smooth margin. Flowering stem can bear a single flower or many flowers (up to 14) organized in cymes with elongated branches. Epicalyx is formed by 2–8 (rarely 12–16) scales, appressed to the base of the calyx; sometimes scale-like bracteoles are present on the flower pedicel. Upper epicalyx scales are often obtuse, rarely acute, usually overlapping, 6.32 ± 1.09 mm long, 6.61 ± 1.22 mm wide, with a mucro 0.79 ± 0.56 mm long. When present, lower epicalyx scales are mainly acute, rarely obtuse, usually non-overlapping, 5.11 ± 1.08 mm long, 3.94 ± 0.90 mm wide, with a mucro 1.06 ± 0.61 mm long. Calyx is 23.06 ± 3.54 mm long and 4.33 ± 0.70 mm wide. Calyx teeth are 4.89 ± 0.96 long, always with an acute apex, overlapping or not at the base, sometimes with a visible membranaceous margin. Corolla is 20.26 ± 3.94 mm wide, composed by five pink petals 31.56 ± 5.17 mm long, with a limb 9.79 ± 2.17 mm long and 7.69 ± 1.63 wide. Petals have 5–11 (up to 20) teeth, 0.88 ± 0.45 mm long. Androecium is composed by 10 stamens bearing anthers 2.72 ± 0.50 mm long. Gynoecium is composed by a pistil with a 6.81 ± 1.22 mm long ovary.

■ AUTHOR CONTRIBUTIONS

LP and JF conceived the study, LP supervised and coordinated the study, JF, GA, FB, LB, FC, DI, MI, LM, and LP sampled the populations, FB and FC provided the occurrence data, JF, LB, DI, LM, and AT made the morphometric measurements, GC performed the ecological niche comparison analyses, SF generated the genetic dataset and assisted analyses, JF performed the morphometric and genetic analyses, prepared the figures, and drafted the manuscript. All authors contributed and reviewed advanced versions of the manuscript.

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■ LITERATURE CITED

Bacchetta, G. & Brullo, S. 2000. *Dianthus mossanus* (Caryophyllaceae), a new species from Sardinia. *Portugaliae Acta Biol.* 19: 295–301.

Bacchetta, G., Brullo, S., Casti, M. & Giusso del Galdo, G.P. 2010. Taxonomic revision of the *Dianthus sylvestris* group (Caryophyllaceae) in central-southern Italy, Sicily and Sardinia. *Nordic J. Bot.* 28: 137–173. https://doi.org/10.1111/j.1756-1051.2009.00459.x

Bartolucci, F., Peruzzi, L., Galasso, G., Alessandrini, A., Ardenghi, N.M.G., Bacchetta, G., Banfi, E., Barberis, G., Bernardo, L., Bouvet, D., Bovio, M., Calvia, G., Castello, M., Cecchi, L., Del Guacchio, E., Domina, G., Fascetti, S., Gallo, L., Gottschlich, G., Guarino, R., Gubellini, L., Hofmann, N., Iberite, M., Jiménez-Mejías, P., Longo, D., Marchetti, D., Martini, F., Masin, R.R., Medagli, P., Peccenini, S., Prosser, F., Roma-Marzio, F., Rosati, L., Santangelo, A., Scoppola, A., Selvaggi, A., Selvi, F., Soldano, A., Stinca, A., Wagensommer, R.P., Wilhalm, T. & Conti, F. 2024. A second update to the checklist of the vascular flora native to Italy. *Pl. Biosyst.* 158(2): 219–296. https://doi.org/10.1080/11263504.2024.2320126

Bradburd, G.S., Coop, G.M. & Ralph, P.L. 2018. Inferring continuous and discrete population genetic structure across space. *Genetics* 210: 33–52. https://doi.org/10.1534/genetics.118.301333

Bresadola, L., Link, V., Buerkle, C.A., Lexer, C. & Wegmann, D. 2020. Estimating and accounting for genotyping errors in RAD-seq experiments. *Molec. Ecol. Resources* 20: 856–870. https://doi.org/10.1111/1755-0998.13153

Broennimann, O., Fitzpatrick, M.C., Pearman, P.B., Petitpierre, B., Pellissier, L., Yoccoz, N.G., Thuiller, W., Fortin, M.-J., Randin, C., Zimmermann, N.E., Graham, C.H. & Guisan, A. 2012. Measuring ecological niche overlap from occurrence and spatial environmental data: Measuring niche overlap. *Global Ecol. Biogeogr.* 21: 481–497. https://doi.org/10.1111/j.1466-8238.2011.00698.x

Broennimann, O., Di Cola, V. & Guisan, A. 2023. ecospat: Spatial Ecology Miscellaneous Method: Version 4.0.0. https://CRAN.R-project.org/package=ecospat

Brullo, S. & Guarino, R. 2017. Complesso di *Dianthus sylvestris*. Pp. 200–205 in: Pignatti, S. (ed.), *Flora d'Italia*, 2nd ed., vol. 2. Milan: New Business Media.

Brullo, S. & Guarino, R. 2019. Complesso di *Dianthus sylvestris*. Pp. 77–81 in: Pignatti, S. (ed.), *Flora d'Italia*, 2nd ed., vol. 4. Milan: New Business Media.

Brullo, S., Brullo, C., Colombo, P., Galdo, G.G.D., Ilardi, V. & Perrone, R. 2015. *Dianthus borbonicus* (Caryophyllaceae), a new species from Sicily. *Phytotaxa* 233: 49–60. https://doi.org/10.11646/phytotaxa.233.1.3

Carolin, R.C. 1957. Cytological and hybridization studies in the genus *Dianthus*. *New Phytol.* 56: 81–97. https://doi.org/10.1111/j.1469-8137.1957.tb07451.x

Castro, I., Rocha, J., Martins, M., Carnide, V., Martín, J.P., Veiga, P., Serafim, A.B., Amich, F., Ramírez-Rodríguez, R., Colombo, G. & Crespí, A.L. 2022. The redundancy effect under morphogenetic and environmental fluctuations. The case of the *Dianthus pungens* group. *Pl. Biosyst.* 156: 292–306. https://doi.org/10.1080/11263504.2020.1857864

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A. & Cresko, W.A. 2013. Stacks: An analysis tool set for population genomics. *Molec. Ecol.* 22: 3124–3140. https://doi.org/10.1111/mec.12354

Cohen, J. 1988. *Statistical power analysis for the behavioral sciences*, 2nd ed. Hillsdale, NJ: Erlbaum.

Conti, F. & Bartolucci, F. 2015. *The vascular flora of the National Park of Abruzzo, Lazio and Molise (Central Italy): An annotated checklist*. Geobotany Studies: Basics, Methods and Case Studies. Cham: Springer. https://doi.org/10.1007/978-3-319-09701-5

Cramér, H. 1946. *Mathematical methods of statistics*. Princeton Mathematical Series 9. Princeton: Princeton University Press.

Dag, O., Dolgun, A. & Konar, N.M. 2018. onewaytests: An R package for one-way tests in independent groups designs. *R Journal* 10: 175–199. https://doi.org/10.32614/RJ-2018-022

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R. & 1000 Genomes Project Analysis Group 2011. The variant call format and VCFtools. *Bioinformatics* 27: 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T., McCarthy, S.A., Davies, R.M. & Li, H. 2021. Twelve years of SAMtools and BCFtools. *GigaScience* 10: giab008. https://doi.org/10.1093/gigascience/giab008

Domina, G., Astuti, G., Bacchetta, G., Barone, G., Rešetnik, I., Terlević, A., Thiébaut, M. & Peruzzi, L. 2021a. Typification of 14 names in the *Dianthus virgineus* group (Caryophyllaceae). *Phytokeys* 187: 1–14. https://doi.org/10.3897/phytokeys.187.75534

Domina, G., Astuti, G., Barone, G., Gargano, D., Minuto, L., Varaldo, L. & Peruzzi, L. 2021b. Lectotypification of the Linnaean name *Dianthus virgineus* (Caryophyllaceae) and its taxonomic consequences. *Taxon* 70: 1096–1100. https://doi.org/10.1002/tax.12563

Dray, S. & Dufour, A.-B. 2007. The ade4 package: Implementing the duality diagram for ecologists. *J. Statist. Softw.* 22(4): 1–20. https://doi.org/10.18637/jss.v022.i04

Earl, D.A. & vonHoldt, B.M. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genet. Resources* 4: 359–361. https://doi.org/10.1007/s12686-011-9548-7

Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molec. Ecol.* 14: 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Falush, D., Stephens, M. & Pritchard, J.K. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* 164: 1567–1587. https://doi.org/10.1093/genetics/164.4.1567

Falush, D., Stephens, M. & Pritchard, J.K. 2007. Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molec. Ecol. Notes* 7: 574–578. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Fassou, G., Korotkova, N., Nersesyan, A., Koch, M.A., Dimopoulos, P. & Borsch, T. 2022. Taxonomy of *Dianthus* (Caryophyllaceae) – Overall phylogenetic relationships and assessment of species diversity based on a first comprehensive checklist of the genus. *Phytokeys* 196: 91–214. https://doi.org/10.3897/phytokeys.196.77940

Franzoni, J., Astuti, G. & Peruzzi, L. 2023. Weak genetic isolation and putative phenotypic selection in the wild carnation *Dianthus virgineus* (Caryophyllaceae). *Biology (Basel)* 12: 1355. https://doi.org/10.3390/biology12101355

Franzoni, J., Astuti, G., Bacchetta, G., Barone, G., Bartolucci, F., Bernardo, L., Carta, A., Conti, F., Domina, G., Frajman, B., Giusso del Galdo, G.P., Iamonico, D., Iberite, M., Minuto, L., Sarigu, M., Terlević, A., Turini, A., Varaldo, L., Volgger, D. & Peruzzi, L. 2024. A cytosystematic study of the *Dianthus virgineus* complex (Caryophyllaceae) in the Central Mediterranean. *J. Syst. Evol.* 62: 589–602. https://doi.org/10.1111/jse.13025

Gargano, D., Franzoni, J., Luqman, H., Fior, S., Rovito, S. & Peruzzi, L. 2023. Phenotypic correlates of genetic divergence suggest at least three species in the complex of *Dianthus virgineus* (Caryophyllaceae). *Taxon* 72: 1019–1033. https://doi.org/10.1002/tax.13073

Garrison, E. & Marth, G. 2012. Haplotype-based variant detection from short-read sequencing. *arXiv* 1207.3907. https://doi.org/10.48550/arXiv.1207.3907

Garrison, E., Kronenberg, Z.N., Dawson, E.T., Pedersen, B.S. & Prins, P. 2022. A spectrum of free software tools for processing the VCF variant call format: vcflib, bio-vcf, cyvcf2, hts-nim and slivar. *PLoS Computat. Biol.* 18: e1009123. https://doi.org/10.1371/journal.pcbi.1009123

Gonzalo-Turpin, H. & Hazard, L. 2009. Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *J. Ecol.* 97: 742–751. https://doi.org/10.1111/j.1365-2745.2009.01509.x

Goudet, J. 2005. hierfstat: A package for R to compute and test hierarchical F-statistics. *Molec. Ecol. Notes* 5: 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x

Halbritter, A.H., Fior, S., Keller, I., Billeter, R., Edwards, P.J., Holderegger, R., Karrenberg, S., Pluess, A.R., Widmer, A. & Alexander, J.M. 2018. Trait differentiation and adaptation of plants along elevation gradients. *J. Evol. Biol.* 31: 784–800. https://doi.org/10.1111/jeb.13262

Hamzaoğlu, E., Koç, M., Büyük, İ., Aksoy, A. & Soydam Aydin, S. 2015. A new serpentine-adapted carnation (Caryophyllaceae) from Turkey: *Dianthus serpentinus* sp. nov. *Nordic. J. Bot.* 33: 57–63. https://doi.org/10.1111/njb.00582

Hardion, L., Perrier, A., Martinez, M., Navrot, N., Gaquerel, E., Tournay, F., Nguefack, J. & Combroux, I. 2020. Integrative revision of *Dianthus superbus* subspecies reveals different degrees of differentiation, from plasticity to species distinction. *Syst. Biodivers.* 18: 255–268. https://doi.org/10.1080/14772000.2020.1737979

Herve, M. 2023. RVAideMemoire: Testing and plotting procedures for biostatistics: R package version 0.9-83-7. https://CRAN.R-project.org/package=RVAideMemoire

Hijmans, R.J. 2016. Geosphere: Spherical trigonometry. Version 1.5.18. https://CRAN.R-project.org/package=geosphere

Hubisz, M.J., Falush, D., Stephens, M. & Pritchard, J.K. 2009. Inferring weak population structure with the assistance of sample group information. *Molec. Ecol. Resources* 9: 1322–1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x

Karger, D.N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R.W., Zimmermann, N.E., Linder, H.P. & Kessler, M. 2017. Climatologies at high resolution for the earth's land surface areas. *Sci. Data* 4: 170122. https://doi.org/10.1038/sdata.2017.122

Kassambara, A. & Mundt, F. 2020. factoextra: Extract and visualize the results of multivariate data analyses. Version 1.0.7. https://CRAN.R-project.org/package=factoextra

Knyazeva, S.G. & Hantemirova, E.V. 2020. Comparative analysis of genetic and morpho-anatomical variability of common juniper (*Juniperus communis* L.). *Russ. J. Genet.* 56: 48–58. https://doi.org/10.1134/S102279542001007X

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A. & Mayrose, I. 2015. CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across K. *Molec. Ecol. Resources* 15: 1179–1191. https://doi.org/10.1111/1755-0998.12387

Lê, S., Josse, J. & Husson, F. 2008. FactoMineR: An R package for multivariate analysis. *J. Statist. Softw.* 25(1): 1–18. https://doi.org/10.18637/jss.v025.i01

Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv* 1303.3997v2. https://doi.org/10.48550/arXiv.1303.3997

Lischer, H.E.L. & Excoffier, L. 2012. PGDSpider: An automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics* 28: 298–299. https://doi.org/10.1093/bioinformatics/btr642

Luqman, H., Wegmann, D., Fior, S. & Widmer, A. 2023. Climate-induced range shifts drive adaptive response via spatio-temporal sieving of alleles. *Nature Commun.* 14: 1080. https://doi.org/10.1038/s41467-023-36631-9

Mangiafico, S. 2023. rcompanion: Functions to support extension education program evaluation. Version 2.4.34. Rutgers Cooperative Extension. New Brunswick, New Jersey. https://CRAN.R-project.org/package=rcompanion

Marhold, K. 2011. Caryophyllaceae. In: The Euro+Med Plantbase Project – The information resource for Euro-Mediterranean plant diversity. http://ww2.bgbm.org/EuroPlusMed/PTaxonDetail.asp?NameCache=Dianthus&PTRefFk=7200000 (accessed 8 Mar 2022).

Meyer, F.K. 2011. *Beiträge zur Flora von Albanien*. Oberhof, Germany: Thüringische Botanische Gesellschaft e.V.

Navarro, D. 2015. *Learning statistics with R: A tutorial for psychology students and other beginners*. Version 0.6. University of New South Wales, Sydney, Australia. https://learningstatisticswithr.com

Nei, M. 1987. *Molecular evolutionary genetics*. New York: Columbia University Press.

Oberprieler, C. 2023. The Wettstein tesseract: A tool for conceptualising species-rank decisions and illustrating speciation trajectories. *Taxon* 72: 1–7. https://doi.org/10.1002/tax.12825

Pagès, J. 2004. Analyse factorielle de données mixtes. *Rev. Statist. Appl.* 4: 93–111.

Pålsson, A., Walther, U., Fior, S. & Widmer, A. 2023. Life history traits mediate elevational adaptation in a perennial alpine plant. *bioRxiv*. https://doi.org/10.1101/2023.10.17.562199

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S. & Hoekstra, H.E. 2012. Double Digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE* 7: e37135. https://doi.org/10.1371/journal.pone.0037135

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959. https://doi.org/10.1093/genetics/155.2.945

Puritz, J.B., Hollenbeck, C.M. & Gold, J.R. 2014. dDocent: A RADseq, variant-calling pipeline designed for population genomics of non-model organisms. *PeerJ* 2: e431. https://doi.org/10.7717/peerj.431

R Core Team 2023. *R: A language and environment for statistical computing, version 4.3.1*. Vienna: R Foundation for Statistical Computing. https:// www.R-project.org/

Roda, F., Ambrose, L., Walter, G.M., Liu, H.L., Schaul, A., Lowe, A., Pelser, P.B., Prentis, P., Rieseberg, L.H. & Ortiz-Barrientos, D. 2013. Genomic evidence for the parallel evolution of coastal forms in the *Senecio lautus* complex. *Molec. Ecol.* 22: 2941–2952. https://doi.org/10.1111/mec.12311

Rovito, S., Bernardo, L., Passalacqua, N.G. & Gargano, D. 2022. Variazioni morfologiche in relazione all'altitudine in *Dianthus virgineus* s.l. (Caryophyllaceae) nell'Appennino meridionale: Dalla morfometria tradizionale e geometrica, alla biometria funzionale. *Notiz. Soc. Bot. Ital.* 6(2): 139–140.

Schoener, T.W. 1970. Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology* 51: 408–418. https://doi.org/10.2307/1935376

Stuessy, T.F. 2009. *Plant taxonomy: The systematic evaluation of comparative data*, 2nd ed. New York: Columbia University Press.

Tarasov, A., Vilella, A.J., Cuppen, E., Nijman, I.J. & Prins, P. 2015. Sambamba: Fast processing of NGS alignment formats. *Bioinformatics* 31: 2032–2034. https://doi.org/10.1093/bioinformatics/btv098

Terlević, A., Bogdanović, S., Frajman, B. & Rešetnik, I. 2022. Genome size variation in *Dianthus sylvestris* Wulfen sensu lato (Caryophyllaceae). *Plants (Switzerland)* 11: 1481. https://doi.org/10.3390/plants11111481

Terlević, A., Temunović, M., Bogdanović, S., Grgurev, M., Ljubičić, I. & Rešetnik, I. 2023. Morphological and environmental variability of *Dianthus sylvestris* (Caryophyllaceae) in the Balkan Peninsula. *Bot. J. Linn. Soc.* 201: 377–389. https://doi.org/10.1093/botlinnean/boac058

Tison, J.-M. & de Foucault, B. 2014. *Flora Gallica: Flore de France*. Mèze: Biotope Éditions.

Trucchi, E., Frajman, B., Haverkamp, T.H.A., Schönswetter, P. & Paun, O. 2017. Genomic analyses suggest parallel ecological divergence in *Heliosperma pusillum* (Caryophyllaceae). *New Phytol.* 216: 267–278. https://doi.org/10.1111/nph.14722

Tutin, T.G. & Walters, S.M. 1993. *Dianthus* L. Pp. 227–246 in: Tutin, T.G., Burges, N.A., Chater, A.D., Edmondson, J.R., Heywood, V.H., Moore, D.M., Valentine, D.H., Walters, S.M. & Webb, D.A. (eds.), *Flora Europaea*, 2nd ed., vol. 1. Cambridge, U.K.: Cambridge University Press.

Valente, L.M., Savolainen, V. & Vargas, P. 2010. Unparalleled rates of species diversification in Europe. *Proc. Roy. Soc. London, Ser. B, Biol. Sci*. 277: 1489–1496. https://doi.org/10.1098/rspb.2009.2163

Wang, J. 2017. The computer program STRUCTURE for assigning individuals to populations: Easy to use but easier to misuse. *Molec. Ecol. Resources* 17: 981–990. https://doi.org/10.1111/1755-0998.12650

Warren, D.L., Glor, R.E. & Turelli, M. 2008. Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution* 62: 2868–2883. https://doi.org/10.1111/j.1558-5646.2008.00482.x

Westergaard, K.B., Zemp, N., Bruederle, L.P., Stenøien, H.K., Widmer, A. & Fior, S. 2019. Population genomic evidence for plant glacial survival in Scandinavia. *Molec. Ecol.* 28: 818–832. https://doi.org/10.1111/mec.14994

Wigginton, J.E., Cutler, D.J. & Abecasis, G.R. 2005. A note on exact tests of Hardy-Weinberg equilibrium. *Amer. J. Human Genet.* 76: 887–893. https://doi.org/10.1086/429864

Yu, G., Smith, D.K., Zhu, H., Guan, Y. & Lam, T.T. 2017. GGTREE: An R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Meth. Ecol. Evol.* 8: 28–36. https://doi.org/10.1111/2041-210X.12628

Zheng, X., Levine, D., Shen, J., Gogarten, S.M., Laurie, C. & Weir, B.S. 2012. A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics* 28: 3326–3328. https://doi.org/10.1093/bioinformatics/bts606

Appendix 1. Voucher specimens of the studied populations of *Dianthus brachycalyx*, *D. inodorus* and *D. virgineus*.

Taxon name, locality, date, collector(s), collector number, voucher number (herbarium).

Dianthus brachycalyx A.Huet & É.Huet ex Bacch., Brullo, Casti & Giusso, Italy, Abruzzo, Vado di Corno, 21 Jul 2020, *F. Bartolucci, F. Conti, M. Chidozie Ogwu s.n.* PI 062137, 064671–064689 (PI); ***D. brachycalyx***, Italy, Basilicata, Serra del Prete, 6 Aug 2015, *D. Gargano s.n.* (Z+ZT); ***D. brachycalyx***, Italy, Calabria, Monte Caramolo, 4 Aug 2015, *D. Gargano s.n.* (Z+ZT); ***D. brachycalyx***, Italy, Calabria, Monte Dolcedorme, 13 Aug 2015, *D. Gargano s.n.* (Z*ZT); ***D. brachycalyx***, Italy, Calabria, Monte Dolcedorme, 28 Jun 2019, *L. Bernardo s.n.* PI 062135, 064406–064424 (PI); ***D. brachycalyx***, Italy, Marche, Monte Vettore, 22 Jul 2020, *F. Bartolucci, F. Conti, M. Chidozie Ogwu s.n.* PI 062138, 064634–064652 (PI); ***Dianthus inodorus*** (L.) Gaertn., Italy, Veneto, Busi di Avesa, 18 Jul 2020, *L. Minuto s.n.*, PI 041593–041609 (PI); ***Dianthus virgineus*** L., France, Auvergne-Rhône-Alpes, Sahune, 7 Jun 2017, *H. Luqman s.n.* (Z+ZT); ***D. virgineus***, France, Provence-Alpes-Côte d'Azur, Veaux, 6 Jun 2017, *H. Luqman s.n.* (Z+ZT); ***D. virgineus***, France, Occitanie, Montferrier sur Lez, 9 Jul 2020, *L. Varaldo s.n.* PI 041610–041619 (PI); ***D. virgineus***, France, Provence-Alpes-Côte d'Azur, La Turbie, 1 Jul 2020, *L. Varaldo s.n.* PI 062198, 063997–064015 (PI); ***D. virgineus***, France, Provence-Alpes-Côte d'Azur, Col de Castillon, 7 Aug 2020, *L. Varaldo s.n.* PI 062199, 064481–064494 (PI); ***D. virgineus***, France, Provence-Alpes-Côte d'Azur, Gorges de Daluis, 14 Jul 2022, *J. Franzoni & L. Peruzzi s.n.* PI 062177, 062178, 065148–065166 (PI); ***D. virgineus***, France, Provence-Alpes-Côte d'Azur, Mont Chiran, 6 Jun 2017, *H. Luqman s.n.* (Z+ZT); ***D. virgineus***, France, Provence-Alpes-Côte d'Azur, Saint-Vallier-de-Thiey, 5 Jun 2017, *H. Luqman s.n.* (Z+ZT); ***D. virgineus***, Italy, Abruzzo, Monti Simbruini, 15 Jul 2020, *F. Bartolucci, F. Conti, P. Paris s.n.* PI 062210, 064690–064698 (PI); ***D. virgineus***, Italy, Abruzzo, Monte Morrone, 17 Jul 2020, *F. Bartolucci, F. Conti, M. Chidozie Ogwu s.n.* PI 062208, 064653–064670 (PI); ***D. virgineus***, Italy, Calabria, Caccuri, 31 Jul 2020, *L. Bernardo s.n.* PI 062205, 064387–064405 (PI); ***D. virgineus***, Italy, Calabria, Civita, 21 Jul 2015, *D. Gargano s.n.* (Z+ZT); ***D. virgineus***, Italy, Calabria, Morano, 22 Jul 2015, *D. Gargano s.n.* (Z+ZT); ***D. virgineus***, Italy, Calabria, Saracena, 30 Jul 2015, *D. Gargano s.n.* (Z+ZT); ***D. virgineus***, Italy, Campania, Monte Faito, 15 Jul 2021, *M. Innangi s.n.* PI 062136, 064425–064442 (PI); ***D. virgineus***, Italy, Emilia-Romagna, Monte Prinzera, 8 Apr 2011, *H. Luqman s.n.* (Z+ZT); ***D. virgineus***, Italy, Lazio, Litorale di Caprolace, 24 Jun 2020, *D. Iamonico, M. Iberite s.n.* PI 062206, 064443–064461 (PI); ***D. virgineus***, Italy, Lazio, Castel Porziano, 15 Jun 2021, *D. Iamonico s.n.* PI 062196, 064462–064480 (PI); ***D. virgineus***, Italy, Liguria, Andagna, 1 Jul 2020, *L. Varaldo s.n.* PI 062197, 064016–064033 (PI); ***D. virgineus***, Italy, Liguria, Cogoleto, 20 May 2020, *L. Varaldo s.n.* PI 062200, 064495–064501 (PI); ***D. virgineus***, Italy, Toscana, Monte Pisano, 3 Jun 2020, *G. Astuti & J. Franzoni s.n.* PI 061231–061250 (PI); ***D. virgineus***, Italy, Toscana, Poggio Pelato, 8 Jun 2020, *G. Astuti & A. Giacò s.n.* PI 061120–061134 (PI); ***D. virgineus***, Italy, Toscana, Corno al Bufalo, 19 Jun 2020, *G. Astuti & J. Franzoni s.n.* PI 061193–061212 (PI); ***D. virgineus***, Italy, Toscana, Monte Argentario, 27 Jun 2020, *J. Franzoni & M. Franzoni s.n.* PI 041621, 057908, 061213–061230 (PI); ***D. virgineus***, Italy, Toscana, Stribugliano, 28 Jun 2020, *J. Franzoni & M. Franzoni s.n.* PI 043074, 057910, 061269–061286 (PI); ***D. virgineus***, Italy, Toscana, Resceto, 14 Jul 2020, *J. Franzoni & A. Giacò s.n.* PI 043072, 057909, 061251–061268 (PI); ***D. virgineus***, Italy, Toscana, Monte Le Coste, 16 Jul 2020, *J. Franzoni & A. Giacò s.n.* PI 061155–061174 (PI); ***D. virgineus***, Italy, Toscana, Sasso di Castro, 16 Jul 2020, *J. Franzoni & A. Giacò s.n.* PI 061135–061154 (PI); ***D. virgineus***, Italy, Toscana, Pania di Corfino, 20 Jul 2020, *G. Astuti & J. Franzoni s.n.* PI 041622, 061101–061119 (PI); ***D. virgineus***, Italy, Toscana, Libro Aperto, 31 Jul 2020, *J. Franzoni & L. Peruzzi s.n.* PI 043073, 061082–061100 (PI).