# PHYLOGEOGRAPHY AND TAXONOMY OF Dianthus sylvestris WULFEN s.l. ON THE BALKAN PENINSULA

**Terlević, Ana**

**Doctoral thesis / Disertacija**

**2023**

Sveučilište u Zagrebu

Faculty of Science
Department of Biology

Ana Terlević

# PHYLOGEOGRAPHY AND TAXONOMY OF *Dianthus sylvestris* WULFEN s.l. ON THE BALKAN PENINSULA

DOCTORAL THESIS

Zagreb, 2022

University of Zagreb

Faculty of Science

Department of Biology

Ana Terlević

# PHYLOGEOGRAPHY AND TAXONOMY OF *Dianthus sylvestris* WULFEN s.l. ON THE BALKAN PENINSULA

DOCTORAL THESIS

Supervisors:

1. Ivana Rešetnik, PhD
2. Prof. Sandro Bogdanović, PhD

Zagreb, 2022

Sveučilište u Zagrebu

Prirodoslovno-matematički fakultet

Biološki odsjek

Ana Terlević

# FILOGEOGRAFIJA I TAKSONOMIJA VRSTE *Dianthus sylvestris* WULFEN s.l. NA BALKANSKOM POLUOTOKU

DOKTORSKI RAD

Mentori:

1. Dr. sc. Ivana Rešetnik
2. Prof. dr. sc. Sandro Bogdanović

Zagreb, 2022

This doctoral dissertation was carried out as a part of the postgraduate programme at the University of Zagreb, Faculty of Science, Department of Biology – Botany, under the supervision of Ivana Rešetnik, PhD, and Prof. Sandro Bogdanović, PhD. The research was performed within the project "Phylogeography and evolution of three ecologically divergent amphi-Adriatic plant groups", supported by the Croatian Science Foundation (project number UIP-2017-05-2882); project leader Ivana Rešetnik. Both the experimental part of the research and the analyses were carried out at three institutions: Department of Biology of the Faculty of Science, University of Zagreb (Croatia), Institute of Botany, Innsbruck University (Austria), and Institute of Integrative Biology, ETH Zürich (Switzerland).

## Acknowledgements

*My deepest gratitude goes to my supervisor Dr. Ivana Rešetnik. Thank you, Ivana, for your support and patience during my PhD study and related research, and especially for your dedication and help in writing of this Thesis. Your guidance was an integral part of me being able to make it to the end.*

*My second supervisor, Prof. Sandro Bogdanović, merits my deepest gratitude as well. Thank you, Sandro, for motivating and guiding me through this journey. The emotional support you gave me, helped me to move forward.*

*I would like to thank Prof. Zlatko Liber, Prof. Toni Nikolić and Prof. Peter Schönswetter, for being a part of my dissertation committee thus providing their insightful comments and encouragement.*

*My sincere thanks go to Martina Temunović and Božo Frajman, for sharing their immense knowledge and challenging me to widen mine, while making my dissertation so much richer. I thank Simone Fior and Hirzi Luqman, for co-creating this exciting research on Dianthus, and for the fruitful discussions on Zoom call meetings.*

*I thank all the colleagues and fellow lab mates from the University of Innsbruck and ETH Zürich, for welcoming me and letting me learn new things. Additionally, I thank the Italian co-authors on research publications involved in this Thesis.*

*I owe it to my closest colleagues that were involved in the Dianthus research. Ivica, thank you for all those memorable field trips, it would not be so fun without your positive attitude. Marko, thank you for your big heart, for being here for two years and asking me every morning how I'm doing. Marin, thank you for motivating me to take all those R courses and expand my statistical knowledge. Maja, thank you for being such a brilliant bioinformatician, and for being here when the deadlines catch up with us. All my fellows at the Marulić square 20/II, thank you for all the fun we had in the last four years. Thank you for your presence, company and support!*

*A big thanks goes to my best friends. Magdalena, thank you for asking me the right questions which encouraged me to think deeper about what I want in life. Irena, thank you for motivating me to look at plants from another perspective. Sandra, although physically distant most of the time, thank you for being spiritually here with me, your advice meant a lot. Zuzana, thank you for listening and encouraging me, you always have the right words and cheer me up. Lara, thank you for your presence, your critical thinking about everything and unique humour that were always an inspiration to me.*

*In the end, I thank my family, my parents, sister and grandmas, for their support without understanding the meaning of my work! Thank you for believing in me and for giving me space to develop myself in such a way!*

University of Zagreb Doctoral thesis

Faculty of Science

Department of Biology

# PHYLOGEOGRAPHY AND TAXONOMY OF *Dianthus sylvestris* WULFEN s.l. ON THE BALKAN PENINSULA

ANA TERLEVIĆ

Faculty of Science, Department of Biology

The Balkan Peninsula is one of the diversity centres for the morphologically highly variable and taxonomically inconsistently treated *Dianthus sylvestris* (Caryophyllaceae). In this thesis, an array of methods ranging from nomenclatural revision and morphometrics, following with phylogeographic methods (RADseq) and environmental niche analyses, to genome size estimates, were combined to explore the intraspecific relationships within Balkan populations of *D. sylvestris*, to discuss phylogeographic and historical processes that contributed to the observed genetic divergence patterns in this species, and to propose a sensible taxonomic solution. Patterns of genetic variation and species distribution models (SDM) suggested that *D. sylvestris* in the Balkan Peninsula survived the Pleistocene glaciations in two separate glacial refugia located along the eastern Adriatic coast. Two genetic groups with the split occurring around the Neretva River valley have been identified. These groups correspond to the two discerned morphological entities, the north-western and the south-eastern group of populations, distinct by the epicalyx scales shape, calyx teeth incision and petal denticulation. Migration analyses revealed relatively high rates of gene flow within each of the two groups, whereas there was almost no gene flow between them. The genetic differentiation did not support the current taxonomy, and the morphometric analyses revealed a continuous variability of quantitative morphological characters and an absence of clear-cut qualitative morphological differences among the subspecies. However, there was an obvious discontinuity in both morphological and genetic clines, therefore a taxonomic subspecies level was attributed to the two resulting groups, *D. sylvestris* subsp. *sylvestris* in the north and *D. sylvestris* subsp. *bertisceus* in the south. The thermophilous and earlier-flowering *D. sylvestris* subsp. *tergestinus* formed a separate evolutionary lineage, thus a new species-level treatment was proposed for this taxon.

(40 pages, 1 figure, 216 references, original in English)

Keywords: morphometrics, environmental niche analyses, flow cytometry, genetic variation, cline, glacial refugia

Supervisors: Ivana Rešetnik, PhD

Prof. Sandro Bogdanović, PhD

Reviewers: Prof. Zlatko Liber, PhD

Prof. Toni Nikolić, PhD

Prof. Peter Schönswetter, PhD

Sveučilište u Zagrebu
Prirodoslovno-matematički fakultet
Biološki odsjek

Doktorski rad

# FILOGEOGRAFIJA I TAKSONOMIJA VRSTE *Dianthus sylvestris* WULFEN s.l. NA BALKANSKOM POLUOTOKU

ANA TERLEVIĆ

Prirodoslovno-matematički fakultet, Biološki odsjek

Balkanski poluotok jedno je od središta raznolikosti morfološki vrlo varijabilne i taksonomski nedosljedno tretirane vrste *Dianthus sylvestris* (Caryophyllaceae). U sklopu ove disertacije korišten je niz metoda, od nomenklaturne revizije i morfometrije, preko filogeografskih metoda (RADseq) i analiza ekoloških niša, do procjene veličine genoma, kako bi se istražili unutarvrsni odnosi balkanskih populacija vrste *D. sylvestris* i utvrdili mogući filogeografski i povijesni procesi koji su pridonijeli uočenim uzorcima genetske divergencije ove vrste, te kako bi se predložilo novo taksonomsko rješenje. Obrasci genetske varijabilnosti i modeli povoljnosti staništa (SDM) upućuju da je vrsta *D. sylvestris* na Balkanskom poluotoku preživjela pleistocenske glacijacije u dva odvojena glacijalna refugija smještena duž istočne obale Jadranskog mora. Identificirane su dvije genetske skupine, gdje se granica između njih nalazi oko doline rijeke Neretve. Ove skupine odgovaraju dvjema uočenim morfološkim entitetima, sjeverozapadnoj i jugoistočnoj skupini populacija, koje se razlikuju po obliku ljuski epikaliksa, urezu zubaca čaške i nazubljenosti latice. Migracijske analize pokazale su relativno visoke stope protoka gena unutar svake od dviju skupina, dok između njih protoka gena gotovo nije bilo. Genetska diferencijacija nije u skladu s trenutačnom taksonomijom, a morfometrijske analize pokazale su kontinuiranu varijabilnost kvantitativnih morfoloških osobina i odsutnost jasnih kvalitativnih morfoloških razlika između podvrsta. Međutim, postoji jedan očiti diskontinuitet u morfološkom i genetskom gradijentu, stoga je taksonomski status podvrste pripisan dvjema skupinama populacija, *D. sylvestris* subsp. *sylvestris* na sjeveru i *D. sylvestris* subsp. *bertisceus* na jugu. Termofilni i ranije cvjetajući *D. sylvestris* subsp. *tergestinus* tvori zasebnu evolucijsku liniju te je za ovu svojtu predložen novi taksonomski tretman na razini vrste.

(40 stranica, 1 slika, 216 literaturnih navoda, jezik izvornika: engleski)

Ključne riječi: morfometrija, analize ekološke niše, protočna citometrija, genetska varijabilnost, gradijent, glacijalni refugiji

| | |
|---|---|
| Mentori: | Dr. sc. Ivana Rešetnik |
| | Prof. dr. sc. Sandro Bogdanović |
| Ocjenjivači: | Prof. dr. sc. Zlatko Liber |
| | Prof. dr. sc. Toni Nikolić |
| | Prof. dr. sc. Peter Schönswetter |

## Table of content

# LIST OF PUBLICATIONS

I. **Terlević A**, Rešetnik I. 2020. Inventory of the historical *Dianthus sylvestris* herbarium materials from Herbarium Croaticum and Herbarium Ivo and Marija Horvat. *Glasnik Hrvatskog botaničkog društva* 8: 8–14. doi:10.46232/glashbod.8.1.2.

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

III. **Terlević A**, Bogdanović S, Frajman B, Rešetnik I. 2022. Genome Size Variation in *Dianthus sylvestris* Wulfen sensu lato (Caryophyllaceae). *Plants* 11: 1481. doi:10.3390/plants11111481.

IV. **Terlević A**, Temunović M, Bogdanović S, Grgurev M, Ljubičić I, Rešetnik I. 2022. Morphological and environmental variability of *Dianthus sylvestris* Wulfen (Caryophyllaceae) on the Balkan Peninsula. *Botanical Journal of the Linnean Society (In press).* doi:10.1093/botlinnean/boac058.

V. Temunović M, **Terlević A**, Fior S, Luqman H, Rešetnik I, Widmer A. Genomic divergence of *Dianthus sylvestris* Wulfen s.l. on the Balkan Peninsula. *In preparation*.

# LIST OF APPENDICES

I. **Terlević A**, Temunović M, Bogdanović S, Grgurev M, Ljubičić I, Rešetnik I. 2022. Environmental variability of *Dianthus sylvestris* Wulfen (Caryophyllaceae) on the Balkan Peninsula.

II. **Terlević A**, Bogdanović S, Rešetnik I. 2022. Taxonomic treatment of *Dianthus sylvestris* Wulfen (Caryophyllaceae) on the Balkan Peninsula.

# THESIS SUMMARY

Phylogeographic studies across Europe have revealed Balkan, Apennine, and Iberian Peninsulas as the three main refugia for European flora and fauna of the temperate zone during Quaternary climatic fluctuations, and that these Peninsulas acted as sources for the recolonization of northern Europe after the glaciations. Moreover, due to the long-term environmental stability, these refugial areas often have high biodiversity and endemism. The hypothesis that the origin of most genera and species dates back to the Pliocene and Miocene periods is widely supported. However, the evolution within complexes of sister species and the evolution of intraspecific lineages are closely related with migration dynamics and changes in their distributions caused by cyclical climate changes during the Pleistocene. With the accumulation of evidence from phylogeographic studies, it has become clear that the Mediterranean Peninsulas harbour genetically divergent evolutionary lineages, which suggests the isolation of populations in geographically different microrefugia ('refugia-within-refugia' hypothesis). The Balkan Peninsula is a topographically and ecologically very heterogeneous area, characterised with high interspecific and intraspecific diversity. Therefore, its complex geological and climatic history, combined with heterogenous habitats, provides exceptional material for phylogeographic research. Concordant patterns of genetic variability and distribution have been observed in different species on the Peninsula, suggesting the existence of common patterns in biogeographical history. It is postulated that ecologically divergent species with different habitat requirements had different migration dynamics and distribution shifts in response to oscillations of favourable and unfavourable time periods. Specifically, the thermophilous species shifted their distribution along latitudes, while cold-adapted species probably experienced elevational range shifts. Both thermophilous and cold-adapted species experienced range expansion during favourable conditions, and range contraction during unfavourable conditions, according to their requirements. However, data on migration dynamics are lacking for species occupying habitats spanning wide latitudinal and elevational ranges. One such species is the taxonomically intricate *Dianthus sylvestris* Wulfen.

*Dianthus* L. is a taxonomically very complex genus and represents one of the most diverse plant groups in Europe. It is characterised by many endemic species with small and geographically very limited distributions, which indicates recent diversification. *Dianthus sylvestris* is distributed across the central Mediterranean, where it grows primarily on rocky grasslands, from lowland coastal to alpine environments. According to the available literature, numerous taxa at the species or subspecies level belonging to *D. sylvestris* s.l. have been

described across its distribution area. However, botanists have not yet agreed upon its taxonomy. Identification keys at the intraspecific level exist (e.g., Trinajstić 1979; Micevski 1993; Tutin and Walters 1993; Brullo and Guarino 2019), but the identification of subspecies is often ambiguous. *Dianthus sylvestris* on the Balkan Peninsula is currently considered as a species with seven subspecies: *D. sylvestris* subsp. *alboroseus* F. K. Mey, *D. sylvestris* subsp. *bertisceus* Rech. f., *D. sylvestris* subsp. *kozjakensis* Micevski, *D. sylvestris* subsp. *longicaulis* (Ten.) Greuter et Burdet, *D. sylvestris* subsp. *nodosus* (Tausch) Hayek, *D. sylvestris* subsp. *sylvestris* and *D. sylvestris* subsp. *tergestinus* (Rchb.) Hayek.

The main aim of this thesis was to disentangle the intraspecific relationships within *D. sylvestris* on the Balkan Peninsula, combining traditional (morphometrics) and contemporary approaches (new generation sequencing, NGS, and the application of single-nucleotide polymorphism, SNP, genome size estimations and species distribution models, SDMs). The research objectives were to: (1) Conduct morphometric and genetic analysis with the aim of solving the taxonomic problems of *D. sylvestris* s.l. and to gain insight into phylogeographic processes at the local and regional level; (2) Develop models of habitat suitability for the present and the past and to analyse the ecological niches of *D. sylvestris* subspecies with the aim of investigating the relationship between their morphological, genetic and environmental divergence; (3) Conduct a taxonomic revision of *D. sylvestris* s.l. on the Balkan Peninsula. Moreover, the following research hypotheses were tested: (1) There is genetic variability between populations of *D. sylvestris* s.l. associated with the existence of microrefugia on the Balkan Peninsula; (2) Populations belonging to different subspecies, evolutionary lineages and/or different habitat types can be identified according to their morphological diagnostic characters.

In this thesis, represented through four publications (**I – IV**), one scientific paper in preparation (**Publication V**), and two unpublished results (**Appendix I – II**), an array of complementary methods was used to disentangle relationships within *D. sylvestris* s.l.

Genomic data based on the comprehensive sampling across the Balkan Peninsula was combined with species distribution models (SDMs) generated for the present and the past (Last Glacial Maximum; LGM) to identify putative glacial refugia and assess putative migration patterns on the Balkan Peninsula during Pleistocene climatic oscillations. Several areas of pronounced relative genome size (RGS) variation at small geographic distances were revealed among the widespread diploid populations, likely indicating the intraspecific genetic divergence, whereas spatially restricted tetraploid populations of *D. sylvestris* were recorded for the first time on the Peninsula (**Publication III**). Their restricted distribution in the northern Adriatic region may be an indication of the existence of a refugium in this area. Genomic data showed a clear divergence of *D. sylvestris* subsp. *tergestinus* from the rest of the Balkan populations, despite their co-occurrence in sympatry. Furthermore, two groups of populations, the north-western and the south-eastern group, were revealed as two separately evolving evolutionary lineages, giving evidence for the existence of two separated glacial refugia on the Balkan Peninsula (**Publication V**). The two lineages might have come into contact during expansions, probably occurring throughout interglacials, in the intermediate area of Montenegro where this event resulted in the observed admixed populations. However, since *D. sylvestris* comprises both low-elevation (valleys) and high-elevation (alpine) populations, an alternative hypothesis of Pleistocene migration pathways was also suggested. It implies that the observed complex genetic structure may be a result of both range contraction of low-altitude populations and range expansion of high-altitude populations during the cold periods (**Publication V**). However, these hypotheses need to be tested in future research.

As a first step towards understanding the taxonomy of *D. sylvestris* and its tangled nomenclature synonymy, an inventory of historical herbarium material in the Croatian herbaria was conducted (**Publication I**). Furthermore, since the names *D. sylvestris* Wulfen and *D. virgineus* var. *tergestinus* Rchb. were published before 1958 (Art. 40.1; Turland *et al.* 2018), they did not have a *type*, so *types* relative to this period were chosen as *lectotypes* amongst the original material used by the authors of these names (**Publication II**). Also, a *lectotype* was chosen among the *syntypes* for the name *D. sylvestris* subsp. *bertisceus* (**Appendix II**). A taxonomic approach integrating RGS analyses, morphometrics, environmental niche analyses, phylogenetics and population genetics was applied. A continuous variability, where morphological states gradually change both with environment and geography was revealed (**Publication IV, Appendix I**). The continuity, i.e., a cline, was found in genomic data as well, except for a subset of populations identified as *D. sylvestris* subsp. *tergestinus* showing genetic divergence from all other Balkan populations (**Publication V**). Furthermore, Balkan populations showed a genetic split around the area of Neretva River valley, and this split is reflected in one morphological character, that is the shape of epicalyx scales (**Publication IV**). All above-mentioned evidence advocates for a reduction of the number of subspecies on the Balkan Peninsula (**Appendix II**).

The scientific contribution of this thesis is the advancing of general knowledge about migration routes of plants during the Pleistocene and the clarification of the role of refugia and ‘refugia-within-refugia’ in shaping high biodiversity in the Balkan Peninsula. A contemporary approach of integrating different types of data and methodologies enables testing of the hypothesis of species movement along latitudes or elevations in response to cyclical changes in Pleistocene. Moreover, this thesis contributes to the reinforcement of state-of-the-art methodology and analyses in the Croatian research community focused on evolutionary and phylogeographic issues, such as the NGS and the application of SNPs for exploring biodiversity. Finally, populations of *D. sylvestris* s.l. on the Balkan Peninsula are morphologically, ecologically, and genetically characterised, which enables defining a sensible taxonomy. Resolved taxonomic relationships contribute to the proper management and protection of natural populations.

# PROŠIRENI SAŽETAK

Filogeografska istraživanja diljem Europe otkrila su Balkanski, Apeninski i Pirinejski poluotok kao tri glavna utočišta za europsku floru i faunu umjerenog pojasa tijekom klimatskih fluktuacija u Kvartaru, te da su ti poluotoci djelovali kao ishodišta za rekolonizaciju sjeverne Europe nakon glacijacija. Štoviše, zbog dugoročne stabilnosti okoliša, ova refugijalna područja često imaju visoku stopu biološke raznolikosti i endemizma. Široko je potvrđena hipoteza da podrijetlo većine rodova i vrsta seže u razdoblje Pliocena i Miocena. Međutim, evolucija unutar kompleksa sestrinskih vrsta i evolucija unutarvrsnih linija usko su povezane s migracijskom dinamikom i promjenama u njihovoj distribuciji uzrokovanom cikličkim klimatskim promjenama tijekom Pleistocena. Akumulacijom dokaza iz filogeografskih istraživanja postalo je jasno da Sredozemni poluotoci kriju genetski različite evolucijske linije, što sugerira izolaciju populacija u geografski različitim mikrorefugijima (hipoteza 'refugija unutar refugija'). Balkanski poluotok je topografski i ekološki vrlo heterogeno područje, obilježeno visokom međuvrsnom i unutarvrsnom raznolikošću. Složena geološka i klimatska povijest, u kombinaciji s heterogenim staništima, pruža izuzetan materijal za filogeografska istraživanja ovog područja. Sukladni obrasci genetske varijabilnosti i distribucije uočeni su kod različitih vrsta na Poluotoku, što ukazuje na postojanje zajedničkih obrazaca u biogeografskoj povijesti. Pretpostavlja se da su ekološki divergentne vrste s različitim zahtjevima staništa imale različitu migracijsku dinamiku i pomake distribucije kao odgovor na oscilacije povoljnih i nepovoljnih vremenskih razdoblja. Točnije, termofilne vrste pomicale su svoju distribuciju duž geografske širine, dok su vrste prilagođene nižim temperaturama vjerojatno doživjele pomake u rasponu nadmorske visine. I termofilne i vrste prilagođene nižim temperaturama doživjele su proširenje areala tijekom povoljnih uvjeta, a smanjenje areala tijekom nepovoljnih uvjeta, u skladu sa svojim zahtjevima. Međutim, nedostaju podaci o dinamici migracije vrsta koje zauzimaju staništa širokog raspona geografskih širina i nadmorskih visina. Jedna takva je taksonomski kompleksna vrsta *Dianthus sylvestris* Wulfen.

*Dianthus* L. taksonomski je veoma složen rod i predstavlja jednu od najraznovrsnijih biljnih skupina u Europi. Karakteriziraju ga mnoge endemske vrste s malom i geografski vrlo ograničenom distribucijom, što ukazuje na nedavnu diverzifikaciju. Vrsta *Dianthus sylvestris* rasprostranjena je u središnjem Sredozemlju, gdje raste prvenstveno na kamenjarskim travnjacima, od nizinskih obalnih do alpskih staništa. Prema dostupnoj literaturi, brojne svojte na razini vrste ili podvrste koje pripadaju vrsti *D. sylvestris* s.l. opisane su na području distribucije. Međutim, botaničari još nisu usuglašeni oko taksonomije tih svojti. Identifikacijski ključevi na unutarvrsnoj razini postoje (npr. Trinajstić 1979; Micevski 1993; Tutin and Walters 1993; Brullo and Guarino 2019), no identifikacija podvrsta često je dvosmislena. Trenutno se *D. sylvestris* na Balkanskom poluotoku smatra vrstom koja obuhvaća sedam podvrsta: *D. sylvestris* subsp. *alboroseus* F. K. Mey, *D. sylvestris* subsp. *bertisceus* Rech. f., *D. sylvestris* subsp. *kozjakensis* Micevski, *D. sylvestris* subsp. *longicaulis* (Ten.) Greuter et Burdet, *D. sylvestris* subsp. *nodosus* (Tausch) Hayek, *D. sylvestris* subsp. *sylvestris* i *D. sylvestris* subsp. *tergestinus* (Rchb.) Hayek.

Glavni cilj ove disertacije bio je razjasniti unutarvrsne odnose vrste *D. sylvestris* na Balkanskom poluotoku, kombinirajući tradicionalne (morfometrija) i suvremene pristupe (sekvencioniranje nove generacije, NGS, i primjena polimorfizma jednog nukleotida, SNP, procjena veličine genoma i modeliranje povoljnosti staništa, SDM). Ciljevi istraživanja bili su: (1) Provesti morfometrijsku i genetičku analizu s ciljem rješavanja taksonomskih problema vrste *D. sylvestris* s.l. te stjecanje uvida u filogeografske procese na lokalnoj i regionalnoj razini; (2) Razviti modele povoljnosti staništa za sadašnjost i prošlost i analizirati ekološke niše podvrsta vrste *D. sylvestris* s ciljem istraživanja odnosa između njihove morfološke, genetske i okolišne divergencije; (3) Provesti taksonomsku reviziju vrste *D. sylvestris* s.l. na Balkanskom poluotoku. Nadalje, ispitane su sljedeće istraživačke hipoteze: (1) Postoji genetska varijabilnost između populacija vrste *D. sylvestris* s.l. povezana s postojanjem mikrorefugija na Balkanskom poluotoku; (2) Populacije koje pripadaju različitim podvrstama, evolucijskim linijama i/ili različitim tipovima staništa mogu se identificirati prema njihovim morfološkim dijagnostičkim osobinama.

U ovoj disertaciji, predstavljenoj kroz četiri publikacije (**I – IV**), jedan znanstveni rad u pripremi (**Publikacija V**) i dva neobjavljena rezultata (**Dodatak I – II**), upotrijebljen je niz komplementarnih metoda kako bi se razjasnilo odnose unutar vrste *D. sylvestris* s.l.

Genomski podaci temeljeni na sveobuhvatnom uzorku na Balkanskom poluotoku kombinirani su s modelima povoljnosti staništa (SDM) generiranim za sadašnjost i prošlost (posljednji glacijalni maksimum; LGM) kako bi se identificirali pretpostavljeni glacijalni refugiji i procijenili pretpostavljeni migracijski obrasci na Balkanskom poluotoku tijekom klimatskih oscilacija u Pleistocenu. Među široko rasprostranjenim diploidnim populacijama otkrivena su područja izražene varijabilnosti relativne veličine genoma (RGS) na malim geografskim udaljenostima, što vjerojatno ukazuje na unutarvrsnu genetsku divergenciju, dok su prostorno ograničene tetraploidne populacije vrste *D. sylvestris* prvi put zabilježene na poluotoku (**Publikacija III**). Njihova ograničena rasprostranjenost na području sjevernog

Jadrana može biti pokazatelj postojanja refugija na tom području. Populacije podvrste *D. sylvestris* subsp. *tergestinus* u genomskim su podacima pokazale jasnu divergenciju od ostalih balkanskih populacija, unatoč njihovom pojavljivanju u simpatriji. Nadalje, dvije skupine populacija, sjeverozapadna i jugoistočna skupina, otkrivene su kao dvije zasebno razvijajuće evolucijske linije, dajući dokaze u prilog postojanju dvaju odvojenih glacijalnih refugija na Balkanskom poluotoku (**Publikacija V**). Ove su dvije linije mogle doći u kontakt tijekom ekspanzija, koje su se vjerojatno događale tijekom interglacijala, na području Crne Gore gdje je ovaj događaj rezultirao populacijama koje su pokazale miješanu genetsku strukturu. Međutim, budući da *D. sylvestris* obuhvaća i nizinske (doline) i visoko planinske (alpske) populacije, također je predložena alternativna hipoteza o Pleistocenskim migracijskim putovima, koja implicira da uočena složena genetska struktura može biti rezultat smanjivanja područja rasprostranjenosti nizinskih populacija i širenja rasprostranjenosti visoko planinskih populacija tijekom hladnih razdoblja (**Publikacija V**). Međutim, te hipoteze treba testirati u budućim istraživanjima.

Kao prvi korak prema razumijevanju taksonomije vrste *D. sylvestris* i njezine zamršene nomenklaturne sinonimije, provedena je inventarizacija povijesne herbarijske građe u hrvatskim herbarijima (**Publikacija I**). Nadalje, budući da su imena *D. sylvestris* Wulfen i *D. virgineus* var. *tergestinus* Rchb. objavljena prije 1958. (čl. 40.1; Turland *et al.* 2018) i nemaju tipski primjerak, ilustracije iz izvorne građe koju su autori ovih naziva koristili, odabrani su kao lektotipovi (**Publikacija II**). Također, među sintipovima je odabran lektotip za ime *D. sylvestris* subsp. *bertisceus* (**Dodatak II**). Primijenjen je taksonomski pristup koji uključuje RGS analizu, morfometriju, analizu ekoloških niša, filogeografiju i populacijsku genetiku. Otkrivena je kontinuirana varijabilnost, gdje se morfološka stanja postupno mijenjaju s okolišem i geografijom (**Publikacija IV, Dodatak I**). Kontinuitet, tj. gradijent, također je pronađen u genomskim podacima, osim podskupa populacija identificiranih kao *D. sylvestris* subsp. *tergestinus* koji je pokazao genetsku divergenciju od svih ostalih balkanskih populacija (**Publikacija V**). Balkanske su populacije pokazale genetsku podjelu oko područja doline rijeke Neretve, a ta podjela se ogleda u jednoj morfološkoj osobini, a to je oblik ljuski epikaliksa (**Publikacija IV**). Svi gore navedeni dokazi govore u prilog smanjenju broja podvrsta na Balkanskom poluotoku (**Dodatak II**).

Znanstveni doprinos ove disertacije je unaprjeđenje općeg znanja o migracijskim putovima biljaka tijekom Pleistocena i pojašnjenje uloge refugija i 'refugija unutar refugija' u oblikovanju visoke bioraznolikosti na Balkanskom poluotoku. Suvremeni pristup integriranja različitih tipova podataka i metodologija omogućuje testiranje hipoteze o kretanju vrsta duž geografskih širina ili nadmorskih visina kao odgovor na cikličke promjene u Pleistocenu. Nadalje, ovaj rad pridonosi razvoju analitike i najsuvremenije metodologije u hrvatskoj istraživačkoj zajednici, usmjerene na evolucijska i filogeografska pitanja, kao što su NGS i primjena SNP-ova za istraživanje bioraznolikosti. Konačno, populacije vrste *D. sylvestris* s.l. na Balkanskom poluotoku su morfološki, ekološki i genetski karakterizirane, što omogućuje definiranje smislene taksonomije. Razriješeni taksonomski odnosi doprinose pravilnom gospodarenju i zaštiti prirodnih populacija.

# INTRODUCTION

# *INTRODUCTION*

## *Integrative approach to systematic botany*

The advancement of quantitative approaches to classification in the second half of the 20th century was a consequence of dissatisfaction with the previous intuitive approaches to classification and to science in general (Stuessy and Hörandl 2014). Phenetics appeared as a first attempt of quantitative classification (Michener and Sokal 1957), and after systematists realised that classifying species based solely on morphology has no sense, cladistics came into this challenge (Hennig 1966). Systematics is a discipline dealing with change, and the study of evolution is crucial for contemporary systematics. However, controversy regarding how taxonomists should use the results of phylogenetic analyses and incorporate the diversity of evolutionary patterns and processes into classification still exists. First, evolutionary processes characteristic for plants, such as polyploidization and hybridization, result in non-dichotomous branching patterns (Soltis and Soltis 2009), and thus generate incongruences among phylogenetic trees. However, incongruence does not have to be considered a limitation, but it may rather be regarded as a source of information for a deeper understanding of evolutionary processes (Wendel and Doyle 1998). Second, there are various concepts of species available, and different user groups use different concepts (Turelli *et al.* 2001; Fujita *et al.* 2012). Speciation is usually a process, not a singular event in time, and as such, approaches using genomic data mostly delineate lineages (fine-scale genetic structure), rather than species (Sukumaran and Knowles 2017). The confusion about species limits and the potential of taxonomic overinflation could create problems in all fields that rely on species as units of analysis, from studies of macroevolutionary dynamics to conservation biology (Sukumaran and Knowles 2017). Therefore, results based on genomic data should be considered as only one of the multiple lines of evidence which requires validation from other sources of information, such as morphology/phenotype and ecology. Overall, present-day systematic botany is a complex methodical discipline which benefits from technical development and new data sources. Only by integrating the information obtained from evidence-based multi-layered results can we discover relationships between species and/or lineages and determine underlying processes (Stuessy and Hörandl 2014). By incorporating up-to-date systematic botany, the intriguing questions on biodiversity of a certain area and on the processes involved in its creation can be uncovered.

## *Phylogeography*

Phylogenetic analysis is the central theme of higher-level systematics, whereas population genetics permeate studies of intraspecific evolution. Throughout most of the 20th century, there was a gap between those two fields and phylogeography nowadays is helping to bridge this gap by synthesising micro- and macroevolutionary thought (Avise 2007). Phylogeography, a term coined by Avise *et al.* (1987), is a discipline concerned with the geographical distribution of genetic lineages within and among closely related species. To explore the genetic structure, phylogeography uses both information on contemporary forces of genetic exchange, such as reproductive ecology and gene flow, and historical information (Schaal *et al.* 1998). Thus, phylogeographic studies help to understand the evolutionary processes that have driven population expansion and contraction and gene movement, and that were impacted by climate fluctuations and geographic and environmental boundaries. Empirically, most intraspecific genetic lineages have proved to be non-overlapping or nearly so in geographic distribution, i.e., allopatric (e.g., Kuzmanović *et al.* 2013; Španiel, Marhold, *et al.* 2017; Šlenker *et al.* 2021). Moreover, the geographic distributions of genetic lineages frequently correspond with obvious environmental barriers to gene flow or the locations of Pleistocene refugia (Schönswetter *et al.* 2005; Slovák *et al.* 2012; Grdiša *et al.* 2014; Rešetnik *et al.* 2020; Záveská *et al.* 2021; Ninčević *et al.* 2021). Phylogeography can be also used as an insight into the relationship between genetic and morphological/taxonomic differentiation. For example, the concordance between morphological differentiation and genetic lineages was found in *Cyanus tuberosus* group (Skokanová *et al.* 2019) and in *Cardamine acris* (Perný *et al.* 2004), where genetic lineages were recognised as subspecies. On the other hand, clades inferred by chloroplast sequences in *Heliosperma pusillum* (Frajman and Oxelman 2007), and genetic groups inferred by amplified fragment length polymorphism (AFLP) in *Knautia drymeia* (Rešetnik, Frajman, *et al.* 2016), were geographically, rather than taxonomically correlated. Continuing and deepening phylogeographic research has great potential to better understand the genesis of biodiversity hotspots, and thus to help in their conservation (Radosavljević *et al.* 2012; Theodoridis *et al.* 2019; Liber *et al.* 2020).

## *Taxonomy*

The integration of different types of data and methodologies is currently widely used for disentangling taxonomically intricate groups of organisms (Pante *et al.* 2015; Caković *et al.* 2018; Frajman *et al.* 2019; Hardion *et al.* 2020). Correct identification of a specimen is a basic prerequisite and a first step for all botanical scientific research. Thus, misidentifications of taxa can be fatal to the research results or nature protection actions (Mace 2004). Many taxa show exceptional variability associated with various evolutionary and ecological phenomena, such as polyploidy (Balao *et al.* 2009), phenotypic plasticity (Pfennig *et al.* 2010), different ecotypes (Hardion *et al.* 2020), etc., making their taxonomy very complex. Classification systems developed for such groups are temporary and prone to changes. Depending on the preferences of the authors, the taxonomic approach can result in splitting a taxon into many smaller subgroups (“splitters” approach, e.g., Lin *et al.* 2021) or combining several taxa into one large group (“lumpers” approach, e.g., Caković *et al.* 2015). Continuous splitting and lumping taxa based on subjective criteria generates an unstable taxonomy (Eriksson *et al.* 2022).

Throughout the history of plant taxonomy, the accumulation of nomenclatural synonyms is very common. The *International Code of Nomenclature for algae, fungi and plants* defines current plant nomenclature practices and describes how to name plants assigned to ranked categories (Turland *et al.* 2018). The requirement to deal with names and to solve nomenclatural issues of historical botanical research occupies a large proportion of the taxonomist’s working time. The main reason is that typification works are often published in minor or general publications, making it difficult to know whether a name of a taxon has already been typified. Consequently, herbarium collections are repeatedly studied from scratch by individual researchers studying the history of a name. Historical and geographical information attached to historical herbarium collections are often vague, which makes them challenging to study. However, modern technologies, such as digitising and cataloguing, are making the collections widely accessible to scientists, and thus enable their reinterpretations (Hardy *et al.* 2020).

### ***Intraspecific diversity***

All plants display some degree of morphological variability among populations. Swedish botanist Göte W. Turesson was one of the first to show that slightly different phenotypes are the result of adaptations to local environmental conditions (Turesson 1925). To describe these mutually different populations, Turesson introduced the term *ecotype*. The biosystematists that agreed with Turesson stressed the importance of reproductive isolating barriers that limit gene flow between groups on the path to speciation (Clausen 1951). On the other hand, Huxley (1938) was concerned that the continuously distributed phenotypic variation was overlooked by classification systems of his era and thus introduced the term *cline*. In contrast to initial Turesson's concept of *ecotype* suggesting that intraspecific plant variation could be classified into discrete units, Huxley's *cline* implies the graduation of variation in traits over space. It is true that some traits can vary gradually, others discontinuously, depending on a number of factors, e.g., gene flow, number of genes involved, environmental stressors, etc. (Linhart and Grant 1996), but *ecotypes* reflect the composite response of multiple traits to the local selection pressures, whereas *clines* describe single character variation across space. The relationship of *ecotypes* to the taxonomic hierarchy and the practical need to name plants is diverse. According to some authors, it is justified for *ecotypes* to receive a status in the taxonomic hierarchy and to be nomenclaturally resolved usually at the level of subspecies, for example in *Orchis papilionacea* L. (Cozzolino *et al.* 2021) and *Dianthus superbus* L. (Hardion *et al.* 2020). On the other hand, *cline* represents a gradient in phenotypic or genetic character which is developed in the absence of barriers to gene flow (Endler 1977), which thus cannot be fitted into discrete units. The revolutionary impacts of the use of DNA sequence data and their interpretation using genomic tools, coupled with population structure and geographical patterns analyses (e.g., Dyer *et al.* 2010) has contributed to the understanding how genetic variation is partitioned within plants species (Abbott 2017; Halbritter *et al.* 2018). With new data and discoveries, we gain evidence that plant species are not just phyletic lineages that need to be taxonomically recognised but consist of dynamically evolving populations whose relationships can be fully understood by integrating different types of data and methodologies.

## ***Data used in systematics / integrative taxonomy***

### *Morphology*

Morphological data are still the most used in plant taxonomy since morphological features can be recorded immediately and allow for a rapid identification. Morphological data can be classified in macromorphological and micromorphological characters, where the former is the most used as diagnostic in identification keys (Stuessy 2009; Beentje 2016). The latter have gained importance with the improvement of scanning electron microscopy techniques, for example characters related to trichomes (Carnicero 2017) and seeds (Bogdanović *et al.* 2016) are successfully used as diagnostic. Morphological features can also be classified into vegetative and reproductive data characters. Vegetative organs often perform several functions simultaneously (physical support, assimilation, water conduction, etc.), and therefore have greater adaptive capacity and phenotypic plasticity (Halbritter *et al.* 2018), leading to their reduced usefulness in taxonomy. However, leaf characters are often successfully used in the taxonomy of tree species (Jensen *et al.* 2002; Morales-Saldaña *et al.* 2021). Characters of reproductive organs are easy to observe and they are effective when creating identification keys and in description of taxa. These characters display less variability in relation to vegetative characters, they are more numerous, and even small differences in the structure of flower often provide reliable insight into phylogenetic relationships and classification (Stuessy 2009).

One of the principal tools in assessing morphological features is multivariate morphometrics, which in the second half of the 20th century became standard for analysing large sets of measurements from different samples, and became desirable to validate morphological descriptions of taxa (e.g., Trigas *et al.* 2018). The morphometric analyses are frequently used to explore diversity patterns at intraspecific level (Marhold 2011a). When combined with other methodological approaches such as molecular systematics and/or ploidy level estimations, multivariate morphometrics is successfully applied to disentangle complexes of closely related taxa (Đurović *et al.* 2014; Španiel, Zozomová-Lihová, *et al.* 2017; Buzurović *et al.* 2020), re-examine poorly described or dubious taxa (Rooks *et al.* 2011) and describe intraspecific variability, such as morphological differentiation between populations in heterogeneous environments (Abdusalam and Li 2018) and morphological differentiation between cytotype (Španiel *et al.* 2011). Taxonomic hypotheses can be generated using ordination methods such as principal components analysis (PCA), whereas they are tested using discriminant analyses (DAs). Principal components analysis is a technique that reduces dimensionality of the original character space. As the first two dimensions capture most of the variation of the original dataset, it is possible to visualise the relationships of individual objects on a two-dimensional plane. Although PCA was developed for quantitative characters with normal distribution, it can be used with certain reservations also for binary and semiquantitative characters and is also considerably robust with respect to departures of characters from normal distribution. The strict limitation of PCA is with regard to the number of analysed characters which should be lower than the number of analysed objects (Marhold 2011a). On the other hand, to view relationships among predefined groups in space defined by canonical axes, canonical discriminant analysis (CDA) is used. For example, groupings based on genetic markers (Kučera *et al.* 2013), genome size or ploidy levels (Koutecký *et al.* 2012) or habitat characteristics (Španiel *et al.* 2011) can be interpreted. CDA maximises separation of predefined groups and thus allows answering two questions (Marhold 2011a): 1) based on available characters, to what extent can predefined groups be morphologically distinguished, and 2) which characters contribute to this differentiation. However, it is important that the characters that define the groups should not be involved in the analysis to avoid circular argumentation.

### *Genomic data*

Molecular/genomic data are applied in population genetic studies (geographic variability, course of gene flow, etc.; Temunović *et al.* 2012), determination of boundaries between species and higher categories (Caković *et al.* 2015), and construction of phylogenetic hypotheses on all taxonomic levels (Fassou *et al.* 2022). The recent proliferation of DNA sequencing technologies has led to a rapid increase in the volume of genomic data. Obtaining high-coverage, well-assembled and thoroughly annotated genomes is still very expensive and time-consuming, therefore subsampling methods that yield a specific set of markers chosen *a priori* provide a cost-effective solution for achieving deep sequence coverage across the sampled sites of the genome (Harvey *et al.* 2016). Restriction site associated DNA sequencing (RAD-Seq) is the most widespread method for obtaining genomic datasets from non-model organisms (Harvey *et al.* 2016). RAD-Seq is a family of methods using restriction enzymes which are often selected to cut at sites widely distributed across the genome, and thus RAD-Seq sites may come from diverse coding and non-coding regions, but not from repetitive regions (DaCosta and Sorenson 2014). Most RAD-Seq techniques focus on collecting short sequences or single nucleotide polymorphism (SNP) data from groups of short sequences.

SNP data are genomic markers used in population genetics and phylogeographic studies aimed at elucidating the history of natural populations (Trucchi *et al.* 2017; Binks *et al.* 2021; Liang *et al.* 2022; Prata *et al.* 2022). The development of efficient analytical methods has allowed variation across multiple genomes of sampled populations to be investigated and species histories to be inferred. In any project on population genetics, multiple methods have to be used and compared since this approach will provide a more comprehensive overview of the evolutionary processes acting (Bourgeois and Warren 2021). To visualize genetic distances among individuals and populations, multivariate approach such as Principal Components Analysis (PCA) can be used. This method is suitable for analysing polyploid species, since it does not have any underlying population genetic-based assumptions, such as the Hardy-Weinberg (HW) equilibrium (Dufresne *et al.* 2014). Unlike these model-free approaches, model-based approaches provide an estimate of the proportion of an individual genome originating from multiple ancestral gene pools, given a predefined number of clusters ($K$). Some of these tools, based on a Bayesian framework, are STRUCTURE (Pritchard *et al.* 2000) and

FASTSTRUCTURE (Raj *et al.* 2014), which group individuals in clusters maximizing HW equilibrium and linkage disequilibrium between loci. An alternative to STRUCTURE may be the Discriminant analysis of principal components (DAPC, ADEGENET; Jombart *et al.* 2010), which maximizes divergence between groups identified by PCA and it does not require that populations are in HW equilibrium. However, methods for assessing population structure that make use of genotype information are in principle more powerful than multivariate approaches (Dufresne *et al.* 2014). Tools for demographic inference that are based on allele frequencies are, for example, TREEMIX (Pickrell and Pritchard 2012), which infers most likely admixture events in a tree, and divMigrate (Sundqvist *et al.* 2016), which estimates directional relative migration (=gene flow) and tests asymmetry between migrating rates. Asymmetric migration is common in natural populations and information about the symmetry of gene flow becomes important for understanding genetic structuring of populations and make inference on past demographic changes (Sundqvist *et al.* 2016).

Phylogenetic trees are commonly used to reconstruct the relationships between populations, and can be constructed based on restriction-digest methods that primarily focus on SNPs (Herrera and Shank 2016; Harvey *et al.* 2016; Bombonato *et al.* 2020; Bateman 2021). SNP data has been once considered reserved for population genetic studies, however their potential to both resolve phylogenetic problems and investigate population demographics, makes them an important source of genomic information (Leaché and Oaks 2017). One tool for phylogenetic inference based on the maximum likelihood (ML) search criterion is IQ-TREE (Minh *et al.* 2020), which estimates divergence time and phylogenetic relationships. The task of phylogenetics is to find the best way to translate evolutionary changes in DNA sequences and other characters into models that effectively represent the underlying mechanisms for genome-scale variation (Bravo *et al.* 2019).

### *Cytology*

Out of the multitude of cytological characteristics, data related to chromosomes are mainly used in plant taxonomy – their number and morphology, aspects of polyploidy and number and position of satellite chromosomes (Behroozian *et al.* 2012). The use of flow cytometry (FCM) for estimating genome size of plants has rapidly expanded in taxonomic studies since the late 1980s (Suda, Kron, *et al.* 2007). It is generally used for ploidy estimation of individuals (Greilhuber *et al.* 2005; Suda and Trávníček 2006), in which DNA content of a sample is compared to a reference of known ploidy and expressed as multiples of a single chromosome complement. Understanding the course of evolution, adaptive radiation, the emergence of new species etc., is often directly related to cytological changes. FCM is now an essential tool for quantifying these changes and identifying cryptic taxonomic structure by providing distinction of morphologically indiscernible taxa (Suda, Krahulcová, *et al.* 2007; Šmarda *et al.* 2008; Slovák *et al.* 2009; Kolár *et al.* 2009; Frajman *et al.* 2015; Hodálová *et al.* 2020; Martínez-Sagarra *et al.* 2021). Combining DNA content measurements with chromosome counts allows direct verification of the estimated ploidy level. Information on ploidy is often important in guiding taxonomic delineation of plants since allopolyploids are often morphologically distinct and reproductively isolated from their diploid progenitors. Consequently, allopolyploids are frequently circumscribed at the species level (Soltis *et al.* 1993). On the other hand, autopolyploids often morphologically resemble their diploid ancestors and hence they are not recognised taxonomically (Soltis *et al.* 2007) or in some cases are identified at subspecific level (Mosquin 1967). Additional benefit of FCM is that it can be used to differentiate between taxa with the same chromosome number (i.e., homoploid taxa) but different DNA amount (i.e., genome size; Murray 2005). However, the methods of FCM for distinguishing related homoploid taxa must be more stringent than when estimating ploidy, as the differences in DNA content are often small (Nunvářová Kabátová *et al.* 2019).

### *Environmental data*

To avoid mistaken description of taxa, the integrative taxonomy approach considers also using evidence from ecology. Ecological data are different from other data used in systematic botany because they do not come from the plants themselves, but from the interactions of plants with the environment and other plants and animals. Knowledge from the field of ecology is often used to interpret the distribution of taxa (Guisan and Thuiller 2005; Soberón 2007; Barve *et al.* 2011; Sikora *et al.* 2013; Đurović *et al.* 2021), variability in plants (Knight and Ackerly 2002; Souza *et al.* 2019; Alcántara-Ayala *et al.* 2020; Li *et al.* 2020; Stephens *et al.* 2022), development of adaptive traits (Guzmán *et al.* 2009; Halbritter *et al.* 2018; Ninčević *et al.* 2021), etc. Furthermore, ecological data helps to describe the emergence of *ecotypes* (Bertel *et al.* 2018; Cozzolino *et al.* 2021), specialisation on certain soil types (edaphic; Piedallu *et al.* 2016; Konečná *et al.* 2020), the effect of pollination mechanisms (Shrestha *et al.* 2018), certain types of reproductive isolation (Owens and Samuk 2020), etc. Ecological indicators have major influence on classification systems and phylogenetic interpretations at lower taxonomic levels, primarily below the genus (Aguirre-Gutiérrez *et al.* 2015; Jaime *et al.* 2015; Janišová *et al.* 2018).

The recent availability of environmental data from databases containing climate, soil and topographic GIS layers (Geographic Information System; e.g., Chelsa, SoilGrids and EarthEnv; Hengl *et al.* 2017; Karger *et al.* 2017, 2020; Amatulli *et al.* 2018) allow the quantification of species ecological niches, as well as niche overlap between different species (Rödder and Engler 2011; Broennimann *et al.* 2012). Niche quantification and niche comparison between species are usually performed with an ordination method in environmental space (Broennimann *et al.* 2012). Ecological niches of taxa can be characterised and the output used to answer fundamental questions about niche evolution and speciation and for validating taxon boundaries (Dagnino *et al.* 2017; Collart *et al.* 2020). Consequently, the generated knowledge provides additional line of evidence for disentangling taxonomically challenging groups (Mota-Vargas and Rojas-Soto 2016; Otero *et al.* 2019; Lin *et al.* 2021). Furthermore, niche equivalency and niche similarity tests assess the statistical significance of a measured niche difference against null model niches taken randomly within a given background area (Warren *et al.* 2008). These tests are used for interpreting the significance of ecological niche differentiation among species or subspecies, and for testing specific hypotheses about niche conservatism/divergence (Warren *et al.* 2008). The above-mentioned analyses and tests are also known as pre-modelling analyses. On the other hand, core-modelling analyses are based on the quantification of species-environment relationship, calibrating the models and using them to make spatial predictions (Guisan and Zimmermann 2000; Di Cola *et al.* 2017). This later approach of bringing together the quantified ecological niche of a species and then projecting it onto geography to identify regions where the species' requirements are manifested is known as 'species distribution modelling' (SDM; Barve *et al.* 2011).

SDM is also known as 'ecological (or environmental) niche modelling' (ENM) or habitat suitability modelling. Two primary types of SDM techniques are regression methods, such as Generalized Linear and Generalized Additive Models (GLM/GAM, Guisan *et al.* 2002), and machine learning (ML) methods, such as Maximum Entropy (MaxEnt, Merow *et al.* 2013), Random Forest (Evans *et al.* 2011) and Boosted Regression Trees (BRT/GBM, Elith *et al.* 2008). Its correlative/statistical models are used for understanding and explaining species distributions (Glasnović *et al.* 2018), predicting rare species occurrences (McCune 2016), predicting the impacts of climate change on species distribution (Đurović *et al.* 2021), assessing invasion risk (Jaźwa *et al.* 2018), understanding niche dynamics and evolution (Yesson and

Culham 2006; Luqman *et al.* 2022) and in landscape genetics for evaluating the effects of landscape features on gene flow patterns (Cruzan and Hendrickson 2020). In the context of climate change, a large number of general circulation models (GCMs) have been developed, representing physical processes in the atmosphere, ocean and land surface, allowing to simulate the climate responses in the past and in the future. In phylogeographic studies, GCMs/climate data usually used for modelling potential habitat suitability are those developed for the Last Glacial Maximum period (LGM, ~21 kya, Karger *et al.* 2017). Hence, SDM is increasingly used in identifying potential refugia along the Quaternary climatic oscillations for past populations (Pinto-Carrasco *et al.* 2022). The integration of both molecular and past and present ecological data are required for getting a better understanding of the possible mechanisms that generated species' diversity patterns and their current distribution patterns (Kutnjak *et al.* 2014; Nieto Feliner 2014; Đurović *et al.* 2021; Reich *et al.* 2021).

### *Phylogeography of the Balkan Peninsula*

A myriad of genetic and species diversity is found on the three Mediterranean peninsulas, the Iberian, Apennine and Balkan Peninsulas (Hewitt 2011). Among them, the Balkan Peninsula is one of the biodiversity hotspots containing more endemics than other peninsulas (Hewitt 2011; Nieto Feliner 2014), accumulated in a comparative small space over extended time (Médail and Diadema 2009). Its geological history included several cycles of land connections and disconnections, from Miocene to Pleistocene (Tzedakis 2004), including events such as the Messinian Salinity Crisis (MSC = 5.96–5.33 Mya; Krijgsman *et al.* 1999) and the inland formation of lakes of various sizes following the desiccation of the Pannonian Sea occurring on the same time (Krstić *et al.* 2012). The majority of genera diversified during these events (Vargas *et al.* 2018), however, the within species diversification most likely occurred in response to Pleistocene glacial oscillations.

During recent years, an accumulation of phylogeographic studies on species complexes from the Balkan Peninsula have revealed various intricate phylogeographic patterns, where different lineages occurring in separate microrefugia correspond to phylogeographic breaks within the Peninsula (Španiel and Rešetnik 2022). Barriers such as mountains, river canyons and valleys fostered the formation of multiple microrefugia within the Peninsula during Pleistocene glacial cycles, in which multiple genetic lineages have diverged (e.g., Surina *et al.* 2011; Kutnjak *et al.* 2014; Caković *et al.* 2015; Đurović *et al.* 2017, 2021). This pattern has become known as 'refugia within refugia' (Gómez and Lunt 2007; Médail and Diadema 2009).

The mode and tempo of postglacial recolonisation of genetic lineages is difficult to generalise, since different species inhabited different geographic locations of microrefugia and were isolated by different barriers. Moreover, the genetic differentiation during population expansions and/or reductions underwent different selection processes. However, two main patterns of genetic differentiation during postglacial recolonisation events have been considered. One is the 'leading edge' hypothesis (Hewitt 2000), and the second one is the hypothesis of glacial persistence in two separate, i.e., the north-western and the south-eastern Balkan refugia. The first pattern implies higher genetic diversity in southern populations and a corresponding reduction towards north due to repeated founder events from long-distance colonisers, and it was observed in e.g., *Edraianthus tenuifolius* (Surina *et al.* 2011) and *Euphorbia myrsinites* (Falch *et al.* 2019). The second pattern implies the persistence in two different refugia hosting populations with similar genetic diversity, and it was documented in e.g., *Viola suavis* s.l. (Mereďa *et al.* 2011).

In the western Balkan Peninsula, specifically along the Dinaric Mountains, most studied taxa exhibit the differentiation into two or three genetic lineages, as seen in e.g., *Tanacetum cinerariifolium* (Grdiša *et al.* 2014), *Salvia officinalis* (Rešetnik, Baričevič, *et al.* 2016; Jug-Dujaković *et al.* 2020), *Silene saxifraga* (Đurović *et al.* 2017), *Cerastium grandiflorum* and *C. decalvans* (Đurović *et al.* 2021). The Dinaric Mountains have several deep and narrow canyons formed by karstic rivers which likely acted as barriers to gene flow. Evidence for such allopatric diversification has been found between populations of e.g., *T. cinerariifolium* (Grdiša *et al.* 2014) separated by the Zrmanja canyon in the Northern Dinaric Mountains; *Alyssum austrodalmaticum* (Španiel, Zozomová-Lihová, *et al.* 2017; Zozomová-Lihová *et al.* 2020), *Euphorbia myrsinites* (Falch *et al.* 2019) and *Campanula pyramidalis* and *C. austroadriatica* (Lakušić *et al.* 2013) separated by Neretva river valley in the Central Dinaric Mountains; and *Heliosperma pusillum* group (Frajman and Oxelman 2007) separated by the karst rivers Drina and Sutjeska in the Southern Dinaric Mountains. Secondary postglacial contacts between populations that have experienced some differentiation during glacial or interglacial periods, without developing a complete reproductive barrier, lead to admixture of lineages and thus reflect complex patterns (Rešetnik *et al.* 2020; Zozomová-Lihová *et al.* 2020; Reich *et al.* 2021).

## *Dianthus sylvestris* Wulfen (Caryophyllaceae)

*Dianthus* is a taxonomically difficult genus (Tutin and Walters 1993) with over 100 species occurring in Europe, and with more than 70 endemics in geographically restricted areas (Valente *et al.* 2010). Tutin and Walters (1993) largely follow the systematic treatment of *Dianthus* by Pax and Hoffmann (1934), who estimated the number of species to be close to 300. Moreover, recent taxonomic research has been largely focused on the description of new taxa (Mazzola *et al.* 2004; Meyer 2011; Brullo *et al.* 2015).

According to the scenario proposed by Valente *et al.* (2010), *Dianthus* diversified with a constant rate until 2.0−1.3 Myr ago (Early−Mid-Pleistocene), when an acceleration in diversification took place, coinciding with a period of profound climatic transformations in the Pleistocene (Willis 1994). Furthermore, the diversification of the Eurasian lineage of *Dianthus* is assumed to be still an ongoing process (Valente *et al.* 2010), which is reflected in the taxonomic complexity due to uncertainties in species boundaries. Its rapid radiation did not yet allow for the accumulation of mutations in the sequenced genomic regions, while phenotypic characters evolved more quickly (Fassou *et al.* 2022). Since most of the available species' treatments are based on classical morphology, most of the subgenera and sections do not represent natural groups (Fassou *et al.* 2022). The most widely used intrageneric classification has been that of Pax and Hoffmann (1934), until the work of Fassou *et al.* (2022), which showed little correlation with previous systematic treatments.

One of the taxonomically intricate species of the genus is *Dianthus sylvestris* (Figure 1). This species has divergent habitat preferences, and it grows from the sea level to the alpine zone and it is associated with both carbonate and serpentinite flora (Gammella 2016). Due to its high morphological and ecological variability, it has been getting more attention recently from taxonomists (Bacchetta *et al.* 2010; Domina *et al.* 2021) and researches in the field of plant ecological genetics (Gammella 2016; Luqman *et al.* 2022). *Dianthus sylvestris* was described and named as a species in 1786 by Wulfen. One or more forms of this plant were previously known to Séguier (1745), who recorded its occurrence between Trent and Verona, and formed a phrase: "*Caryophyllus silvestris flore rubro inodoro*". It is probably the plant mentioned by Linné (1753) as an unscented variety of *D. Caryophyllus;* Linne cited the Séguier's depiction for *D. Caryophillus* var. *inodorus,* and extracted the name "*inodorus*" from the Séguier's phrase. The majority of Wulfen's collection is deposited at W (herbarium acronyms follow Thiers 2021). However, twenty percent of collections at W have been lost in World War II, including parts of Caryophyllaceae, and it is assumed that the Wulfen's original material of *D. sylvestris* was destroyed. Consequently, the original material used for the description of several taxa was unknown. The first push forward to elucidating the taxonomic uncertainties of this taxon came from Bacchetta *et al.* (2010), who treat *D. sylvestris* occurring on the central and southern Apennine Peninsula, Sardinia and Sicily as a species complex.

**Figure 1.** *Dianthus sylvestris* from Jacquin (1781-1787, Tav. 82).

Regarding the area of the Balkan Peninsula, numerous intraspecific entities on different taxonomic levels (subspecies, varieties, forms) were reported by many authors over the last 200 years, and the authors' opinions on their taxonomic treatment is often conflicting (Schlosser and Vukotinović 1869; Borbás 1876; Beck-Mannagetta 1909; Hayek 1924; Gjurašin 1933; Mayer and Trpin 1965; Trinajstić 1979). According to Marhold (2011b), seven subspecies have been recorded for the Balkans: *D. sylvestris* subsp. *bertisceus* Rech. f., *D. sylvestris* subsp. *kozjakensis* Micevski, *D. sylvestris* subsp. *longicaulis* (Ten.) Greuter et Burdet, *D. sylvestris* subsp. *nodosus* (Tausch) Hayek, *D. sylvestris* subsp. *siculus* (C. Presl) Tutin, *D. sylvestris* subsp. *sylvestris* and *D. sylvestris* subsp. *tergestinus* (Rchb.) Hayek. Four subspecies have been recorded on the Croatian territory (Flora Croatica Database – FCD, Nikolić 2020); *D. sylvestris* subsp. *longicaulis*, *D. sylvestris* subsp. *nodosus*, *D. sylvestris* subsp. *sylvestris*, *D. sylvestris* subsp. *tergestinus*). However, there is still a dispute concerning the number of taxa and in-depth analyses are required for acquiring a sensible taxonomic circumscription of Balkan populations. Type specimens are known for five taxa. The iconography from Jacquin (1781-1787, Tav. 82) was designated by Bacchetta *et al.* (2010) as a neotype for the typical subspecies; syntypes of *D. sylvestris* subsp. *bertisceus* are kept in G (Geneva, Switzerland) and K (Kew, London UK); the holotype of *D. sylvestris* subsp. *kozjakensis* is in the University Herbarium in Skopje; the lectotype of *D. sylvestris* subsp. *longicaulis* is in NAP (Naples, Italy); and the holotype of the last described subspecies *D. sylvestris* subsp. *alboroseus* is in JE (Jena, Germany). For *D. sylvestris* subsp. *nodosus* the type specimen has not been designated yet.

## THESIS OUTLINE

This thesis summarizes four published scientific publications (**I – IV**), one scientific paper in preparation (**Publication V**), and two unpublished results (**Appendix I – II**) in which all the research hypotheses and objectives are addressed.

**Research hypotheses**: (1) There is genetic variability between populations of *D. sylvestris* s.l. associated with the existence of microrefugia on the Balkan Peninsula; (2) Populations belonging to different subspecies, evolutionary lineages and/or different habitat types can be identified according to their morphological diagnostic characters.

**Research objectives**: (1) Conduct morphometric and genetic analysis with the aim of solving the taxonomic problems of *D. sylvestris* s.l. and gaining insight into phylogeographic processes at the local and regional level; (2) Develop models of habitat suitability for the present and the past and analyse the ecological niches of *D. sylvestris* subspecies with the aim of investigating the relationship between their morphological, genetic and environmental divergence; (3) Conduct a taxonomic revision of *D. sylvestris* s.l. on the Balkan Peninsula.

Publications contributing to the first and second objectives are **III-V**, and all the included publications contribute to some extent to the third objective.

**First objective:** A detailed exploration of morphological characters (**Publication IV**) coupled with genetic analyses (**Publication V**) revealed a gradual change that follows geography, that is a southeast – northwest cline of both morphological diversity and genetic structure. The morphometric analyses based on 15 morphological characters did not show any clear groupings of populations that would correspond to the subspecies, but rather revealed two groups, namely one group consisting of north-western, and the other group including the south-eastern Balkan populations. Although showing morphological overlap with two sympatric subspecies, populations identified as *D. sylvestris* subsp. *tergestinus* were genetically divergent from all other Balkan populations. **Publication V** explores the potential phylogeographic process that could have driven the diversification of *D. sylvestris* on the Balkan Peninsula, that is allopatric diversification and speciation promoted mainly by geographic barriers.

**Second objective**: By quantifying niche overlap and performing niche equivalency and similarity tests (**Appendix I**), niche conservatism was revealed to be more common, since there was not a single significant case of niche divergence. In **Publication V**, two separated glacial refugia are identified and potential migration patterns are discussed, contributing to understanding the phylogeography of *D. sylvestris* s.l. on the Balkan Peninsula. The finding of tetraploid populations only within the north-western Balkan group (**Publication III**) implies that the northern Balkan Peninsula was under more pronounced climatic oscillations compared to the more stable conditions in the south, and that these climatic transitions could have been the driving force for polyploidization.

**Third objective:** Firstly, nomenclatural issues were addressed within the first two publications. Results of **Publication I** represent a detailed inventory of the historical herbarium material, the current composition of *D. sylvestris* taxa in Croatian herbarium collections (ZA and ZAHO) and their distributions across the Balkans. In **Publication II** lectotypes were chosen for the names *D. sylvestris* Wulfen and *D. virgineus* var. *tergestinus* Rchb., strengthening the applications of names lacking type material. Also, in **Appendix II** a lectotype was chosen among the syntypes for the name *D. sylvestris* subsp. *bertisceus*. **Publication III** revealed several areas of pronounced genome size variation at small geographic distances on the Peninsula, likely indicating intraspecific divergence. However, these divergences are not indicative of the subspecies, thus genome size did not show taxonomic value. **Publication IV** indicates that some morphological characters are variable even within the population level, and thus their taxonomic importance becomes questionable. **Publication V** gives the final evidence which advocates for a comprehensive reduction of the number of subspecies on the Balkan Peninsula. Based on all obtained results, a new taxonomic treatment and a key for the identification of *Dianthus sylvestris* taxa on the Balkan Peninsula were proposed in **Appendix II**.

# INDIVIDUAL PUBLICATIONS

# Publication I

# Inventory of the historical *Dianthus sylvestris* herbarium materials from Herbarium Croaticum and Herbarium Ivo and Marija Horvat

ANA TERLEVIĆ*, IVANA REŠETNIK

University of Zagreb, Faculty of Science, Department of Biology, Division of Botany, Marulićev trg 20/II, HR-10000 Zagreb, Croatia

***Autor za dopisivanje / corresponding author:** ana.terlevic@biol.pmf.hr

**Tip članka / article type:** kratko znanstveno priopćenje / short scientific communication
**Povijest članka / article history:** primljeno / received: 4.12.2019., prihvaćeno / accepted: 26.4.2020.
**URL:** https://doi.org/10.46232/glashbod.8.1.2

**Terlević, A., Rešetnik, I. (2020): Inventory of the historical *Dianthus sylvestris* herbarium materials from Herbarium Croaticum and Herbarium Ivo and Marija Horvat. Glas. Hrvat. bot. druš. 8(1): 8-14.**

## Abstract

In order to initiate a taxonomical revision of the *Dianthus sylvestris* group, an inventory of historical herbarium materials from ZA and ZAHO collections was conducted. Herbarium specimens of *D. sylvestris* group from these two herbaria were digitized and the data from the original herbarium labels were inserted in the Flora Croatica Database. A total of 344 herbarium sheets were digitized and six taxa (*D. sylvestris* Wulfen in Jacq. ssp. *sylvestris*, *D. sylvestris* ssp. *tergestinus* (Rchb.) Hayek, *D. sylvestris* ssp. *longicaulis* (Ten.) Greuter et Burdet, *D. sylvestris* ssp. *nodosus* (Tausch) Hayek, *D. siculus* C. Presl and *D. arrosti* C. Presl) were registered within studied collections. Inventory of herbarium sheets from ZA and ZAHO historical collections provided a significant insight into historical distributional data of *D. sylvestris* taxa related to the area of the Balkan Peninsula, which is a prerequisite for accurate taxonomic/geographic sampling for further morphological and molecular analyses.

**Keywords:** Balkan Peninsula, digitization, distribution, Flora Croatica Database

**Terlević, A., Rešetnik, I. (2020): Pregled povijesnog herbarijskog materijala vrste *Dianthus sylvestris* u zbirkama Herbarium Croaticum i Herbarij Ive i Marije Horvat. Glas. Hrvat. bot. druš. 8(1): 8-14.**

## Sažetak

Kako bi se započelo s taksonomskom revizijom grupe svojti koje pripadaju *Dianthus sylvestris*, provedeno je popisivanje povijesnog herbarijskog materijala iz zbirki ZA i ZAHO. Herbarijski primjerci vrsta koje pripadaju grupi *D. sylvestris* su digitalizirani, a podaci s originalnih herbarijskih etiketa su uneseni u bazu podataka Flora Croatica. Digitalizirano je ukupno 344 herbarijskih listova i zabilježeno je šest svojti (*D. sylvestris* Wulfen in Jacq. ssp. *sylvestris*, *D. sylvestris* ssp. *tergestinus* (Rchb.) Hayek, *D. sylvestris* ssp. *longicaulis* (Ten.) Greuter et Burdet, *D. sylvestris* ssp. *nodosus* (Tausch) Hayek, *D. siculus* C. Presl i *D. arrosti* C. Presl). Popisivanje herbarijskih listova ovih svojti iz ZA i ZAHO pruža uvid u povijesne podatke o rasprostranjenosti svojti iz grupe *D. sylvestris* na području balkanskog poluotoka, što je preduvjet za ispravno taksonomsko/geografsko uzorkovanje za daljnje morfološke i molekularne analize.

**Ključne riječi:** Balkan, baza podataka Flora Croatica, digitalizacija, rasprostranjenost

## Introduction

*Dianthus sylvestris* group is considered to be one of the most complex groups within the genus *Dianthus* L. (Caryophyllaceae). An important diversity centre is the central Mediterranean area (Balkan and Apennine Peninsula) where the number of taxa belonging to this group is high. They mainly occur in rupestrian habitats, as well as on rocky and sunny grasslands, garigues, steppes and mesic meadows (Bacchetta et al. 2010). Real taxonomical value of taxa belonging to this group is often doubtful, and synonymy confusion is common. The group is morphologically characterized by woody stocks, shortly branched, usually with dense terminal leaf rosettes, linear and acute leaves, flowers not or slightly fragrant, arranged in loose cymes, epicalyx scales 2-6(-8), glabrous, abruptly contracted into a short mucro, 3-5 times shorter than calyx, glabrous petals with limb denticulate at apex or entire (Pignatti 1982, Tutin & Walters 1993). Subspecies listed by Marhold (2011), regarding the Euro-Mediterranean area, are *Dianthus sylvestris* ssp. *bertisceus* Rech. f., *D. sylvestris* ssp. *kozjakensis* Micevski, *D. sylvestris* ssp. *longicaulis* (Ten.) Greuter et Burdet, *D. sylvestris* ssp. *nodosus* (Tausch) Hayek, D. sylvestris ssp. *siculus* (C. Presl) Tutin, *D. sylvestris* ssp. *tergestinus* (Rchb.) Hayek and *D. sylvestris* ssp. *sylvestris*. In addition, *D. sylvestris* ssp. *alboroseus* F.K. Meyer, a pale pink to white flowering form, was described from southern Albania (Meyer 2011). According to Bacchetta et al. (2010), in central and southern Italy, Sardinia and Sicily this group is represented by 17 morphologically and ecologically well differentiated species.

The *D. sylvestris* group is very polymorphic and no reasonable key on subspecific level can be presented. Since an in-depth taxonomical review of this group needs to be done, the first step is to analyse the existing herbarium data from ZA (Herbarium Croaticum) and ZAHO (Herbarium of Ivo and Marija Horvat) collections. Therefore, the aim of this article is to present: (i) an update of the distribution data based on herbarium data, (ii) taxa analysis in terms of spatial distribution, collectors and collecting dates.

## Materials and methods

The herbarium specimens of *D. sylvestris* group were searched for and used for the purpose of digitization within two Croatian herbaria, ZA and ZAHO (acronyms are according to Thiers 2019). A regular procedure within these herbaria include the following steps: mounting, systematisation and digitization. According to Šegota et al. (2017), first the plant material was placed on a new paper, mounted with pH neutral adhesive tape on herbarium sheets while the herbarium labels were glued with Gaylord pH neutral white adhesive. The original metadata for *D. sylvestris* group herbarium sheets were inserted within the Flora Croatica Database (Nikolić 2019). The metadata include: herbarium ID, taxon name, locality, habitat, geographical coordinates, collector(s) and identifier(s) name, and collection dates. Most of these data were provided from the original label's text, while data such as geographical coordinates were derived from an up-to-date interpretation of collection locality. Finally, the prepared sheets were scanned using available scanning equipment (inversed Epson Expression 11000XL Pro A3 scanner) with the image resolution of 300 dpi (tiff). A stamp with the ZA

**Figure 1.** Herbarium sheets of *Dianthus sylvestris* group taxa after digital imaging, (a) ZA 47545, (b) ZAHO 46581.

and ZAHO herbarium ID, colour plate and ruler were added to each sheet prior to scanning (Fig. 1). Finally, the images were uploaded in the Flora Croatica Database and are accessible on virtual portal of the Herbarium Croaticum.

## Results and discussion

In total, 344 herbarium sheets were found and digitized within the two studied collections. In the ZA *Herbarium Generale* (collection formed via exchange with other world herbaria) 25 specimens of *D. sylvestris* group were discovered. The ZA collection holds 207 herbarium sheets (60%) and ZAHO collection holds 137 sheets (40%). Altogether, six taxa belonging to *D. sylvestris* group were registered, four of them being native to Croatia. Specimens determined as *D. sylvestris* s.l. occurred on the largest number of herbarium sheets (235), followed by *D. sylvestris* ssp. *tergestinus* (69), *D. sylvestris* ssp. *nodosus* (33), *D. sylvestris* ssp. *longicaulis* (3), *D. arrosti* C. Presel (2), *D. siculus* C. Presel (1) and *D. sylvestris* ssp. *sylvestris* (1) (Tab. 1).

*Dianthus arrosti* and *D. siculus* are not native to Croatia. The species *D. arrosti* occurs in Sicily, Sardinia and southern Calabria (Bacchetta et al. 2010), and according to Marhold (2011) it is also native to Algeria and Morocco. One herbarium specimen found in ZA *Herbarium Generale* originates from Dr. L. Nicotra – Herbarium Siculum (PAL, Palermo, Italy), and the other from Dr. C. Baenitz, Herbarium Europeum (MNHM, Paris, France). However, locality descriptions on herbarium labels are not clear. *D. siculus* is common in Italy, Corse, Spain, Morocco, Algeria and Tunisia (Bacchetta et al. 2010, Marhold 2011). In Euro+Med PlantBase (Marhold 2011) it is registered as *D. sylvestris* ssp.

**Table 1.** The number of digitized herbarium sheets for each taxon across the studied collections.

| Taxon name | ZA | ZAHO | total |
|---|---|---|---|
| *Dianthus sylvestris* Wulfen in Jacq. | 103 | 132 | 235 |
| *Dianthus sylvestris* Wulfen in Jacq. ssp. *tergestinus* (Rchb.) Hayek | 66 | 3 | 69 |
| *Dianthus sylvestris* Wulfen in Jacq. ssp. *nodosus* (Tausch) Hayek | 32 | 1 | 33 |
| *Dianthus sylvestris* Wulfen in Jacq. ssp. *longicaulis* (Ten.) Greuter et Burdet | 3 | 0 | 3 |
| *Dianthus arrosti* C. Presl | 2 | 0 | 2 |
| *Dianthus sylvestris* Wulfen in Jacq. ssp. *sylvestris* | 0 | 1 | 1 |
| *Dianthus siculus* C. Presl | 1 | 0 | 1 |
| **Grand total** | **207** | **137** | **344** |

*siculus* (C. Presl) Tutin. The single herbarium specimen found in ZA Herbarium Generale originate from the collection of O. Debeaux – Plantes d`Algerie.

Herbarium specimens originate from 12 European countries and from Algeria (Fig. 2); however, the majority were collected in Croatia (57%). As much as 23 herbarium sheets in ZA, and 7 herbarium sheets in ZAHO collection could not be georeferenced

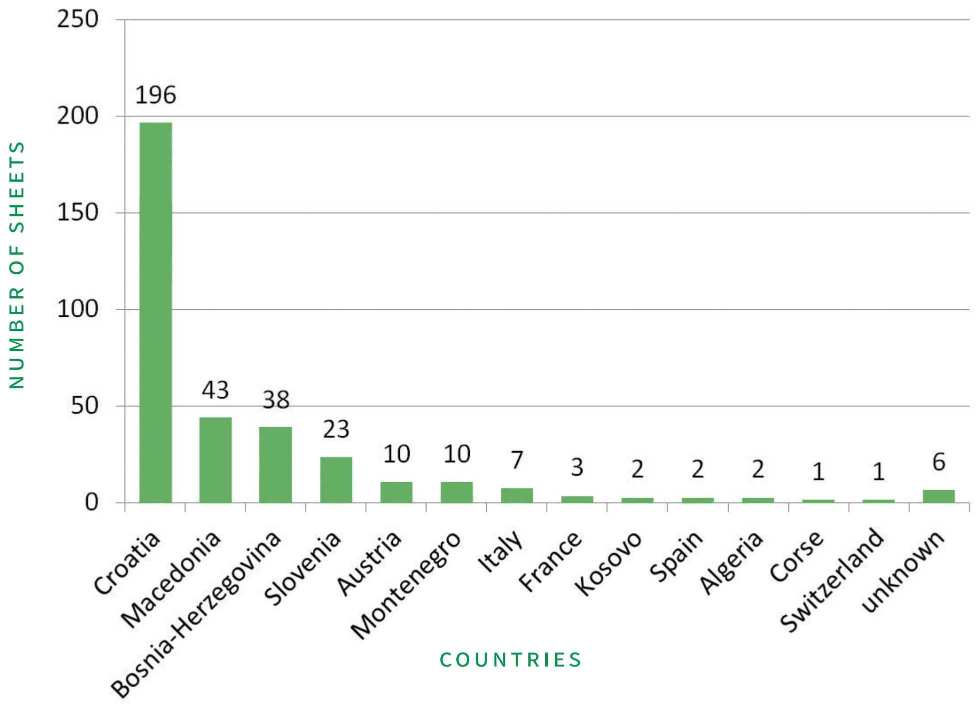


**Figure 2.** Geographical origin of *D. sylvestris* group herbarium specimens stored across studied collections.

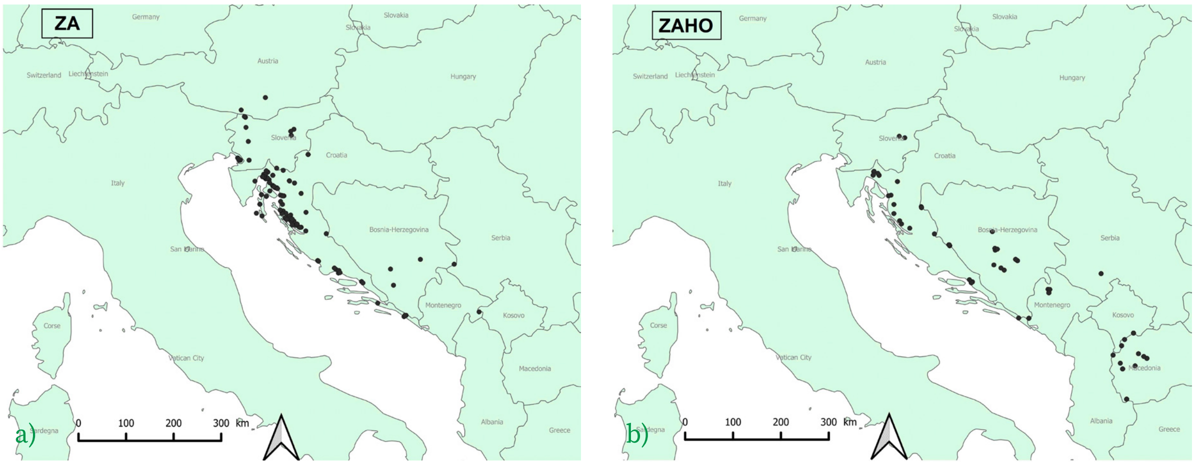


**Figure 3.** Distribution maps of *D. sylvestris* group herbarium specimens from (a) ZA and (b) ZAHO collection.

due to ambiguous locality description or due to the lack of any locality description, except the country. Likewise, all of the 25 herbarium sheets from *Herbarium Generale* have not been georeferenced for the same reasons. Regarding the Croatian territory, the collection sites of taxa are localized mostly on Mt Velebit, which reflects field activities of collectors of those two collections (Fig. 3). The distribution of taxa in Croatia based on historical herbarium data (Fig. 4) showed overlaps with the field record data made by the authors during 2018 and 2019 fieldworks (location data not shown).

The most productive collector of the studied herbarium materials was Ivo Horvat, the lone collector of ZAHO, with 135 herbarium sheets. Ljudevit Rossi stored 40 herbarium sheets in ZA, Dragutin Hirc 30, Ivo Pevalek 17, Josip Klasancije Schlosser 13, Ljudevit Vukotinović 12, while other 44 collectors contributed with less than 10 herbarium sheets each. The majority of specimens were collected between 1910s and 1940s (Fig. 5) which is in line with the previous reports (Strgulc Krajšek et al. 2009, Šegota et al. 2017). As much as 49 herbarium specimens lack data on collecting period, i.e. the year of collecting was either missing from the label or it was illegible. The oldest specimen kept at ZAHO was collected in 1920, whereas the oldest specimens from ZA date back to 1853 for *Herbarium Generale* (unknown collector), and to 1852 for the main ZA collection (specimen collected by Lj. Vukotinović and K. Schlosser).

Taxa of *D. sylvestris* group which have not been found in ZA and ZAHO collections, but are known from the Balkan Peninsula, are *D. sylvestris* ssp.

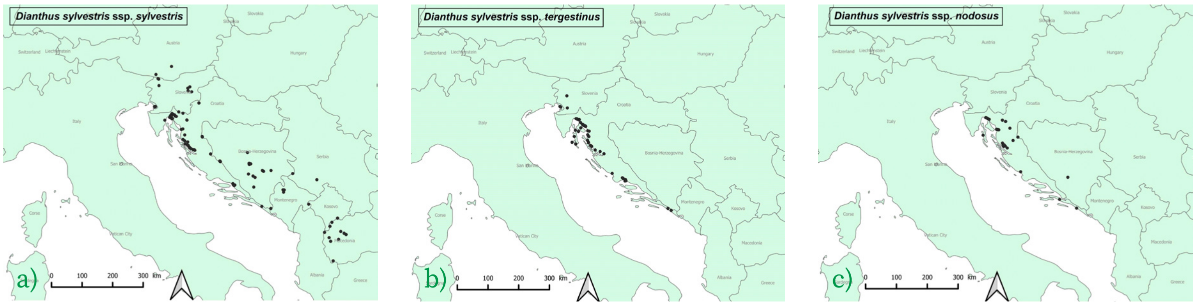


**Figure 4.** Individual distribution maps for (a) *D. sylvestris* (including the single herbarium specimen stored as *D. sylvestris* Wulfen in Jacq. ssp. *sylvestris*), (b) *D. sylvestris* ssp. *tergestinus*, and (c) *D. sylvestris* ssp. *nodosus* stored across studied collections.

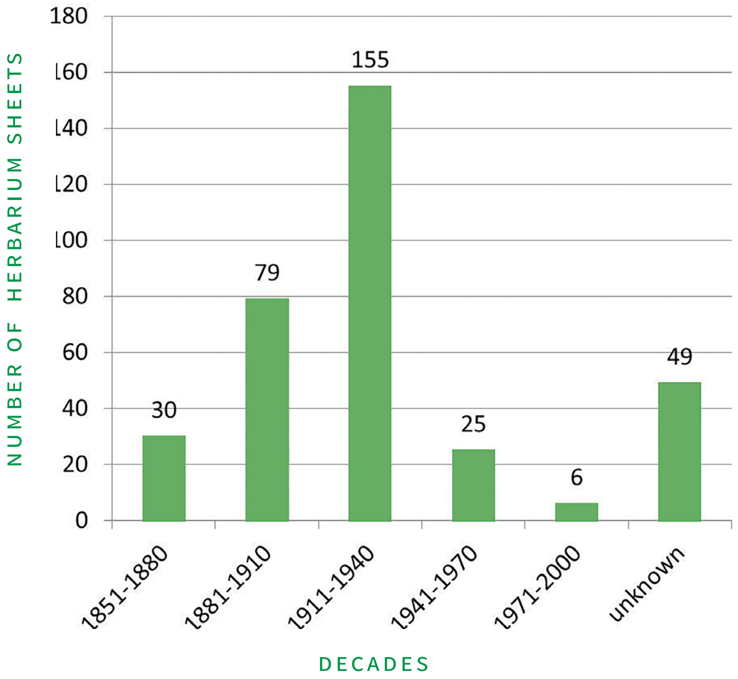


**Figure 5.** Temporal distribution of the herbarium specimens based on collection period shown in decadal scale.

*alboroseus, D. sylvestris* ssp. *bertisceus* and *D. sylvestris* ssp. *kozjakensis*. However, these taxa have rather restricted distribution areas that were rarely covered by field activities of main ZA collectors. Moreover, taxa *D. sylvestris* ssp. *kozjakensis* and *D. sylvestris* ssp. *alboroseus* were described only recently by Micevski (1990) and Meyer (2011), respectively.

These results represent the current state of *D. sylvestris* group taxa composition within ZA and ZAHO collections. Apart from historical herbarium specimens, *D. sylvestris* collections from ZA have recently been growing due to extensive sampling of *D. sylvestris* taxa throughout the Balkans (Croatia and neighbouring countries). Sampling of *D. sylvestris* material for morphological and molecular analysis is under way, in order to resolve taxonomic, phylogenetic as well as phylogeographic relationships among taxa. After the morphological characterisation of taxa, it will be possible to carry out a thorough taxonomic revision, from which a different view on taxa composition in these herbaria could be obtained.

## Acknowledgment

This work has been supported in part by Croatian Science Foundation under the project UIP-2017-05-2882 (AmphiAdriPlant). The work of doctoral student Ana Terlević has been fully supported by the “Young researchers’ career development project – training of doctoral students” of the Croatian Science Foundation funded by the European Union from the European Social Fund. Authors are grateful to students Marina Babić, Ana Kordić and Petra Stić for their help during material digitizing process.

## References

Bacchetta, G., Brullo, S., Casti, M., Pietro Giusso del Galdo, G. (2010): Taxonomic revision of the Dianthus sylvestris group (Caryophyllaceae) in central-southern Italy, Sicily and Sardinia. – Nordic Journal of Botany 28(2): 137-173.

Horvat, M., Plazibat, M. (2007): Prikaz Horvatova herbarija (ZAHO) u Zagrebu. Natura Croatica 16(4): 267-408.

Strgulc Krajšek, S., Šegedin, T., Jogan, N. (2009):

Revision of *Epilobium* and *Chamerion* in the Croatian herbaria ZA and ZAHO. Natura Croatica 18 (1): 155-167.

Marhold, K. (2011): Caryophyllaceae. In: Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity. http://ww2.bgbm.org/EuroPlusMed/ (accessed October 12, 2019).

Meyer, F. K. (2011): Beiträge zur Flora von Albanien. Haussknechtia Beih 15: 1-220.

Micevski, K. (1990): Novitäten in der Gattung Dianthus L. (Caryophyllaceae) in der Flora der SR Makedonien. Prilozi, Oddelenie za Bioloshki i Meditsinski Nauki. Makedonska Akademija na Naukite i Umetnostite 8(1-2): 31-46.

Micevski, K. (1993): Flora na Republika Makedonija, 1,2. Macedonian Academy of Sciences and Arts. Skopje: 153-391.

Nikolić, T. (ed.) (2019): Flora Croatica database. Department of Botany, Faculty of Science, University of Zagreb. http://hirc.botanic.hr/fcd (accessed October 12, 2019).

Pignatti, S. (1982): Flora d’Italia, 1. Edagricole, Bologna, 265-273.

Strgulc Krajšek, S., Šegedin, T. i Jogan, N. (2009): Revision of *Epilobium* and *Chamerion* in the Croatian herbaria ZA and ZAHO. Natura Croatica 18(1): 155-167.

Šegota, V., Buzjak, S., Vilović, T., Sedlar, Z., Rešetnik, I., Bogdanović, S. (2017): Curators in action: intricate genus Fritillaria L. (Liliaceae) from ZA, ZAHO, CNHM and ZAGR revised and digitized. Glasnik Hrvatskog Botaničkog Društva 5(2): 4-14.

Thiers, B. (2017): Index Herbariorum. A global directory of public herbaria and associated staff. New York Botanical Garden’s Virtual Herbarium. http://sweetgum.nybg.org/ih/ (accessed October 2019).

Trinajstić, I. (1979): Analitička flora Jugoslavije. Svezak 1, Broj 5: 732-734.

Tutin, T. G., Walters, S. M. (1993): Carex L. In: Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., Webb, D. A. (eds.): Flora Europaea 1. Cambridge University Press, Cambridge, 227-240.

# Publication II

PhytoKeys 187: 1–14 (2021)
doi: 10.3897/phytokeys.187.75534
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

# Typification of 14 names in the *Dianthus virgineus* group (Caryophyllaceae)

Gianniantonio Domina[1], Giovanni Astuti[2], Gianluigi Bacchetta[3], Giulio Barone[1], Ivana Rešetnik[4], Ana Terlevic[4], Melanie Thiébaut[5], Lorenzo Peruzzi[2]

**1** *Department of Agriculture, Food and Forest Sciences, University of Palermo, Viale delle Scienze ed. 4, I-90128 Palermo, Italy* **2** *Dipartimento di Biologia, Unità di Botanica, Università di Pisa, Via Derna 1, 56126, Pisa, Italy* **3** *Dipartimento di Scienze della Vita e dell'Ambiente, Centro Conservazione Biodiversità (CCB), Università di Cagliari, V.le S. Ignazio da Laconi 13, 09123, Cagliari, Italy* **4** *Faculty of Science, Department of Biology, University of Zagreb, Marulićev trg 20/II, 10000, Zagreb, Croatia* **5** *Herbier de l'Université Claude Bernard Lyon 1, FR-CERESE, UMR 5023 LEHNA, Lyon, France*

Corresponding author: Gianniantonio Domina (gianniantonio.domina@unipa.it)

Academic editor: A. Sukhorukov | Received 20 September 2021 | Accepted 10 November 2021 | Published 13 December 2021

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

## Abstract

The nomenclature of 14 taxa from Central and Southern Europe within the *Dianthus virgineus* group is discussed. *Dianthus aggericola* Jord., *D. collivagus* Jord., *D. consimilis* Jord., *D. orophilus* Jord., *D. saxicola* Jord., *D. juratensis* Jord. are here lectotypified by specimens from the Jordan herbarium in LY, while *D. godronianus* Jord. by a specimen in P. *Dianthus subacaulis* Vill. is neotypified by a specimen collected on Mont Ventoux (S. France) and housed in MPU. For *D. sylvestris* Wulfen, a lectotype is here designated and its previous neotypification is discussed. *Dianthus caryophyllus* var. *tenuifolius* Moris, *D. caryophyllus* f. *minor* Moris and *D. sylvestris* var. *garganicus* Ten. are lectotypified by specimens housed in herbarium Moris (TO) and herbarium Tenore (K). *Dianthus virgineus* var. *tergestinus* Rchb. is lectotypified by a drawing from the *Icones florae Germanicae & Helveticae,* while *D. contractus* var. *evolutus* Lojac. is neotypified by a specimen in P. For each taxon the currently accepted name is provided including new synonymies. The type indication is followed by nomenclatural and taxonomic notes, in which the original material found is commented and the reasons for the identification of the types are discussed.

## Keywords

*Dianthus*, France, Italy, Slovenia, nomenclature

## Introduction

*Dianthus* L. (Caryophyllaceae) includes about 300 species from the temperate regions of the Old World, many of which are narrow endemics (Hardion et al. 2020). This genus still shows challenging systematics. A large part of recent taxonomic research, in fact, has been focused on the description of new taxa. Since 2000, 64 new species and subspecies have been described for the Euro-Mediterranean area, and a total of 98 new names have been published (IPNI 2021). Conversely, the taxonomic contributions on *Dianthus* that have taken into consideration groups of species with statistical analyses of morphological data or molecular investigations are very few (Domina et al. 2017; Hardion et al. 2020). Furthermore, the genus-level taxonomic treatments date back to more than 50 years ago (Williams 1890, 1893; Pax and Hoffmann 1934; Reeve 1967), and the recently published treatments of *Dianthus* in national flora (Bernal et al. 1990; Tison and de Foucault 2014; Vangjeli 2015; Brullo and Guarino 2017; Nikolić 2020) have not brought any significant change to the taxonomy of this genus. In several cases, the nomenclatural types for these taxa are not yet designated (Domina & al. 2021). This created a situation of taxonomic uncertainty. *Dianthus* is an interesting genus, both from a biological and economical point of view (Hardion et al. 2020). Hence, an integrated approach to the taxonomy of this genus is needed.

The *D. virgineus* L. group can be considered as one of the richest and most complex in the genus. Many taxa have been described from Central and Southern Europe, albeit their actual taxonomical value is often doubtful. The genus has undergone extensive taxonomic investigations since the 18th century (Smith 1794), but in many cases the original material used for the description of the taxa is not known and the nomenclatural types have not been designated yet. The lectotype of *Dianthus virgineus* L., the oldest available name that applies to wild plants in this group, has been designated only recently (Domina et al. 2021). The nomenclature and morphology of the large part of taxa described from Southern Italy, Sardinia, and Sicily have been investigated by Bacchetta et al. (2010). Other typifications were published by Camarda and Corrias (1987), Brullo et al. (2000), Arrigoni (2006), and Clementi et al. (2015). However, most of the taxa described in this group are still to be investigated.

In this study, the nomenclature of 14 taxa is discussed: *Dianthus aggericola* Jord., *D. collivagus* Jord., *D. consimilis* Jord, *D. godronianus* Jord., *D. orophilus* Jord., *D. saxicola* Jord., *D. juratensis* Jord., and *D. subacaulis* Vill. from S. France; *D. sylvestris* Wulfen from NE Italy/SW Slovenia; *D. virgineus* var. *tergestinus* Rchb. from NE Italy; *D. caryophyllus* var. *tenuifolius* Moris and *D. caryophyllus* f. *minor* Moris from Sardinia; *D. sylvestris* var. *garganicus* Ten. from S Italy; and *D. contractus* var. *evolutus* Lojac. from Sicily.

As part of an ongoing project aimed to push forward the taxonomic knowledge on selected genera of the Italian vascular flora, this study aims to lay the foundations for further taxonomic investigations by an integrated approach based on morphometric and molecular data (Domina et al. 2021; Giacò et al. 2021).

## Material and methods

We examined the scientific literature for the effective place of publication of surveyed *Dianthus* names described from Central and South Europe. The bibliographic data was searched in the available digital sources and in the libraries of the European institutions, while the original material of the investigated species was searched in the main European herbaria: B, C, FI, G, K, MA, MPU, NAP, P, PAL, RAB, RO, TO, W, WU, and ZA; herbarium acronyms follow Thiers (2021). A start-up online screening was possible thanks to digital herbarium specimens' images provided by GBIF (https://www.gbif.org), Jstor (http://plants.jstor.org), and ReColNat (https://www.recolnat.org/fr/). More thorough investigations were conducted in the Jordan herbarium at LY. The articles of the International Code of Nomenclature for Algae, Fungi, and Plants (hereafter ICN) follow Turland et al. (2019). Until more in-depth, integrated morphometric, genetic, karyological, and ecological information comes to light, our judgments should be considered provisionally accepted, according to current knowledge. In this group, the characters that have been proven to best discriminate species (Bacchetta et al. 2010) are: leaf length and width, number of flowers per scape, shape and length of outer and inner bracts. These characters have been used to check the morphological features of the selected types. The anther and petal length have been proposed as additional discriminant characters, but these can be easily appreciated on fresh plants and not on herbarium samples.

## Typification of the names

### *Dianthus aggericola* Jord. in Billot, Annot.: 48. 1856. [December 1856]

= *D. virgineus* L., Sp. Pl. 1: 412. 1753. Ind. Loc.: "du Reculet (Ain)".

**Type.** (lectotype here designated): *Dianthus aggericola* Jord., du Reculet, 8 July 1854 [A. Jordan], LY0079734!

**Note.** No other original material was found in the surveyed herbaria. According to the label, this plant was originally collected in Reculet and then grown in Jordan's garden, where it was collected in July. As a matter of fact, besides his huge herbarium and library, Alexis Jordan owned a one-hectare experimental garden. We know that he used it to sow most species every year, while maintaining alive perennial ones, and that he regularly made herbarium sheets from these cultivated plants. In this context, the notion of original material requires particular attention (Thiébaut and Tison 2016).

The lectotype designated here matches the protologue and corresponds to the current application of the name, which is considered a heterotypic synonym of *D. godronianus* Jord. in Kerguélen (1993), in turn, currently considered a heterotypic synonym of *D. virgineus* (Domina et al. 2021). The lectotype of *D. aggericola* and that of *D. virgineus*, have the same leaf length and width, uniflowered scapes, the same length and shape of outer and inner bracts, the same calyx length and shape. We, therefore, confirm this synonymy.

### ***Diantbus caryophyllus* var. *tenuifolius* Moris, Fl. Sardoa 1: 231. 1837. [April 1837]**

≡ *D. siculus* subsp. *tenuifolius* (Moris) Arrigoni, Parlatorea 7: 20. 2005.
= *D. genargenteus* Bacch., Brullo, Casti & Giusso, Nordic J. Bot. 28(2): 145. 2010.
Ind. Loc.: “In sterilibus frequens [Sardinia]”.

**Type.** (lectotype here designated): *Dianthus caryophyllus tenuifolia*, prope Belvì, July, inter rupe / Mus. Bot. Horti Taurinensis, Herb. Moris Barbey Cat Sard. N.156, TO!

**Note.** Three herbarium sheets are kept in TO, with several individuals each. All three specimens bear labels handwritten by Moris but lack the year of collection. Two of them come from generic localities (“in arenis maritimis” and “in collibus”), while one is from Belvì in the centre of Sardinia (Nuoro). All the specimens are complete and in good condition but refer to different collections: two specimens have been collected in inner localities, whereas another one comes from the coast. Moris reports that the scape bears a single flower and that another taxon (*D. caryophyllus* var. *tenuifolius* f. *minor*) grows in arenosis maritimis [sandy coast]. Thus, here we propose the specimen from Belvì as lectotype, despite not being dated, assuming that the herbarium in TO hosts the original material by Moris as already done by Arrigoni (1979), Rizzotto (1989), Escobar García et al. (2010) in other similar cases,

Based on the specimen in TO coming from the coast (referring actually to f. *minor*), Valsecchi (1985), and then Bacchetta et al. (2010), refer *D. caryophyllus* var. *tenuifolius* to *D. morisianus* Vals. Based on the diagnosis and the lectotype designated here, *D. caryophyllus* var. *tenuifolius* does not belong to *D. morisianus*. The former taxon shows short scapes bearing one or few flowers and epicalyx scales with mucro 0.5–1.5 mm long, while the latter shows longer multiflowered scapes with epicalyx scales with mucro 2.0–3.5 mm long. This interpretation agrees with Arrigoni (2010). According to the lectotype of *D. caryophyllus* var. *tenuifolius*, which shows woody stocks contracted with branches, epicalyx scales with an evident mucro, and small calyx, this taxon is a heterotypic synonym of *D. genargenteus* Bacch., Brullo, Casti & Giusso.

### ***Dianthus caryophyllus* f. *minor* Moris, Fl. Sardoa 1: 231. 1837. [April 1837]**

= *D. morisianus* Vals., Boll. Soc. Sarda Sci. Nat. 24: 333. 1985. Ind. Loc.: “In arenosis maritimis [Sardinia]”.

**Type.** (lectotype here designated): *Dianthus caryophyllus* var *tenuifolia*, in arenosis maritimis, S. Nicolai flumini major Majo junio / Mus. Bot. Horti Taurinensis, Herb. Moris Barbey Cat Sard. N.156, TO!

**Note.** A single sheet was found in TO. Albeit it may represent the holotype, it is cautiously designated here as a lectotype.

The selected specimen, uniflorous, has fixed seven portions of plants whose leaves and flower scapes are smaller than those of the typical form. All other characters of the flowers correctly match the protologue. This taxon is a heterotypic synonym of

*D. morisianus*, a species described by Valsecchi (1985) for the same area and habitat (Peruzzi et al. 2015), that shows the same leaf length and width and, albeit with multiflowered scapes, the same length and shape of outer and inner bracts, and the same calyx length and shape.

### ***Dianthus collivagus* Jord. in Billot, Annot.: 46. 1856. [December 1856]**

≡ *D. caryophyllus* var. *collivagus* (Jord.) Cariot & St-Lager Étude Fl., éd. 8, 2: 104. 1889.
=? *D. sylvestris* Wulfen in Jacq., Coll. 1: 237. 1786. [January-September 1786]. Ind. Loc.: “abonde sur les côteaux du Rhône près de Lyon”.

**Type.** (lectotype here designated): *Dianthus scheuchzeri* Rchb., *Dianthus sylvestris* auct. Gall. ex parte non Wulf, Lyon a Néron, Jordan, odor levis, folia ramis trigemina semper angustissima; *Dianthus scheuchzeri* Jord. non Rchb., *Dianthus collivagus* Jord., Lyon à Neron, ex herbis Jordan, July 1854, CLF056818!

**Note.** Other six specimens collected by Jordan are preserved at LY, but they are not original material, since they are lacking a date or reporting dates later than the protologue.

The lectotype designated here matches the protologue and corresponds to the current application of the name, which is considered as a heterotypic synonym of *D. sylvestris* subsp. *sylvestris* in Kerguélen (1993). The lectotype of *D. collivagus*, concerning the shape of calyx teeth, is very similar to the lectotype of *D. inodorus* (L.) Gaertn., which in turn is currently included within the variability of *D. sylvestris* (Domina et al. 2021).

### ***Dianthus consimilis* Jord. in Billot, Annot. 47. 1856. [December 1856]**

≡ *D. caryophyllus* var. *consimilis* (Jord.) Rouy & Foucaud in Rouy, Fl. Fr. 3: 195. 1896.
=? *D. sylvestris* Wulfen in Jacq., Coll. 1: 237. 1786. [January-September 1786]. Ind. Loc.: “Alpes de l’Oisans”.

**Type.** (lectotype here designated): *Dianthus consimilis* Jord., June-July 1855, [A. Jordan] Roux, Herbier Jordan, LY0079676!

**Note.** At LY we found another specimen citing “Lautaret (H. Alpes, May, June-July 1855, ex Horto Alexis Jordan, LY0079674!” but lacking basal leaves.

The lectotype designated here matches the protologue and corresponds to the current application of the name, which is considered as a heterotypic synonym of *D. sylvestris* subsp. *sylvestris* in Kerguélen (1993). The lectotype of *D. consimilis*, concerning the shape of calyx teeth, is very similar to the lectotype of *D. inodorus* (L.) Gaertn., which, in turn, is currently included within the variability of *D. sylvestris* (Domina et al. 2021).

### ***Diantus contractus* var. *evolutus* Lojac., Fl. Sicul. 1(1): 165. 1888. [September 1888]**

= *D. arrostoi* C.Presl, Delic. Prag. 60. 1822. Ind. Loc.: “Sulle più alte vette delle Nebrodi sui terreni ghiaiosi o sulle rupi calcaree di Serre di Quacedda. Juntera Minà Pal!”.

**Type.** (neotype here designated): *Dianthus contractus* Jan., *Dianthus constrictus* Janka, In asperis calcareis elatioribus montis Nebrodes, Julio, M. Lojacono Pojero, P05052873 (photo!).

**Note.** Neither the original material nor traces of this taxon were found in the herbaria consulted and among the documents accompanying the centuries distributed by Lojacono (Aghababyan et al. 2012; Domina et al. 2014). We chose to designate as a neotype the single specimen found, which is at least collected by Lojacono.

The neotype designated here matches the protologue and allows to consider this name as an heterotypic synonym of *D. arrostoi* C.Presl. Compared to the lectotype of *D. contractus* designated by Bacchetta et al. (2010: 151: s.l., s.d., Jan, NAP-GUSS!), and to the lectotype of *D. arrostoi* designated by Camarda and Corrias (1987: 417), this variety differs only by the more elongated scapes.

### ***Dianthus godronianus* Jordan in Mém. Acad. Roy. Sci. Lyon, Sect. Sci., ser. 2, 1: 241. 1851. [January 19851]**

≡ *D. caryophyllus* subsp. *godronianus* (Jord.) P.Martin, Soc. Ech. Pl. Vasc. Eur. Bassin Médit. 19: 93. 1984.

≡ *D. sylvestris* var. *godronianus* (Jord.) Kerguélen, Lejeunia, Nouv. Sér., 120: 81. 1987.

= *D. virgineus* L., Sp. Pl. 1: 412. 1753. Ind. Loc.: Coteaux stériles de la région des oliviers. Provence, Hyères, Marseille, Toulon, Apt, mont Ventoux, Vaucluse, Villeneuve; Dauphinée, Rabou près de Gap, Valence, Avignon, Languedoc, Viviers, pont du Gard, Uzés, Montpellier, Mende, Perpignan Corse, Calvi, Bastia, Cervione, Evisa, Otta, Campitello.

**Type.** (lectotype here designated): Soleirol, Herb. Cors., 959 *Dianthus virgineus* L. (Gren. et Godr.), *Dianthus sylvestris* Duby, Bastia - mai 1823, P05000349 (photo!).

**Note.** – Jordan (1851, 1856) believed that the plants referred by Godron (1847, 1848) to *D. virgineus* L. actually represent a different species, which he renamed *D. godronianus*. According to Godron (1848), this species grows in the surroundings of Montpellier, South France, and Corsica. A duplicate of the collection no. 959 by Soleirol, explicitly cited as seen by Godron (1848), was chosen as lectotype.

This specimen corresponds with the protologue and with the current application of the name. In Kerguélen (1993), this taxon is considered accepted at varietal rank (*D. sylvestris* subsp. *longicaulis* var. *godronianus*). In Jauzein (2014), this taxon is instead included in *D. caryophyllus* subsp. *longicaulis* (Ten.) Arcang., but the author argues that it could constitute a distinct subspecies (*D. caryophyllus* subsp. *godronianus* (Jord.) P.Martin). *Dianthus godronianus* is instead considered a distinct species by Tison and de Foucault (2014), although these authors note that some coastal populations in Provence differ for a few morphological features. According to the lectotype features and the recent lectotypification of the latter name (Domina et al. 2021), this species can be regarded as a heterotypic synonym of *D. virgineus*.

### ***Dianthus orophilus*** **Jord. in Billot, Annot.: 43. 1856 [December 1856]**

≡ *D. caryophyllus* var. *orophilus* (Jord.) Rouy & Foucaud, Fl. Fr. 3: 195. 1896 [July-August 1896]
=? *D. sylvestris* Wulfen in Jacq., Coll. 1: 237. 1786. [January-September 1786]. Ind. Loc.: "schistes au Lautaret et dans le province de Maurienne (Savoie)".

**Type.** (lectotype here designated): *Dianthus orophilus*, *Dianthus sylvestris* an var. *gracilior*, du Lautaret May [18]53-June [18]55 […], LY0825955!

**Note.** Two syntypes from Col de Lautaret are housed at LY: LY0825955 and LY0087623, both in good condition. We have designated here the most complete one as lectotype. The selected type comes from Jordan's garden, where it was cultivated since its first collection in 1853.

This specimen conforms to the description of the protologue and corresponds to the current application of the name, which is considered as a heterotypic synonym of *D. sylvestris* subsp. *sylvestris* in Kerguélen (1993). The lectotype of *D. orophilus* concerning the shape of calyx teeth, is very similar to the lectotype of *D. inodorus* (L.) Gaertn., which in turn is currently included within the variability of *D. sylvestris* (Domina et al. 2021).

### ***Dianthus saxicola*** **Jord., Pugill. Pl. Nov.: 29. 1852 [October 1852]**

≡ *D. caryophyllus* var. *saxicola* (Jord.) Cariot & St-Lager, Étude Fl., éd. 8, 2: 103. 1889.
=? *D. sylvestris* Wulfen in Jacq., Coll. 1: 237. 1786. [January-September 1786]. Ind. Loc.: "in lapidosis et rupestribus calcareis Beugesi et Delphinatus prope Lyon ubi eum legi".

**Type.** (lectotype here designated): *Dianthus saxicola* Jord., Serrières (Ain) près de Lyon, 7 June 1852, A. Jordan, LY0682162!

**Note.** Two specimens belonging to the original material are housed at LY: LY0682162 and LY0088790. Both are in good condition. We have designated here the most complete one as the lectotype.

This specimen conforms to the description of the protologue and corresponds to the current application of the name, which is considered a distinct species by Tison and de Foucault (2014). The lectotype of *D. saxicola* has 10–15 cm long basal leaves and multiflorous scapes; concerning the shape of calyx teeth, it is very similar to the lectotype of *D. inodorus* (L.) Gaertn., which in turn is currently included within the variability of *D. sylvestris* (Domina et al. 2021). Further research is needed to clarify the relationships between these two taxa.

### ***Dianthus juratensis*** **Jord. in Billot, Annot.: 47. 1856. [December 1856]**

≡ *D. caryophyllus* var. *juratensis* (Jord.) Gren., Fl. Chaîne Jurass.: 105. 1865.
=? *D. sylvestris* Wulfen in Jacq., Coll. 1: 237. 1786. [January-September 1786]. Ind. Loc.: “du Mont Reculet (Ain)”.

**Type.** (lectotype here designated): *Dianthus juratensis* Jord., mont Reculet (Ain), 24 August 1854, [A. Jordan], LY0083755!

**Note.** Another herbarium sheet (LY08259243) is preserved at LY; it contains plants collected in 1855 in Villeurbanne, where they were cultivated after being originally collected in the wild at Reculet (Ain).

The lectotype designated here matches the protologue and corresponds to the current application of the name, which is considered as a heterotypic synonym of *D. sylvestris* subsp. *sylvestris* in Kerguélen (1993). The lectotype of *Dianthus juratensis*, concerning the shape of calyx teeth, is very similar to the lectotype of *D. inodorus* (L.) Gaertn., which in turn is currently included within the variability of *D. sylvestris* (Domina et al. 2021).

### ***Dianthus subacaulis*** **Vill., Hist. Pl. Dauphiné 3(2): 597. 1789. [September-October 1789]**

≡ *D. sylvestris* var. *subacaulis* (Vill.) W.D.J.Koch, Syn. Fl. Germ. Helv. 1: 97. 1835.
≡ *D. virgineus* var. *subacaulis* (Vill.) Ser., Prodr. [A. P. de Candolle] 1: 361. 1824.
≡ *D. pungens* subsp. *subacaulis* (Vill.) Bernal, Laínz, Muñoz Garmendia & Pedrol, Anales Jard. Bot. Madrid 44(2): 571. 1987.
=? *D. sylvestris* Wulfen in Jacq., Coll. 1: 237. 1786. [January-September 1786]. Ind. Loc.: “aux environs du Buis, sur le Mont Ventoux”.

**Type.** (neotype here designated): Herbier A. Dubuis, *Dianthus subacaulis* Vill. subsp. *subacaulis*, Pentes rocailleuses dénudées près du somment du Mont Ventoux (1912 m). (Vaucluse), 7 July 1955, MPU329773 (photo!).

**Note.** No original material was found in GRM and in the other surveyed herbaria. Also A. P. V. Mutel’s Herbarium was checked because he used to include Villars specimens in his own herbarium (M. Lefebvre, pers. comm.).

The neotype designated here matches the protologue and corresponds to the current application of the name, which is accepted by both Kerguélen (1993) and Tison and de Foucault (2014). This species is characterized by having 1 cm long basal leaves, very short, 1–5 cm long single-flowered scapes and epicalyx scales lanceolate with a linear mucro. Concerning the shape of calyx teeth, it is very similar to the lectotype of *D. inodorus* (L.) Gaertn., which in turn is currently included within the variability of *D. sylvestris* (Domina et al. 2021). Further research is needed to clarify the relationships between these two taxa.

## ***Dianthus sylvestris*** **Wulfen in Jacq., Coll. 1: 237. 1786. [January-September 1786]**

≡ *D. caryophyllus* subsp. *sylvestris* (Wulfen) Rouy & Foucaud, Fl. France 3: 193. 1896. Ind. Loc.: – “in montibus illis prope Ponewitsch Baronis Wolkensberg in Carniolia, tum in M. Utocsek prope Pillichgraz; in iis. Vallis Rablensis; denique & in iis Vallis Canalensis &c.”.

**Types.** (lectotype here designated): The water-coloured iconography published by Jacquin (1781–1786, t. 82, the small individual on the right).

**Note.** The iconography designated by Bacchetta et al. (2010) as neotype is actually part of the original material as uncited illustration (Art. 9.12 of the ICN), since Jacquin’s Icones and Collectanea work are interrelated. Therefore this neotypification must be corrected in lectotypification. This illustration depicts two individuals: one small with a 2 branched single-flowered stem and one large, unbranched but with multiflowered stems and basal leaves three times longer, exemplifying morphological variation in this species. In the protologue, it is clearly stated that the larger plant was seen only once in Monte Re, near Lake of Predil, NE Italy (“Uno duntaxat, quod miratus sum, loco Montis regii Rablensis, giganteum inveni, caulibus cubitalibus bi- & trifloris”), while smaller plants are common elsewhere in Carniola. Accordingly, we can conclude that the two drawings depict plants originating from two different areas, thus belonging to two different gatherings. Consequently, the type designated by Bacchetta et al. (2010: 143), neotype or lectotype, belongs to more than one gathering and cannot be accepted as a type (Art. 8.1, 8.2, 9.3 of the ICN). Thus, the name remains to be typified. No other original material for this name exists (de Langen et al. 1984), so that we select here as lectotype only the small specimen of the water-coloured iconography published by Jacquin at table 86 that better fits the description “folia ... pollicari aut circiter longitudine… Caulis subquinquepollicaris... Flos plerumque unicus [Leaves … one inch or about one inch long, stem less than 5 inches ... flower generally single]”.

The lectotype here selected agrees with the current application of the name by numerous authors, e.g., Kerguélen (1993), Bacchetta et al. (2010), Tison and de Foucault (2014), Brullo and Guarino (2017), who consider *D. sylvestris* as an accepted species. The overall size of the plant, and the length of the leaves are not stable characters for taxonomic discrimination. The shape and relative size of calyx and epicalyx scales are better discriminating taxonomic characters and are evident in the lectotype. These features allow to distinguish *D. sylvestris* subsp. *sylvestris* from *D. sylvestris* subsp. *tergestinus* (Bacchetta et al. 2010).

## ***Dianthus sylvestris*** **var.** ***garganicus*** **Ten., Fl. Napol. Syll.: 208. 1831. [July-August 1831]**

≡ *D. caryophyllus* subsp. *garganicus* (Ten.) Grande, Boll. Soc. Bot. Ital. 1912: 178. 1912.

≡ *D. caryophyllus* var. *garganicus* (Ten.) Fiori, Nuova Fl. Italia 1: 512. 1924.
≡ *D. sylvestris* subsp. *garganicus* (Ten.) Pignatti, Giorn. Bot. Ital. 107: 211. 1973.
≡ *D. garganicus* (Ten.) Brullo, Braun-Blanquetia 2: 31. 1988.
= *D. tarentinus* Lacaita, Nuovo Giorn. Bot. Ital. n.s., 18(4): 511. 1911. Ind. Loc.: "Gargano".

**Type.** (lectotype here designated): *Dianthus sylvaticus*, *D. sylvestris* Ten. Fl. Neap. Prodr. (1811) p. xxv. - Eiusd. Fl. Nap. I (1811–1815) p. 231, Gargano, Tenore misit Nov 1827 / Herb. J. Gay., Presented by Dr. Hooker, February 1868, K000725365 (photo!).

**Note.** In the same herbarium sheet three herbarium specimens, sent by Michele Tenore to Jaques Étienne Gay, are mounted. K000725363 was collected by Tenore from Calmaldoli (Campania, Italy) in November 1825; K000725364 by Nicolas Bové from La Calle (Algeria) in June 1839, and K000725365 by Tenore from Gargano (Apulia, Italy) in November 1827. In NAP there is a specimen from Gargano with the handwriting by Michele Tenore, lacking a date.

The lectotype designated here matches the protologue and corresponds to the current application of the name, which is considered as a heterotypic synonym of *D. tarentinus* Lacaita (Bacchetta et al. 2010; Brullo and Guarino 2017; Bartolucci et al. 2018). This synonymy is here confirmed based on the shape and size of the leaves, of the scales of the epicalyx and of the calyx which are observable on the types of the two taxa.

### ***Dianthus virgineus* var. *tergestinus* Rchb., Icon. Fl. Germ. Helv. 6: 47, pl. 266 fig. 5049β?. 1842–1844. [1844 publ. 1842–1844]**

≡ *D. tergestinus* (Rchb.) A.Kern., Sched. Fl. Exs. Austro-Hung. 2: 71. 1883.
≡ *D. caryophyllus* var. *tergestinus* (Rchb.) Tanfani in Caruel, Fl. Ital. 9(2): 283. 1892.
≡ *D. sylvestris* subsp. *tergestinus* (Rchb.) Hayek, Repert. Spec. Nov. Regni Veg. Beih. 30(1, 2): 247. 1924. Ind. Loc.: none [but Trieste, Italy, can be easily inferred from the epithet "tergestinus" that means "from Trieste"].

**Type.** (lectotype here designated): Rchb., Icon. Fl. Germ. Helv. 6: pl. 266 fig. 5049β. 1842–1844.

**Note.** The main text (Icon. Fl. Germ. Helv. 6: 47. 1842–1844. [1844 publ. 1842–1844]) lacks a written diagnosis or description, and, in any case, it is not clear if the plate was published simultaneously with the main text. Stafleu and Cowan (1983) reports that the volume 6 was published between 1842 and 1844, even though the title page shows 1844. However, this name was validly published on plate CCLXVI (= 266) by an illustration with analysis (Arts. 38.7 and 38.8 of the ICN), which is obviously part of the original material.

This taxon is considered as a subspecies of *D. sylvestris* by Vangjeli (2015), Brullo and Guarino (2017), Bartolucci et al. (2018), Peruzzi et al. (2019), and Nikolić (2020). It differs from *D. sylvestris* subsp. *sylvestris* by having a poorly developed mucro of the epicalyx scales and entire petals. Its distribution (Trieste area and along the north-eastern Adriatic coast), separated from the main range of *D. sylvestris* subsp. *sylvestris*, is compatible with the rank of subspecies.

## Acknowledgements

We thank Hans-Joachim Esser (M), Laura Guglielmone (TO), Matthieu Lefebvre (GRM) and the other curators of herbaria listed in Material and Methods section for their help with searching for the original material. A special thanks to W. Greuter (PAL) for nomenclatural advice. This work was supported by the "Progetto di Ricerca di Rilevante Interesse Nazionale" (PRIN) "PLAN.T.S. 2.0 – towards a renaissance of PLANt Taxonomy and Systematics" led by the University of Pisa, under the grant number 2017JW4HZK (Principal Investigator: Lorenzo Peruzzi). The work of Ana Terlević and Ivana Rešetnik has been supported by Croatian Science Foundation under the project UIP-2017-05-2882 (AmphiAdriPlant), as well as by the "Young researchers' career development project – training of doctoral students" of the Croatian Science Foundation funded from the European Social Fund.

## References

Aghababyan M, Greuter W, Raimondo FM (2012) Michele Lojacono-Pojero's Centuriae in the herbaria and archives in Geneva. Bocconea 24: 177–193.

Arrigoni PV (1979) Le piante endemiche della Sardegna, 40–53. Bollettino della Società Sarda di Scienze Naturali 18(1978): 223–295.

Arrigoni PV (2006) Taxonomical and chorological contribution to the Sardinian flora. Bocconea 19: 33–48.

Arrigoni PV (2010) Flora dell'Isola di Sardegna (Vol. 2). C. Delfino ed., Sassari.

Bacchetta G, Brullo S, Casti M, Giusso del Galdo GP (2010) Taxonomic revision of the *Dianthus sylvestris* group (Caryophyllaceae) in Central-Southern Italy, Sicily and Sardinia. Nordic Journal of Botany 28(2): 137–173. https://doi.org/10.1111/j.1756-1051.2009.00459.x

Bartolucci F, Peruzzi L, Galasso G, Albano A, Alessandrini A, Ardenghi NMG, Astuti G, Bacchetta G, Ballelli S, Banfi E, Barberis G, Bernardo L, Bouvet D, Bovio M, Cecchi L, Di Pietro R, Domina G, Fascetti S, Fenu G, Festi F, Foggi B, Gallo L, Gottschlich G, Gubellini L, Iamonico D, Iberite M, Jiménez-Mejías P, Lattanzi E, Marchetti D, Martinetto E, Masin RR, Medagli P, Passalacqua NG, Peccenini S, Pennesi R, Pierini B, Poldini L, Prosser F, Raimondo FM, Roma-Marzio F, Rosati L, Santangelo A, Scoppola A, Scortegagna S, Selvaggi A, Selvi F, Soldano A, Stinca A, Wagensommer RP, Wilhalm T, Conti F (2018) An updated checklist of the vascular flora native to Italy. Plant Biosystems 152(2): 179–303. https://doi.org/10.1080/11263504.2017.1419996

Bernal M, Laínz M, Muñoz Garmendia F (1990) *Dianthus*. In: Castroviejo S, Laínz M, López González G, Montserrat P, Muñoz Garmendia F, Paiva J, Villar L (Eds) Flora Iberica vol. 2. C.S.I.C, Madrid.

Brullo S, Guarino R (2017) Complesso di *D. sylvestris*. In: Pignatti S (Ed.) Flora d'Italia (Vol. 2). New Business Media, Milano, 200–205.

Brullo S, Scelsi F, Spampinato G (2000) New taxa belonging to *Dianthus vulturius* Guss. & Ten. group (Caryophyllaceae) from S Calabria (Italy). Portugaliae Acta Biologica 19: 303–317.

Camarda I, Corrias B (1987) Tipificazione di *Dianthus siculus* C.Presl e di *D. arrosti* C.Presl in J. & C.Presl. Informatore Botanico Italiano 19: 415–421.

Clementi M, Vukojičić S, Lakušić D, Kuzmanović N (2015) Typification of the names published by Roberto de Visiani and Josif Pančić in Plantae Serbicae Rariores aut Novae – Decas I. Phytotaxa 202(2): 121–134. https://doi.org/10.11646/phytotaxa.202.2.4

de Langen FR, Oost EH, Jarvis CE (1984) Lectotypification of *Dianthus caryophyllus* L. and *D. chinensis* L. (Caryophyllaceae). Taxon 33(4): 716–724. https://doi.org/10.2307/1220794

Domina G, Greuter W, Mazzola P, Raimondo FM (2014) Names of Italian vascular plants published by Michele Lojacono Pojero. Flora Mediterranea 24: 215–232. https://doi.org/10.7320/FlMedit24.215

Domina G, Scibetta S, Scafidi F, Giovino A (2017) Contribution to the identification of *Dianthus rupicola* (Caryophyllaceae) subspecies using morphological and molecular approaches. Phytotaxa 291(1): 17–32. https://doi.org/10.11646/phytotaxa.291.1.2

Domina G, Astuti G, Barone G, Gargano D, Minuto L, Varaldo L, Peruzzi L (2021) Lectotypification of the name *Dianthus virgineus* and consequences within the *D. sylvestris* group (Caryophyllaceae). Taxon 70(5): 1096–1100. https://doi.org/10.1002/tax.12563

Escobar García P, Mascia F, Bacchetta G (2010) Typification of the name *Lavatera triloba* subsp. *pallescens* (Moris) Nyman and reassessment of *L. minoricensis* Cambess. (*L. triloba* subsp. *minoricensis* comb. nov.). Anales del Jardin Botanico de Madrid 67(2): 79–86. https://doi.org/10.3989/ajbm.2244

Giacò A, Astuti G, Peruzzi L (2021) Typification and nomenclature of the names in the *Santolina chamaecyparissus* species complex (Asteraceae). Taxon 70(1): 189–201. https://doi.org/10.1002/tax.12429

Godron M (1847) Observations sur le *Dianthus virgineus* de Linné. Mémoires de la Société Royale des Sciences, Lettres et arts de Nancy 1846: 1–16.

Godron M (1848) *Dianthus*. In: Grenier M, Godron M (Eds) Flore de France ou description des plantes qui croissent naturellement en France et en Corse. 1. J.-B. Baillière, Paris et De Sainte-Agathe Aîné Besançon, 228–242.

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. Systematics and Biodiversity 18(3): 255–268. https://doi.org/10.1080/14772000.2020.1737979

IPNI (2021) International Plant Names Index. Published on the Internet http://www.ipni.org, The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Botanic Gardens. [Retrieved 20 October 2021]

Jacquin NJ (1781–1786) Icones plantarum rariorum 1 Christianum Fridericum Wappler, Vindobonae.

Jauzein Ph (2014) *Dianthus*. In: Tison JM, Jauzein Ph, Michaud H (Eds) Flore de la France méditerranéenne continentale. Naturalia, Turriers, 1114–1121.

Jordan MA (1851) Pugillus plantarum novarum. Mémoires de l'Académie Royale des Sciences, Belles-Lettres et Arts de Lyon, sér. 2, section des sciences 1: 212–358.

Jordan MA (1856) Notices sur plusieurs plantes nouvelles in Billot PC, Annotations a la Flora de France ed d'Allemagne. Edler, Haguenau, 43–49.

Kerguélen M (1993) Index synonymique de la flore de France. Muséum national d'histoire naturelle, Paris.

Nikolić T (2020) Flora Croatica. Vaskularna flora Republike Hrvatske (Vol. 2). Alfa d.d., Zagreb.

Pax F, Hoffmann K (1934) *Caryophyllaceae*. In: Engler AK, Prantl K (Eds) Die Natürlichen Pflanzenfamilien, 2nd ed. (Vol. 16c). Engelmann, Leipzig, 275–364.

Peruzzi L, Domina G, Bartolucci F, Galasso G, Peccenini S, Raimondo FM, Albano A, Alessandrini A, Banfi E, Barberis G, Bernardo L, Bovio M, Brullo S, Brundu G, Brunu A, Camarda I, Carta L, Conti F, Croce A, Iamonico D, Iberite M, Iiriti G, Longo D, Marsili S, Medagli P, Pistarino A, Salmeri C, Santangelo A, Scassellati E, Selvi F, Soldano A, Stinca A, Villani M, Wagensommer RP, Passalacqua NG (2015) An inventory of the names of vascular plants endemic to Italy, their loci classici and types. Phytotaxa 196(1): 1–217. https://doi.org/10.11646/phytotaxa.196.1.1

Peruzzi L, Galasso G, Domina G, Bartolucci F, Santangelo A, Alessandrini A, Astuti G, D'Antraccoli M, Roma-Marzio F, Ardenghi NMG, Barberis G, Conti F, Bernardo L, Peccenini S, Stinca A, Wagensommer RP, Bonari G, Iamonico D, Iberite M, Viciani D, Del Guacchio E, Giusso Del Galdo G, Lastrucci L, Villani M, Brunu A, Magrini S, Pistarino A, Brullo S, Salmeri C, Brundu G, Clementi M, Carli E, Vacca G, Marcucci R, Banfi E, Longo D, Di Pietro R, Passalacqua NG (2019) An inventory of the names of native, non-endemic vascular plants described from Italy, their loci classici and types. Phytotaxa 410(1): 1–215. https://doi.org/10.11646/phytotaxa.410.1.1

Reeve H (1967) *Dianthus* L. In: Davis PH (Ed.) Flora of Turkey and the East Aegean islands, 2. Edinburgh University Press, Edinburgh, 99–131.

Rizzotto M (1989) Nomenclatural notes and lectotypification of *Alkanna lutea* Moris (*Boraginaceae*). Taxon 38(4): 653–656. https://doi.org/10.2307/1222659

Smith E (1794) Remarks on the genus *Dianthus*. Transaction of the Linnean Society of London 2(1): 292–304. https://doi.org/10.1111/j.1096-3642.1794.tb00262.x

Stafleu A, Cowan RS (1983) Taxonomic literature (2nd ed., Vol. 6). Bohn, Scheltema & Holkema, Utrecht.

Thiebaut M, Tison J-M (2016) Typification of thirty-two names given by Alexis Jordan in the genus *Biscutella* (Brassicaceae). Phytotaxa 269(2): 103–112. https://doi.org/10.11646/phytotaxa.269.2.3

Thiers B (2021) Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's virtual herbarium. http://sweetgum.nybg.org/science/ih/ [accessed 15.06.2019]

Tison J-M, de Foucault B (2014) Flora Gallica. Flore de France. Biotope Éditons, Mèze.

Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber W-H, Li D-Z, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF [Eds] (2019) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159: 1–254. https://doi.org/10.12705/ Code.2018

Valsecchi F (1985) Le piante endemiche della Sardegna. 179. Bollettino della Società Sarda di Scienze Naturali 24: 333–337.

Vangjeli J (2015) Excursion Flora of Albania. Koeltz, Königstein.

Williams FN (1890) Pinks of Central Europe. West, Newman and co., London.

Williams FN (1893) A monograph of the genus *Dianthus*, Linn. Journal of the Linnean Society of London. Botany 29: 346–478. https://doi.org/10.1111/j.1095-8339.1893.tb02037.x

# Publication III

plants

*Article*

# Genome Size Variation in *Dianthus sylvestris* Wulfen sensu lato (Caryophyllaceae)

**Ana Terlević [1], Sandro Bogdanović [2,3], Božo Frajman [4,†] and Ivana Rešetnik [1,*,†]**

[1] Department of Biology, Faculty of Science, University of Zagreb, Trg Marka Marulića 20/II, 10000 Zagreb, Croatia; ana.terlevic@biol.pmf.hr
[2] Department of Agricultural Botany, Faculty of Agriculture, University of Zagreb Svetošimunska cesta 25, 10000 Zagreb, Croatia; sbogdanovic@agr.hr
[3] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska cesta 25, 10000 Zagreb, Croatia
[4] Department of Botany, Institute of Botany, University of Innsbruck, Sternwartestraße 15, A-6020 Innsbruck, Austria; bozo.frajman@uibk.ac.at
* Correspondence: ivana.resetnik@biol.pmf.hr
† These authors contributed equally to this work.

**Abstract:** Genome size (GS) is an important characteristic that may be helpful in delimitation of taxa, and multiple studies have shown correlations between intraspecific GS variation and morphological or environmental factors, as well as its geographical segregation. We estimated a relative GS (RGS) of 707 individuals from 162 populations of *Dianthus sylvestris* with a geographic focus on the Balkan Peninsula, but also including several populations from the European Alps. *Dianthus sylvestris* is morphologically variable species thriving in various habitats and six subspecies have been recognized from the Balkan Peninsula. Our RGS data backed-up with chromosome counts revealed that the majority of populations were diploid ($2n = 30$), but ten tetraploid populations have been recorded in *D. sylvestris* subsp. *sylvestris* from Istria (Croatia, Italy). Their monoploid RGS is significantly lower than that of the diploids, indicating genome downsizing. In addition, the tetraploids significantly differ from their diploid counterparts in an array of morphological and environmental characteristics. Within the diploid populations, the RGS is geographically and only partly taxonomically correlated, with the highest RGS inferred in the southern Balkan Peninsula and the Alps. We demonstrate greater RGS variation among the Balkan populations compared to the Alps, which is likely a result of more pronounced evolutionary differentiation within the Balkan Peninsula. In addition, a deep RGS divergence within the Alps likely points to persistence of the alpine populations in different Pleistocene refugia.

**Keywords:** genome size; Balkan Peninsula; European Alps; tetraploids; glacial refugia

check for updates

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

Academic Editors: Sonja Siljak-Yakovlev, Oriane Hidalgo and Joan Vallès

Received: 11 April 2022
Accepted: 28 May 2022
Published: 31 May 2022

## 1. Introduction

Genome size (GS; size of the monoploid chromosome set, [1]) is an important cytogenetic characteristic that may be helpful in delimitation of taxa [2–10]. Numerous studies using GS data, often in combination with chromosome counts, have explored diversification of polyploid species complexes [11–14] or genera with high incidence of polyploidy [15–18]. Flow cytometric GS estimation has become an established method, as it allows rapid estimation of nuclear DNA content of large numbers of individuals in either absolute or relative units [2,19]. It is a faster and more convenient method for ploidy level estimations compared to conventional chromosome counting, and it can be used for detecting rare cytotypes [20] or to provide evidence of GS intraspecific variability [21].

The existence of intraspecific variation in GS has been acknowledged [7,22–24] and reported for many species, e.g., *Festuca pallens* Host [23], *Senecio carniolicus* Willd. [25], *Tephroseris longifolia* (Jacq.) Griseb. & Schenk [26], and *Minuartia verna* (L.) Hiern [8], albeit sometimes argued to be of minor evolutionary relevance [24] or suggested to be a result of an experimental artefact [27]. Nevertheless, this variation can be a result of microevolutionary differentiation and can reflect taxonomic heterogeneity [28]. The reasons for GS variation in the absence of polyploidy may be sought in the increased activity of retrotransposons [29] and the accumulation of retrotransposons and other repetitive elements are considered the main factors of GS increase in angiosperms [7].

Multiple studies have shown correlations between intraspecific variation of GS and morphological or environmental factors, as well as geographical distribution [5,26,30–35], but exact causes of this variation, and thus the interpretation of GS heterogeneity, remains a challenging task [35–37]. For instance, GS is a characteristic that may be related to the variation in plant phenology [38] and water availability [36], and may affect morphological characteristics such as seed size, nuclear and cell volumes, and duration of mitotic and meiotic cycles [39]. Evidence concerning GS variation across environmental gradients may point to the involvement of GS in adaptive evolution [18,34] or speciation and diversification [40]. Thus, GS data can facilitate taxon delimitation at sectional, specific, and intraspecific levels [3,4,6,9,12,15].

*Dianthus* is one of the largest genera of Caryophyllaceae comprising over 300 species distributed throughout Eurasia and northern Africa [41]. This high diversity is a result of rapid radiation and diversification, which was pronounced especially in the Mediterranean Basin [42], where several polymorphic *Dianthus* groups with high intraspecific diversity, and thus unresolved taxonomy exist [43–48]. One of them is *Dianthus sylvestris* Wulfen s.l., which is one of the most taxonomically challenging groups of the European flora [49,50]. Its main diversity centers are the Balkan and the Apennine Peninsulas, where large morphological variation has led to description of several taxa growing in various habitats from the Mediterranean coast to the alpine belt [44,45,47,51]. Due to high morphological variability and subtle morphological transitions, there is a dispute concerning the number of taxa. Several authors have tried to develop a sensible intraspecific classification for *D. sylvestris* on the Balkan Peninsula [52–56], leading to recognition of six subspecies [57–59]: *D. sylvestris* subsp. *alboroseus* F.K. Mey., *D. sylvestris* subsp. *bertisceus* Rech. f., *D. sylvestris* subsp. *kozjakensis* Micevski, *D. sylvestris* subsp. *nodosus* (Tausch) Hayek, *D. sylvestris* subsp. *sylvestris*, and *D. sylvestris* subsp. *tergestinus* (Rchb.) Hayek. The morphometric study of Terlević et al. (submitted) [60] performed on 97 populations of *D. sylvestris* s.l. across its range on the Balkan Peninsula showed that the states of several morphological traits deemed diagnostic for subspecies, i.e., number and shape of epicalyx scales, calyx length, petal characteristics, and indumentum density, frequently overlap, making the reliable identification of subspecies often difficult and ambiguous. However, the combination of morphological characteristics (i.e., entire or slightly eroded petals and usually one pair of epicalyx scales) and different flowering time clearly distinguishes the thermophilus *D. sylvestris* subsp. *tergestinus* from all other subspecies including the sympatric *D. sylvestris* subsp. *sylvestris* and *D. sylvestris* subsp. *nodosus*.

Within *Dianthus*, the most frequent chromosome number is diploid ($2n = 2x = 30$) [61–64], although polyploid taxa including series with up to seven ploidy levels have been documented ($2n = 2x$, $3x$, $4x$, $5x$, $6x$, $8x$, and $12x$) [11,65]. In the Chromosome Counts Database (CCDB) [63], chromosome numbers for 162 *Dianthus* taxa have been registered, of which 89 (55%) are diploid ($2n = 30$), 17 (10%) tetraploid ($2n = 60$), and 11 (7%) hexaploid ($2n = 90$), whereas for 45 taxa (28%) multiple ploidy levels have been recorded. The karyological features of *Dianthus* chromosomes have been rarely reported due to their small size (0.6–2.7 μm long) and large number in polyploids, but most of them are metacentric and of similar size [61,66–68].

For *D. sylvestris* two ploidy levels have been reported: diploids ($2n = 30$) throughout the distribution area [61,62,69] and tetraploids ($2n = 60$) from Gorges de Daluis in the Maritime Alps in France [70]. For diploids from Mt. Jahorina in Bosnia and Herzegovina, the GS of 1C = 0.61 pg has been reported by Siljak-Yakovlev et al. [69] and consequently by Pellicer & Leitch [64]. Due to the low number of chromosomally investigated populations, precise information about the incidence of polyploidy within *D. sylvestris* remains unclear and it is unknown how both ploidies, as well as GS variation, correlate to taxonomic entities within the species and if there is a geographic pattern of GS variation that could be of evolutionary significance.

The main aim of this study was thus to investigate GS and ploidy-level variation within *D. sylvestris* s.l., with a geographic focus on the Balkan Peninsula and to a lesser extent the Alps. To this end, we intersect the GS data of 162 populations, calibrated with chromosome counts, with taxonomic entities and explore its geographic variation. More specifically, we (i) ask if there are polyploid populations present in the area and how they are distributed, (ii) explore whether the pattern of relative genome size (RGS) variation correlates to current taxonomic treatment and geography, and (iii) investigate if there is a relationship between RGS and environmental variation. The obtained data, together with other evidence, will help to disentangle the complex relationships within *D. sylvestris* s.l.

## 2. Results

### 2.1. Chromosome Numbers

We estimated the diploid chromosome numbers $2n = 2x = 30$ for two populations from Karlobag (D32) and Krk island (D185) in Croatia and the tetraploid numbers $2n = 4x = 60$ for three populations (D12, D20, and D21) from Istria (Italy and Croatia; Figure 1, Table S1). Chromosomes were small, 1–2 µm long.

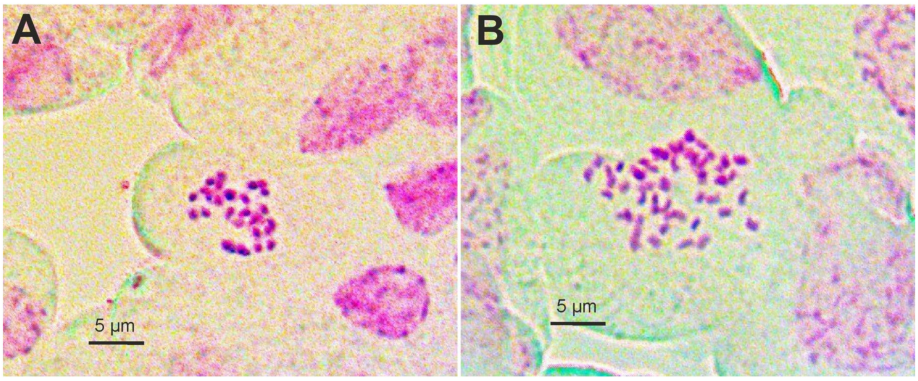


**Figure 1.** Mitotic chromosomes from *Dianthus sylvestris* root tips. (**A**) A diploid ($2n = 2x = 30$) from the population D185 (Punat, Krk island, Croatia) and (**B**) a tetraploid ($2n = 4x = 60$) from the population D12 (Plomin, Mt Učka, Croatia).

### 2.2. Relative Genome Size Estimation and DNA Ploidy Level

RGS was analyzed for 707 individuals from 162 populations of *D. sylvestris* s.l. from the Balkan Peninsula and the Alps (Figure 2, Figure S1). High-resolution histograms of DNA content comprised two large G1 peaks representing nuclei of the sample and the reference (Figure 3), and the ratio of their positions determined the sample's RGS. In addition to the main peaks, the minor peaks of the sample corresponded to endopolyploid nuclei [71] that are common in Caryophyllaceae [72]. The coefficient of variation (CV) of the sample's G0/G1 peak of the majority of 707 measurements was between 1.36 to 6 (4.7 on average). In further analyses, we also included 17 populations that exceeded this threshold and had a CV of up to 10, as their peaks were clearly visible and their RGS values fitted well to the remaining data.

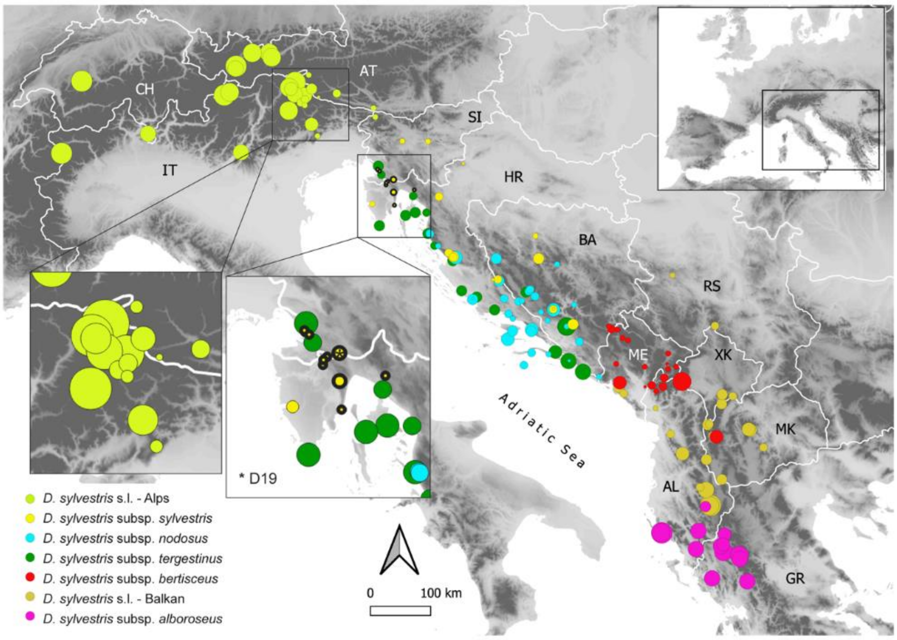


**Figure 2.** Geographical distribution of the monoploid relative genome size (RGS) variation of 162 diploid and tetraploid (circles with thick black outline) populations of *Dianthus sylvestris* s.l. in the Balkan Peninsula and the Alps. Only populations with at least three measured individuals and a standard deviation of RGS < 0.01 are shown. The size of the dots is proportional to the mean monoploid RGS of the corresponding populations. Asterisk indicates the ploidy-mixed population D19. Color coding indicates different taxa.

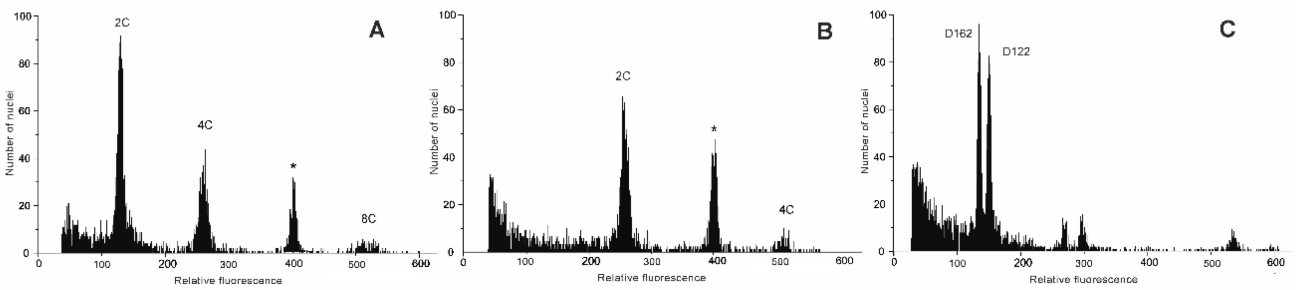


**Figure 3.** Histograms of fluorescence intensities of diploid (**A**) (population D19) and tetraploid (**B**) (population D17) accessions of *D. sylvestris*, together with the internal reference standard *Bellis perennis* (asterisk), from which relative genome size (RGS) was calculated. The peaks are labelled following Greilhuber et al. [1]. Multiple peaks of the sample correspond to nuclei after one (4C) and two (8C) rounds of endoreplication. (**C**) Histogram showing genuine difference between diploid populations D162 (0.325 ± 0.001) and D122 (0.360 ± 0.004).

Flow-cytometry screening resulted in two discrete groups of RGS values that corresponded to the estimated diploid and tetraploid chromosome numbers. Diploid populations occurred throughout the sampled area and all subspecies, at elevations from 8 to 2274 m. On the other hand, all tetraploid populations belonged to *D. sylvestris* subsp. *sylvestris*, and were limited to Istria and Kvarner (Croatia and Italy), from 240 to 941 m (Figure 2). A total of 152 populations (94%) were DNA-diploid, with RGS ranging from 0.324 to 0.376 (mean: 0.341 ± 0.011), whereas ten populations (6%) were DNA-tetraploid with RGS ranging from 0.640 to 0.657 (mean: 0.649 ± 0.006; Table 1). A 1.16-fold variation in RGS was thus revealed among diploids with a 1.03-fold variation among tetraploids (Figure 3C, Table 1). Only one population from Istria (Vodice-D19; Figure 2) was ploidy-mixed, with two individuals being DNA-diploid and eight DNA-tetraploid. The monoploid RGS of 15 DNA-diploid populations of *D. sylvestris* subsp. *sylvestris* ranged from 0.162 to

0.188 and was significantly higher compared to ten DNA-tetraploid populations with values between 0.160 and 0.164 (Figure 4, Kruskal–Wallis test = 10.58, $p < 0.01$).

**Table 1.** Descriptive statistics of relative genome size (RGS) variation in diploid (2*x*) and tetraploid (4*x*) populations of *Dianthus sylvestris* s.l., for which at least three individuals were analyzed. SD, standard deviation; mRGS, monoploid RGS.

| Ploidy | No. Measurements | Populations | Individuals | Min. No. Individuals | Max. No. Individuals | Mean No. Individuals | Mean RGS | SD RGS | Min RGS | Max RGS | Mean mRGS | SD mRGS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2*x* | 536 | 152 | 657 | 3 | 12 | 4.3 | 0.341 | 0.011 | 0.324 | 0.376 | 0.17 | 0.005 |
| 4*x* | 45 | 10 | 50 | 3 | 8 | 5 | 0.649 | 0.006 | 0.64 | 0.657 | 0.162 | 0.001 |

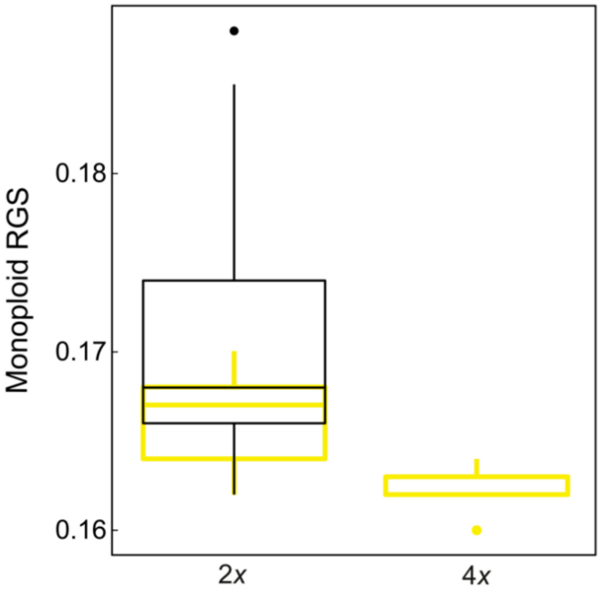


**Figure 4.** Monoploid relative genome size (RGS) variation in *Dianthus sylvestris* s.l. in the Balkan Peninsula and the Alps. Black colored box indicates the variation of the complete diploid dataset (152 DNA-diploids, 2*x*), whereas yellow color corresponds to 15 DNA-diploids (2*x*), and 10 DNA-tetraploid (4*x*) populations of *D. sylvestris* subsp. sylvestris. Boxes correspond to 25 and 75 percentiles, horizontal lines to medians, whiskers 5 to 95 percentiles, and circles to outliers.

### 2.3. Morphological and Environmental Differences between Diploids and Tetraploids

Comparison of morphological characteristics from the study of Terlević et al. (submitted) [60] between the nine diploid and seven tetraploid populations of *D. sylvestris* subsp. *sylvestris* showed a statistically significant difference in five vegetative characteristics (Table S2). The first two PCA axes explained 75.64% and 18.06% of the total morphological variation (Figure 5A) and the characteristics contributing most to the separation along the first axis were plant height (PH) and height of the first branching (FBH; component scores 0.49 and 0.50). The same variables had the highest scores in the DA (0.80 and 0.93), even though there was overlap between the scores of the discriminant functions (Figure 5B). The tetraploid plants were thus higher and had their lowermost lateral shoots higher. In addition, they had longer cauline and basal leaves (CLL, BLL), as well as longer internodes (UIL).

Although the correlation test failed to show any association between RGS of 152 diploid populations and environmental variables, the Kruskal–Wallis test showed significant difference in 14 environmental variables between 15 diploid and ten tetraploid populations of *D. sylvestris* subsp. *sylvestris* (Table S3 and Figure S2). The environmental variables contributing most to the separation of diploid and tetraploid populations were those describing temperature and precipitation preferences, as well as the terrain geomorphology. The first two PCA axes explained 40.32% and 18.24% of the total environmental variation

(Figure 5C) and the variables contributing most to the separation along the first axis were soil clay content and number of snow days in a year (component scores 0.50 and −0.53). Number of snow days in a year, slope and eastness had the highest scores in the DA (−0.59 and −0.64), without overlap between diploids and tetraploids (Figure 5D).

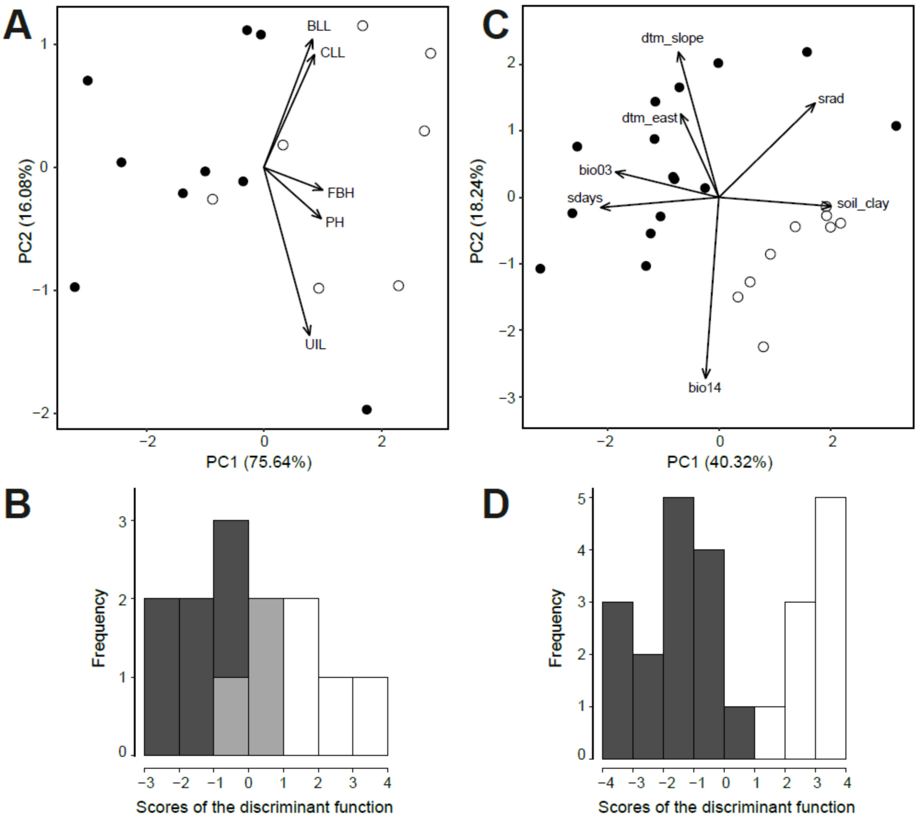


**Figure 5.** Morphological and environmental differentiation between diploid (black) and tetraploid (white) populations of *Dianthus sylvestris* subsp. *sylvestris*, with their overlap in B shown in grey. (**A**) Principal component analysis (PCA) and (**B**) histogram of discriminant analysis (DA) based on five morphological characteristics. (**C**) PCA and (**D**) histogram of DA based on seven environmental variables. Characteristic abbreviations in (**A**,**C**) are explained in Tables S2 and S3.

### *2.4. RGS Variation across Intraspecific Entities*

Differences in RGS among the five subspecies and two geographic groups of populations of *D. sylvestris* s.l. were significant (Kruskal–Wallis = 54.1, $p < 0.01$, Figure 6A). The Tukey post-hoc test showed that populations of *D. sylvestris* subsp. *alboroseus* and those of *D. sylvestris* s.l. from the Alps had significantly larger monoploid RGS values than other groups ($p < 0.01$, Figure 6A). Additionally, the RGS of *D. sylvestris* subsp. *tergestinus* was significantly higher than the RGS of *D. sylvestris* subsp. *sylvestris*, whereas there was no significant difference between *D. sylvestris* s.l. from the Balkans, *D. sylvestris* subsp. *bertisceus*, *D. sylvestris* subsp. *nodosus*, and *D. sylvestris* subsp. *sylvestris*.

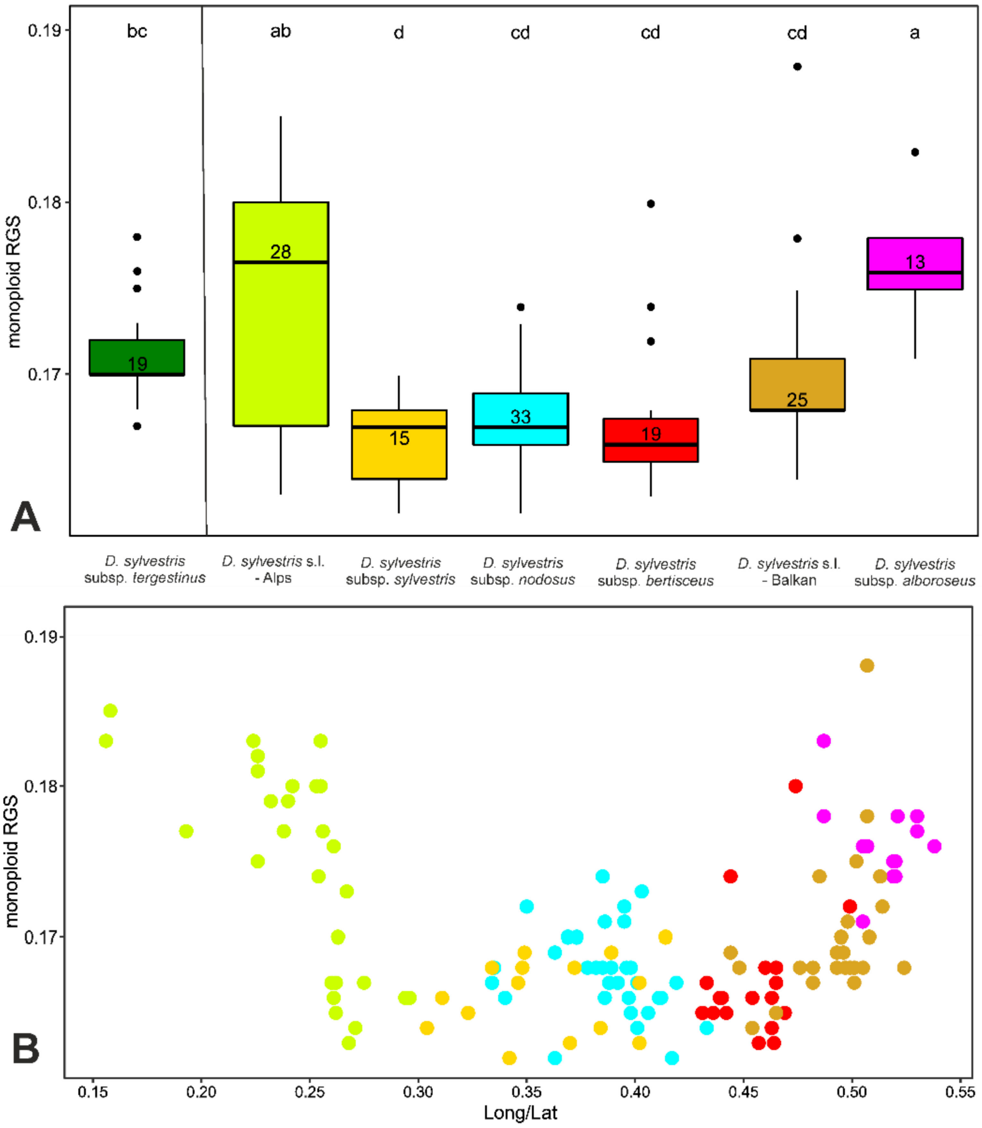


**Figure 6.** Monoploid RGS variation based on population means of 152 DNA-diploid populations of *Dianthus sylvestris* s.l. Color coding for taxa corresponds to Figure 2. (**A**) Boxes in the boxplot and (**B**) points in the scatterplot are arranged in geographical order from the north-west to the south-east. Being the most clearly distinguished subspecies, D. sylvestris subsp. tergestinus is singled out on the left of plot (**A**) and excluded from the plot (**B**). Boxes correspond to 25 and 75 percentiles, horizontal lines to medians, whiskers 5 to 95 percentiles, and circles to outliers. Means not significantly different at $p < 0.01$, according to the Tukey post-hoc test, are indicated by the same letter. Numbers adjacent to the median lines represent the sample size.

## 3. Discussion

### 3.1. Tetraploidization within D. sylvestris Populations in the Northern Balkan Peninsula

The extensive RGS measurements combined with confirmatory chromosome number estimations revealed the prevalence of diploid populations of *D. sylvestris* throughout the investigated area, as well as the occurrence of tetraploid populations in the northwesternmost Balkan Peninsula (Istria and Kvarner in Croatia and Italy; Figure 2, Table S1). Therefore, this is the first report of tetraploid populations within *D. sylvestris* s.l. in the Balkan Peninsula, which likely originated separately from the tetraploid populations reported from France [70], given the geographic distance between them. The confirmation of independent origin of these two groups of tetraploid populations requires additional evidence based on genetic data or detailed examination of RGS values of French populations as their different values might indicate a separate origin. However, multiple polyploidization events within single species are common and have been reported, for example in *Astragalus onobrychis* [73], *Cerastium decalvans* [74], and *Euphorbia montenegrina* [10]. In addition, within *Dianthus*, multiple and independent origin of polyploids within the *Dianthus broteri* complex [75,76] and several heteroploid species of *Dianthus* section *Plumaria* [11], that even occur sympatrically, have been reported. Diploidization, a process following polyploidization, is commonly accompanied by elimination of parts of the genome [77,78], termed genome downsizing [79]. Reduction of monoploid GS has been observed in many different plant groups (e.g., [8,15,79,80]) and our data suggest that it is occurring also in tetraploid *D. sylvestris*, as its monoploid RGS was significantly smaller compared to the diploids (Kruskal–Wallis test = 10.58, $p < 0.01$).

Given the morphological similarity of tetraploid populations to their diploid counterparts occurring in the same area, both being identified as *D. sylvestris* subsp. *sylvestris*, we suggest an autopolyploid origin of tetraploids, as it was also suggested for polyploids within *Dianthus* sect. *Plumaria* [11]. A tetraploid origin from the diploid *D. sylvestris* subsp. *sylvestris*, rather from *D. sylvestris* subsp. *tergestinus* that also occurs in the same area (Figure 2), is further supported by more similar monoploid RGS of the tetraploids with the former taxon. Alternatively, an allopolyploid origin involving putative diploid parents of northern i.e., alpine and eastern i.e., Balkan provenance is possible. Despite a clear identification of tetraploid populations as *D. sylvestris* subsp. *sylvestris*, we demonstrated that tetraploid individuals differ from diploids in stem and leaf size characteristics. This is in line with other studies, where it has been shown that polyploidization can substantially affect morphological variation and, although the relationship between ploidy and body/organ size is complex, polyploid plants are often larger than their diploid parents [81,82].

In addition to morphological divergence, ecological differentiation among different ploidies within the same species has also been evidenced [76,83,84]. Given that polyploids may be more competitive compared to diploids [85], they are expected to have higher dispersal potential and may thrive in different ecological niches. It has been suggested that diploids tend to be restricted to refugia, whereas polyploids show better ability to re-colonize deglaciated regions [86]. However, contradicting cytogeographic patterns were also observed [8,87], and in *D. sylvestris* the tetraploids were also found to be geographically and environmentally restricted compared to the diploids that thrive in a broad range of environments. Diploid and tetraploid populations of *D. sylvestris* subsp. *sylvestris* were environmentally segregated by different temperature and precipitation preferences, as well as different geomorphology of the terrain. Tetraploids were collected in warmer habitats with less fluctuation in temperature, and more precipitation during the driest month. Furthermore, they thrive on significantly less steep, westerly exposed slopes with higher clay content in the soil and in habitats with a lower number of frost and snow days per year and with higher surface solar radiation. Nevertheless, one mixed-ploidy population from north-west Croatia (Vodice-D19) provides evidence that tetraploids and their diploid progenitors can occupy similar environments [88] and further studies are needed to reveal whether the observed ecological divergence is adaptive or simply a result of much wider distribution of diploids inhabiting a broader array of environments. Interestingly, the area of Istria, where tetraploids occur, is known as an important Pleistocene refugium for plants [89,90]. It is likely that Pleistocene climatic oscillation also triggered polyploidization in *D. sylvestris*, given the Pleistocene radiation in Eurasian *Dianthus* [42].

### *3.2. Geographic and Intraspecific Variation of RGS within Diploid D. sylvestris s.l.*

Despite the fact that RGS estimations were performed with DAPI, which is an AT-content dependent, and cannot be, with 100% reliability, translated to absolute values and thus used in comparative studies [91], such a conversion based on the GS of our standard *Bellis perennis* (2C = 3.38 pg, [92]) revealed a variation of 1C in *D. sylvestris* s.l. ranging between 0.55 and 0.64 pg (mean: 0.58 pg, N = 152 populations). These values therefore correspond well to the estimated absolute GS of 1C = 0.61 pg [69]. *Dianthus sylvestris* is thus a small-genome species and it has been suggested that plants with small genomes display more pronounced morphological variation and thrive in wide environmental gradients, compared to large-genome taxa [37]. *Dianthus sylvestris* exhibits a high morphological variability and grows from the coastal Mediterranean environment to the alpine belt both in the Alps as well as the Balkan Peninsula [60] and hence supports the available evidence, but further studies across flowering plants are needed to bring more evidence for this hypothesis.

Our comprehensive sampling revealed an indicative spatial pattern of RGS variation within diploid populations of *D. sylvestris* (Figures 2 and 6B). Although the general pattern is complex and often populations with clearly different RGS occur in vicinity, larger RGS values predominate in the southern Balkan Peninsula and the central part of the Alps, i.e., at the south-eastern and northern margin of the species distribution. A clear geographic pattern in RGS variation with smaller monoploid RGS in the distribution center and its increase towards the distribution margins were also observed at the genus level in *Knautia* [15] and *Sesleria* [16]. Causes for such patterns remain unclear, but it has been suggested that larger GS can limit adaptive and competitive abilities of populations at the distribution margins and might thus represent a factor limiting further range expansion [37], however further studies are needed to rigorously test this hypothesis.

The southernmost populations with large RGS correspond to *D. sylvestris* subsp. *alboroseus*. Therefore, this subspecies also exhibited highest RGS among the Balkan taxa (Figure 6B). Towards the north-west, the RGS decreases in populations morphologically intermediate between *D. sylvestris* subsp. *alboroseus* and *D. sylvestris* subsp. *bertisceus* and thus not clearly classified to any subspecies and reaches the smallest values in *D. sylvestris* subsp. *bertisceus*. Further to the north-west, a slight, although statistically non-significant, increase in geographically adjacent *D. sylvestris* subsp. *nodosus* and *D. sylvestris* subsp. *sylvestris* can be observed. The latter two subspecies can hardly be distinguished morphologically, they have similar environmental niches [60] and their highly similar RGS renders their recognition as two subspecies questionable. Interestingly, these two subspecies with similar morphology and RGS appear to form a unique phylogenomic cluster based on preliminary analyses of the RADseq data (Temunović et al., unpublished), separated from more southern populations, which are genetically more diverse. More pronounced genetic differentiation of southern compared to northern populations has been observed in several plant groups (e.g., [10,74,93–95]) and is, in *D. sylvestris*, also reflected in more pronounced RGS variation in this geographic region. Along the same line, ecologically and morphologically distinct *D. sylvestris* subsp. *tergestinus* [60] growing along the Adriatic coast, had higher RGS compared to geographically partly sympatric *D. sylvestris* subsp. *nodosus* and *D. sylvestris* subsp. *sylvestris*. Preliminary analyses of the genomic RADseq data (Temunović et al., unpublished) suggests that *D. sylvestris* subsp. *tergestinus* forms an evolutionary lineage distinct from all other Balkan populations of *D. sylvestris*. Its divergent RGS, which is significantly different from *D. sylvestris* subsp. *sylvestris*, is thus likely a result of divergent evolutionary histories.

Also, in the Alps, there is a pronounced variation in RGS within *D. sylvestris*, with a clear trend in its geographical distribution (Figure 2). Whereas the majority of the analyzed samples scattered across the western parts of the Eastern Alps, and a few populations from the Western Alps, exhibit larger RGS ranging between 0.346 and 0.369, the easternmost alpine populations (most of them from the eastern part of the Southern Alps) have smaller RGS ranging between 0.326 and 0.339 that correspond to the RGS of the populations from the north-western Balkan Peninsula. Therefore, the most prominent RGS variation among all groups analyzed was within the alpine group (Figure 6A). This relatively abrupt change in RGS within the Alps could be a result of divergence due to persistence of the species in two (or more) separate glacial refugia. It is likely that the eastern populations from the Southern Alps shared their refugium with the northern Balkan populations of *D. sylvestris* subsp. *sylvestris* in the north-western Balkan Peninsula, whereas the other alpine populations with clearly higher RGS survived the glacial cycles in more western refugia; several isolated refugia along the southern margin of the Alps have also been suggested for other alpine plants by Schönswetter et al. [96]. The observed RGS divergence in *D. sylvestris* within the Alps corresponds to a genetic discontinuity (Luqman et al., unpublished; Temunović et al., unpublished), but the exact border between the two lineages and their relation to the GS remains to be determined. In South Tyrol and adjacent Veneto (Italy) as well as East Tyrol (Austria), populations with relatively high RGS grow in close vicinity with the populations with lower RGS, suggesting that there might be a hybrid zone between the alpine and the Balkan lineage.

Multiple studies have suggested that environmental conditions may place constraints on the evolution of GS [34,37], hence genome size can either be directly associated with temperature and precipitation [97] or indirectly associated through elevation [34] or latitude [17,37,98]. However, the lack of association of RGS with environmental variables in our study on one hand, and a clear geographic trend in the RGS variation in *D. sylvestris* on the other hand, suggest that it was likely the phylogeographic rather than the environmental divergence that shaped the RGS variation in our study species.

## 4. Materials and Methods

### 4.1. Plant Material

We sampled 134 populations of *D. sylvestris* s.l. throughout the Balkan Peninsula and 28 populations from the European Alps between 2018 and 2021. At each locality, basal leaves from 3 to 12 individual plants were desiccated in silica gel for RGS measurements, and an herbarium specimen was collected. For chromosome number estimations, we collected mature seeds from several localities in July and August of 2020 and 2021. The seeds were air-dried and dry-stored in darkness at room temperature. Vouchers are deposited in the herbarium ZA (Table S1). We identified the plants and assigned them to subspecies based on identification keys in national and regional floras [44,99–105], and treated the populations that we could not assign to any of the known subspecies based on their morphology as *D. sylvestris* s.l.

### 4.2. Chromosome Counts

We determined chromosome numbers for five populations (Table S1). The seeds were germinated at the surface of a peat medium in plastic pots with regular watering at room temperature. Root tips were harvested at about noon and pre-treated with 0.002 M 8-hydroxyquinoline for 4 h in darkness at 4 °C. Subsequently, material was fixed in 3:1 ethanol–glacial acetic acid for 12–24 h at 4 °C. The fixed root tips were hydrolyzed in 5 M HCl at room temperature for 45 min, and then washed in distilled water. The root tips were stained in Schiff's reagent for 2 h. Finally, we squashed the stained root tips on a slide glass in a drop of 45% (*v*/*v*) acetic acid. Photomicrographs of chromosomes at mitotic metaphase were taken with Zeiss Lab. A1 AXIO microscope (Carl Zeiss Microscopy, Jena, Germany) equipped with ToupCam 5.1 MP digital camera. Snapshots were exported and studied using ImageJ software.

### 4.3. Flow Cytometry

Silica-gel-dried leaves were analyzed using flow cytometry (FCM) of 4′,6-diamidino-2-phenylindole (DAPI; final concentration 0.036 M) stained nuclei [106] to estimate RGS and DNA ploidy levels of sampled populations. We used *Bellis perennis* as the primary internal standard [92]. Desiccated green leaf tissue (c. 0.5 cm$^2$) of one to two plant individuals from the same population was chopped together using a sharp razor blade in a plastic Petri dish, with an appropriate amount of fresh reference standard and processed as described in Suda et al. [20]. The relative fluorescence intensity of 3000 nuclei was recorded using a Partec CyFlow Space flow cytometer (Sysmex Partec, Münster, Germany). We used Partec FloMax software to evaluate histograms and to calculate coefficients of variation (CV) of the standard and sample peaks. We calculated RGS as the ratio between the mean relative fluorescence of sample and standard. Samples with CV of the G1 peak >10% were re-analyzed until sufficient quality was achieved [21]. The number of measured individuals per population yielding high quality FCM histograms is given in Table S1.

### *4.4. Statistical Analyses*

We calculated mean RGS value and standard deviation for each population from individual measurements of at least three individuals and we inferred the DNA ploidy levels [107] for all analyzed populations. We performed the Kruskal–Wallis test and Tukey post-hoc tests on population means, to evaluate statistically significant differences. The difference in monoploid RGS between diploids and tetraploids was tested for significance on a subset of 25 populations of *D. sylvestris* subsp. *sylvestris*, whereas the difference in RGS among the subspecies was tested for all diploid populations (N = 152). Due to the very small sample size (only one population known from the locus classicus), *D. sylvestris* subsp. *kozjakensis* was not included in these tests. All statistical analyses were performed using R-4.0.2 [108].

We performed principal component analysis (PCA) and discriminant analysis (DA) to explore the variability and the relative importance of characteristics/variables discriminating between the diploid and the tetraploid populations of *D. sylvestris* subsp. *sylvestris*. Both PCA and DA were performed for the morphological and the environmental dataset. The morphometric PCA and DA were performed using five morphological characteristics showing significant difference between the two ploidy levels (Table S2), whereas the environmental PCA and DA were based on seven environmental variables showing significant difference between the ploidy levels (Table S3) and without collinearity issues.

We used morphological data of the populations from the Balkan Peninsula from Terlević et al. ([60], Table S1) and tested the differences between nine diploid and seven tetraploid populations of *D. sylvestris* subsp. *sylvestris* (N = 16). We downloaded environmental data from three databases: climate data from the Chelsa database [109,110], soil properties from the SoilGrids [111], and topographic variables from the EarthEnv database [112]. The environmental space of each studied population was depicted by extracting environmental data from the points defined by longitude (N) and latitude (E; Table S1), and the differences were tested between 15 diploid and 10 tetraploid populations of *D. sylvestris* subsp. *sylvestris* (N = 25). The association between DNA-ploidy levels and environmental variables was also visualized with the package 'ggplot2'. Environmental variables were standardized to meet the assumption of homogeneity of variance and linearity. The correlation between environmental and RGS variability of 152 diploid populations in the studied area was tested employing Pearson correlation coefficients.

## 5. Conclusions

By analyzing the RGS variation of *Dianthus sylvestris* in the Balkan Peninsula and to a lesser extent in the Alps, our results reveal complex patterns of RGS in widespread diploid populations and spatially restricted tetraploid populations in the north-western Balkan Peninsula. The populations in the central and western parts of the Alps, as well as those at the southern distribution limit in the Balkan Peninsula, exhibit higher RGS, likely corresponding to discrete evolutionary lineages. In addition, two areas of more pronounced RGS variation at small geographic distances are revealed. One is located in the eastern Alps and the other in southern Dinaric Mountains. We suggest that the observed RGS differences are a result of evolutionary divergence due to persistence in separate glacial refugia. However, only upcoming integration of phylogenomic data will show the correlation of observed morphological (taxonomic) and GS variation with evolutionary differentiation.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11111481/s1. **Table S1.** Relative genome size (RGS) and ploidy level (2*x*, DNA-diploids; 4*x*, DNA-tetraploids) of 162 populations of *Dianthus sylvestris* from the Balkan Peninsula and the Alps, including their provenance and voucher data. For each population, DNA ploidy estimates, mean RGS of a holoploid genome and standard deviation are given. Number of individuals analyzed for RGS in each population (N) and the range of CV values are also provided. Ploidy: 2*x* and 4*x*. Asterisk (*) indicates the populations for which confirmatory chromosome counts were performed. Plus (+) in the column Morphometrics indicates the morphometrically analyzed populations in the previous study by Terlević et al. (submitted) [60]. **Figure S1.** Relative genome size (RGS) in diploid populations of *Dianthus sylvestris* sorted by increasing RGS values. Population means (dots) with corresponding standard deviation (vertical lines) are presented. Population names correspond to Table S1. Colors correspond to subspecies, as in Figure 2. **Table S2.** Results of Kruskal–Wallis test between diploid (2*x*, N = 9) and tetraploid (4*x*, N = 7) populations of *Dianthus sylvestris* subsp. *sylvestris*. Morphological characteristics with significant differences between ploidy levels at $p < 0.05$ are shown in bold. **Table S3.** Results of Kruskal–Wallis test between diploid (2*x*, N = 15) and tetraploid (4*x*, N = 10) populations of *Dianthus sylvestris* subsp. *sylvestris*. Environmental variables with significant differences between ploidy levels at $p < 0.05$ are shown in bold. **Figure S2.** Boxplots showing environmental differences along the 14 environmental variables between diploid (2*x*) and tetraploid (4*x*) populations of *Dianthus sylvestris* subsp. *sylvestris*. Only variables with significant differences ($p < 0.05$), as revealed by Kruskal–Wallis test (Table S3), are shown.

**Author Contributions:** I.R. and A.T. conceived and designed the study; I.R., S.B., A.T. and B.F. collected plant material; A.T., I.R. and S.B. performed RGS measurements; A.T. and S.B. performed chromosome counting; A.T. analyzed the data; and A.T., B.F. and I.R. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** The research was funded by the Croatian Science Foundation under the project name "Phylogeography and evolution of three ecologically divergent amphi-Adriatic plant groups" (project No. UIP-2017-05-2882) to I.R., and by the Austrian Agency for International Cooperation and the Croatian Ministry of Science and Education—Austria–Croatia bilateral project "Genome size evolution and polyploidization in amphi-Adriatic *Aurinia* (Brassicaceae) and the *Cerastium tomentosum* and *Dianthus sylvestris* species groups (Caryophyllaceae)" (HR17/2020) to B.F. and S.B. This manuscript is part of A.T.'s Ph.D. thesis, and her work has been fully supported by the "Young researchers' career development project—training of doctoral students" (ESF-DOK-01-2018) of the Croatian Science Foundation funded by the European Union from the European Social Fund.

**Data Availability Statement:** Data are contained within the article or the Supplementary Materials.

**Acknowledgments:** We thank all collectors listed in the Table S1 who helped with sampling and S. Fior and H. Luqman for providing some samples from the Alps and for sharing their preliminary genomic data with us. We are grateful to D. Pirkebner and M. Magauer for their assistance in the laboratory and to D. Volgger, M. Doboš, and M. Temunović, for measuring RGS of some populations. Collecting permits were issued for the territory of Croatia by the Ministry of Environment and Nature Protection of Croatia.

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

## References

1. Greilhuber, J.; Doležel, J.; Lysák, M.A.; Bennett, M.D. The Origin, Evolution and Proposed Stabilization of the Terms 'Genome Size' and 'C-Value' to Describe Nuclear DNA Contents. *Ann. Bot.* **2005**, *95*, 255–260. [CrossRef]
2. Doležel, J.; Bartoš, J. Plant DNA Flow Cytometry and Estimation of Nuclear Genome Size. *Ann. Bot.* **2005**, *95*, 99–110. [CrossRef] [PubMed]
3. Suda, J.; Krahulcová, A.; Trávníček, P.; Rosenbaumová, R.; Peckert, T.; Krahulec, F. Genome Size Variation and Species Relationships in *Hieracium* Sub-Genus *Pilosella* (Asteraceae) as Inferred by Flow Cytometry. *Ann. Bot.* **2007**, *100*, 1323–1335. [CrossRef] [PubMed]
4. Šmarda, P.; Bureš, P.; Horová, L.; Rotreklová, O. Intrapopulation Genome Size Dynamics in *Festuca pallens*. *Ann. Bot.* **2008**, *102*, 599–607. [CrossRef] [PubMed]
5. Slovák, M.; Vít, P.; Urfus, T.; Suda, J. Complex Pattern of Genome Size Variation in a Polymorphic Member of the Asteraceae. *J. Biogeogr.* **2009**, *36*, 372–384. [CrossRef]
6. Ladner, J.; Mayfield, M.H.; Prather, L.A.; Ferguson, C.J. Polyploidy and Genome Size Variation in *Phlox nana* (Polemoniaceae) from the Pecos Plains of New Mexico and the Davis Mountains of West Texas, USA. *J. Bot. Res. Inst. Tex.* **2017**, *11*, 351–362. [CrossRef]
7. Pellicer, J.; Hidalgo, O.; Dodsworth, S.; Leitch, I. Genome Size Diversity and Its Impact on the Evolution of Land Plants. *Genes* **2018**, *9*, 88. [CrossRef]

8. Nunvářová Kabátová, K.; Kolář, F.; Jarolímová, V.; Krak, K.; Chrtek, J. Does Geography, Evolutionary History or Ecology Drive Ploidy and Genome Size Variation in the *Minuartia verna* Group (Caryophyllaceae) across Europe? *Plant Syst. Evol.* **2019**, *305*, 1019–1040. [CrossRef]
9. Hodálová, I.; Mártonfiová, L.; Skokanová, K.; Majerová, M.; Somlyay, L.; Mereďa, P. The Utility of Genome Size in Plant Identification: A Case Study on *Sesleria* (Poaceae) from Croatia and Slovenia. *Plant Syst. Evol.* **2020**, *306*, 87. [CrossRef]
10. Caković, D.; Cresti, L.; Stešević, D.; Schönswetter, P.; Frajman, B. High Genetic and Morphological Diversification of the *Euphorbia verrucosa* Alliance (Euphorbiaceae) in the Balkan and Iberian Peninsulas. *Taxon* **2021**, *70*, 286–307. [CrossRef]
11. Weiss, H.; Dobeš, C.; Schneeweiss, G.M.; Greimler, J. Occurrence of Tetraploid and Hexaploid Cytotypes between and within Populations in *Dianthus* Sect. *Plumaria* (Caryophyllaceae). *New Phytol.* **2002**, *156*, 85–94. [CrossRef]
12. Kolár, F.; Stech, M.; Trávnícek, P.; Rauchová, J.; Urfus, T.; Vít, P.; Kubesová, M.; Suda, J. Towards Resolving the *Knautia arvensis* Agg. (Dipsacaceae) Puzzle: Primary and Secondary Contact Zones and Ploidy Segregation at Landscape and Microgeographic Scales. *Ann. Bot.* **2009**, *103*, 963–974. [CrossRef] [PubMed]
13. Niketić, M.; Siljak-Yakovlev, S.; Frajman, B.; Lazarević, M.; Stevanović, B.; Tomović, G.; Stevanović, V. Towards Resolving the Systematics of *Cerastium* Subsection *Cerastium* (Caryophyllaceae): A Cytogenetic Approach. *Bot. J. Linn. Soc.* **2013**, *172*, 205–224. [CrossRef]
14. Frajman, B.; Schönswetter, P.; Weiss-Schneeweiss, H.; Oxelman, B. Origin and Diversification of South American Polyploid *Silene* Sect. *Physolychnis* (Caryophyllaceae) in the Andes and Patagonia. *Front. Genet.* **2018**, *9*, 639. [CrossRef]
15. Frajman, B.; Rešetnik, I.; Weiss-Schneeweiss, H.; Ehrendorfer, F.; Schönswetter, P. Cytotype Diversity and Genome Size Variation in *Knautia* (Caprifoliaceae, Dipsacoideae). *BMC Evol. Biol.* **2015**, *15*, 140. [CrossRef] [PubMed]
16. Lazarević, M.; Kuzmanović, N.; Lakušić, D.; Alegro, A.; Schönswetter, P.; Frajman, B. Patterns of Cytotype Distribution and Genome Size Variation in the Genus *Sesleria* Scop. (Poaceae). *Bot. J. Linn. Soc.* **2015**, *179*, 126–143. [CrossRef]
17. Souza, G.; Costa, L.; Guignard, M.S.; Van-Lume, B.; Pellicer, J.; Gagnon, E.; Leitch, I.J.; Lewis, G.P. Do Tropical Plants Have Smaller Genomes? Correlation between Genome Size and Climatic Variables in the *Caesalpinia* Group (Caesalpinioideae, Leguminosae). *Perspect. Plant Ecol.* **2019**, *38*, 13–23. [CrossRef]
18. Trávníček, P.; Čertner, M.; Ponert, J.; Chumová, Z.; Jersáková, J.; Suda, J. Diversity in Genome Size and GC Content Shows Adaptive Potential in Orchids and is Closely Linked to Partial Endoreplication, Plant Life-History Traits and Climatic Conditions. *New Phytol.* **2019**, *224*, 1642–1656. [CrossRef]
19. Suda, J.; Trávníček, P. Estimation of Relative Nuclear DNA Content in Dehydrated Plant Tissues by Flow Cytometry. *Curr. Protoc. Cytom.* **2006**, *38*, 7.30.1–7.30.14. [CrossRef]
20. Suda, J.; Kron, P.; Husband, B.C.; Trávníček, P. Flow Cytometry and Ploidy: Applications in Plant Systematics, Ecology and Evolutionary Biology. In *Flow Cytometry with Plant Cells*; Doležel, J., Greilhuber, J., Suda, J., Eds.; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2007; pp. 103–130. ISBN 978-3-527-61092-1.
21. Greilhuber, J.; Temsch, E.M.; Loureiro, J.C.M. Nuclear DNA Content Measurement. In *Flow Cytometry with Plant Cells*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2007; pp. 67–101. ISBN 978-3-527-61092-1.
22. Piegu, B.; Guyot, R.; Picault, N.; Roulin, A.; Saniyal, A.; Kim, H.; Collura, K.; Brar, D.S.; Jackson, S.; Wing, R.A.; et al. Doubling Genome Size without Polyploidization: Dynamics of Retrotransposition-Driven Genomic Expansions in *Oryza australiensis*, a Wild Relative of Rice. *Genome Res.* **2006**, *16*, 1262–1269. [CrossRef]
23. Šmarda, P.; Bureš, P. Intraspecific DNA Content Variability in *Festuca pallens* on Different Geographical Scales and Ploidy Levels. *Ann. Bot.* **2006**, *98*, 665–678. [CrossRef]
24. Šmarda, P.; Bureš, P. Understanding Intraspecific Variation in Genome Size in Plants. *Preslia* **2010**, *82*, 41–61.
25. Sonnleitner, M.; Hülber, K.; Flatscher, R.; García, P.E.; Winkler, M.; Suda, J.; Schönswetter, P.; Schneeweiss, G.M. Ecological Differentiation of Diploid and Polyploid Cytotypes of *Senecio carniolicus* Sensu Lato (Asteraceae) is Stronger in Areas of Sympatry. *Ann. Bot.* **2016**, *117*, 269–276. [CrossRef] [PubMed]
26. Janišová, M.; Skokanová, K.; Hlásny, T. Ecological Differentiation, Speciation, and Rarity: How Do They Match in *Tephroseris longifolia* Agg. (Asteraceae)? *Ecol. Evol.* **2018**, *8*, 2453–2470. [CrossRef] [PubMed]
27. Doležel, J.; Greilhuber, J.; Lucretti, S.; Meister, A.; Lysák, M.A.; Nardi, L.; Obermayer, R. Plant Genome Size Estimation by Flow Cytometry: Inter-Laboratory Comparison. *Ann. Bot.* **1998**, *82*, 17–26. [CrossRef]
28. Murray, B.G. When Does Intraspecific C-Value Variation Become Taxonomically Significant? *Ann. Bot.* **2005**, *95*, 119–125. [CrossRef] [PubMed]
29. Kumar, A.; Bennetzen, J.L. Plant Retrotransposons. *Annu. Rev. Genet.* **1999**, *33*, 479–532. [CrossRef]
30. Bureš, P.; Wang, Y.-F.; Horova, L.; Suda, J. Genome Size Variation in Central European Species of *Cirsium* (Compositae) and Their Natural Hybrids. *Ann. Bot.* **2004**, *94*, 353–363. [CrossRef]
31. Bennett, M.D.; Leitch, I.J. Genome Size Evolution in Plants. In *The Evolution of the Genome*; Gregory, T.R., Ed.; Academic Press: Burlington, MA, USA, 2005; pp. 89–162. ISBN 978-0-12-301463-4.
32. Vidic, T.; Greilhuber, J.; Vilhar, B.; Dermastia, M. Selective Significance of Genome Size in a Plant Community with Heavy Metal Pollution. *Ecol. Appl.* **2009**, *19*, 1515–1521. [CrossRef]
33. Kang, M.; Wang, J.; Huang, H. Nitrogen Limitation as a Driver of Genome Size Evolution in a Group of Karst Plants. *Sci. Rep.* **2015**, *5*, 11636. [CrossRef]

34. Bilinski, P.; Albert, P.S.; Berg, J.J.; Birchler, J.A.; Grote, M.N.; Lorant, A.; Quezada, J.; Swarts, K.; Yang, J.; Ross-Ibarra, J. Parallel Altitudinal Clines Reveal Trends in Adaptive Evolution of Genome Size in *Zea mays*. *PLoS Genet.* **2018**, *14*, e1007162. [CrossRef] [PubMed]
35. Cacho, N.I.; McIntyre, P.J.; Kliebenstein, D.J.; Strauss, S.Y. Genome Size Evolution Is Associated with Climate Seasonality and Glucosinolates, but Not Life History, Soil Nutrients or Range Size, across a Clade of Mustards. *Ann. Bot.* **2021**, *127*, 887–902. [CrossRef] [PubMed]
36. Bottini, M.C.J.; Greizerstein, E.J.; Aulicino, M.B.; Poggio, L. Relationships among Genome Size, Environmental Conditions and Geographical Distribution in Natural Populations of NW Patagonian Species of *Berberis* L. (Berberidaceae). *Ann. Bot.* **2000**, *86*, 565–573. [CrossRef]
37. Knight, C.A.; Molinari, N.A.; Petrov, D.A. The Large Genome Constraint Hypothesis: Evolution, Ecology and Phenotype. *Ann. Bot.* **2005**, *95*, 177–190. [CrossRef]
38. Grime, J.P.; Shacklock, J.M.L.; Brand, S.R. Nuclear DNA Contents, Shoot Phenology and Species Co-Existence in a Limestone Grassland Community. *New Phytol.* **1985**, *100*, 435–445. [CrossRef]
39. Leitch, I.J.; Bennett, M.D. Genome Size and Its Uses: The Impact of Flow Cytometry. In *Flow Cytometry with Plant Cells*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2007; pp. 153–176. ISBN 978-3-527-61092-1.
40. Kraaijeveld, K. Genome Size and Species Diversification. *Evol. Biol.* **2010**, *37*, 227–233. [CrossRef] [PubMed]
41. Bittrich, V. Caryophyllaceae. In *Flowering Plants, Dicotyledons: Magnoliid, Hamamelid and Caryophyllid Families*; Kubitzki, K., Rohwer, J.G., Eds.; Springer: Berlin/Heidelberg, Germany; London, UK, 1993; Volume 2, pp. 206–236. ISBN 978-3-642-08141-5.
42. Valente, L.M.; Savolainen, V.; Vargas, P. Unparalleled Rates of Species Diversification in Europe. *Proc. R. Soc. B Biol. Sci.* **2010**, *277*, 1489–1496. [CrossRef] [PubMed]
43. Bernal, M.; Laínz, M.; Munoz Garmendia, F. *Dianthus* L. In *Flora Iberica*; Bolibar, S.C., Ed.; Real Jardín Botánico, C.S.I.C.: Madrid, Spain, 1990; Volume 2, pp. 426–462.
44. Tutin, T.G.; Walters, S.M. Dianthus L. In *Flora Europaea*; Tutin, T.G., Ed.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 1993; Volume 1, pp. 227–246. ISBN 978-0-521-41007-6.
45. Bacchetta, G.; Brullo, S.; Casti, M.; del Galdo, G.P.G. Taxonomic Revision of the *Dianthus sylvestris* Group (Caryophyllaceae) in Central-Southern Italy, Sicily and Sardinia. *Nord. J. Bot.* **2010**, *28*, 137–173. [CrossRef]
46. Domina, G.; Scibetta, S.; Scafidi, F.; Giovino, A. Contribution to the Identification of *Dianthus rupicola* (Caryophyllaceae) Subspecies Using Morphological and Molecular Approaches. *Phytotaxa* **2017**, *291*, 17–32. [CrossRef]
47. Brullo, S.; Guarino, R. Dianthus. In *Flora d'Italia*; Pignatti, S., Ed.; Edagricole: Milano, Italy, 2019; Volume 4, ISBN 978-88-506-5245-7.
48. Hardion, L.; Perrier, A.; Martinez, M.; Navrot, N.; Gaquerel, E.; Tournay, F.; Nguefack, J.; Combroux, I. Integrative Revision of *Dianthus superbus* Subspecies Reveals Different Degrees of Differentiation, from Plasticity to Species Distinction. *Syst. Biodivers.* **2020**, *18*, 255–268. [CrossRef]
49. Domina, G.; Astuti, G.; Bacchetta, G.; Barone, G.; Rešetnik, I.; Terlević, A.; Thiébaut, M.; Peruzzi, L. Typification of 14 Names in the *Dianthus virgineus* Group (Caryophyllaceae). *PhytoKeys* **2021**, *187*, 1–14. [CrossRef] [PubMed]
50. Domina, G.; Astuti, G.; Barone, G.; Gargano, D.; Minuto, L.; Varaldo, L.; Peruzzi, L. Lectotypification of the Linnaean Name *Dianthus virgineus* (Caryophyllaceae) and Its Taxonomic Consequences. *Taxon* **2021**, *70*, 1096–1100. [CrossRef]
51. Gammella, M. Local Adaptation and Gene Flow in Serpentine and Limestone Populations of *D. sylvestris*. Doctoral dissertation, Università Degli Studi di Napoli Federico II, Naples, Italy, 2016.
52. Beck-Mannagetta, G. *Flora Bosne, Hercegovine i Novopazarskog Sandžaka*; Glasnik Zemaljskog muzeja u Bosni i Hercegovini: Sarajevo, Bosnia and Herzegovina, 1909; Volume 21.
53. Von Hayek, A. *Prodromus Florae Peninsulae Balcanicae*; Verlag des Repertoriums: Dahlem bei Berlin, Germany, 1924.
54. Gjurašin, S. *Rod Dianthus u Flori Hrvatske i Slavonije*, 18th ed.; Prirodoslovna istraživanja Kraljevine Jugoslavije; Jugoslavenska Akademija Znanosti i Umjetnosti: Zagreb, Croatia, 1933.
55. Mayer, E.; Trpin, D. *Dianthus Sylvestris*—Kompleks v Jugoslaviji. *Biološki Vestnik* **1965**, *13*, 53–59.
56. Trinajstić, I. *Analitička Flora Jugoslavije*; Institut za botaniku Sveučilišta u Zagrebu: Zagreb, Croatia, 1979.
57. Greuter, W.; Burdet, H.M.; Long, G. *Med-Checklist. 1*; des Conservatoire et Jardin Botaniques de la Ville de Genève: Geneva, Switzerland, 1984; ISBN 978-2-8277-0151-3.
58. Marhold, K. Caryophyllaceae. Euro+Med Plantbase—The Information Resource for Euro-Mediterranean Plant Diversity. Available online: https://www.emplantbase.org/home.html (accessed on 20 December 2020).
59. Meyer, F.K. *Beiträge zur Flora von Albanien*; Thüringische Botanische Gesellschaft e.V.: Oberhof, Germany, 2011.
60. Terlević, A.; Temunović, M.; Bogdanović, S.; Grgurev, M.; Ljubičić, I.; Rešetnik, I. Morphological and Ecological Variability of *Dianthus sylvestris* Wulfen (Caryophyllaceae) on the Balkan Peninsula. *Bot. J. Linn. Soc.* 2022, *submitted*.
61. Carolin, R.C. Cytological and Hybridization Studies in the Genus *Dianthus*. *New Phytol.* **1957**, *56*, 81–97. [CrossRef]
62. Moore, D.M. *Flora Europaea Check-List and Chromosome Index*; Cambridge University Press: Cambridge UK; New York, NY, USA, 1982; ISBN 978-0-521-23759-8.
63. Rice, A.; Glick, L.; Abadi, S.; Einhorn, M.; Kopelman, N.M.; Salman-Minkov, A.; Mayzel, J.; Chay, O.; Mayrose, I. The Chromosome Counts Database (CCDB)—A Community Resource of Plant Chromosome Numbers. *New Phytol.* **2015**, *206*, 19–26. [CrossRef] [PubMed]

64. Pellicer, J.; Leitch, I.J. The Plant DNA C-Values Database (Release 7.1): An Updated Online Repository of Plant Genome Size Data for Comparative Studies. *New Phytol.* **2020**, *226*, 301–305. [CrossRef]
65. Balao, F.; Casimiro-Soriguer, R.; Talavera, M.; Herrera, J.; Talavera, S. Distribution and Diversity of Cytotypes in *Dianthus broteri* as Evidenced by Genome Size Variations. *Ann. Bot.* **2009**, *104*, 965–973. [CrossRef]
66. Behroozian, M.; Vaezi, J.; Joharchi, M.R. A Karyological Study of Some *Dianthus* L. Species (Caryophyllaceae) in Northeast of Iran. *Feddes Repert.* **2012**, *123*, 265–272. [CrossRef]
67. Şahin, E.; Eroğlu, H.E.; Hamzaoğlu, E.; Koç, M. Karyotype Analysis of Four Species of *Dianthus* Section *Fimbriati* (Caryophyllaceae, Sileneae). *Caryologia* **2016**, *69*, 267–272. [CrossRef]
68. Gatt, M.K.; Hammett, K.R.W.; Markham, K.R.; Murray, B.G. Yellow Pinks: Interspecific Hybridization between *Dianthus plumarius* and Related Species with Yellow Flowers. *Sci. Hortic.* **1998**, *77*, 207–218. [CrossRef]
69. Siljak-Yakovlev, S.; Pustahija, F.; Šolić, E.M.; Bogunić, F.; Muratović, E.; Bašić, N.; Catrice, O.; Brown, S.C. Towards a Genome Size and Chromosome Number Database of Balkan Flora: C-Values in 343 Taxa with Novel Values for 242. *Adv. Sci. Lett.* **2010**, *3*, 190–213. [CrossRef]
70. Löve, Á. IOPB Chromosome Number Reports. XVIII. *Taxon* **1968**, *17*, 419–422. [CrossRef]
71. Doležel, J.; Greilhuber, J.; Suda, J. Estimation of Nuclear DNA Content in Plants Using Flow Cytometry. *Nat. Protoc.* **2007**, *2*, 2233–2244. [CrossRef] [PubMed]
72. Barow, M.; Jovtchev, G. Endopolyploidy in Plants and Its Analysis by Flow Cytometry. In *Flow Cytometry with Plant Cells*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2007; pp. 349–372. ISBN 978-3-527-61092-1.
73. Záveská, E.; Maylandt, C.; Paun, O.; Bertel, C.; Frajman, B.; The STEPPE Consortium; Schönswetter, P. Multiple Auto- and Allopolyploidisations Marked the Pleistocene History of the Widespread Eurasian Steppe Plant *Astragalus onobrychis* (Fabaceae). *Mol. Phylogenet. Evol.* **2019**, *139*, 106572. [CrossRef] [PubMed]
74. Niketić, M.; Đurović, S.Z.; Tomović, G.; Schönswetter, P.; Frajman, B. Diversification within Ploidy-Variable Balkan Endemic *Cerastium decalvans* (Caryophyllaceae) Reconstructed Based on Genetic, Morphological and Ecological Evidence. *Bot. J. Linn. Soc.* **2021**, *199*, 578–608. [CrossRef]
75. Balao, F.; Valente, L.M.; Vargas, P.; Herrera, J.; Talavera, S. Radiative Evolution of Polyploid Races of the Iberian Carnation *Dianthus broteri* (Caryophyllaceae). *New Phytol.* **2010**, *187*, 542–551. [CrossRef]
76. López-Jurado, J.; Mateos-Naranjo, E.; Balao, F. Niche Divergence and Limits to Expansion in the High Polyploid *Dianthus broteri* Complex. *New Phytol.* **2019**, *222*, 1076–1087. [CrossRef]
77. Soltis, P.S.; Marchant, D.B.; Van de Peer, Y.; Soltis, D.E. Polyploidy and Genome Evolution in Plants. *Curr. Opin. Genet. Dev.* **2015**, *35*, 119–125. [CrossRef]
78. Soltis, D.E.; Visger, C.J.; Marchant, D.B.; Soltis, P.S. Polyploidy: Pitfalls and Paths to a Paradigm. *Am. J. Bot.* **2016**, *103*, 1146–1166. [CrossRef]
79. Leitch, I.J.; Bennett, M.D. Genome Downsizing in Polyploid Plants. *Biol. J. Linn. Soc.* **2004**, *82*, 651–663. [CrossRef]
80. Weiss-Schneeweiss, H.; Greilhuber, J.; Schneeweiss, G.M. Genome Size Evolution in Holoparasitic *Orobanche* (Orobanchaceae) and Related Genera. *Am. J. Bot.* **2006**, *93*, 148–156. [CrossRef]
81. Tsukaya, H. Controlling Size in Multicellular Organs: Focus on the Leaf. *PLoS Biol.* **2008**, *6*, e174. [CrossRef] [PubMed]
82. Flatscher, R.; García, P.E.; Hülber, K.; Sonnleitner, M.; Winkler, M.; Saukel, J.; Schneeweiss, G.M.; Schönswetter, P. Underestimated Diversity in One of the World's Best Studied Mountain Ranges: The Polyploid Complex of *Senecio carniolicus* (Asteraceae) Contains Four Species in the European Alps. *Phytotaxa* **2015**, *213*, 1–21. [CrossRef] [PubMed]
83. Sonnleitner, M.; Flatscher, R.; García, P.E.; Rauchová, J.; Suda, J.; Schneeweiss, G.M.; Hülber, K.; Schönswetter, P. Distribution and Habitat Segregation on Different Spatial Scales among Diploid, Tetraploid and Hexaploid Cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps. *Ann. Bot.* **2010**, *106*, 967–977. [CrossRef]
84. Duchoslav, M.; Jandová, M.; Kobrlová, L.; Šafářová, L.; Brus, J.; Vojtěchová, K. Intricate Distribution Patterns of Six Cytotypes of *Allium oleraceum* at a Continental Scale: Niche Expansion and Innovation Followed by Niche Contraction With Increasing Ploidy Level. *Front. Plant Sci.* **2020**, *11*, 1885. [CrossRef]
85. Weiss-Schneeweiss, H.; Emadzade, K.; Jang, T.-S.; Schneeweiss, G.M. Evolutionary Consequences, Constraints and Potential of Polyploidy in Plants. *Cytogenet. Genome Res.* **2013**, *140*, 137–150. [CrossRef]
86. Parisod, C.; Besnard, G. Glacial in Situ Survival in the Western Alps and Polytopic Autopolyploidy in *Biscutella laevigata* L. (Brassicaceae). *Mol. Ecol.* **2007**, *16*, 2755–2767. [CrossRef]
87. Kolář, F.; Lučanová, M.; Vít, P.; Urfus, T.; Chrtek, J.; Fér, T.; Ehrendorfer, F.; Suda, J. Diversity and Endemism in Deglaciated Areas: Ploidy, Relative Genome Size and Niche Differentiation in the *Galium pusillum* Complex (Rubiaceae) in Northern and Central Europe. *Ann. Bot.* **2013**, *111*, 1095–1108. [CrossRef]
88. Castro, M.; Loureiro, J.; Serrano, M.; Tavares, D.; Husband, B.C.; Siopa, C.; Castro, S. Mosaic Distribution of Cytotypes in a Mixed-Ploidy Plant Species, *Jasione montana*: Nested Environmental Niches but Low Geographical Overlap. *Bot. J. Linn. Soc.* **2019**, *190*, 51–66. [CrossRef]
89. Magri, D.; Vendramin, G.G.; Comps, B.; Dupanloup, I.; Geburek, T.; Gömöry, D.; Latałowa, M.; Litt, T.; Paule, L.; Roure, J.M.; et al. A New Scenario for the Quaternary History of European Beech Populations: Palaeobotanical Evidence and Genetic Consequences. *New Phytol.* **2006**, *171*, 199–221. [CrossRef]

90. Médail, F.; Diadema, K. Glacial Refugia Influence Plant Diversity Patterns in the Mediterranean Basin. *J. Biogeogr.* **2009**, *36*, 1333–1345. [CrossRef]
91. Doležel, J.; Sgorbati, S.; Lucretti, S. Comparison of Three DNA Fluorochromes for Flow Cytometric Estimation of Nuclear DNA Content in Plants. *Physiol. Plant.* **1992**, *85*, 625–631. [CrossRef]
92. Schönswetter, P.; Lachmayer, M.; Lettner, C.; Prehsler, D.; Rechnitzer, S.; Reich, D.S.; Sonnleitner, M.; Wagner, I.; Hülber, K.; Schneeweiss, G.M.; et al. Sympatric Diploid and Hexaploid Cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps Are Separated along an Altitudinal Gradient. *J. Plant Res.* **2007**, *120*, 721–725. [CrossRef] [PubMed]
93. Caković, D.; Stešević, D.; Schönswetter, P.; Frajman, B. How Many Taxa? Spatiotemporal Evolution and Taxonomy of *Amphoricarpos* (Asteraceae, Carduoideae) on the Balkan Peninsula. *Org. Divers. Evol.* **2015**, *15*, 429–445. [CrossRef]
94. Falch, M.; Schönswetter, P.; Frajman, B. Both Vicariance and Dispersal Have Shaped the Genetic Structure of Eastern Mediterranean *Euphorbia myrsinites* (Euphorbiaceae). *Perspect. Plant Ecol.* **2019**, *39*, 125459. [CrossRef]
95. Đurović, S.; Schönswetter, P.; Niketić, M.; Tomović, G.; Frajman, B. Disentangling Relationships among the Members of the *Silene saxifraga* Alliance (Caryophyllaceae): Phylogenetic Structure Is Geographically Rather than Taxonomically Segregated. *Taxon* **2017**, *66*, 343–364. [CrossRef]
96. Schönswetter, P.; Stehlik, I.; Holderegger, R.; Tribsch, A. Molecular Evidence for Glacial Refugia of Mountain Plants in the European Alps. *Mol. Ecol.* **2005**, *14*, 3547–3555. [CrossRef]
97. Qiu, F.; Baack, E.J.; Whitney, K.D.; Bock, D.G.; Tetreault, H.M.; Rieseberg, L.H.; Ungerer, M.C. Phylogenetic Trends and Environmental Correlates of Nuclear Genome Size Variation in *Helianthus* Sunflowers. *New Phytol.* **2019**, *221*, 1609–1618. [CrossRef]
98. Knight, C.A.; Ackerly, D.D. Variation in Nuclear DNA Content across Environmental Gradients: A Quantile Regression Analysis. *Ecol. Lett.* **2002**, *5*, 66–76. [CrossRef]
99. Micevski, K. *Flora na SR Makedonija*; Makedonska Akademija na Naukite i Umetnostite: Skopje, North Macedonia, 1993; Volume 1.
100. Martinčič, A. *Mala Flora Slovenije*, 4th ed.; Tehniška Založba Slovenije: Ljubljana, Slovenia, 2007; ISBN 978-961-251-026-8.
101. Dimopoulos, P.; Raus, T.; Bergmeier, E.; Constantinidis, T.; Iatrou, G.; Kokkini, S.; Strid, A.; Tzanoudakis, D. (Eds.) *Vascular Plants of Greece: An Annotated Checklist*; Englera; Botanic Garden and Botanical Museum Berlin-Dahlem, Berlin and Hellenic botanical society: Athens, Greek, 2013; ISBN 978-3-921800-88-1.
102. Stešević, D.; Caković, D. *Katalog Vaskularne Flore Crne Gore*; Crnogorska Akademija Nauka i Umjetnosti: Podgorica, Montenegro, 2013; Volume 1.
103. Vangjeli, J. *Excursion Flora of Albania*; Koeltz Scientific Books: Oberreifenberg, Germany, 2015; ISBN 978-3-87429-477-5.
104. Barina, Z.; Somogyi, G.; Pifkó, D.; Rakaj, M. Checklist of vascular plants of Albania. *Phytotaxa* **2018**, *378*, 1–339. [CrossRef]
105. Nikolić, T. Flora Croatica Database. Prirodoslovno-Matematički Fakultet, Sveučilište u Zagrebu. Available online: http://hirc.botanic.hr/fcd (accessed on 7 June 2020).
106. Suda, J.; Trávníček, P. Reliable DNA Ploidy Determination in Dehydrated Tissues of Vascular Plants by DAPI Flow Cytometry—New Prospects for Plant Research. *Cytom. Part A* **2006**, *69A*, 273–280. [CrossRef]
107. Suda, J.; Krahulcová, A.; Trávníek, P.; Krahulec, F. Ploidy Level versus DNA Ploidy Level: An Appeal for Consistent Terminology. *Taxon* **2006**, *55*, 447–450. [CrossRef]
108. R Core Team. *R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing*; R Core Team: Vienna, Austria, 2020.
109. Karger, D.N.; Conrad, O.; Böhner, J.; Kawohl, T.; Kreft, H.; Soria-Auza, R.W.; Zimmermann, N.E.; Linder, H.P.; Kessler, M. Climatologies at High Resolution for the Earth's Land Surface Areas. *Sci. Data* **2017**, *4*, 170122. [CrossRef] [PubMed]
110. Karger, D.N.; Schmatz, D.R.; Dettling, G.; Zimmermann, N.E. High-Resolution Monthly Precipitation and Temperature Time Series from 2006 to 2100. *Sci. Data* **2020**, *7*, 248. [CrossRef] [PubMed]
111. Hengl, T.; de Jesus, J.M.; Heuvelink, G.B.M.; Gonzalez, M.R.; Kilibarda, M.; Blagotić, A.; Shangguan, W.; Wright, M.N.; Geng, X.; Bauer-Marschallinger, B.; et al. SoilGrids250m: Global Gridded Soil Information Based on Machine Learning. *PLoS ONE* **2017**, *12*, e0169748. [CrossRef]
112. Amatulli, G.; Domisch, S.; Tuanmu, M.-N.; Parmentier, B.; Ranipeta, A.; Malczyk, J.; Jetz, W. A Suite of Global, Cross-Scale Topographic Variables for Environmental and Biodiversity Modeling. *Sci. Data* **2018**, *5*, 180040. [CrossRef]

**Table S1.** Relative genome size (RGS) and ploidy level (2*x*, DNA-diploids; 4*x*, DNA-tetraploids) of 162 populations of *Dianthus sylvestris* from the Balkan Peninsula and the Alps, including their provenance and voucher data. For each population, DNA ploidy estimates, mean RGS of a holoploid genome and standard deviation are given. Number of individuals analyzed for RGS in each population (N) and the range of CV values are also provided. Ploidy: 2*x* and 4*x*. Asterisk (*) indicate the populations for which confirmatory chromosome counts were performed. Plus (+) for Morphometrics indicate the morphometrically analyzed populations in the previous study by Terlević et al. (submitted) [60].

| ID | Population name | Taxon | Country | Altitude (m a.s.l.) | Long (N) | Lat (E) | Collector | Collection date | Voucher | Mean RGS | SD RGS | N | Range of CV | Ploidy | Morpho-metrics |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D131 | Pindus Theodoriana | *D. sylvestris* subsp. *alboroseus* | Greece | 1023 | 39.42647 | 21.21591 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 21/06/2019 | ZA-54934 | 0.352 | 0.003 | 5 | 1.99–2.82 | 2x | |
| D132 | Mt Gorilla | *D. sylvestris* subsp. *alboroseus* | Greece | 664 | 39.49754 | 20.54052 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 21/06/2019 | ZA-54935 | 0.351 | 0.002 | 6 | 2.14–2.46 | 2x | + |
| D133 | Lygeres Siolades | *D. sylvestris* subsp. *alboroseus* | Greece | 744 | 39.76638 | 21.09354 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 22/06/2019 | ZA-54936 | 0.353 | 0.003 | 5 | 2.15–4.74 | 2x | + |
| D134 | Lygeres Aoos | *D. sylvestris* subsp. *alboroseus* | Greece | 1398 | 39.82082 | 21.08762 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 22/06/2019 | ZA-54937 | 0.357 | 0.003 | 6 | 2.84–4.43 | 2x | |
| D135 | Kapesovo | *D. sylvestris* subsp. *alboroseus* | Greece | 1135 | 39.88615 | 20.78638 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 22/06/2019 | ZA-54938 | 0.356 | 0.002 | 5 | 2.84–4.09 | 2x | + |
| D137 | Pindus Pyrgos | *D. sylvestris* subsp. *alboroseus* | Greece | 631 | 40.14424 | 20.82399 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 22/06/2019 | ZA-54940 | 0.348 | 0.006 | 4 | 2.93–3.84 | 2x | + |
| D138 | Pindus Mikropapingo | *D. sylvestris* subsp. *alboroseus* | Greece | 1296 | 39.97295 | 20.74076 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 23/06/2019 | ZA-54941 | 0.350 | 0.003 | 6 | 2.02–2.57 | 2x | + |
| D139 | Pindus Drakolimni | *D. sylvestris* subsp. *alboroseus* | Greece | 2093 | 39.99494 | 20.78599 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 23/06/2019 | ZA-54942 | 0.349 | 0.005 | 5 | 2.77–4.72 | 2x | |
| D140 | Mt Dhembel | *D. sylvestris* subsp. *alboroseus* | Albania | 1523 | 40.21507 | 20.31646 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 24/06/2019 | ZA-54943 | 0.351 | 0.000 | 6 | 1.98–2.31 | 2x | + |
| D141 | Gjirokaster | *D. sylvestris* subsp. *alboroseus* | Albania | 400 | 39.94504 | 20.25109 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 25/06/2019 | ZA-54944 | 0.353 | 0.002 | 6 | 2.54–2.59 | 2x | + |
| D142-1 | Llogara village | *D. sylvestris* subsp. *alboroseus* | Albania | 950 | 40.21436 | 19.57959 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 25/06/2019 | ZA-54946 | 0.355 | 0.003 | 4 | 3.01–4.41 | 2x | |
| D142-2 | Llogara park | *D. sylvestris* subsp. *alboroseus* | Albania | 1600 | 40.20721 | 19.59952 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 25/06/2019 | ZA-54945 | 0.365 | 0.010 | 4 | 4.43–5.55 | 2x | |
| D78 | Mt Ostrovice | *D. sylvestris* subsp. *alboroseus* | Albania | 1386 | 40.5741 | 20.47551 | D. Lakušić, N. Kuzmanović, I. Janković, M. Zbiljić | 11/07/2018 | ZA-48633 | 0.342 | 0.004 | 3 | 2.46–4.70 | 2x | |
| D150 | Rrapsh | *D. sylvestris* subsp. *bertisceus* | Albania | 773 | 42.41509 | 19.50371 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 27/06/2019 | ZA-54956 | 0.336 | 0.005 | 6 | 2.19–2.80 | 2x | + |
| D151 | Thethi Shtegut | *D. sylvestris* subsp. *bertisceus* | Albania | 1735 | 42.38844 | 19.73021 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 28/06/2019 | ZA-54957 | 0.336 | 0.002 | 6 | 3.63–5.02 | 2x | + |
| D153 | Rruga Thethe | *D. sylvestris* subsp. *bertisceus* | Albania | 665 | 42.32486 | 19.59345 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 28/06/2019 | ZA-54959 | 0.332 | 0.004 | 5 | 3.25–4.62 | 2x | + |
| D154 | Cijevna | *D. sylvestris* subsp. *bertisceus* | Montenegro | 139 | 42.39835 | 19.36711 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 28/06/2019 | ZA-54960 | 0.326 | 0.001 | 5 | 4.70–5.09 | 2x | + |
| D158 | Slano jezero | *D. sylvestris* subsp. *bertisceus* | Montenegro | 900 | 42.75111 | 18.79916 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 29/06/2019 | ZA-54964 | 0.334 | 0.001 | 5 | 3.55–3.60 | 2x | + |
| D169 | Mt Shkelzeni | *D. sylvestris* subsp. *bertisceus* | Albania | 2108 | 42.45568 | 20.11681 | D. Shuka | 24/07/2019 | ZA-54975,<br>ZA-54976 | 0.361 | 0.006 | 5 | 4.19–5.99 | 2x | + |
| D245 | Bistra | *D. sylvestris* subsp. *bertisceus* | North Macedonia | 1700 | 41.60719 | 20.75689 | I. Rešetnik, M. Doboš, I. Ljubičić | 20/07/2021 | ZA-62635 | 0.344 | 0.007 | 4 | 4.20–5.84 | 2x | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D250 | Grahovo road | *D. sylvestris* subsp. *bertisceus* | Montenegro | 800 | 42.47019 | 18.85884 | I. Rešetnik, M. Doboš, I. Ljubičić | 24/07/2021 | ZA-62639 | 0.347 | 0.008 | 4 | 4.52–5.63 | 2x | |
| D86 | Sutjeska | *D. sylvestris* subsp. *bertisceus* | Bosnia and Herzegovina | 638 | 43.31548 | 18.66821 | S. Bogdanović, I. Ljubičić | 11/07/2018 | ZA-48662 | 0.330 | 0.003 | 4 | 2.55–5.47 | 2x | + |
| D87 | Mt Maglic | *D. sylvestris* subsp. *bertisceus* | Bosnia and Herzegovina | 1580 | 43.27309 | 18.71955 | S. Bogdanović, I. Ljubičić | 12/07/2018 | ZA-48663 | 0.334 | 0.001 | 3 | 4.66–4.96 | 2x | + |
| D88 | Piva river | *D. sylvestris* subsp. *bertisceus* | Montenegro | 735 | 43.26326 | 18.84523 | S. Bogdanović, I. Ljubičić | 12/07/2018 | ZA-48664 | 0.329 | 0.006 | 3 | 3.22–4.92 | 2x | + |
| D89 | Pivska planina | *D. sylvestris* subsp. *bertisceus* | Montenegro | 1680 | 43.13713 | 18.94089 | S. Bogdanović, I. Ljubičić | 13/07/2018 | ZA-48665 | 0.331 | 0.006 | 3 | 2.15–4.03 | 2x | + |
| D90 | Mt Durmitor | *D. sylvestris* subsp. *bertisceus* | Montenegro | 2002 | 43.10045 | 19.05084 | S. Bogdanović, I. Ljubičić | 13/07/2018 | ZA-48666 | 0.330 | 0.004 | 3 | 3.04–4.70 | 2x | + |
| D91 | Berane | *D. sylvestris* subsp. *bertisceus* | Montenegro | 711 | 42.88203 | 19.86147 | S. Bogdanović, I. Ljubičić | 13/07/2018 | ZA-48612 | 0.328 | 0.002 | 3 | 3.03–4.01 | 2x | + |
| D92 | Andrijevica | *D. sylvestris* subsp. *bertisceus* | Montenegro | 746 | 42.72892 | 19.82458 | S. Bogdanović, I. Ljubičić | 13/07/2018 | ZA-48667 | 0.325 | 0.003 | 3 | 3.04–3.69 | 2x | + |
| D93 | Mt Prokletije Zeletin | *D. sylvestris* subsp. *bertisceus* | Montenegro | 2011 | 42.64971 | 19.84412 | S. Bogdanović, I. Ljubičić | 14/07/2018 | ZA-48668 | 0.330 | 0.007 | 3 | 2.71–3.78 | 2x | + |
| D94 | Mt Prokletije Popadija W | *D. sylvestris* subsp. *bertisceus* | Montenegro | 1989 | 42.52064 | 19.76142 | S. Bogdanović, I. Ljubičić | 14/07/2018 | ZA-48669 | 0.333 | 0.009 | 3 | 2.96–3.97 | 2x | + |
| D95 | Mt Prokletije Popadija SW | *D. sylvestris* subsp. *bertisceus* | Montenegro | 1948 | 42.67997 | 20.01136 | S. Bogdanović, I. Ljubičić | 15/07/2018 | ZA-48670 | 0.331 | 0.002 | 5 | 2.73–4.94 | 2x | + |
| D96 | Moraca river | *D. sylvestris* subsp. *bertisceus* | Montenegro | 278 | 42.69683 | 19.37431 | S. Bogdanović, I. Ljubičić | 15/07/2018 | ZA-48613 | 0.332 | 0.007 | 3 | 4.01–4.60 | 2x | + |
| D178 | Mt Kozjak | *D. sylvestris* subsp. *kozjakensis* | North Macedonia | 1292 | 41.40914 | 21.67842 | S. Bogdanović, I. Rešetnik | 14/07/2019 | ZA-54985 | 0.336 | 0.003 | 5 | 1.82–2.36 | 2x | + |
| D106 | Sitnica | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 915 | 42.56432 | 18.44312 | S. Bogdanović, I. Ljubičić | 17/7/2018, 29/6/2019 | ZA-48623 | 0.328 | 0.002 | 5 | 1.79–5.00 | 2x | + |
| D124 | Lastovo | *D. sylvestris* subsp. *nodosus* | Croatia | 48 | 42.75519 | 16.92379 | I. Rešetnik, S. Bogdanović | 04/06/2019 | ZA-54927 | 0.336 | 0.004 | 12 | 1.61–4.88 | 2x | |
| D127 | Brac | *D. sylvestris* subsp. *nodosus* | Croatia | 150 | 43.26608 | 16.69359 | I. Ljubičić, A. Terlević, M. Grgurev | 04/06/2019 | ZA-54930 | 0.343 | 0.006 | 4 | 4.17–4.50 | 2x | |
| D128 | Mosor | *D. sylvestris* subsp. *nodosus* | Croatia | 810 | 43.52237 | 16.61158 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević, M. Grgurev | 05/06/2019 | ZA-54931 | 0.337 | 0.004 | 5 | 2.73–4.41 | 2x | |
| D160 | Peljesac | *D. sylvestris* subsp. *nodosus* | Croatia | 160 | 42.91527 | 17.42674 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 29/06/2019 | ZA-54966 | 0.331 | 0.005 | 5 | 4.28–5.03 | 2x | + |
| D183 | Smricnjak | *D. sylvestris* subsp. *nodosus* | Croatia | 83 | 43.75222 | 15.87861 | L. Laca, S. Bogdanović | 17/06/2020 | ZA-56692 | 0.324 | 0.004 | 5 | 2.43–4.04 | 2x | + |
| D192 | Vrsak | *D. sylvestris* subsp. *nodosus* | Croatia | 1713 | 43.71589 | 16.8893 | M. Doboš | 22/07/2020 | ZA-56821 | 0.331 | 0.002 | 5 | 2.61–4.10 | 2x | + |
| D193 | Gornja Korita | *D. sylvestris* subsp. *nodosus* | Croatia | 906 | 43.70998 | 16.8192 | S. Bogdanović | 22/07/2020 | ZA-56822 | 0.337 | 0.009 | 5 | 3.93–5.57 | 2x | |
| D2 | Korcula | *D. sylvestris* subsp. *nodosus* | Croatia | 250 | 42.94086 | 17.08055 | I. Rešetnik, S. Bogdanović | 11/05/2018 | ZA-46277 | 0.337 | 0.004 | 4 | 2.89–4.75 | 2x | |
| D254 | Hrgud | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 1069 | 43.09763 | 18.05759 | I. Rešetnik, M. Doboš, I. Ljubičić | 25/07/2021 | ZA-62640 | 0.334 | 0.003 | 3 | 3.98–6.29 | 2x | |
| D27 | Mt Velebit Plesevica | *D. sylvestris* subsp. *nodosus* | Croatia | 1211 | 44.70794 | 14.96583 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 15/06/2018 | ZA-47427 | 0.335 | 0.007 | 3 | 2.88–4.93 | 2x | |
| D275 | Hvar | *D. sylvestris* subsp. *nodosus* | Croatia | 610 | 43.14478 | 16.5971 | S. Bogdanović | 28/07/2021 | ZA-62642 | 0.348 | 0.003 | 5 | 3.20–5.61 | 2x | |
| D28 | Mt Velebit Alan | *D. sylvestris* subsp. *nodosus* | Croatia | 1020 | 44.7153 | 14.9569 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 15/06/2018 | ZA-47428 | 0.334 | 0.002 | 10 | 2.70–4.73 | 2x | + |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D33 | Mt Velebit Baske Ostarije | *D. sylvestris* subsp. *nodosus* | Croatia | 905 | 44.52948 | 15.14316 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 15/06/2018 | ZA-47433 | 0.332 | 0.005 | 3 | 2.79–4.93 | 2x | + |
| D42 | Mt Velebit Brundo | *D. sylvestris* subsp. *nodosus* | Croatia | 1605 | 44.36112 | 15.51313 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 17/06/2018 | ZA-47442 | 0.344 | 0.004 | 5 | 3.00–6.03 | 2x | |
| D46 | Sibenik | *D. sylvestris* subsp. *nodosus* | Croatia | 18 | 43.73736 | 15.87176 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 18/06/2018 | ZA-47456 | 0.338 | 0.008 | 6 | 2.80–5.16 | 2x | + |
| D47-1 | Mt Biokovo low | *D. sylvestris* subsp. *nodosus* | Croatia | 325 | 43.25955 | 17.07843 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 18/06/2018 | ZA-47457 | 0.342 | 0.005 | 6 | 4.22–4.91 | 2x | + |
| D47-2 | Mt Biokovo high | *D. sylvestris* subsp. *nodosus* | Croatia | 1116 | 43.28407 | 17.08662 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 18/06/2018 | ZA-47459 | 0.344 | 0.009 | 7 | 2.75–5.73 | 2x | + |
| D52 | Mostar | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 382 | 43.32759 | 17.80412 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 19/06/2018 | ZA-47463 | 0.331 | 0.009 | 4 | 2.87–4.60 | 2x | + |
| D55 | Konjic | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 360 | 43.64998 | 17.96701 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 19/06/2018 | ZA-47466 | 0.332 | 0.001 | 6 | 2.24–4.75 | 2x | + |
| D56 | Vranic | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 549 | 43.41747 | 17.41643 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 | ZA-47467 | 0.327 | 0.004 | 4 | 4.00–4.65 | 2x | + |
| D57 | Blidinje | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 1298 | 43.58668 | 17.48789 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 | ZA-47468 | 0.334 | 0.004 | 5 | 2.49–5.07 | 2x | + |
| D6 | Mironja | *D. sylvestris* subsp. *nodosus* | Croatia | 332 | 42.81569 | 17.84349 | I. Rešetnik, S. Bogdanović | 13/5/2018, 29/6/2019 | ZA-46281 | 0.325 | 0.003 | 9 | 2.60–4.85 | 2x | + |
| D60 | Mt Cvrsnica Cavkarice | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 1556 | 43.57896 | 17.54072 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 | ZA-47471 | 0.346 | 0.008 | 4 | 2.72–4.92 | 2x | + |
| D61 | Trobukva | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 904 | 43.54695 | 17.27952 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 | ZA-47472 | 0.331 | 0.001 | 4 | 1.36–3.96 | 2x | + |
| D63 | Mt Cincar Krug | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 1197 | 43.85963 | 17.03066 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 21/06/2018 | ZA-47474 | 0.334 | 0.006 | 5 | 1.84–2.76 | 2x | + |
| D64 | Mt Cincar Begovaca | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 1397 | 43.88544 | 17.05981 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 21/06/2018 | ZA-47475 | 0.336 | 0.006 | 4 | 3.95–4.70 | 2x | + |
| D65 | Livno Jurina glava | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 1096 | 43.78286 | 17.16035 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 21/06/2018 | ZA-47476 | 0.334 | 0.005 | 5 | 2.13–4.49 | 2x | + |
| D66 | Drvar | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 716 | 44.34866 | 16.35956 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 22/06/2018 | ZA-47477 | 0.340 | 0.002 | 3 | 4.52–4.99 | 2x | + |
| D8 | Omis | *D. sylvestris* subsp. *nodosus* | Croatia | 63 | 43.45025 | 16.69853 | I. Rešetnik, S. Bogdanović | 13/5/2018, 29/6/2019 | ZA-46283 | 0.328 | 0.004 | 6 | 2.44–3.13 | 2x | + |
| D80 | Mt Dinara Glavas | *D. sylvestris* subsp. *nodosus* | Croatia | 740 | 44.01427 | 16.41704 | S. Bogdanović, I. Ljubičić | 30/06/2018 | ZA-48607 | 0.340 | 0.008 | 6 | 4.28–6.00 | 2x | + |
| D82 | Mt Svilaja | *D. sylvestris* subsp. *nodosus* | Croatia | 934 | 43.75119 | 16.52322 | S. Bogdanović, I. Ljubičić | 01/07/2018 | ZA-48608 | 0.336 | 0.003 | 5 | 3.53–4.66 | 2x | + |
| D85 | Mt Vlasic | *D. sylvestris* subsp. *nodosus* | Bosnia and Herzegovina | 1100 | 44.25852 | 17.62419 | S. Bogdanović, I. Ljubičić | 10/07/2018 | ZA-48611 | 0.329 | 0.005 | 3 | 3.72–4.65 | 2x | + |
| D12 | Nacinovici | *D. sylvestris* subsp. *sylvestris* | Croatia | 240 | 45.12395 | 14.20468 | S. Bogdanović, I. Ljubičić | 19/5/2018, 13/7/2019 | ZA-46416 | 0.641 | 0.007 | 8 | 2.43–4.66 | 4x* | + |
| D130 | Krcic | *D. sylvestris* subsp. *sylvestris* | Croatia | 345 | 44.03398 | 16.29247 | S. Bogdanović, I. Ljubičić | 14/06/2019 | ZA-54933 | 0.327 | 0.002 | 5 | 2.48–3.89 | 2x | + |
| D162 | Ostrc | *D. sylvestris* subsp. *sylvestris* | Croatia | 740 | 45.76377 | 15.6486 | A. Terlević, M. Grgurev | 05/07/2019 | ZA-54968 | 0.325 | 0.001 | 5 | 1.85–1.91 | 2x | + |
| D165 | Litija | *D. sylvestris* subsp. *sylvestris* | Slovenia | 295 | 46.08895 | 14.8873 | A. Terlević, I. Ljubičić | 09/07/2019 | ZA-54971 | 0.331 | 0.003 | 5 | 3.31–4.29 | 2x | |
| D166 | Polhograjska Grmada | *D. sylvestris* subsp. *sylvestris* | Slovenia | 900 | 46.08407 | 14.33474 | A. Terlević, I. Ljubičić | 10/07/2019 | ZA-54972 | 0.333 | 0.006 | 4 | 2.18–3.34 | 2x | + |
| D17 | Mt Spacatto | *D. sylvestris* subsp. *sylvestris* | Italy | 300 | 45.64227 | 13.8311 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 | ZA-47417 | 0.640 | 0.004 | 3 | 2.00–2.54 | 4x | + |
| D184 | Lim bay | *D. sylvestris* subsp. *sylvestris* | Croatia | 136 | 45.13314 | 13.73433 | A. Terlević | 21/06/2020 | ZA-56693 | 0.328 | 0.007 | 5 | 2.96–6.55 | 2x | + |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D19-2 | Vodice tetrapl | *D. sylvestris* subsp. *sylvestris* | Croatia | 660 | 45.47917 | 14.04971 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/6/2018,<br>13/7/2019 | ZA-47419 | 0.651 | 0.006 | 8 | 1.80–3.57 | 4x | + |
| D196 | Brkini | *D. sylvestris* subsp. *sylvestris* | Slovenia | 625 | 45.50694 | 14.16806 | B. Frajman | 15/08/2020 | ZA-56825 | 0.657 | 0.006 | 5 | 1.85–2.80 | 4x | |
| D20 | Zbevnica | *D. sylvestris* subsp. *sylvestris* | Croatia | 941 | 45.45721 | 14.01632 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/6/2018,<br>19/7/2020 | ZA-47420 | 0.651 | 0.004 | 3 | 3.55–4.50 | 4x* | + |
| D205 | Klecice | *D. sylvestris* subsp. *sylvestris* | Croatia | 968 | 45.26685 | 15.1413 | I. Ljubičić, A. Terlević | 16/06/2021 | ZA-62605 | 0.337 | 0.007 | 4 | 4.36–5.37 | 2x | |
| D206 | Mt Calvo | *D. sylvestris* subsp. *sylvestris* | Italy | 360 | 45.65316 | 13.82246 | I. Rešetnik, S. Bogdanović, M. Temunović, A. Terlević | 28/06/2021 | ZA-62606 | 0.646 | 0.008 | 4 | 2.57–3.58 | 4x | |
| D207 | Hervati | *D. sylvestris* subsp. *sylvestris* | Italy | 345 | 45.6259 | 13.86941 | I. Rešetnik, S. Bogdanović, M. Temunović, A. Terlević | 28/06/2021 | ZA-62607 | 0.652 | 0.009 | 6 | 2.70–4.30 | 4x | |
| D21 | Slum | *D. sylvestris* subsp. *sylvestris* | Croatia | 553 | 45.4234 | 14.01213 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/6/2018,<br>19/7/2020 | ZA-47421 | 0.650 | 0.009 | 4 | 2.26–5.01 | 4x* | + |
| D22 | Mt Ucka | *D. sylvestris* subsp. *sylvestris* | Croatia | 525 | 45.31681 | 14.17734 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 | ZA-47422 | 0.656 | 0.010 | 4 | 2.02–4.90 | 4x | + |
| D24 | Gornje Jelenje | *D. sylvestris* subsp. *sylvestris* | Croatia | 862 | 45.36157 | 14.61724 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 14/06/2018 | ZA-47424 | 0.648 | 0.003 | 5 | 2.35–4.87 | 4x | + |
| D256 | Velez | *D. sylvestris* subsp. *sylvestris* | Bosnia and Herzegovina | 1912 | 43.35755 | 17.93495 | I. Rešetnik, M. Doboš, I. Ljubičić | 25/07/2021 | ZA-62641 | 0.340 | 0.009 | 4 | 4.23–5.21 | 2x | |
| D34 | Mt Velebit Visocica | *D. sylvestris* subsp. *sylvestris* | Croatia | 1458 | 44.4293 | 15.3643 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 16/06/2018 | ZA-47434 | 0.334 | 0.004 | 4 | 3.14–4.43 | 2x | |
| D37 | Mt Velebit Buljma | *D. sylvestris* subsp. *sylvestris* | Croatia | 1218 | 44.36597 | 15.46143 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 17/06/2018 | ZA-47437 | 0.336 | 0.004 | 5 | 3.77–4.93 | 2x | + |
| D38 | Mt Paklenica Struge | *D. sylvestris* subsp. *sylvestris* | Croatia | 1368 | 44.37109 | 15.46567 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 17/06/2018 | ZA-47438 | 0.339 | 0.006 | 3 | 2.74–4.53 | 2x | + |
| D58 | Mt Cvrsnica SW | *D. sylvestris* subsp. *sylvestris* | Bosnia and Herzegovina | 1928 | 43.59503 | 17.54076 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 | ZA-47469 | 0.325 | 0.001 | 3 | 2.97–4.59 | 2x | |
| D59 | Mt Cvrsnica Siroka kosa | *D. sylvestris* subsp. *sylvestris* | Bosnia and Herzegovina | 1732 | 43.58652 | 17.53378 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 | ZA-47470 | 0.334 | 0.007 | 4 | 3.72–5.07 | 2x | + |
| D81 | Mt Dinara Osljak | *D. sylvestris* subsp. *sylvestris* | Croatia | 1418 | 44.03867 | 16.39343 | S. Bogdanović, I. Ljubičić | 30/06/2018 | ZA-48606 | 0.336 | 0.008 | 3 | 4.39–4.93 | 2x | + |
| D83 | Banja Luka | *D. sylvestris* subsp. *sylvestris* | Bosnia and Herzegovina | 207 | 44.68547 | 17.1797 | S. Bogdanović, I. Ljubičić | 10/07/2018 | ZA-48609 | 0.329 | 0.005 | 3 | 2.99–4.99 | 2x | + |
| D84 | Jajce | *D. sylvestris* subsp. *sylvestris* | Bosnia and Herzegovina | 380 | 44.3468 | 17.24253 | S. Bogdanović, I. Ljubičić | 10/07/2018 | ZA-48610 | 0.338 | 0.009 | 3 | 2.12–4.87 | 2x | |
| D1 | Klek village | *D. sylvestris* subsp. *tergestinus* | Croatia | 80 | 42.94874 | 17.56451 | I. Rešetnik, S. Bogdanović | 09/05/2018 | ZA-46276 | 0.345 | 0.005 | 3 | 4.58–5.00 | 2x | + |
| D11 | Gornji Kamenjak | *D. sylvestris* subsp. *tergestinus* | Croatia | 55 | 44.81097 | 13.90088 | S. Bogdanović, I. Ljubičić | 17/05/2018 | ZA-46415 | 0.339 | 0.005 | 4 | 2.96–4.68 | 2x | + |
| D111 | Cres | *D. sylvestris* subsp. *tergestinus* | Croatia | 40 | 44.97721 | 14.445 | S. Bogdanović, S. Cambria | 21/10/2018 | ZA-48628 | 0.342 | 0.002 | 4 | 2.05–4.25 | 2x | |
| D129 | Gradina | *D. sylvestris* subsp. *tergestinus* | Croatia | 186 | 43.77399 | 15.95608 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević, M. Grgurev | 05/06/2019 | ZA-54932 | 0.340 | 0.004 | 4 | 2.98–4.02 | 2x | + |
| D13 | Krizisce | *D. sylvestris* subsp. *tergestinus* | Croatia | 513 | 45.2676 | 14.59441 | S. Bogdanović, I. Ljubičić | 19/05/2018 | ZA-46417 | 0.333 | 0.004 | 4 | 3.75–4.68 | 2x | + |
| D16 | Sezana | *D. sylvestris* subsp. *tergestinus* | Italy | 344 | 45.70153 | 13.83729 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 | ZA-47416 | 0.341 | 0.007 | 4 | 3.31–3.82 | 2x | + |
| D18 | Petrinje | *D. sylvestris* subsp. *tergestinus* | Slovenia | 422 | 45.57284 | 13.90681 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 | ZA-47418 | 0.336 | 0.008 | 5 | 2.66–4.99 | 2x | + |
| D185 | Krk | *D. sylvestris* subsp. *tergestinus* | Croatia | 220 | 45.02389 | 14.64694 | I. Rešetnik | 14/06/2020 | ZA-56694 | 0.340 | 0.004 | 5 | 3.30–6.93 | 2x* | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D25 | Bunica | *D. sylvestris* subsp. *tergestinus* | Croatia | 8 | 45.02484 | 14.88473 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 14/06/2018 | ZA-47425 | 0.337 | 0.001 | 4 | 2.67–4.62 | 2x | |
| D29 | Donji Bileni | *D. sylvestris* subsp. *tergestinus* | Croatia | 274 | 44.71196 | 14.9225 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 15/06/2018 | ZA-47429 | 0.340 | 0.007 | 5 | 2.46–4.92 | 2x | |
| D32 | Karlobag | *D. sylvestris* subsp. *tergestinus* | Croatia | 12 | 44.53338 | 15.06519 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 15/06/2018 | ZA-47432 | 0.335 | 0.005 | 3 | 4.09–4.66 | 2x* | |
| D36 | Mt Velebit Paklenica | *D. sylvestris* subsp. *tergestinus* | Croatia | 397 | 44.31347 | 15.45328 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 17/06/2018 | ZA-47436 | 0.340 | 0.006 | 4 | 2.86–4.77 | 2x | + |
| D4 | Dubrovnik | *D. sylvestris* subsp. *tergestinus* | Croatia | 279 | 42.64521 | 18.12021 | I. Rešetnik, S. Bogdanović | 12/05/2018 | ZA-46279 | 0.352 | 0.005 | 3 | 2.75–4.26 | 2x | + |
| D43 | Vransko jezero | *D. sylvestris* subsp. *tergestinus* | Croatia | 35 | 43.86523 | 15.6423 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 18/06/2018 | ZA-47453 | 0.341 | 0.008 | 4 | 2.58–4.72 | 2x | |
| D50 | Mostar Vukodol | *D. sylvestris* subsp. *tergestinus* | Bosnia and Herzegovina | 250 | 43.32542 | 17.79292 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 19/06/2018 | ZA-47461 | 0.353 | 0.005 | 5 | 3.32–4.18 | 2x | |
| D53 | Mostar Hum | *D. sylvestris* subsp. *tergestinus* | Bosnia and Herzegovina | 385 | 43.32755 | 17.80407 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 19/06/2018 | ZA-47464 | 0.355 | 0.009 | 5 | 2.11–4.87 | 2x | |
| D62 | Livno | *D. sylvestris* subsp. *tergestinus* | Bosnia and Herzegovina | 821 | 43.84762 | 16.9733 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 21/06/2018 | ZA-47473 | 0.340 | 0.008 | 3 | 2.81–4.33 | 2x | + |
| D7 | Mravinica Doli | *D. sylvestris* subsp. *tergestinus* | Croatia | 351 | 42.81084 | 17.83079 | I. Rešetnik, S. Bogdanović | 13/05/2018 | ZA-46282 | 0.351 | 0.007 | 3 | 2.49–4.33 | 2x | + |
| D9 | Malacka | *D. sylvestris* subsp. *tergestinus* | Croatia | 602 | 43.58147 | 16.32579 | I. Rešetnik, S. Bogdanović | 13/05/2018 | ZA-46284 | 0.339 | 0.003 | 3 | 4.57–5.88 | 2x | + |
| 16866 | Ahrntal | *D. sylvestris* s.l. - Alps | Italy | 1075 | 46.89278 | 11.96194 | D. Volgger, F. Faltner, B. Frajman | 12/07/2021 | IB-16866 | 0.366 | 0.001 | 3 | 5.99–8.21 | 2x | |
| 16867 | Pustertal Niederdorf | *D. sylvestris* s.l. - Alps | Italy | 1850 | 46.70778 | 12.17528 | D. Volgger, A. Seyer, B. Frajman | 12/07/2021 | IB-16867 | 0.352 | 0.010 | 3 | 2.71–3.08 | 2x | |
| 16868 | Karnische Alpen | *D. sylvestris* s.l. - Alps | Austria | 1960 | 46.69444 | 12.49306 | D. Volgger, B. Frajman | 13/07/2021 | IB-16868 | 0.326 | 0.005 | 3 | 4.30–5.60 | 2x | |
| 16869 | Dolomiti Passo Tre Croci | *D. sylvestris* s.l. - Alps | Italy | 1810 | 46.5575 | 12.19944 | D. Volgger, B. Frajman | 13/07/2021 | IB-16869 | 0.330 | 0.004 | 3 | 4.89–9.03 | 2x | |
| 16870 | Dolomiti Fiames | *D. sylvestris* s.l. - Alps | Italy | 1410 | 46.59667 | 12.11222 | D. Volgger, B. Frajman | 13/07/2021 | IB-16870 | 0.334 | 0.001 | 3 | 4.80–8.79 | 2x | |
| 16871 | Dolomiti Furkelpass | *D. sylvestris* s.l. - Alps | Italy | 2140 | 46.71139 | 11.97028 | B. Frajman | 13/07/2021 | IB-16871 | 0.354 | 0.003 | 3 | 6.76–7.30 | 2x | |
| 16872 | Pustertal Issing | *D. sylvestris* s.l. - Alps | Italy | 870 | 46.81 | 11.84306 | D. Volgger, B. Frajman | 13/07/2021 | IB-16872 | 0.359 | 0.004 | 3 | 6.80–9.45 | 2x | |
| 16875 | Pustertal Burgkofl | *D. sylvestris* s.l. - Alps | Italy | 935 | 46.79278 | 11.88111 | F. Faltner | 12/07/2021 | IB-16875 | 0.348 | 0.008 | 3 | 8.83–10.19 | 2x | |
| 16927 | Villgrater Berge | *D. sylvestris* s.l. - Alps | Austria | 1500 | 46.80917 | 12.33083 | B. Frajman, M. Doboš | 02/08/2021 | IB-16927 | 0.339 | 0.001 | 5 | 2.34–5.62 | 2x | |
| 16972 | Pordenone | *D. sylvestris* s.l. - Alps | Italy | 900 | 46.28 | 12.36778 | P. Schönswetter, T. Zeni | 31/07/2021 | IB-16972 | 0.346 | 0.006 | 5 | 3.81–5.44 | 2x | |
| 16973 | Prealpi Bellunesi | *D. sylvestris* s.l. - Alps | Italy | 1515 | 46.11278 | 12.50778 | P. Schönswetter, T. Zeni | 01/08/2021 | IB-16973 | 0.328 | 0.001 | 3 | 6.65–9.27 | 2x | |
| 16977 | Dolomiti Strudelkopf | *D. sylvestris* s.l. - Alps | Italy | 2170 | 46.64417 | 12.2 | A. Seyr | 13/08/2021 | IB-16977 | 0.335 | 0.005 | 6 | 3.82–4.90 | 2x | |
| D121 | Oberes Gericht | *D. sylvestris* s.l. - Alps | Austria | 1160 | 47.03669 | 10.64664 | M. Falch | 16/07/2018 | ZA-54924 | 0.351 | 0.003 | 3 | 2.47–3.43 | 2x | |
| D122 | Innsbruck S | *D. sylvestris* s.l. - Alps | Austria | 880 | 47.24425 | 11.41853 | M. Falch | 03/09/2018 | ZA-54925 | 0.360 | 0.004 | 3 | 1.87–4.49 | 2x | |
| D164 | Vrsic | *D. sylvestris* s.l. - Alps | Slovenia | 1954 | 46.43132 | 13.73659 | A. Terlević, I. Ljubičić | 09/07/2019 | ZA-54970 | 0.333 | 0.000 | 6 | 2.24–2.87 | 2x | |
| D197 | Achberg | *D. sylvestris* s.l. - Alps | Austria | 785 | 47.28972 | 10.98667 | B. Frajman | 07/07/2020 | ZA-56826 | 0.359 | 0.004 | 5 | 2.49–6.76 | 2x | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D199 | Gailtal Arnoldstein | *D. sylvestris* s.l. - Alps | Austria | 545 | 46.56528 | 13.69083 | B. Frajman, P. Schönswetter | 22/05/2020 | ZA-56828 | 0.333 | 0.002 | 5 | 2.62–6.11 | 2x | |
| D67 | Prutz | *D. sylvestris* s.l. - Alps | Austria | 904 | 47.07507 | 10.65361 | I. Rešetnik | 27/06/2018 | ZA-47478 | 0.363 | 0.008 | 3 | 1.94–4.48 | 2x | |
| D68 | Mals | *D. sylvestris* s.l. - Alps | Italy | 1039 | 46.67989 | 10.55889 | I. Rešetnik | 27/06/2018 | ZA-47479 | 0.361 | 0.002 | 3 | 2.04–3.96 | 2x | |
| D69 | Gailtal alps | *D. sylvestris* s.l. - Alps | Austria | 1971 | 46.75922 | 12.88028 | I. Rešetnik | 29/06/2018 | ZA-47480 | 0.335 | 0.005 | 5 | 1.93–2.89 | 2x | |
| D70 | Zentralalpen | *D. sylvestris* s.l. - Alps | Austria | 2030 | 47.01306 | 12.25111 | I. Rešetnik | 30/06/2018 | ZA-47481 | 0.333 | 0.004 | 3 | 2.48–2.90 | 2x | |
| D71 | Innsbruck Nordkette | *D. sylvestris* s.l. - Alps | Austria | 1906 | 47.30494 | 11.35861 | I. Rešetnik | 08/07/2018 | ZA-47482 | 0.358 | 0.005 | 3 | 2.51–4.57 | 2x | |
| Lago di Fedaia | Lago di Fedaia | *D. sylvestris* s.l. - Alps | Italy | 2220 | 46.46606 | 11.85929 | H. Luqman | 04/08/2016 | ZT-Lago di Fedaia | 0.359 | 0.000 | 3 | 6.69–9.79 | 2x | |
| Mt Caslano | Mt Caslano | *D. sylvestris* s.l. - Alps | Switzerland | 332 | 45.96053 | 8.88331 | H. Luqman | 26/06/2017 | ZT-Mt Caslano | 0.355 | 0.005 | 3 | 6.86–7.55 | 2x | |
| Mustair Low | Mustair Low | *D. sylvestris* s.l. - Alps | Switzerland | 1300 | 46.63216 | 10.44699 | H. Luqman | 03/08/2017 | ZT-Mustair Low | 0.366 | 0.003 | 3 | 4.89–5.81 | 2x | |
| Rifugio Graziani | Rifugio Graziani | *D. sylvestris* s.l. - Alps | Italy | 1566 | 45.79879 | 10.89384 | H. Luqman | 15/07/2017 | ZT-Rifugio Graziani | 0.353 | 0.005 | 3 | 3.81–4.64 | 2x | |
| Schwarzenmatt | Schwarzenmatt | *D. sylvestris* s.l. - Alps | Switzerland | 1220 | 46.63409 | 7.349496 | H. Luqman | 23/07/2016 | ZT-Schwarzenmatt | 0.369 | 0.004 | 3 | 5.28–7.11 | 2x | |
| Thumel | Thumel | *D. sylvestris* s.l. - Alps | Italy | 1940 | 45.53495 | 7.10119 | H. Luqman | 26/08/2017 | ZT-Thumel | 0.367 | 0.008 | 3 | 3.98–4.75 | 2x | |
| D102 | Obzovica | *D. sylvestris* s.l. - Balkan | Montenegro | 931 | 42.30581 | 18.93189 | S. Bogdanović, I. Ljubičić | 16/07/2018 | ZA-48619 | 0.335 | 0.001 | 4 | 2.82–4.14 | 2x | + |
| D103 | Mt Lovcen | *D. sylvestris* s.l. - Balkan | Montenegro | 1681 | 42.4003 | 18.83807 | S. Bogdanović, I. Ljubičić | 16/07/2018 | ZA-48620 | 0.337 | 0.007 | 4 | 3.95–4.88 | 2x | + |
| D108 | Gramsh | *D. sylvestris* s.l. - Balkan | Albania | 969 | 40.86694 | 20.38444 | B. Frajman, P. Schönswetter | 12/07/2018 | ZA-48625 | 0.336 | 0.002 | 4 | 4.19–5.26 | 2x | |
| D109 | Maja e Ostrovice | *D. sylvestris* s.l. - Balkan | Albania | 1406 | 40.57972 | 20.47722 | B. Frajman, P. Schönswetter | 13/07/2018 | ZA-48626 | 0.336 | 0.007 | 3 | 2.92–4.93 | 2x | |
| D110 | Mt Jablanica | *D. sylvestris* s.l. - Balkan | North Macedonia | 1759 | 41.27278 | 20.535 | B. Frajman, P. Schönswetter | 15/07/2018 | ZA-48627 | 0.342 | 0.003 | 3 | 2.94–4.92 | 2x | |
| D119 | Mt Valamara | *D. sylvestris* s.l. - Balkan | Albania | 1670 | 40.82167 | 20.50167 | M. Duchon | 16/07/2018 | ZA-54922 | 0.351 | 0.008 | 3 | 1.70–4.23 | 2x | |
| D145 | Mat | *D. sylvestris* s.l. - Balkan | Albania | 145 | 41.67954 | 19.84739 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 26/06/2019 | ZA-54947 | 0.338 | 0.007 | 5 | 3.73–4.76 | 2x | + |
| D147 | Shkoder | *D. sylvestris* s.l. - Balkan | Albania | 129 | 42.07219 | 19.56423 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 27/06/2019 | ZA-54953 | 0.330 | 0.002 | 8 | 3.16–4.50 | 2x | + |
| D170 | Mt Kopaonik | *D. sylvestris* s.l. - Balkan | Serbia | 1787 | 43.26151 | 20.83653 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 10/07/2019 | ZA-54977 | 0.334 | 0.001 | 6 | 2.14–2.48 | 2x | + |
| D171 | Ljuboten peak | *D. sylvestris* s.l. - Balkan | North Macedonia | 2255 | 42.19948 | 21.12385 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 11/07/2019 | ZA-54978 | 0.335 | 0.005 | 5 | 1.83–4.03 | 2x | + |
| D172 | Ljuboten peak scardicus | *D. sylvestris* s.l. - Balkan | North Macedonia | 2255 | 42.19948 | 21.12385 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 11/07/2019 | ZA-54979 | 0.336 | 0.004 | 5 | 2.31–4.65 | 2x | + |
| D173 | Mt Bistra | *D. sylvestris* s.l. - Balkan | North Macedonia | 1687 | 41.6293 | 20.68481 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 12/07/2019 | ZA-54980 | 0.336 | 0.001 | 6 | 1.86–2.17 | 2x | + |
| D174 | Mt Galicica | *D. sylvestris* s.l. - Balkan | North Macedonia | 1510 | 40.96566 | 20.81879 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 12/07/2019 | ZA-54981 | 0.339 | 0.000 | 5 | 2.37–4.61 | 2x | + |
| D175 | Mt Korab Nistrovski | *D. sylvestris* s.l. - Balkan | North Macedonia | 2112 | 41.7919 | 20.59291 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 13/07/2019 | ZA-54982 | 0.337 | 0.004 | 5 | 2.98–4.02 | 2x | + |
| D176 | Mt Korab Radika | *D. sylvestris* s.l. - Balkan | North Macedonia | 1379 | 41.79959 | 20.62801 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 13/07/2019 | ZA-54983 | 0.337 | 0.001 | 5 | 2.39–2.41 | 2x | + |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D179 | Mt Jakupica Solunska glava | *D. sylvestris* s.l. - Balkan | North Macedonia | 2274 | 41.69279 | 21.40381 | S. Bogdanović, M. Grgurev, I. Rešetnik | 17/07/2019 | ZA-54986 | 0.347 | 0.005 | 5 | 2.02–3.73 | 2x | + |
| D180 | Mt Jakupica Ismailica | *D. sylvestris* s.l. - Balkan | North Macedonia | 1235 | 41.68044 | 21.43242 | S. Bogdanović, M. Grgurev, I. Rešetnik | 17/07/2019 | ZA-54987 | 0.345 | 0.006 | 5 | 1.75–2.61 | 2x | + |
| D182 | Gjergjevica | *D. sylvestris* s.l. - Balkan | Albania | 1300 | 40.58626 | 20.57514 | L. Shuka | 27/07/2019 | ZA-54989,<br>ZA-54990 | 0.376 | 0.004 | 4 | 4.73–6.00 | 2x | + |
| D204 | Voskopoje | *D. sylvestris* s.l. - Balkan | Albania | 1165 | 40.58247 | 20.56072 | D. Lakušić, N. Kuzmanović, I. Janković, M. Zbiljić | 11/07/2018 | ZA-58876 | 0.355 | 0.004 | 3 | 5.63–8.89 | 2x | |
| D246 | Sar Planina | *D. sylvestris* s.l. - Balkan | Albania | 2119 | 42.09306 | 20.8931 | I. Rešetnik, M. Doboš, I. Ljubičić | 21/07/2021 | ZA-62636 | 0.338 | 0.006 | 3 | 3.26–4.52 | 2x | |
| D248 | Mali Gropa | *D. sylvestris* s.l. - Balkan | Albania | 1616 | 41.37702 | 20.06664 | I. Rešetnik, M. Doboš, I. Ljubičić | 22/07/2021 | ZA-62637 | 0.348 | 0.003 | 4 | 3.85–5.21 | 2x | |
| D76 | Maljen | *D. sylvestris* s.l. - Balkan | Serbia | 640 | 44.04424 | 20.01645 | S. Đurović, G. Tomović | 15/07/2018 | ZA-48631 | 0.328 | 0.006 | 4 | 2.95–4.37 | 2x | |
| D77 | Mt Sar Planina | *D. sylvestris* s.l. - Balkan | Serbia | 1755 | 42.2335 | 20.91537 | D. Lakušić, N. Kuzmanović, I. Janković, M. Zbiljić | 14/07/2018 | ZA-48632 | 0.340 | 0.004 | 3 | 2.23–4.34 | 2x | |
| D79 | Mt Kopaonik Pancicev vrh | *D. sylvestris* s.l. - Balkan | Serbia | 1660 | 43.26149 | 20.83973 | S. Đurović, G. Tomović | 13/07/2018 | ZA-48634 | 0.335 | 0.004 | 3 | 3.69–3.95 | 2x | |

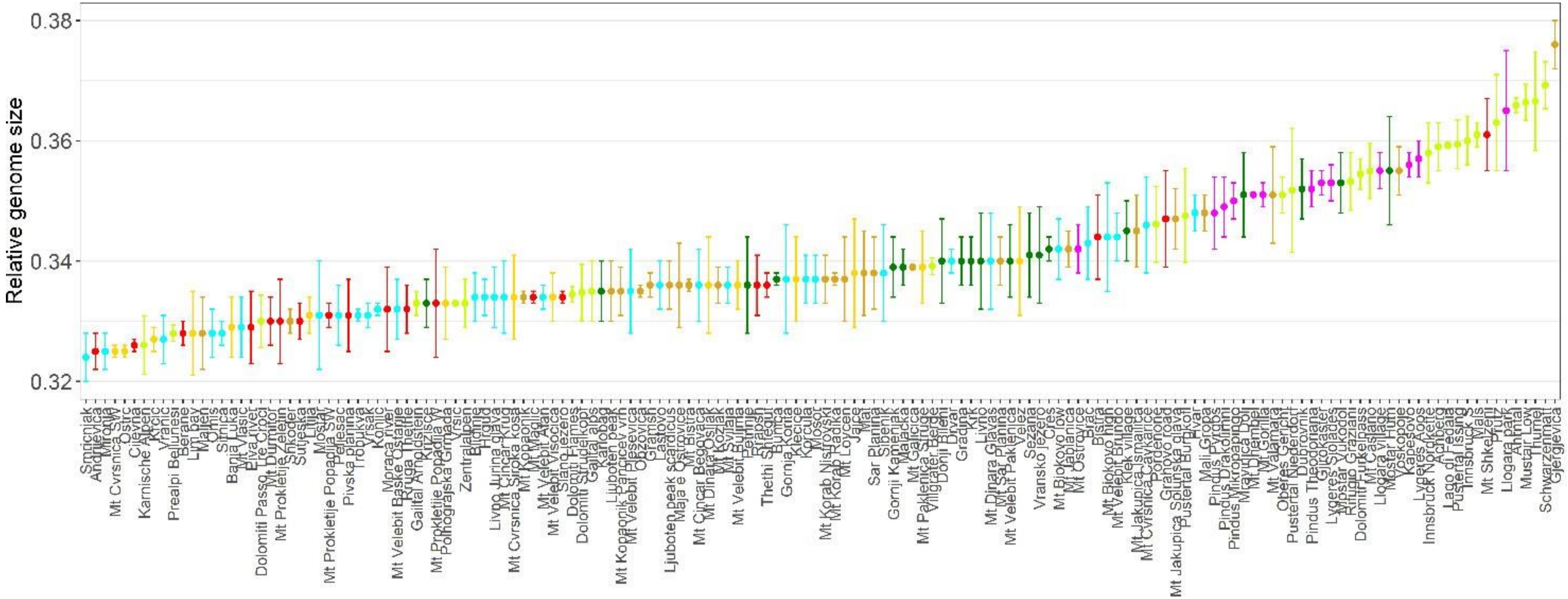


**Figure S1.** Relative genome size (RGS) in diploid populations of *Dianthus sylvestris* sorted by increasing RGS values. Population means (dots) with corresponding standard deviation (vertical lines)are presented. Population names correspond to Table S1. Colors correspond to subspecies, as in Figure 2.

**Table S2.** Results of Kruskal Wallis test between diploid (2*x*, N = 9) and tetraploid (4*x*, N = 7) populations of *Dianthus sylvestris* subsp. *sylvestris*. Morphological characters with significant differences between ploidy levels at $p < 0.05$ are shown in bold.

| **Character** | **Abbreviation** | **statistic** | ***p* value** |
|---|---|---|---|
| Length of the woody part of the stem below basal leaves | BL | 0.025779 | 0.872442 |
| Basal leaf length | BLL | 4.495013 | **0.033994** |
| Calyx length | CL | 0.02521 | 0.873845 |
| Length of the cauline leaf from the second stem node | CLL | 6.463286 | **0.011013** |
| Calyx teeth length | CTL | 1.235294 | 0.26638 |
| Number of epicalyx scales | ESN | 1.153723 | 0.282771 |
| Height of first branching | FBH | 7.868347 | **0.005031** |
| Number of flowers per stem | FPS | 2.072495 | 0.149976 |
| Inflorescence length | IL | 2.876809 | 0.089864 |
| Inner epicalyx scale apex length | ISAL | 0.809524 | 0.368261 |
| Inner epicalyx scale length | ISL | 1.235294 | 0.26638 |
| Inner epicalyx scale width | ISW | 2.691877 | 0.100861 |
| Length of the first stem internode | LIL | 0.002818 | 0.957667 |
| Plant height | PH | 5.9359 | **0.014835** |
| Petal length | PL | 0.226891 | 0.633839 |
| Petal limb length | PLL | 0.02521 | 0.873845 |
| Petal limb width | PLW | 0.02521 | 0.873845 |
| Petal teeth length | PTL | 0.630252 | 0.427263 |
| Maximum number of petal teeth | PTMAX | 0.912933 | 0.339337 |
| Minimum number of petal teeth | PTMIN | 2.707805 | 0.099858 |
| Ratio Calyx teeth length / Calyx length | RCALYX | 2.699817 | 0.10036 |
| Ratio Petal teeth length / Petal limb length | RDENT | 0.280938 | 0.596087 |
| Ratio Calyx length / Claw length | RFL | 1.011204 | 0.314614 |
| Ratio Inner epicalyx scale length without apex / Inner epicalyx scale width | RIS | 1.481793 | 0.223494 |
| Ratio Inner epicalyx scale apex length / Inner epicalyx scale length | RISA | 0.070028 | 0.791296 |
| Ratio Petal limb length / Petal limb width | RLIMB | 1.122098 | 0.289467 |
| Ratio Petal limb length / Petal length | RPETAL | 0.718143 | 0.396754 |
| Length of the last stem internode below branching | UIL | 4.715618 | **0.02989** |

**Table S3.** Results of Kruskal Wallis test between diploid (2*x*, N = 15) and tetraploid (4*x*, N = 10) populations of *Dianthus sylvestris* subsp. *sylvestris*. Environmental variables with significant differences between ploidy levels at $p < 0.05$ are shown in bold.

| **short name** | **long name** | **unit** | **statistic** | ***p* value** |
|---|---|---|---|---|
| bio01 | mean annual air temperature | °C/10 | 5.060548 | **0.024477** |
| bio02 | mean diurnal air temperature range | °C/10 | 4.601475 | **0.031944** |
| bio03 | isothermality | °C/10 | 5.829034 | **0.015764** |
| bio04 | temperature seasonality | °C/10 | 0.249231 | 0.617617 |
| bio05 | mean daily maximum air temperature of the warmest month | °C/10 | 0.943033 | 0.331499 |
| bio06 | mean daily minimum air temperature of the coldest month | °C/10 | 9.484988 | **0.002072** |
| bio07 | annual range of air temperature | °C/10 | 3.673611 | 0.05528 |
| bio08 | mean daily mean air temperatures of the wettest quarter | °C/10 | 1.492675 | 0.221802 |
| bio09 | mean daily mean air temperatures of the driest quarter | °C/10 | 0.249327 | 0.61755 |
| bio10 | mean daily mean air temperatures of the warmest quarter | °C/10 | 4.681801 | **0.030484** |
| bio11 | mean daily mean air temperatures of the coldest quarter | °C/10 | 6.533385 | **0.010587** |
| bio12 | annual precipitation amount | kg m$^{-2}$ | 0.062332 | 0.802848 |
| bio13 | precipitation amount of the wettest month | kg m$^{-2}$ | 0.197151 | 0.657031 |
| bio14 | precipitation amount of the driest month | kg m$^{-2}$ | 5.198299 | **0.022609** |
| bio15 | precipitation seasonality | kg m$^{-2}$ | 2.972931 | 0.084668 |
| bio16 | mean monthly precipitation amount of the wettest quarter | kg m$^{-2}$ | 0.249231 | 0.617617 |
| bio17 | mean monthly precipitation amount of the driest quarter | kg m$^{-2}$ | 4.102386 | **0.042823** |
| bio18 | mean monthly precipitation amount of the warmest quarter | kg m$^{-2}$ | 0.603309 | 0.437318 |
| bio19 | mean monthly precipitation amount of the coldest quarter | kg m$^{-2}$ | 0.012308 | 0.911664 |
| dtm_east | eastness | index | 8.972308 | **0.002741** |
| dtm_north | northness | index | 0.110769 | 0.73927 |
| dtm_slope | slope | ° | 4.68 | **0.030516** |
| nfd | number of frost days | No days | 9.833654 | **0.001713** |
| sdays | number of snow days | No days | 7.240477 | **0.007128** |
| soil_clay | clay content | % | 6.484245 | **0.010883** |
| soil_org_carb | soil organic carbon content | x 5 g kg$^{-1}$ | 1.563563 | 0.211144 |
| soil_ph_h2o | soil pH x 10 in H2O | pH | 1.238427 | 0.265774 |
| soil_sand | sand content | % | 1.236239 | 0.266197 |
| soil_water | soil water content at 33kPa |  | 0.625926 | 0.428854 |
| srad | surface solar radiation downwards | kJ m$^{-2}$ | 4.68 | **0.030516** |

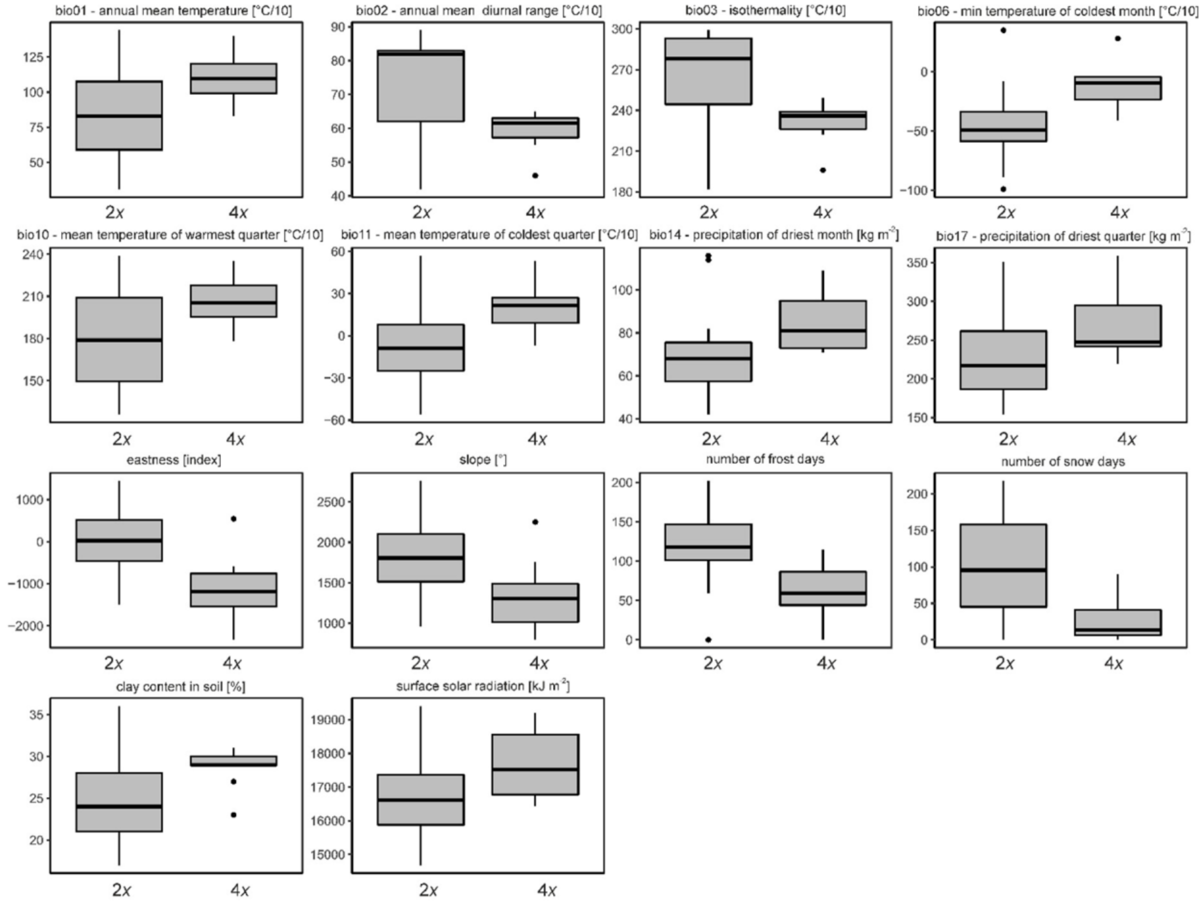


**Figure S2.** Boxplots showing environmental differences along the 14 environmental variables between diploid (2*x*) and tetraploid (4*x*) populations of *Dianthus sylvestris* subsp. *sylvestris*. Only variables with significant differences ($p < 0.05$), as revealed by Kruskal Wallis test (Table S3), are shown.

# Publication IV

CE: LY
QC: RA

*Botanical Journal of the Linnean Society*, 2022, **XX**, 1–13. With 2 figures.

AQ1-AQ4

# Morphological and environmental variability of *Dianthus sylvestris* (Caryophyllaceae) in the Balkan Peninsula

ANA TERLEVIĆ[1], MARTINA TEMUNOVIĆ[2], SANDRO BOGDANOVIĆ[3,4], MARIN GRGUREV[1], IVICA LJUBIČIĆ[3] and IVANA REŠETNIK[1,*]

[1]*Faculty of Science, Department of Biology, University of Zagreb, Trg Marka Marulića 20/II, 10000 Zagreb, Croatia*
[2]*Faculty of Forestry and Wood Technology, Department of Forest Genetics, Dendrology and Botany, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia*
[3]*Faculty of Agriculture, Department of Agricultural Botany, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia*
[4]*Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska cesta 25, 10000 Zagreb, Croatia*

*Received 23 February 2022; revised 23 September 2022; accepted for publication 25 October 2022*

Species with wide ranges often exhibit morphological variability that is mostly shaped by ecological and evolutionary processes, and the resulting diversity is frequently reflected in intraspecific taxonomy. *Dianthus sylvestris* in the Balkan Peninsula encompasses six subspecies (*D. sylvestris* subspp. *alboroseus*, *bertisceus*, *kozjakensis*, *nodosus*, *sylvestris* and *tergestinus*), the identification of which is problematic due to a great morphological variability and complex patterns of environmental variation. To explore morphological variation of described subspecies and to investigate how their morphology varies in relation to environmental variations across their geographical distribution, here we evaluated 97 populations of *D. sylvestris* for variability in 25 morphological characters and 20 environmental variables. Number and shape of epicalyx scales, calyx length and petal denticulation captured the greatest extent of morphological variability between populations. Morphometric analyses revealed a continuous variability in quantitative morphological characters and an absence of clear morphological differentiation between the investigated subspecies. However, two main morphologically distinct entities, the north-western Balkan populations and the south-eastern Balkan populations, were identified and they were split approximately along the Neretva River valley. In addition, our results indicate that environment, rather than geography, plays a potentially more important role in shaping the morphological variability of this taxon. Overall, our results do not support the recognition of the described subspecies or the currently accepted taxonomic concept, instead pointing to a continuous morphological variability of *D. sylvestris* in the Balkan Peninsula.

ADDITIONAL KEYWORDS: cline – geographical variation – morphometrics – redundancy analysis.

## INTRODUCTION

Plant morphological characteristics are influenced by biotic and abiotic factors, and spatio-temporal variation in environmental variables (such as temperature, precipitation, soil) can have profound effects on the adaptation of phenotypic traits to local environments. Morphological and ecological characteristics are both often distributed continuously across geographical gradients (Endler, 1977) and studies across elevation gradients provide evidence of how environmental variation can be one of the drivers behind morphological variability. For example, in many species, reduction in plant height and decrease in specific leaf area with increasing elevation are observed (Scheepens, Frei & Stöcklin, 2010; Wright *et al.*, 2017; Halbritter *et al.*, 2018), and the use of different strategies for pollination and diaspore dispersal along elevation gradients has also been recorded (Pellissier *et al.*, 2010).

Species occupying large areas and diverse habitats exhibit morphological variability that is often reflected in taxonomy, and groups of populations differing from

---

[*] Corresponding author. E-mail: ivana.resetnik@biol.pmf.hr

one another in minor morphological characters, usually occupying distinct geographical areas and being ecologically isolated or phylogenetically separated, are often defined as subspecies (Meikle, 1957; Hamilton & Reichard, 1992). The delimitation of taxonomic categories is not straightforward and requires robust evidence from integrative approaches. However, the main criteria used to circumscribe organisms traditionally are based on quantitative and qualitative morphological characters. Comprehensive evaluation of morphological variability can show that differences among taxa could be related to the combined effect of ecological variables and might not have taxonomic value, as seen in the *Globularia cordifolia* L. species complex (Innangi *et al.*, 2020), or that similar environmental factors can lead to converging leaf morphologies, as observed in *Ternstroemia lineata* DC. species complex (Alcántara-Ayala *et al.*, 2020). Therefore, morphological distinctiveness may not always be a reliable guide to specific or intraspecific classification (Caković *et al.*, 2015; Španiel, Zozomová-Lihová & Marhold, 2017; Buitrago Aristizábal, Oliveira Gouvêa De Figueiredo & André, 2020), and long-standing taxonomic uncertainties may hamper the understanding of species evolutionary history, floristic investigations and conservation actions (Lega *et al.*, 2012; Theodoridis, Nogués-Bravo & Conti, 2019; Ji *et al.*, 2020).

*Dianthus* L. (carnations) is one of the largest genera of Caryophyllaceae with > 300 species native to Eurasia and Africa (Pax & Hoffmann, 1934; Bittrich, 1993; Fassou *et al.*, 2022). Most species have small and geographically restricted distributions, and there are > 70 endemic species in Europe, with the Mediterranean region as the centre of diversity (Bittrich, 1993). The outstanding diversity of the genus originated from a rapid radiation with the fastest rate of speciation known in flowering plants, the onset of which was dated to late Pliocene and probably continued through the Pleistocene (Valente, Savolainen & Vargas, 2010). Rapid diversification is reflected in a complex record of names, diverse taxonomic concepts and a lack of reliable distributional data of individual species, rendering *Dianthus* one of the taxonomically most challenging genera in the European flora (Tutin & Walters, 1993; Brullo & Guarino, 2019).

A detected increase in diversification rates of carnations coincided with increased aridity and seasonality in the Pleistocene, suggesting a link between climate and biodiversity (Valente *et al.*, 2010). Studies associating environmental variations with intraspecific differentiation in *Dianthus* indicated high morphological, environmental and genetic overlaps hampering straightforward intraspecific classification. For example, testing the ecological differentiation of subspecies of *D. superbus* L. based on floral volatile compounds under selection of habitat-specific pollinators showed a lack of phylogenetic support for ecological subspecies, leading the authors to refer to them as ecotypes (Hardion *et al.*, 2020). In the *D. pungens* L. complex a pronounced morpho-environmental overlap, i.e. high redundancy effect, may account for the difficulty of distinguishing subspecies in this taxonomic group, and this was postulated to be a consequence of its recent origin and intense glacial-interglacial fluctuations across the Plio-Pleistocene period (Castro *et al.*, 2021).

*Dianthus sylvestris* Wulfen is considered as one of the most challenging groups in the genus, and the statement by Williams (1893) in the 'Monograph of the genus *Dianthus*' that *D. sylvestris* is a 'species with a polymorphic tendency in which it is very difficult to satisfactorily circumscribe the limits of the specific type' is still true today. *Dianthus sylvestris* is distributed in the Mediterranean area (Balkan and Apennine Peninsulas, Sardinia, Sicily, south-eastern France and Corsica, south-eastern Spain, Algeria and Morocco), the Swiss Jura and the Alps (Bernal, Laínz & Munoz Garmendia, 1990; Tutin & Walters, 1993; Bacchetta *et al.*, 2010; Marhold, 2011; Meyer, 2011; Dimopoulos *et al.*, 2013; Tison & Foucault, 2014; Bornand *et al.*, 2018; Julve, 2018). A recent study showed that *D. sylvestris* comprises three evolutionary lineages, the Alpine, the Apennine and the Balkan lineage, and that they diverged *c.* 200–115 Kya, i.e. during the penultimate glacial-interglacial period (Luqman *et al.*, 2022). To capture the observed morphological diversity of populations on the central Apennine Peninsula, Sardinia and Sicily, Bacchetta *et al.* (2010) recognized up to 17 taxa at specific and intraspecific levels, six of which were described as new to science. The nomenclature of these taxa has been discussed, and the name *D. virgineus* L., being the oldest available name for this group of taxa, has been recently proposed by Domina *et al.* (2021a, b).

The present study focuses on *D. sylvestris* in the Balkan Peninsula. According to Greuter, Burdet & Long (1984), Marhold (2011) and Meyer (2011), seven subspecies are recognized from the Balkan Peninsula: *D. sylvestris* subsp. *alboroseus* F.K.Mey, *D. sylvestris* subsp. *bertisceus* Rech.f., *D. sylvestris* subsp. *kozjakensis* Micevski, *D. sylvestris* subsp. *longicaulis* (Ten.) Greuter & Burdet, *D. sylvestris* subsp. *nodosus* (Tausch) Hayek, *D. sylvestris* subsp. *sylvestris* and *D. sylvestris* subsp. *tergestinus* (Rchb.) Hayek. Numerous other intraspecific taxa (subspecies, varieties and forms) that are now neglected or treated as synonyms have been described and proposed by some authors (Beck-Mannagetta, 1909; Trinajstić, 1979b; Micevski, 1990). During the last two centuries, many authors have tried to develop a workable classification of *D. sylvestris* in the Balkan Peninsula (Schlosser &

Vukotinović, 1869; Borbás, 1876; Beck-Mannagetta, 1909; Hayek, 1924; Gjurašin, 1933; Trinajstić, 1979a), but none of these systems seemed to provide an unambiguous identification key.

The distinction between subspecies of *D. sylvestris* is based on geographical distribution and slight morphological differentiation, whereas their ecological preferences have not yet been investigated. In most studies, habitat descriptions are given in the broad sense as rocky and sunny stands, rocky slopes, mountain and xeric meadows, garrigues and forest fringes (Bacchetta *et al.*, 2010). By studying genetic and phenotypic differentiation between populations of *D. sylvestris* growing on serpentine and limestone soils on the Apennines, Gammella (2016) detected a high amount of gene flow between serpentine and limestone populations. Despite the lack of genetic differentiation, several morphological characters were statistically different and persisted independently from the original soil type, suggesting that these differences could have genetic basis (Gammella, 2016). Aside from this study, little is known about the joint effect of abiotic drivers on the distribution of subspecies of *D. sylvestris*.

By incorporating environmental variation, *D. sylvestris* represents a promising model for exploring the population differentiation regarding the relationships between morphological variability and environmental conditions throughout its distribution range. Hence, the aims of our study are to: (1) explore in detail the morphological differentiation of subspecies of *D. sylvestris*, following the current taxonomic concept, and to identify the most discriminatory morphological characters and (2) analyse how morphology varies in relation to environmental variations across the geographical distribution of *D. sylvestris*, to detect whether environment has influenced the morphological differentiation. To address these questions, a comprehensive morphological and environmental dataset was created on the basis of populations from across the morphological and geographical range of these subspecies in the Balkan Peninsula.

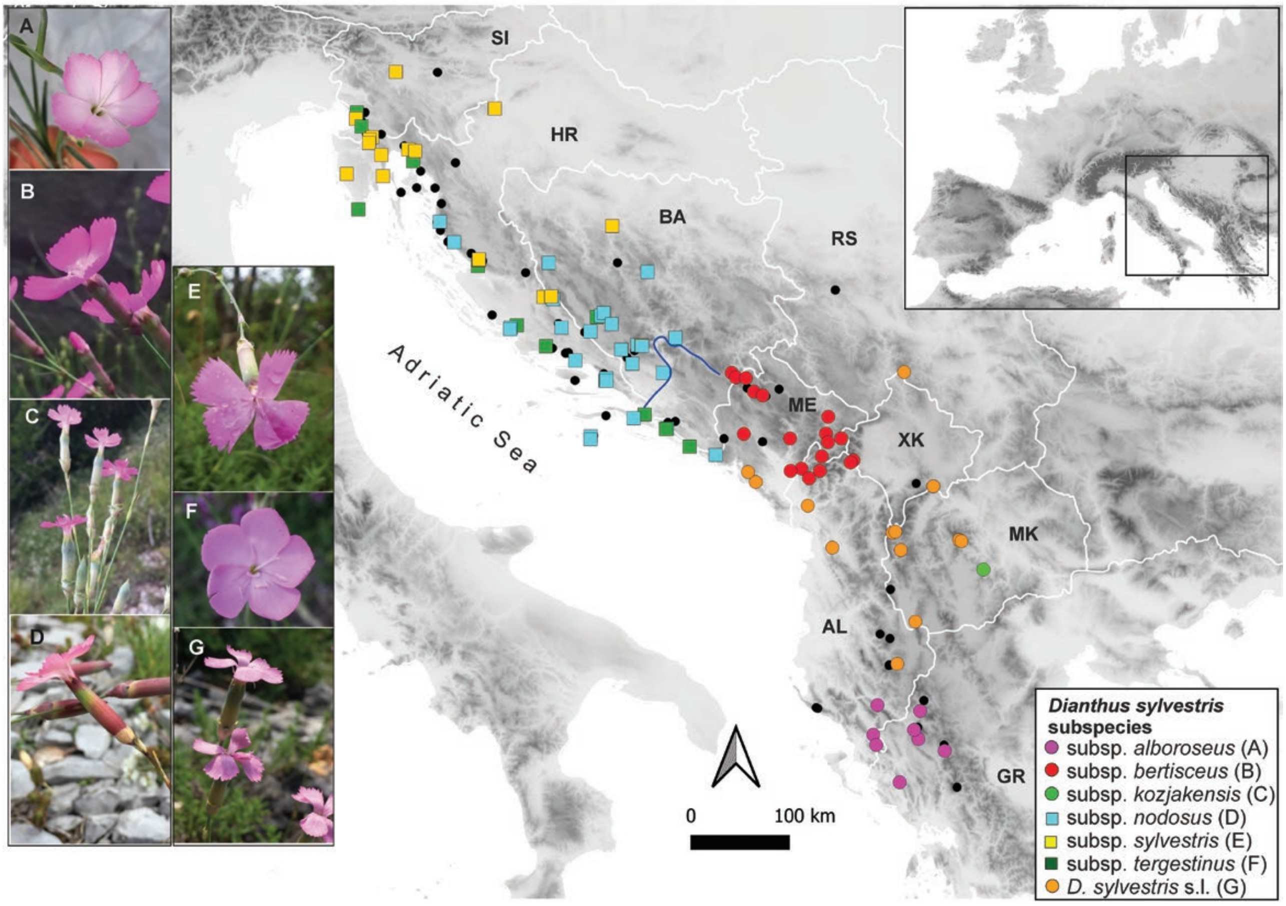
## MATERIAL AND METHODS

### PLANT MATERIAL

*Dianthus sylvestris* is a perennial species with shortly branched woody stocks and dense terminal leaf rosettes from which branches with linear and acute leaves and loose cymose inflorescences with one to 15 flowers grow. The flowers are characterized by two to ten epicalyx scales, abruptly contracted into a short apex and glabrous petals with limb entire to denticulate at the apex (Tutin & Walters, 1993; Bacchetta *et al.*, 2010). The sampling was focused on the area of the Balkan Peninsula where *D. sylvestris* populations have been reported. Information on their distribution was collected from the following sources: (1) herbaria (BEO, BEOU, BP, CAT, CNHM, G, JE, LJU, NHMR, P, PAD, PRC, SARA, TIR, ZA, ZAGR, ZAHO, W and WU; acronyms according to Thiers, 2021), (2) published literature sources, including regional floras of the Balkan countries and the Flora Croatica Database (Beck-Mannagetta, 1909; Josifović, 1970; Trinajstić, 1979a; Strid, 1986; Micevski, 1990, 1993; Martinčič, 2007; Meyer, 2011; Dimopoulos *et al.*, 2013; Stešević & Caković, 2013; Vangjeli, 2015; Nikolić, 2020) and (3) the authors' field observations. The plants were identified and assigned to subspecies on the basis of their morphology and geographical distribution, whereas populations that we could not assign to any of the known subspecies were treated as *D. sylvestris s.l.* The names applied to these populations follow Marhold (2011). On the basis of the used identification keys and regional floras (Micevski, 1993; Tutin & Walters, 1993; Martinčič, 2007; Stešević & Caković, 2013; Dimopoulos *et al.*, 2013; Vangjeli, 2015; Barina *et al.*, 2018; Nikolić, 2020), none of the sampled populations was assigned to *D. sylvestris* subsp. *longicaulis*, although this subspecies was recorded for the Balkan Peninsula (Marhold, 2011). Altogether, 183 populations with 774 individuals were sampled from 2018 to 2020, and a subset of 97 populations was used for the detailed morphometric measurements and analysis. Voucher data are presented in the Supporting Information, Table S1, and the geographical origin of the sampled populations is shown in Figure 1. All voucher specimens are deposited at ZA.

### MORPHOLOGICAL VARIABILITY

The morphological analyses were based on 461 individuals from 97 populations and, depending on the population size, usually five flowering plants were collected per population (Fig. 1, Table S1 in the Supporting Information). The analysed morphological data included 8–27 populations from each of the subspecies of *D. sylvestris*, including populations from the type localities whenever possible, except *D. sylvestris* subsp. *kozjakensis* from which a single population from the type locality was available. Four qualitative (two possible states; Fig. S1, Table S2 in the Supporting Information) and 21 quantitative morphological characters were examined for each individual, and seven ratios were derived (Table S3, Supporting Information). Characters were chosen on the basis of features used in regional floras to distinguish subspecies of *D. sylvestris* (Micevski, 1993; Tutin & Walters, 1993; Martinčič, 2007; Vangjeli, 2015), or used as diagnostic characters for subspecies (Hayek, 1924; Rechinger fil., 1935; Micevski, 1990; Meyer, 2011).



AQ5 **Figure 1.** Localities of sampled populations of *Dianthus sylvestris* in the Balkan Peninsula used for characterizing the environmental variability ($n$ = 183 populations, black dots) and a subset for morphometric analyses ($n$ = 97 populations; for details see Table S1 in the Supporting Information). Squares represent populations from the north-western group according to morphometric PCA, whereas circles represent populations from the south-eastern group. The Neretva River is highlighted by a dark blue line. AQ6

Floral parts of one well-developed flower per plant were attached to transparent foil with adhesive tape, scanned on inverted Epson Expression 11000XL Pro A3 scanner and measured using the ImageJ program (Rueden *et al.*, 2017). In some specimens, one or a few structures were not developed, and the missing character states were replaced by imputation with the 'mice' package (Buuren & Groothuis-Oudshoorn, 2011) and the built-in 'pmm' univariate imputation method, which uses predictive mean matching, i.e. information from other variables in the dataset to predict and impute the missing values. All morphometric analyses were conducted in R v.4.0.2 (R Core Team, 2020).

Each of the four qualitative morphological characters had two possible states, and their total counts per subspecies were obtained to test whether differences of observed counts between subspecies are statistically significant. Chi-square tests were performed on individual plants, using the function 'chisq.test'. Quantitative morphological characters were examined using both univariate and multivariate methods. The Kruskal–Wallis test and Dunn's *post hoc* test with Bonferroni adjustment were performed on population averages for all quantitative morphological characters to evaluate statistically significant differences among subspecies. To visualize the variability between subspecies, boxplots of quantitative morphological characters were generated from population averages. Because of the small sample size (only one population known from the *locus classicus*), *D. sylvestris* subsp. *kozjakensis* was not included in the Chi-square, Kruskal–Wallis and Dunn's *post hoc* tests.

Before multivariate analyses, correlation among quantitative characters was tested using Spearman correlation coefficients ('corr.test' function of the 'psych' R package; Revelle, 2022). Correlation coefficients were based on the matrix including all the studied populations to eliminate pairs of highly correlated characters that may distort downstream analyses. In the case of two highly correlated characters ($r \geq 0.85$ | ), we decided to keep the character that displayed the higher statistical significance (smaller *P* value) among the subspecies according to the Kruskal–Wallis test. Principal component analysis (PCA) was performed based on population averages of selected 13 quantitative (Fig. S2, Supporting Information) and two qualitative morphological characters, turned into dummy variables (IND and EPI; a total of 15 morphological characters) to display the morphological variability between populations of subspecies of *D. sylvestris*. PCA was carried out on the correlation matrix using a set of R functions MorphoTools created by Koutecký (2015).

### ENVIRONMENTAL VARIABILITY

We initially obtained 183 occurrences of *D. sylvestris* for characterizing its environmental variability, which included 97 populations used for the morphometric and redundancy analysis (RDA) analyses (Fig. 1). To characterize the environmental variability of *D. sylvestris* populations, we used environmental data from three databases: climate data from the Chelsa database (Karger *et al*., 2017, 2020); soil properties from the SoilGrids (Hengl *et al.*, 2017); and topographic variables from the EarthEnv database (Amatulli *et al*., 2018, 2019). An initial set of 43 environmental variables were evaluated for collinearity with the 'vifstep' function of the 'usdm' R package (Naimi *et al.*, 2014), and the number of variables was reduced to 20 (Table S4, Supporting Information). The 'vifstep' function calculates the variance inflation factor (VIF) for all variables, excludes one with the highest VIF (greater than the threshold set at 10) and repeats the procedure until no variables with VIF greater than threshold remain. The multivariate environmental space was visualized by the first two axes of the PCA on the basis of values of 20 environmental variables.

### RELATIONSHIP OF MORPHOLOGICAL VARIABILITY WITH ENVIRONMENTAL VARIABLES

To explore the relationship between morphological and environmental variability we used RDA (Legendre & Legendre, 2012) implemented in the 'vegan' R package v.2.5–6 (Oksanen *et al.*, 2018). The 20 environmental variables previously selected for characterizing the environmental variability of populations of *D. sylvestris* (standardized to a zero mean and unit variance), and the geographical coordinates of populations (latitude and longitude) were used as explanatory matrices, and Hellinger-transformed population averages of the morphological characters measurements were used as a response matrix.

To disentangle the unique effects of environment and geography and their joint effects on the variation of morphological characters, we performed three RDA models: (1) the full RDA model with both environmental and geographical variables as predictors; (2) partial RDA in which environmental effects were conditioned on the effects of geography (to obtain the unique effect of the environment) and (3) partial RDA in which geography was conditioned on the effects of environment (to obtain the unique effect of the geography). The significance of the RDA models was assessed using ANOVA with 999 permutations as implemented in 'vegan'. Finally, to identify which environmental and/or geographical variables best explain the overall morphological variability of our populations, we applied a forward selection procedure with the double-stopping criterion (Blanchet, Legendre, & Borcard, 2008) from the 'packfor' R package (Dray, 2013). The variables selected by this procedure as significant were used for performing an additional RDA model (reduced RDA model). We also conducted linear Spearman correlation analyses between the 15 morphological characters used in PCA and the 20 selected environmental variables, with Bonferroni adjustment for multiple tests. Latitude and longitude, representing geographical variables, were also included to detect patterns of morphological variability through latitudinal and longitudinal gradients.

## RESULTS

### MORPHOLOGICAL VARIABILITY

Qualitative morphological characters were analysed on individual plants (Fig. S1, Supporting Information) and the statistically significant differences between the subspecies are shown in Table S2 (Supporting Information). *Dianthus sylvestris* subsp. *bertisceus* showed a statistically significant difference from other subspecies in the arrangement of epicalyx scales (EPI), whereas petal denticulation (DEN) and stem indumentum coverage (IND) had the highest Chi-square values for *D. sylvestris* subsp. *tergestinus* and subsp. *nodosus*, respectively. The range of values of quantitative morphometric characters mostly overlapped between subspecies (Fig. S2, Supporting Information). However, according to the Kruskal–Wallis test (Table S3, Supporting Information), 23 out of 28 investigated characters proved to be significantly different ($P < 0.01$) between the subspecies. Dunn's pairwise *post hoc* tests revealed that, for these 23 characters, at least one pair of subspecies showed a significant difference. Shape of epicalyx scale (RIS, RISA, ISAL, ISW), ratio of petal teeth length and petal limb length (RDENT) and ratio of calyx teeth

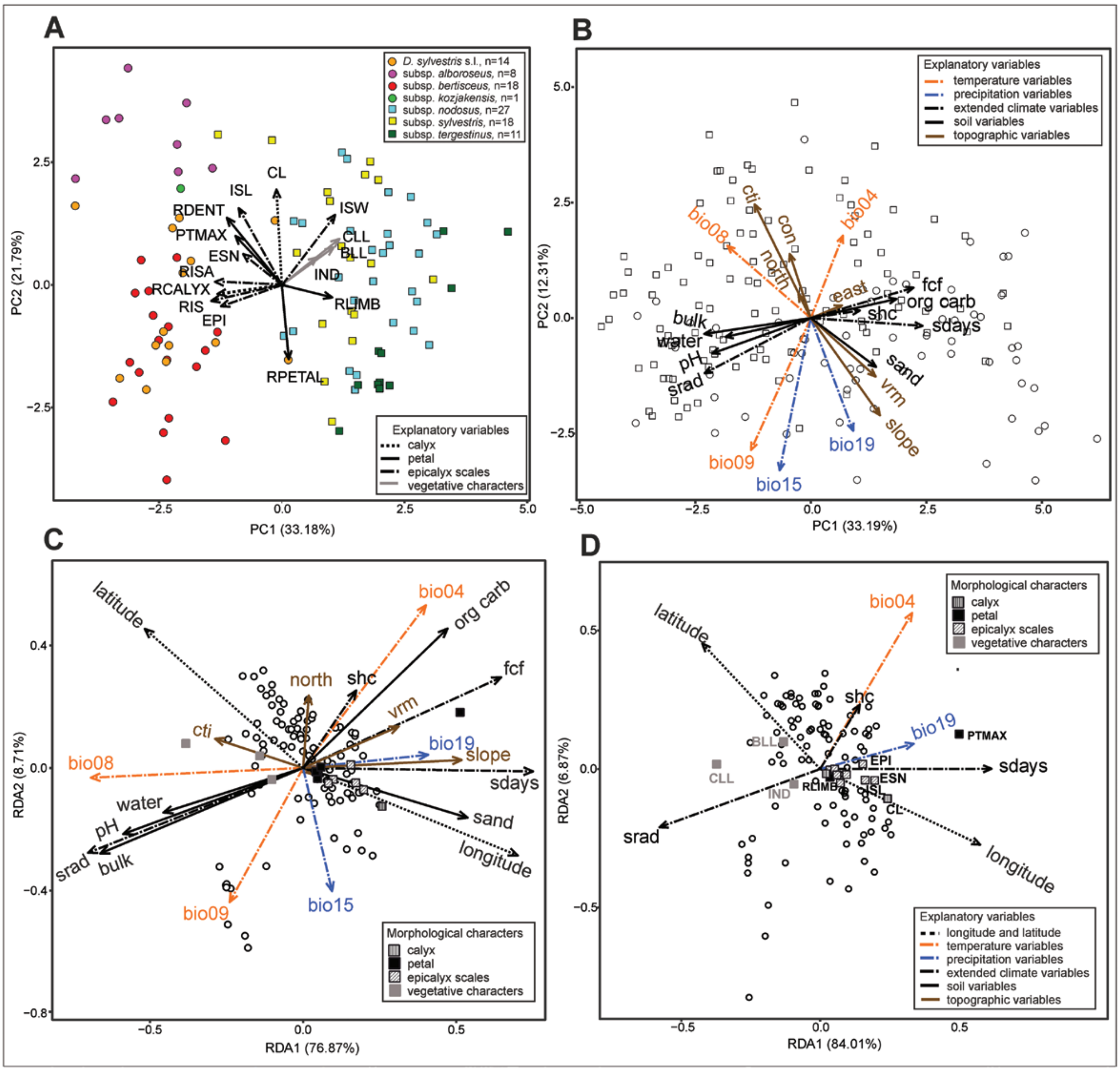


**Figure 2.** A, Principal component analysis (PCA) based on 15 morphological characters examined on 97 populations of *Dianthus sylvestris,* represented by the first two principal components; taxonomic identification at the intraspecific level, based on regional keys and floras, is projected using differently coloured symbols. B, Environmental variability of 183 populations of *D. sylvestris* along the first two axes of the PCA based on 20 environmental variables. Symbols are according to Figure 1. C, Redundancy analysis (RDA) plot of the full RDA model including 20 environmental and two geographical variables (explanatory matrices) in relation to the variability of 15 measured morphological characters (response matrix) examined on 97 populations. D, RDA plot of the reduced RDA model including only the seven variables selected by the forward selection procedure.

length and calyx length (RCALYX) were the six most statistically significant characters (Table S3, Supporting Information).

The PCA was performed on a selection of 15 morphological characters, including two qualitative, five continuous, six ratios and two meristic (Fig. 2A, Fig. S2, Supporting Information). When PCA was performed on averaged measurement values of populations, the first principal component (PC1) explained 33.18% of total variation, the second principal component (PC2) explained 21.79% and the third explained 8.97% (PC3). Characters showing the highest correlation with the first axis (0.73–0.80) in the coefficient matrix were RIS, RISA and RCALYX (listed in decreasing component score value). Characters that showed the highest correlation with the second axis (component scores ranging from 0.69 to 0.88) were CL, ISL and RPETAL. When the populations were considered in two-dimensional morpho-space, a separation of southern Balkan populations and northern Balkan populations along PC1 and a vast amount of overlap along PC2 were detected (Fig. 2A). The gradual change in epicalyx scale shape can be seen in Supplementary Figure S3 (in the Supporting Information). The morphometric PCA plot (Fig. 2A), showed that RIS and RISA had the highest correlation with PC1, separating populations from the northern Balkan Peninsula with more elliptical scales and populations from the southern Balkan Peninsula with scales longer than wide.

### ENVIRONMENTAL VARIABILITY

The first principal component (PC1, Fig. 2B) explained 33.19% of the environmental variability in *D. sylvestris s.l.* It mainly reflected the number of snow days per year (sdays), soil bulk density (soil bulk, indicating the size, shape and arrangement of particles and voids) and solar radiation in the study area (Supporting Information, Table S4; 44, B). PC2 explained 12.31% of the environmental variability corresponding to precipitation seasonality (bio15), the mean daily air temperatures of the driest quarter (bio09), the compound topographic index (cti, a metric of potential ground wetness that is considered steady state) and the mean monthly precipitation of the coldest quarter (bio19; Table S4, Supporting Information; Fig. 2B).

### RELATIONSHIP BETWEEN MORPHOLOGICAL VARIABILITY, ENVIRONMENT AND GEOGRAPHY

The full RDA model with 20 environmental and two geographical variables as predictors was significant ($F = 5.015$, $P = 0.001$) and explained 47.9% of the morphological variation ($R^2_{adj}$, Table 1). Only the first RDA axis was significant ($F = 92.84$, $P < 0.001$), and the first two RDA axes accounted for 76.87 and 8.71% of the explained variance (Fig. 2C). Environmental variation independent of geography explained 39.3% of the morphological variation, 8.7% of the variance was explained only by geography holding the environment constant (unique effect of geography, Table 1) and the interaction between environmental and geographical variables accounted for 17.8% of the variance. The most important environmental and geographical variables explaining the morphological variation of our populations were the number of sdays, longitude and temperature seasonality (bio04), as selected by the forward selection procedure (Table S5, Supporting Information). The first two axes of the reduced RDA model based on seven selected variables from Table S5 (Supporting Information) explained 84.01 and 6.87% of the morphological variation (Fig. 2D). None of the considered soil or topographic variables seemed to significantly affect the overall morphological variation in investigated populations of *D. sylvestris* (Fig. 2C, D).

We conducted linear Spearman correlations between 15 morphological characters used in PCA, 20 environmental and two geographical variables. Longitude, latitude, temperatures of the wettest quarter (bio08) and number of sdays were significantly correlated ($r > |0.60|$, $P < 0.05$) with four morphological characters (RIS, RISA, RCALYX, cauline leaf length (CLL); Fig. S4, Supporting Information) after applying a Bonferroni correction for multiple comparisons.

## DISCUSSION

### MORPHOLOGICAL CLINE HINDERS TAXONOMICAL DELINEATION

The first extensive morphometric study of *D. sylvestris* in the Balkan Peninsula presented here showed extensive overlap in most of the morphological characters of investigated subspecies and a lack of support for current taxonomic intraspecific delineation. Our results indicate two morphologically distinct entities of *D. sylvestris* as seen in PCA ordination space (Fig. 2A). The split between these two groups corresponds to the split between allopatric north-western Balkan populations (including *D. sylvestris* subspp. *nodosus*, *sylvestris* and *tergestinus*) and south-eastern Balkan populations (including *D. sylvestris* subspp. *alboroseus, bertisceus*, *kozjakensis*

**Table 1.** Summary of RDA and partial RDA (pRDA) analyses. Significant *P* values are shown in bold

| Model | Predictors/effect | $R^2$ | $R^2_{adj}$ | *F* | *P* |
|---|---|---|---|---|---|
| $RDA_{full}$ | Environment + Geography | 0.599 | 0.479 | 5.015 | **0.001** |
| $pRDA_{env}$ | Environment \| Geography | 0.319 | 0.215 | 2.9399 | **0.001** |
| $pRDA_{geo}$ | Geography \| Environment | 0.079 | 0.087 | 7.3123 | **0.001** |
| $RDA_{env}$ | Environment | 0.519 | 0.393 | 4.104 | **0.001** |

and *D. sylvestris s.l.*) roughly along the Neretva valley in southern Dalmatia (Croatia, Fig. 1). Although the observed split occurs south of Neretva in the Boka Kotorska region, the populations between Neretva and Boka Kotorska are morphologically intermediate and resemble *D. sylvestris* subsp. *nodosus* and *D. sylvestris* subsp. *bertisceus*. The area adjacent to the present Neretva River valley has been identified as a lineage border in a number of genetic and morphometric studies (Surina, Schönswetter & Schneeweiss, 2011; Mereďa *et al.*, 2011; Falch, Schönswetter & Frajman, 2019; Đurović *et al.*, 2021; Reich *et al.*, 2022). Similar patterns of morphologically hybrid populations in southern Croatia were found in the *Cardamine maritima* DC. group (Kučera, Marhold & Lihová, 2010), whereas molecular and morphological data of the *Campanula pyramidalis* L. complex suggested that individuals occurring south of the Neretva River valley form a distinct entity and only partly overlap with individuals from the northern Adriatic (Lakušić *et al.*, 2013). Likewise, morphometric analysis of the *Alyssum montanum* L. - *A. repens* Baumg. complex revealed this area in southern Croatia to be the border between distribution areas of the closely related *A. austrodalmaticum* Trinajstić and *A. montenegrinum* (Bald.) Španiel, Lihová & Marhold, which are morphologically divergent in several characteristics (Španiel *et al.*, 2017). Lakušić *et al.* (2013) posited that this pattern of splits observed in several plant taxa may mark a border between strongly different ecological conditions due to the Adriatic Sea oscillation in a north–south direction at the time of the last glacial maximum. On the other hand, a recent study of three taxa of *Cerastium* L. from the Dinaric Alps suggested that the deep Neretva valley may have acted as a physical barrier for some plant taxa, rather than an ecological one (Đurović *et al.*, 2021).

Several morphological characters reported in floras (Tutin & Walters, 1993) as diagnostic for subspecies of *D. sylvestris* were considerably variable in our investigated populations. As seen from the results in Table S3 (Supporting Information), most significantly different morphological characters describe the flower, i.e. the reproductive parts of the plant, whereas vegetative characters were less informative. However, in the study by Terlević *et al.* (2022) differences in vegetative characters were found between diploid and tetraploid plants of *D. sylvestris* subsp. *sylvestris,* with tetraploid plants being taller and having longer internodes and leaves.

Mayer & Trpin (1965) identified stem indumentum coverage, number and shape of epicalyx scales, length of calyx, shape of calyx teeth and shape of petals as the most important morphological characters used for taxonomic treatment. They are used in identification keys to distinguish typical *D. sylvestris* from *D. sylvestris* subsp. *nodosus* (Mayer & Trpin, 1965), but the results of our morphometric analyses showed that character values often overlap, and plant identification turned out to be difficult and ambiguous. For example, the proportion of individual plants of *D. sylvestris* subsp. *nodosus* having puberulent stem indumentum is equal to the proportion of plants having glabrous stems (Fig. S1, Supporting Information), thus revealing variability of indumentum coverage within a population and thus questioning the taxonomic usefulness of this character. Although the sizes and shapes of calyx (CL, RCALYX), epicalyx scales (ISL, RIS, RISA) and petals (RPETAL; Fig. 2A) captured the greatest extent of morphological variability among populations, they should not be considered as taxonomically reliable, as noted previously by Mayer & Trpin (1965). Thus, *D. sylvestris* subsp. *nodosus* and subsp. *sylvestris* have similar morphologies and cannot be clearly differentiated. *Dianthus sylvestris* subsp. *bertisceus* can be distinguished from other Balkan subspecies by its divaricated epicalyx scales. However, this character is not discriminatory for this subspecies when populations from the European Alps are considered, as they also have divaricated epicalyx scales (authors' personal observations).

The lack of petal denticulation and usually only one pair of epicalyx scales are the morphological characters frequently used in identification keys to distinguish *D. sylvestris* subsp. *tergestinus* from other subspecies that usually have denticulate petals and two pairs of epicalyx scales (Trinajstić, 1979a; Tutin & Walters, 1993; Vangjeli, 2015). Thus, our results partially confirm that these two diagnostic characters can be used for delimitation of *D. sylvestris* subsp. *tergestinus*. Trinajstić (1979a, b) treated *D. sylvestris* subsp. *tergestinus* as a species and he singled out the populations from the island of Lastovo (Croatia) as a separate endemic taxon *D. tergestinus* (Rchb.) Kerner subsp. *lastovoënsis* Trinajstić. Unlike typical *D. tergestinus*, *D. tergestinus* subsp. *lastovoënsis* has four epicalyx scales (Trinajstić, 1979a), and the plants we collected in early June 2019 from the *locus classicus* had puberulent lower internodes and had just started flowering. These characters led us to identify those plants as *D. sylvestris* subsp. *nodosus*. The populations from Lastovo morphologically resemble *D. sylvestris* subsp. *nodosus* by having slightly smaller petals and two pairs of epicalyx scales, whereas they share the similarity with *D. sylvestris* subsp. *tergestinus* by having entire petal limbs, partly justifying their inclusion under *D. tergestinus* by Trinajstić (1979a).

Although it has not been emphasized in any floras or keys, during our detailed field sampling we observed the flowering time shift between *D. sylvestris* subsp. *tergestinus* on the one hand and *D. sylvestris* subspp. *sylvestris* and *nodosus* on the other. All populations of

*D. sylvestris* subsp. *tergestinus* flowered first from late April to late May and had no overlap in flowering time with populations of *D. sylvestris* subsp. *sylvestris* or subsp. *nodosus* which flowered from late June to late July. Although the variance in flowering time appears to be conserved among plants such that closely related species tend to flower at similar times (Davies *et al.*, 2013), the slightly different flowering time of *D. sylvestris* subsp. *tergestinus* may therefore be indicative of its phylogenetic divergence from *D. sylvestris*. Furthermore, the identification of *D. sylvestris* subsp. *tergestinus* based on the flowering time has been proven to be correct on the basis of preliminary genetic results in which plants identified as *D. sylvestris* subsp. *tergestinus* form a separate lineage (Temunović *et al.*, unpubl. data). Our results thus indicate that *D. sylvestris* subsp. *tergestinus* is a sympatric subspecies with *D. sylvestris* subsp. *sylvestris* and subsp. *nodosus*, differentiated by its flowering time.

### MORPHOLOGICAL VARIABILITY CORRELATES WITH ENVIRONMENTAL VARIABILITY

Bacchetta *et al.* (2010) provided notes on ecology for species/populations belonging to *D. sylvestris* in central and southern Italy, Sicily and Sardinia, but there are no such data describing ecological preferences of subspecies/populations in the Balkan Peninsula. To fill this knowledge gap, we examined the environmental preferences of the Balkan populations and investigated the relationship between morphological variability, environment and geography. Our RDA results indicate that environment plays a potentially more important role in the morphological variability in *D. sylvestris* than geography (Table 1). Number of sdays and temperature seasonality (bio04) turned out to be the two environmental variables that explained most morphological variability (Fig. 2D, Table S5, Supporting Information). *Dianthus sylvestris* occurs from Mediterranean rocky grasslands to high-Alpine habitats, including changes in elevation and its associated shifts in these two variables. Our results showed shorter leaves, and consequently smaller plant biomass, to be common in environments with longer snow cover (Fig. S4B, Supporting Information), which is consistent with previous evidence for the smaller size of Alpine plants compared to lowland plants (Körner, 2003). Declining plant biomass with elevation might result from selection for slower growth, which provides the advantage of warmer microclimatic conditions closer to the ground and protection from wind (Körner, 2003). Additionally, time of flowering is dependent on the length of the season and thus correlated with the duration of snow cover (Halbritter *et al.*, 2018). We hypothesize that the number of sdays encompasses the morphological variability from the thermophilic *D. sylvestris* subsp. *tergestinus* at the one extreme to the Alpine *D. sylvestris* subsp. *bertisceus* on the other end. This is also reflected in phenology, with *D. sylvestris* subsp. *tergestinus* flowering first in May, whereas Alpine populations of *D. sylvestris* subsp. *bertisceus* flower later in July. Similar results were also reported in the study of Neuffer & Hurka (1986) in which populations of *Capsella bursa-pastoris* (L.) Medik. from high elevations generally flowered later than those from lower elevations. The morphological characters describing less stem indumentum (IND, glabrous or puberulent) showed correspondence with the duration of snow coverage. Pubescence in Alpine flora is quite rare (Körner, 2003), in concordance with our result of negative correlation with the number of sdays (Fig. 2C, D). The observed positive correlation with solar radiation (Fig. 2C, D) may be associated with buffering of short-term oscillations of ambient humidity and water saving strategies in drier environments (Körner, 2003) or protection against excess radiation over photosynthetic tissues (Manetas, 2003). Besides these observed correlations, we did not find any clear evidence that would support puberulent indumentum to be taxonomically relevant for any of the subspecies, and thus it may rather represent a structure influenced by environment and/or geography.

Some climatic variables follow latitudinal and/or longitudinal gradients, and hence morphological characters can show patterns of latitudinal and longitudinal variation (Alcántara-Ayala *et al.*, 2020). In our study, longitude was also a major geographical predictor associated with morphological variability (Fig. 2C, D), indicating an effect of geographical isolation. Slope, number of sdays and soil sand content increase eastwards, i.e. from the Adriatic coast towards the Dinaric Alps, whereas mean daily air temperatures of the winter-wettest quarter (bio08) decrease. Thus, the contribution of longitude to morphological variability of investigated populations of *D. sylvestris* might reflect the gradual transition from the coastal Mediterranean environment to the Alpine environment, which is in turn reflected mainly in the CLL and shape of epicalyx scales (RIS and RISA). Specifically, moving eastwards (longitude) calyces have longer teeth, epicalyx scales are less elliptical (ovate to obovate) and have longer apices and cauline and basal leaves are shorter, whereas moving northwards (latitude) calyces have shorter teeth and epicalyx scales are more elliptical (Fig. S3, Supporting Information). Similar morpho-environmental continuity along the eastern Adriatic coast can be observed in *Dianthus ciliatus* Guss. and its three subspecies, with populations identified as *D. ciliatus* subsp. *ciliatus* occurring in northern parts of the distribution area having entire petals and slightly wider scales and populations identified as *D. ciliatus* subspp. *dalmaticus* (Čelak.) Hayek and *medunensis* occurring towards the south having denticulate petals and narrower scales (Tokić, 2022). These examples of subtle morphological modifications combined with uninterrupted distributions reveal the continuous combination of morpho-environmental strategies in spatially heterogenous and temporally dynamic landscapes occurring in young diverse groups.

## CONCLUSIONS

Our morphometric analyses revealed a continuous variability in quantitative morphological characters in *D. sylvestris* and an absence of clear-cut qualitative morphological differences between the subspecies in the Balkan Peninsula. The morphological variation gradually changes with environment and geography, markedly with climate and longitude. Our results do not lend support to the recognition of most of these subspecies or to the current generally accepted taxonomic concept. As an extension to this study and as an integrative approach, molecular analyses of these populations are under way. Until we have access to these data, and due to our desire to make stable taxonomic decisions, we refrain from making comprehensive taxonomic changes at this point.

## ACKNOWLEDGEMENTS

This work was supported by the Croatian Science Foundation under the project number UIP-2017-05-2882 (AmphiAdriPlant). We are grateful to all colleagues who helped us collect samples across the Balkan Peninsula (Božo Frajman, Peter Schönswetter, Dmitar Lakušić, Sanja Đurović and Nevena Kuzmanović), the Ministry of Environment and Nature Protection of Croatia and National Parks chiefs (Triglav National Park) who provided permission to collect plants in protected areas and Lulëzim Shuka for helping us with part of the fieldwork in Albania. We are also grateful to all herbarium curators for consulting plant materials in their collections. This paper is part of Ana Terlević's PhD thesis, with the work supported by the 'Young Researchers' career development project—training of doctoral students' of the Croatian Science Foundation funded by the European Union from the European Social Fund.

AQ8

## DATA AVAILABILITY

The data underlying this article are available in [repository name, e.g. the GenBank Nucleotide Database] at [URL], and can be accessed with [unique identifier, e.g. accession number, deposition number].

## REFERENCES

AQ9

**Alcántara-Ayala O**, **Oyama K**, **Ríos-Muñoz CA**, **Rivas G**, **Ramirez-Barahona S**, **Luna-Vega I. 2020**. Morphological variation of leaf traits in the *Ternstroemia lineata* species complex (Ericales: Pentaphylacaceae) in response to geographic and climatic variation. *PeerJ* **8**: e8307.

**Amatulli G**, **Domisch S**, **Tuanmu MN**, **Parmentier B**, **Ranipeta A**, **Malczyk J**, **Jetz W. 2018**. A suite of global, cross-scale topographic variables for environmental and biodiversity modeling. *Scientific Data* **5**: 180040.

**Amatulli G**, **McInerney D**, **Sethi T**, **Strobl P**, **Domisch S. 2019**. *Geomorpho90m - global high-resolution geomorphometry layers: empirical evaluation and accuracy assessment*. Preprint *PeerJ Preprints*. Preprint: not peer reviewed.

AQ10

**Bacchetta G**, **Brullo S**, **Casti M**, **Pietro Giusso del Galdo G. 2010**. Taxonomic revision of the *Dianthus sylvestris* group (Caryophyllaceae) in central-southern Italy, Sicily and Sardinia. *Nordic Journal of Botany* **28**: 137–173.

**Barina Z**, **Somogyi G**, **Pifkó D**, **Rakaj M. 2018**. Checklist of vascular plants of Albania. *Phytotaxa* **378**: 1.

**Beck-Mannagetta G. 1909**. *Flora Bosne, Hercegovine i Novopazarskog Sandžaka*. Sarajevo: Glasnik Zemaljskog muzeja u Bosni i Hercegovini.

**Bernal M**, **Laínz M**, **Munoz Garmendia F. 1990**. *Dianthus* L. In: Castroviejo Bolibar S, ed. *Flora Iberica*. Madrid: Real Jardín Botánico, C.S.I.C., 426–462.

**Bittrich V. 1993**. Caryophyllaceae. In: Kubitzki K, Rohwer JG, eds, *Flowering plants, dicotyledons: magnoliid, hamamelid and caryophyllid families*. Berlin, London: Springer, 206–236.

**Blanchet FG**, **Legendre P**, **Borcard D. 2008**. Forward selection of explanatory variables. *Ecology* **89**: 2623–2632.

**Borbás V. 1876**. *Symbolae ad 'Caryophylleas' et 'Melanthaceas' florae Croaticae*. Zagreb: Tisak Dioničke Tiskare.

**Bornand C**, **Juillerat P**, **Jutzi M**, **Nyffeler R**, **Santiago H. 2018**. *Flora Helvetica - Exkursionsführer*. Bern: Haupt Verlag.

**Brullo S**, **Guarino R. 2019**. Dianthus. In: Pignatti S, ed. *Flora d'Italia*. *Vol.* **4**. Milan: Edagricole.

**Buitrago Aristizábal MA**, **Oliveira Gouvêa De Figueiredo F**, **André T. 2020**. Accommodating trait overlap and individual variability in species diagnosis of *Ischnosiphon* (Marantaceae). *Botanical Journal of the Linnean Society* **194**: 469–479.

**van Buuren S**, **Groothuis-Oudshoorn K. 2011**. mice: multivariate imputation by chained equations in R. *Journal of Statistical Software* **45**: 1–67.

AQ11

**Caković D**, **Stešević D**, **Schönswetter P**, **Frajman B. 2015**. How many taxa? Spatiotemporal evolution and taxonomy of *Amphoricarpos* (Asteraceae, Carduoideae) on the Balkan Peninsula. *Organisms Diversity & Evolution* **15**: 429–445.

**Castro I**, **Rocha J**, **Martins M**, **Carnide V**, **Martín JP**, **Veiga P**, **Serafim AB**, **Amich F**, **Ramírez-Rodríguez R**, **Colombo G**, **Crespí AL. 2021**. The redundancy effect under morphogenetic and environmental fluctuations. The case of the *Dianthus pungens* group. *Plant Biosystems*

*- An International Journal Dealing with all Aspects of Plant* AQ12 *Biology*: 1–15.

**Davies TJ, Wolkovich EM, Kraft NJB, Salamin N, Allen JM, Ault TR, Betancourt JL, Bolmgren K, Cleland EE, Cook BI, Crimmins TM, Mazer SJ, McCabe GJ, Pau S, Regetz J, Schwartz MD, Travers SE. 2013**. Phylogenetic conservatism in plant phenology. *Journal of Ecology* **101**: 1520–1530.

**Dimopoulos P, Raus T, Bergmeier E, Constantinidis T, Iatrou G, Kokkini S, Strid A, Tzanoudakis D (eds). 2013**. *Vascular plants of Greece: an annotated checklist*. Berlin, Athens: Botanic Garden and Botanical Museum Berlin-Dahlem and Hellenic Botanical Society.

**Domina G, Astuti G, Bacchetta G, Barone G, Rešetnik I, Terlević A, Thiébaut M, Peruzzi L. 2021b**. Typification of 14 names in the *Dianthus virgineus* group (Caryophyllaceae). *PhytoKeys* **187**: 1–14.

**Domina G, Astuti G, Barone G, Gargano D, Minuto L, Varaldo L, Peruzzi L. 2021a**. Lectotypification of the Linnaean name *Dianthus virgineus* (Caryophyllaceae) and its taxonomic consequences. *Taxon* **70**: 1096–1100.

**Dray S. 2013**. *packfor: forward selection with permutation*. https://rdrr.io/rforge/packfor/.

**Đurović SZ, Temunović M, Niketić M, Tomović G, Schönswetter P, Frajman B, Lavergne S. 2021**. Impact of Quaternary climatic oscillations on phylogeographic patterns of three habitat-segregated *Cerastium* taxa endemic to the Dinaric Alps. *Journal of Biogeography* **48**: 2022–2036.

**Endler JA. 1977**. *Geographic variation, speciation and clines*. Princeton: Princeton University Press.

**Falch M, Schönswetter P, Frajman B. 2019**. Both vicariance and dispersal have shaped the genetic structure of Eastern Mediterranean *Euphorbia myrsinites* (Euphorbiaceae). *Perspectives in Plant Ecology, Evolution and Systematics* **39**: 125459.

**Fassou G, Korotkova N, Nersesyan A, Koch MA, 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.

**Gammella M. 2016**. Local adaptation and gene flow in serpentine and limestone populations of *D. sylvestris*.

**Gjurašin S. 1933**. *Rod Dianthus u flori Hrvatske i Slavonije*. Zagreb: Prirodoslovna istraživanja Kraljevine Jugoslavije. Jugoslavenska akademija znanosti i umjetnosti.

**Greuter W, Burdet HM, Long G. 1984**. *Med-checklist. 1*. Geneva: Conservatoire et Jardin Botaniques de la Ville de Genève.

**Halbritter AH, Fior S, Keller I, Billeter R, Edwards PJ, Holderegger R, Karrenberg S, Pluess AR, Widmer A, Alexander JM. 2018**. Trait differentiation and adaptation of plants along elevation gradients. *Journal of Evolutionary Biology* **31**: 784–800.

**Hamilton CW, Reichard SH. 1992**. Current practice in the use of subspecies, variety, and forma in the classification of wild plants. *Taxon* **41**: 485–498.

**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. *Systematics and Biodiversity* **18**: 255–268.

**Hayek A von. 1924**. *Prodromus florae peninsulae Balcanicae*. Dahlem bei Berlin: Verlag des Repertoriums.

**Hengl T, Jesus JM de, Heuvelink GBM, Gonzalez MR, Kilibarda M, Blagotić A, Shangguan W, Wright MN, Geng X, Bauer-Marschallinger B, Guevara MA, Vargas R, MacMillan RA, Batjes NH, Leenaars JGB, Ribeiro E, Wheeler I, Mantel S, Kempen B. 2017**. SoilGrids250m: Global gridded soil information based on machine learning. *PLoS ONE* **12**: e0169748.

**Innangi M, Friščić M, Hazler Pilepić K, Danise T, Conti F, Bartolucci F, Fioretto A, Peruzzi L. 2020**. Explaining intricate morphometric variability with environmental predictors: the case of *Globularia cordifolia* species complex. *Plants* **9**: 314.

**Ji Y, Liu C, Yang J, Jin L, Yang Z, Yang JB. 2020**. Ultra-barcoding discovers a cryptic species in *Paris yunnanensis* (Melanthiaceae), a medicinally important plant. *Frontiers in Plant Science* **11**: 411.

**Josifović M. 1970**. *Flora SR Srbije*. Belgrade: Srpska Akademija nauka i umetnosti. Odeljenje prirodno-matematičkih nauka.

**Julve P. 2018**. *Listes départementales des plantes de France. Version 2018.04 du 24 avril 2018. Programme chorologie départementale de Tela Botanica*. https://www.tela-botanica.org/chorologie-departementale/

**Karger DN, Conrad O, Böhner J, Kawohl T, Kreft H, Soria-Auza RW, Zimmermann NE, Linder HP, Kessler M. 2017**. Climatologies at high resolution for the earth's land surface areas. *Scientific Data* **4**: 170122.

**Karger DN, Schmatz DR, Dettling G, Zimmermann NE. 2020**. High-resolution monthly precipitation and temperature time series from 2006 to 2100. *Scientific Data* **7**: 248.

**Körner C. 2003**. *Alpine plant life: functional plant ecology of high mountain ecosystems*. Berlin, Heidelberg: Springer Science & Business Media.

**Koutecký P. 2015**. MorphoTools: a set of R functions for morphometric analysis. *Plant Systematics and Evolution* **301**: 1115–1121.

**Kučera J, Marhold K, Lihová J. 2010**. *Cardamine maritima* group (Brassicaceae) in the amphi-Adriatic area: a hotspot of species diversity revealed by DNA sequences and morphological variation. *Taxon* **59**: 148–164.

**Lakušić D, Niketić M, Rakić T, Stevanović V. 2013**. *Edraianthus canescens* (Campanulaceae), a new species from the Central Balkan peninsula. *Phytotaxa* **118**: 22.

**Lega M, Fior S, Prosser F, Bertolli A, Li M, Varotto C. 2012**. Application of the unified species concept reveals distinct lineages for disjunct endemics of the *Brassica repanda* (Brassicaceae) complex. *Biological Journal of the Linnean Society* **106**: 482–497.

**Legendre P, Legendre L. 2012**. *Numerical ecology*. Amsterdam: Elsevier.

**Luqman H, Wegmann D, Fior S, Widmer A. 2022**. Climate-induced range shifts drive adaptive response via spatio-temporal sorting of alleles. *bioRxiv*: https://doi.org/10.1101/2022.06.07.495159. Preprint: not peer reviewed.

**Manetas Y. 2003**. The importance of being hairy: the adverse effects of hair removal on stem photosynthesis of *Verbascum speciosum* are due to solar UV-B radiation. *The New Phytologist* **158**: 503–508.

**Marhold K. 2011**. Caryophyllaceae. In: *Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity*. https://www.emplantbase.org/home.html.

**Martinčič A. 2007**. *Mala flora Slovenije*. Ljubljana: Tehniška založba Slovenije.

**Mayer E**, **Trpin D. 1965**. Dianthus sylvestris – kompleks v Jugoslaviji. *Biološki Vestnik* **13**: 53–59.

**Meikle RD. 1957**. What is the subspecies? *Taxon* **6**: 102–105.

**Mereďa P**, **Hodálová I**, **Kučera J**, **Zozomová-Lihová J**, **Letz DR**, **Slovák M. 2011**. Genetic and morphological variation in *Viola suavis s.l.* (Violaceae) in the western Balkan Peninsula: two endemic subspecies revealed. *Systematics and Biodiversity* **9**: 211–231.

**Meyer FK. 2011**. *Beiträge zur Flora von Albanien*. Jena: Thüringische Botanische Gesellschaft e.V.

**Micevski K. 1990**. Novitäten in der Gattung *Dianthus* L. (Caryophyllaceae) in der Flora der SR Makedonien. *Macedonian Academy of Sciences and Arts, Section of Biological and Medical Sciences. Macedonian Academy of Sciences and Arts* **8**: 31–46.

**Micevski K. 1993**. *Flora na SR Makedonija*. Skopje: Makedonska akademija na naukite i umetnostite.

**Naimi B**, **Hamm NAS**, **Groen TA**, **Skidmore AK**, **Toxopeus AG. 2014**. Where is positional uncertainty a problem for species distribution modelling? *Ecography* **37**: 191–203.

**Neuffer B**, **Hurka H. 1986**. Variation of development time until flowering in natural populations of *Capsella bursa-pastoris* (Cruciferae). *Plant Systematics and Evolution* **152**: 277–296.

**Nikolić T. 2020**. *Flora Croatica database*. Zagreb: Prirodoslovno-matematički fakultet, Sveučilište u Zagrebu.

**Oksanen J**, **Blanchet FG**, **Friendly M**, **Kindt R**, **Legendre P**, **McGlinn D**, **Minchin PR**, **O'Hara RB**, **Simpson GL**, **Solymos P**, **Stevens MHH**, **Szoecs E**, **Wagner H. 2018**. vegan: community ecology package. https://cran.r-project.org/web/packages/vegan/index.html.

**Pax F**, **Hoffmann K. 1934**. Caryophyllaceae. In: Engler A, Prantl K, eds, *Die natürlichen Pflanzenfamilen II, 16c*. Leipzig: Engelmann, 275–364.

**Pellissier L**, **Fournier B**, **Guisan A**, **Vittoz P. 2010**. Plant traits co-vary with altitude in grasslands and forests in the European Alps. *Plant Ecology* **211**: 351–365.

**R Core Team. 2020**. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

**Rechinger fil. KH. 1935**. *Ergebnisse einer botanischen Reise in den Bertiscus (Nordalbanische Alpen)*. Berlin-Dahlem: Selbstverlag des Herausgebers, Fabeckstrasse 49.

**Reich D**, **Flatscher R**, **Pellegrino G**, **Hülber K**, **Wessely J**, **Gattringer A**, **Greimler J. 2022**. Biogeography of amphi-adriatic *Gentianella crispata* (Gentianaceae): a northern refugium and recent trans-adriatic migration. *Plant Biosystems* **156**: 754–768.

**Revelle W. 2022**. psych: procedures for psychological, psychometric, and personality research. https://CRAN.R-project.org/package=psych.

**Rueden CT**, **Schindelin J**, **Hiner MC**, **DeZonia BE**, **Walter AE**, **Arena ET**, **Eliceiri KW. 2017**. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* **18**: 529.

**Scheepens JF**, **Frei ES**, **Stöcklin J. 2010**. Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. *Oecologia* **164**: 141–150.

**Schlosser JK**, **Vukotinović Lj. 1869**. *Flora Croatica: exhibens stirpes phanerogamas et vasculares cryptogamas quae in Croatia Slavonia et Dalmatia sponte crescunt nec non illas quae frequentissime coluntur*. Zagreb: Župan (Albrecht et Fiedler).

**Španiel S**, **Zozomová-Lihová J**, **Marhold K. 2017**. Revised taxonomic treatment of the *Alyssum montanum-A. repens* complex in the Balkans: a multivariate morphometric analysis. *Plant Systematics and Evolution* **303**: 1413–1442.

**Stešević D**, **Caković D. 2013**. *Katalog vaskularne flore Crne Gore*. Podgorica: Crnogorska akademija nauka i umjetnosti.

**Strid A (ed.). 1986**. *Mountain flora of Greece*. Cambridge, New York: Cambridge University Press.

**Surina B**, **Schönswetter P**, **Schneeweiss GM. 2011**. Quaternary range dynamics of ecologically divergent species (*Edraianthus serpyllifolius* and *E. tenuifolius*, Campanulaceae) within the Balkan refugium: Quaternary range dynamics within the Balkan refugium. *Journal of Biogeography* **38**: 1381–1393.

**Terlević A**, **Bogdanović S**, **Frajman B**, **Rešetnik I. 2022**. Genome size variation in *Dianthus sylvestris* Wulfen *sensu lato* (Caryophyllaceae). *Plants* **11**: 1481.

**Theodoridis S**, **Nogués-Bravo D**, **Conti E. 2019**. The role of cryptic diversity and its environmental correlates in global conservation status assessments: insights from the threatened bird's-eye primrose (*Primula farinosa* L.). *Diversity and Distributions* **25**: 1457–1471.

**Thiers B. 2021**. *Index Herbariorum. A global directory of public herbaria and associated staff*. The Bronx: New York Botanical Garden's Virtual Herbarium. AQ13

**Tison JM**, **Foucault B (eds). 2014**. *Flora Gallica: flore de France*. Mèze: Biotope Éditions.

**Tokić P. 2022**. *Morfološka varijabilnost Dianthus ciliatus kompleksa (Caryophyllaceae) na području Jadrana*. Master's Thesis, University of Zagreb, Faculty of Agriculture.

**Trinajstić I. 1979a**. *Analitička flora Jugoslavije*. Zagreb: Institut za botaniku Sveučilišta u Zagrebu.

**Trinajstić I. 1979b**. Taxa nova et combinationes novae in flora Jugoslaviae VI. In: *Supplementum ad floram analyticam Jugoslaviae*. Zagreb: Editio Universitatis Zagrebiensis - Liber 7.

**Tutin TG**, **Walters SM. 1993**. *Dianthus* L. In: Tutin TG, …. eds, *Flora Europaea*. Cambridge, New York: Cambridge University Press, 227–246.

**Valente LM**, **Savolainen V**, **Vargas P. 2010**. Unparalleled rates of species diversification in Europe. *Proceedings of the Royal Society B: Biological Sciences* **277**: 1489–1496.

**Vangjeli J. 2015**. *Excursion flora of Albania*. Oberreifenberg: Koeltz Scientific Books.

**Williams FN. 1893**. A monograph of the genus *Dianthus*. *Botanical Journal of the Linnean Society* **29**: 346–469.

**Wright IJ**, **Dong N**, **Maire V**, **Prentice IC**, **Westoby M**, **Díaz S**, **Gallagher RV**, **Jacobs BF**, **Kooyman R**, **Law EA**, **Leishman MR**, **Niinemets U**, **Reich PB**, **Sack L**, **Villar R**, **Wang H**, **Wilf P. 2017**. Global climatic drivers of leaf size. *Science* **357**: 917–921.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

**Figure S1.** The number of plant individuals per state of each qualitative character and per subspecies. Abbreviated labels: *alb* – subsp. *alboroseus*, *bert* – subsp. *bertisceus*, *kozj* – subsp. *kozjakensis*, *nod* – subsp. *nodosus, s.l.* – *D. sylvestris* sensu lato, *sylv* – subsp. *sylvestris*, *terg* – subsp. *tergestinus*.

**Figure S2.** Variation in quantitative characters selected for performing PCA. Abbreviated labels: *alb* – subsp. *alboroseus*, *bert* – subsp. *bertisceus*, *kozj* – subsp. *kozjakensis*, *nod* – subsp. *nodosus*, *s.l.* – *D. sylvestris sensu lato*, *sylv* – subsp. *sylvestris*, *terg* – subsp. *tergestinus.* Means not significantly different at $P < 0.01$, according to Dunn's *post hoc* test, are indicated by the same letter.

**Figure S3.** Sampling area with insertions of one epicalyx scale from the first pair (the closest to the calyx) showing the gradual change of epicalyx scale's shape.

**Figure S4.** Significant correlations ($P < 0.05$) exceeding $r = |0.60|$ between morphological characters and two environmental (B and D) and two geographical variables (A and C), according to Spearman's correlation test with Bonferroni adjustment for multiple comparisons.

**Table S1**. Studied populations of the *Dianthus sylvestris s.l.* including number of individuals collected per population, number of measured individuals used in morphometric analyses, taxon name, voucher, locality data, collectors and collection date. The herbarium acronyms are according to Index Herbariorum.

**Table S2.** Chi-square test between each of the 15 pairs for each qualitative morphological character. Significant differences ($P < 0.01$) are shown in bold. Abbreviated labels: *alb* – subsp. *alboroseus*, *bert* – subsp. *bertisceus*, *nod* – subsp. *nodosus*, *s.l.* – *D. sylvestris* sensu lato, sylv – subsp. *sylvestris*, *terg* – subsp. *tergestinus*.

**Table S3**. Quantitative morphological characters studied in *Dianthus sylvestris s.l.* from the Balkan Peninsula. Morphological differences along 21 quantitative morphological characters and seven ratios between the *D. sylvestris* subspecies as revealed by Kruskal–Wallis test. Significant differences ($P < 0.01$) are shown in bold.

**Table S4.** Environmental variables used for characterizing environmental variability of *Dianthus sylvestris* populations using principal component analysis (PCA) and for RDA analysis, with their abbreviated and full names/definitions, units and types. Shown are also factor loadings of PCA of environmental data related to *D. sylvestris* populations.

**Table S5.** Results of a forward selection procedure with double-stopping criterion ($P$ value and $R^2_{\text{adj}}$), on the RDAfull model.

# SUPPLEMENTARY DATA

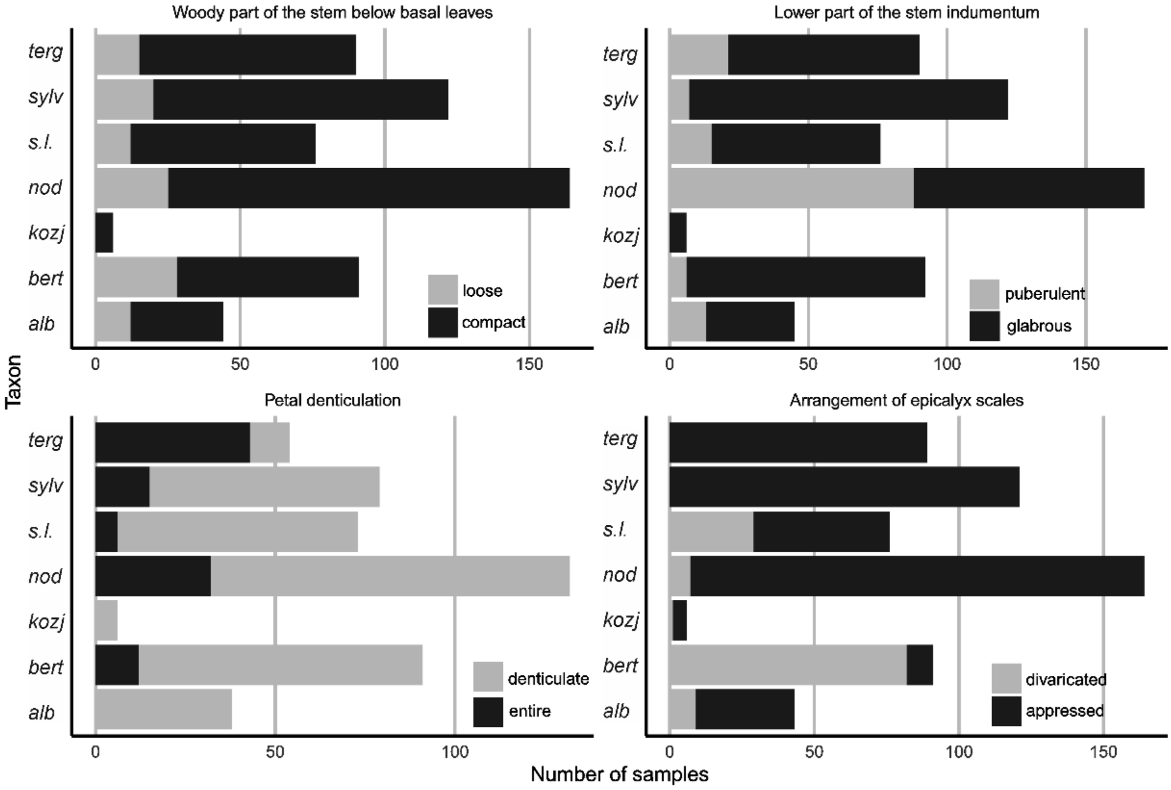


**Figure S1.** Barplots for the four qualitative characters examined on 97 populations of *Dianthus sylvestris* in the Balkan Peninsula, showing the number of plant individuals per character state and per subspecies. Abbreviated labels: *alb* - subsp. *alboroseus*, *bert* - subsp. *bertisceus, kozj* - subsp. *kozjakensis, nod* - subsp. *nodosus, s.l.* - *D. sylvestris* sensu lato, *sylv* - subsp. *sylvestris, terg* - subsp. *tergestinus.*

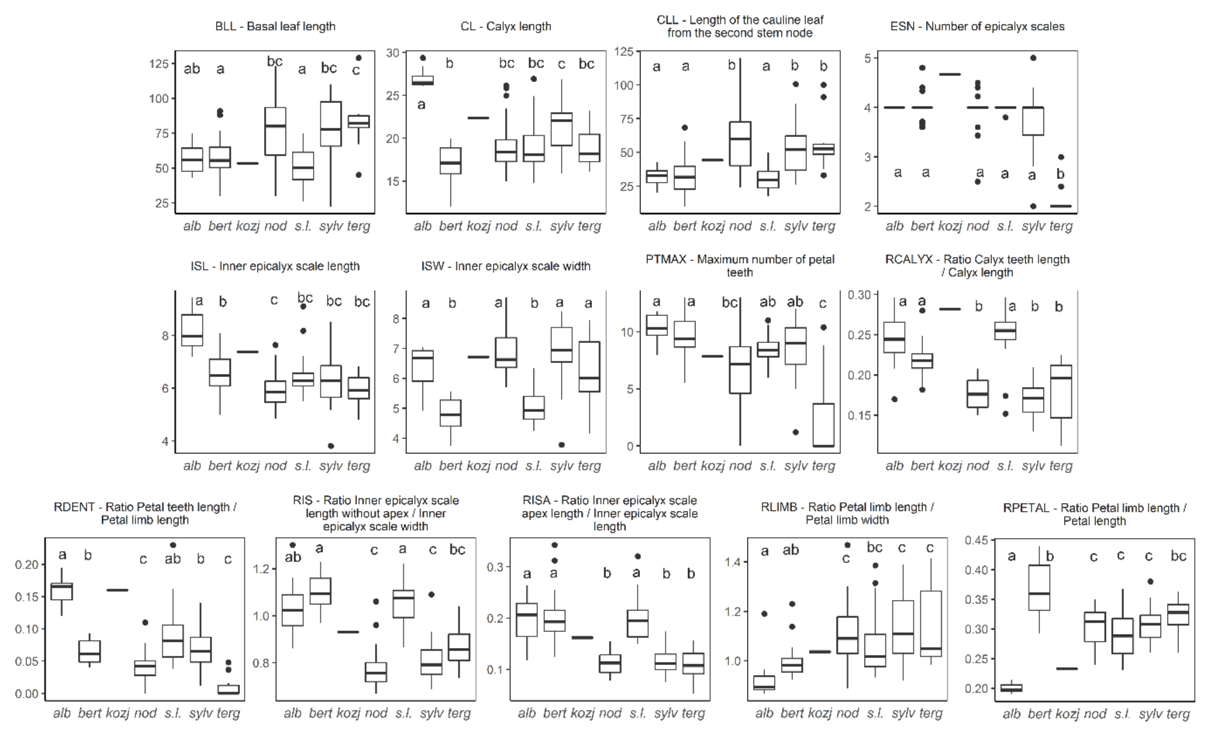


**Figure S2.** Variation in quantitative characters, examined on 97 populations of *Dianthus sylvestris* in the Balkan Peninsula, selected for performing PCA. Abbreviated labels: *alb* - subsp. *alboroseus, bert* - subsp. *bertisceus, kozj* - subsp. *kozjakensis, nod* - subsp. *nodosus, s.l.* - *D. sylvestris sensu lato, sylv* - subsp. *sylvestris, terg* - subsp. *tergestinus.* Means not significantly different at $p < 0.01$, according to Dunn's post-hoc test, are indicated by the same letter.

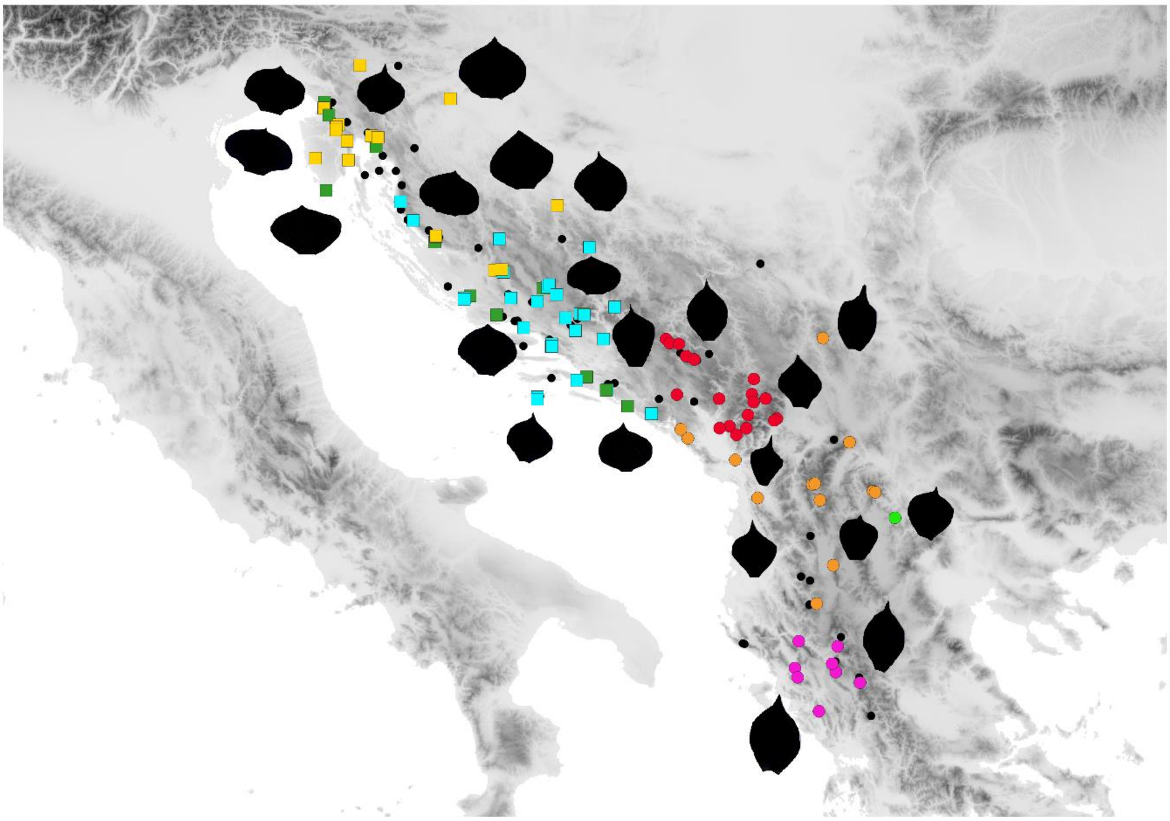


**Figure S3.** Sampling area with insertions of one epicalyx scale from the first pair (the closest to the calyx) showing the gradual change of epicalyx scale's shape in sampled populations of *Dianthus sylvestris*. Symbols are according to Figure 1.

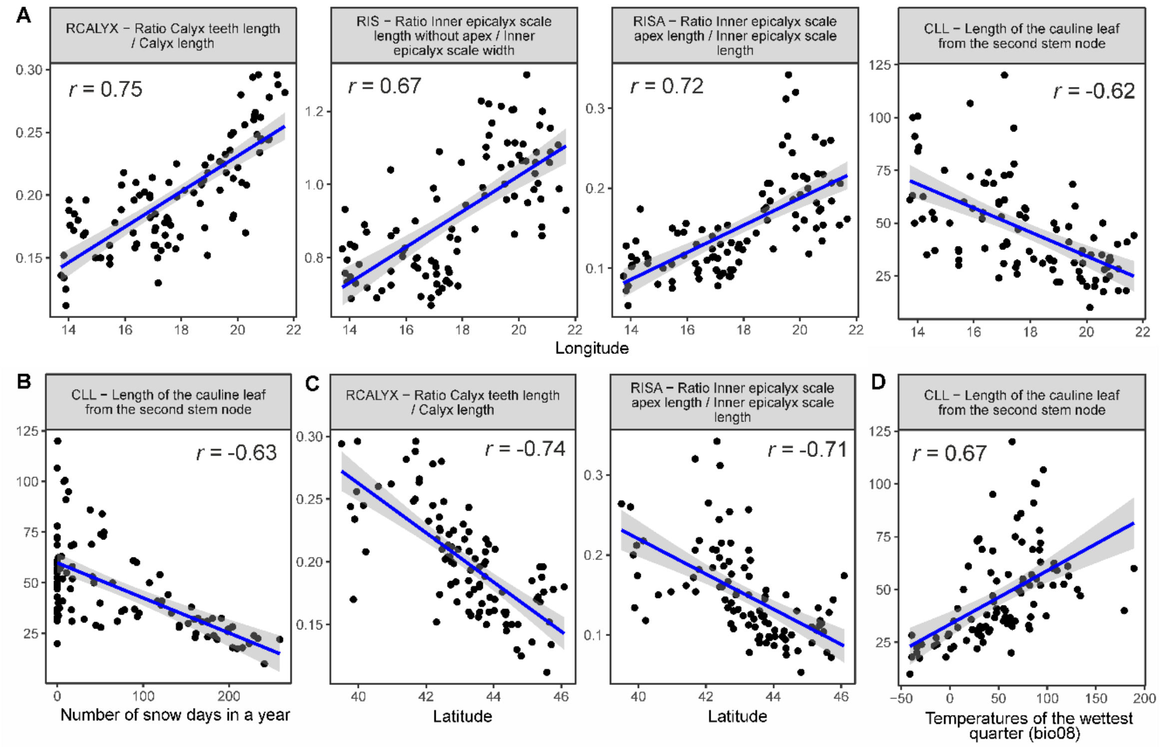


**Figure S4.** Significant correlations ($p < 0.05$) exceeding $r = |0.60|$ between morphological characters and two environmental (B and D) and two geographical variables (A and C), according to the Spearman correlation test with Bonferroni adjustment for multiple comparisons.

**Table S1**. Studied populations of the *Dianthus sylvestris* including number of individuals collected per population, number of measured individuals used in morphometric analyses, taxon name, voucher, locality data, collectors and collection date. The herbarium acronyms are according to Index Herbariorum.

| ID | No of indiv. | Morpho-metrics | Taxon | Voucher | Country | Alt. (m a.s.l.) | Long. (N) | Lat. (E) | Collectors | Collection date |
|---|---|---|---|---|---|---|---|---|---|---|
| D76 | 5 | - | *D. sylvestris s.l.* - Balkan | ZA 48631 | Serbia | 640 | 44.04424 | 20.01645 | S. Đurović, G. Tomović | 15/07/2018 |
| D77 | 5 | - | *D. sylvestris s.l.* - Balkan | ZA 48632 | Serbia | 1755 | 42.2335 | 20.91537 | D. Lakušić, N. Kuzmanović, I. Janković, M. Zbiljić | 14/07/2018 |
| D79 | 5 | - | *D. sylvestris s.l.* - Balkan | ZA 48634 | Serbia | 1660 | 43.26149 | 20.83973 | S. Đurović, G. Tomović | 13/07/2018 |
| D102 | 5 | 5 | *D. sylvestris s.l.* - Balkan | ZA 48619 | Montenegro | 931 | 42.30581 | 18.93189 | S. Bogdanović, I. Ljubičić | 16/07/2018 |
| D103 | 5 | 5 | *D. sylvestris s.l.* - Balkan | ZA 48620 | Montenegro | 1681 | 42.4003 | 18.83807 | S. Bogdanović, I. Ljubičić | 16/07/2018 |
| D108 | 5 | - | *D. sylvestris s.l.* - Balkan | ZA 48625 | Albania | 969 | 40.86694 | 20.38444 | B. Frajman, P. Schönswetter | 12/07/2018 |
| D109 | 5 | - | *D. sylvestris s.l.* - Balkan | ZA 48626 | Albania | 1406 | 40.57972 | 20.47722 | B. Frajman, P. Schönswetter | 13/07/2018 |
| D110 | 4 | - | *D. sylvestris s.l.* - Balkan | ZA 48627 | North Macedonia | 1759 | 41.27278 | 20.535 | B. Frajman, P. Schönswetter | 15/07/2018 |
| D119 | 4 | - | *D. sylvestris s.l.* - Balkan | ZA 54922 | Albania | 1670 | 40.82167 | 20.50167 | M. Duchon | 16/07/2018 |
| D145 | 5 | 5 | *D. sylvestris s.l.* - Balkan | ZA 54947 | Albania | 145 | 41.67954 | 19.84739 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 26/06/2019 |
| D147 | 8 | 8 | *D. sylvestris s.l.* - Balkan | ZA 54953 | Albania | 129 | 42.07219 | 19.56423 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 27/06/2019 |
| D170 | 6 | 6 | *D. sylvestris s.l.* - Balkan | ZA 54977 | Serbia | 1787 | 43.26151 | 20.83653 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 10/07/2019 |
| D171 | 5 | 6 | *D. sylvestris s.l.* - Balkan | ZA 54978 | North Macedonia | 2255 | 42.19948 | 21.12385 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 11/07/2019 |
| D172 | 5 | 5 | *D. sylvestris s.l.* - Balkan | ZA 54979 | North Macedonia | 2255 | 42.19948 | 21.12385 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 11/07/2019 |
| D173 | 6 | 6 | *D. sylvestris s.l.* - Balkan | ZA 54980 | North Macedonia | 1687 | 41.6293 | 20.68481 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 12/07/2019 |
| D174 | 5 | 5 | *D. sylvestris s.l.* - Balkan | ZA 54981 | North Macedonia | 1510 | 40.96566 | 20.81879 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 12/07/2019 |
| D175 | 6 | 6 | *D. sylvestris s.l.* - Balkan | ZA 54982 | North Macedonia | 2112 | 41.7919 | 20.59291 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 13/07/2019 |
| D176 | 5 | 5 | *D. sylvestris s.l.* - Balkan | ZA 54983 | North Macedonia | 1379 | 41.79959 | 20.62801 | S. Bogdanović, M. Doboš, M. Grgurev, I. Rešetnik | 13/07/2019 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| D179 | 5 | 5 | *D. sylvestris s.l.* - Balkan | ZA 54986 | North Macedonia | 2274 | 41.69279 | 21.40381 | S. Bogdanović, M. Grgurev, I. Rešetnik | 17/07/2019 |
| D180 | 5 | 5 | *D. sylvestris s.l.* - Balkan | ZA 54987 | North Macedonia | 1235 | 41.68044 | 21.43242 | S. Bogdanović, M. Grgurev, I. Rešetnik | 17/07/2019 |
| D182 | 5 | 1 | *D. sylvestris s.l.* - Balkan | ZA 54989,<br>ZA 54990 | Albania | 1430 | 40.58883 | 20.57972 | L. Shuka | 27/07/2019 |
| D78 | 5 | - | *D. sylvestris* subsp. *alboroseus* | ZA 48633 | Albania | 1386 | 40.5741 | 20.47551 | D. Lakušić, N. Kuzmanović, I. Janković, M. Zbiljić | 11/07/2018 |
| D131 | 5 | - | *D. sylvestris* subsp. *alboroseus* | ZA 54934 | Greece | 1023 | 39.42647 | 21.21591 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 21/06/2019 |
| D132 | 5 | 5 | *D. sylvestris* subsp. *alboroseus* | ZA 54935 | Greece | 664 | 39.49754 | 20.54052 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 21/06/2019 |
| D133 | 5 | 5 | *D. sylvestris* subsp. *alboroseus* | ZA 54936 | Greece | 744 | 39.76638 | 21.09354 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 22/06/2019 |
| D134 | 7 | - | *D. sylvestris* subsp. *alboroseus* | ZA 54937 | Greece | 1398 | 39.82082 | 21.08762 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 22/06/2019 |
| D135 | 5 | 5 | *D. sylvestris* subsp. *alboroseus* | ZA 54938 | Greece | 1135 | 39.88615 | 20.78638 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 22/06/2019 |
| D136 | 5 | - | *D. sylvestris* subsp. *alboroseus* | ZA 54939 | Greece | 765 | 40.23746 | 20.87395 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 22/06/2019 |
| D137 | 5 | 4 | *D. sylvestris* subsp. *alboroseus* | ZA 54940 | Greece | 631 | 40.14424 | 20.82399 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 22/06/2019 |
| D138 | 6 | 5 | *D. sylvestris* subsp. *alboroseus* | ZA 54941 | Greece | 1296 | 39.97295 | 20.74076 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 23/06/2019 |
| D139 | 5 | - | *D. sylvestris* subsp. *alboroseus* | ZA 54942 | Greece | 2093 | 39.99494 | 20.78599 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 23/06/2019 |
| D140 | 6 | 5 | *D. sylvestris* subsp. *alboroseus* | ZA 54943 | Albania | 1523 | 40.21507 | 20.31646 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 24/06/2019 |
| D141 | 6 | 5 | *D. sylvestris* subsp. *alboroseus* | ZA 54944 | Albania | 400 | 39.94504 | 20.25109 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 25/06/2019 |
| D142-1 | 5 | - | *D. sylvestris* subsp. *alboroseus* | ZA 54946 | Albania | 950 | 40.21436 | 19.57959 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 25/06/2019 |
| D142-2 | 3 | - | *D. sylvestris* subsp. *alboroseus* | ZA 54945 | Albania | 1600 | 40.20721 | 19.59952 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 25/06/2019 |
| D181 | 5 | 1 | *D. sylvestris* subsp. *alboroseus* | ZA 54988 | Albania | 700 | 39.84924 | 20.28111 | D. Shuka | 07/06/2019 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| D86 | 6 | 6 | *D. sylvestris* subsp. *bertisceus* | ZA 48662 | Bosnia and Herzegovina | 638 | 43.31548 | 18.66821 | S. Bogdanović, I. Ljubičić | 11/07/2018 |
| D87 | 5 | 6 | *D. sylvestris* subsp. *bertisceus* | ZA 48663 | Bosnia and Herzegovina | 1580 | 43.27309 | 18.71955 | S. Bogdanović, I. Ljubičić | 12/07/2018 |
| D88 | 5 | 5 | *D. sylvestris* subsp. *bertisceus* | ZA 48664 | Montenegro | 735 | 43.26326 | 18.84523 | S. Bogdanović, I. Ljubičić | 12/07/2018 |
| D89 | 5 | 5 | *D. sylvestris* subsp. *bertisceus* | ZA 48665 | Montenegro | 1680 | 43.13713 | 18.94089 | S. Bogdanović, I. Ljubičić | 13/07/2018 |
| D90 | 5 | 5 | *D. sylvestris* subsp. *bertisceus* | ZA 48666 | Montenegro | 2002 | 43.10045 | 19.05084 | S. Bogdanović, I. Ljubičić | 13/07/2018 |
| D91 | 5 | 5 | *D. sylvestris* subsp. *bertisceus* | ZA 48612 | Montenegro | 711 | 42.88203 | 19.86147 | S. Bogdanović, I. Ljubičić | 13/07/2018 |
| D92 | 6 | 6 | *D. sylvestris* subsp. *bertisceus* | ZA 48667 | Montenegro | 746 | 42.72892 | 19.82458 | S. Bogdanović, I. Ljubičić | 13/07/2018 |
| D93 | 6 | 6 | *D. sylvestris* subsp. *bertisceus* | ZA 48668 | Montenegro | 2011 | 42.64971 | 19.84412 | S. Bogdanović, I. Ljubičić | 14/07/2018 |
| D94 | 5 | 5 | *D. sylvestris* subsp. *bertisceus* | ZA 48669 | Montenegro | 1989 | 42.52064 | 19.76142 | S. Bogdanović, I. Ljubičić | 14/07/2018 |
| D95 | 5 | 5 | *D. sylvestris* subsp. *bertisceus* | ZA 48670 | Montenegro | 1948 | 42.67997 | 20.01136 | S. Bogdanović, I. Ljubičić | 15/07/2018 |
| D96 | 5 | 5 | *D. sylvestris* subsp. *bertisceus* | ZA 48613 | Montenegro | 278 | 42.69683 | 19.37431 | S. Bogdanović, I. Ljubičić | 15/07/2018 |
| D150 | 6 | 6 | *D. sylvestris* subsp. *bertisceus* | ZA 54956 | Albania | 773 | 42.41509 | 19.50371 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 27/06/2019 |
| D151 | 7 | 7 | *D. sylvestris* subsp. *bertisceus* | ZA 54957 | Albania | 1735 | 42.38844 | 19.73021 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 28/06/2019 |
| D153 | 5 | 5 | *D. sylvestris* subsp. *bertisceus* | ZA 54959 | Albania | 665 | 42.32486 | 19.59345 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 28/06/2019 |
| D154 | 5 | 3 | *D. sylvestris* subsp. *bertisceus* | ZA 54960 | Montenegro | 139 | 42.39835 | 19.36711 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 28/06/2019 |
| D157 | 1 | - | *D. sylvestris* subsp. *bertisceus* | ZA 54963 | Montenegro | 600 | 42.67368 | 19.03014 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 28/06/2019 |
| D158 | 5 | 5 | *D. sylvestris* subsp. *bertisceus* | ZA 54964 | Montenegro | 900 | 42.75111 | 18.79916 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 29/06/2019 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| D168 | 5 | 1 | *D. sylvestris* subsp. *bertisceus* | ZA 54974 | Albania | 1115 | 42.47372 | 20.15167 | D. Shuka | 23/07/2019 |
| D169 | 5 | 2 | *D. sylvestris* subsp. *bertisceus* | ZA 54975,<br>ZA 54976 | Albania | 2253 | 42.45741 | 20.12222 | D. Shuka | 24/07/2019 |
| SDM16 | - | - | *D. sylvestris* subsp. *bertisceus* | - | Bosnia and Herzegovina | 620 | 43.30424 | 18.64968 | S. Bogdanović, I. Ljubičić | 11/07/2018 |
| SDM17 | - | - | *D. sylvestris* subsp. *bertisceus* | - | Montenegro | 1526 | 43.25179 | 18.72164 | S. Bogdanović, I. Ljubičić | 12/07/2018 |
| SDM18 | - | - | *D. sylvestris* subsp. *bertisceus* | - | Bosnia and Herzegovina | 1647 | 43.28504 | 18.71911 | S. Bogdanović, I. Ljubičić | 12/07/2018 |
| SDM19 | - | - | *D. sylvestris* subsp. *bertisceus* | - | Montenegro | 809 | 43.17217 | 18.85883 | S. Bogdanović, I. Ljubičić | 13/07/2018 |
| SDM20 | - | - | *D. sylvestris* subsp. *bertisceus* | - | Montenegro | 1692 | 43.09217 | 19.08971 | S. Bogdanović, I. Ljubičić | 13/07/2018 |
| SDM21 | - | - | *D. sylvestris* subsp. *bertisceus* | - | Montenegro | 1346 | 43.15262 | 19.25794 | S. Bogdanović, I. Ljubičić | 13/07/2018 |
| D178 | 6 | 6 | *D. sylvestris* subsp. *kozjakensis* | ZA 54985 | North Macedonia | 1292 | 41.40914 | 21.67842 | S. Bogdanović, I. Rešetnik | 14/07/2019 |
| D2 | 7 | - | *D. sylvestris* subsp. *nodosus* | ZA 46277 | Croatia | 250 | 42.94086 | 17.08055 | I. Rešetnik, S. Bogdanović | 11/05/2018 |
| D6 | 5 | 7 | *D. sylvestris* subsp. *nodosus* | ZA 46281 | Croatia | 332 | 42.81569 | 17.84349 | I. Rešetnik, S. Bogdanović | 13/5/2018, 29/6/2019 |
| D8 | 5 | 4 | *D. sylvestris* subsp. *nodosus* | ZA 46283 | Croatia | 63 | 43.45025 | 16.69853 | I. Rešetnik, S. Bogdanović | 13/5/2018, 29/6/2019 |
| D27 | 5 | - | *D. sylvestris* subsp. *nodosus* | ZA 47427 | Croatia | 1211 | 44.70794 | 14.96583 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 15/06/2018 |
| D28 | 10 | 1 | *D. sylvestris* subsp. *nodosus* | ZA 47428 | Croatia | 1020 | 44.7153 | 14.9569 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 15/06/2018 |
| D33 | 5 | 1 | *D. sylvestris* subsp. *nodosus* | ZA 47433 | Croatia | 905 | 44.52948 | 15.14316 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 15/06/2018 |
| D42 | 6 | - | *D. sylvestris* subsp. *nodosus* | ZA 47442 | Croatia | 1605 | 44.36112 | 15.51313 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 17/06/2018 |
| D46 | 6 | 6 | *D. sylvestris* subsp. *nodosus* | ZA 47456 | Croatia | 18 | 43.73736 | 15.87176 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 18/06/2018 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| D47-1 | 4 | 4 | *D. sylvestris* subsp. *nodosus* | ZA 47457 | Croatia | 325 | 43.25955 | 17.07843 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 18/06/2018 |
| D47-2 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 47459 | Croatia | 899 | 43.261 | 17.10161 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 18/06/2018 |
| D47-3 | 2 | 1 | *D. sylvestris* subsp. *nodosus* | ZA 47459 | Croatia | 535 | 43.26069 | 17.09043 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 18/06/2018 |
| D52 | 7 | 7 | *D. sylvestris* subsp. *nodosus* | ZA 47463 | Bosnia and Herzegovina | 355 | 43.32548 | 17.80283 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 19/06/2018 |
| D55 | 7 | 6 | *D. sylvestris* subsp. *nodosus* | ZA 47466 | Bosnia and Herzegovina | 360 | 43.64998 | 17.96701 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 19/06/2018 |
| D56 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 47467 | Bosnia and Herzegovina | 549 | 43.41747 | 17.41643 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 |
| D57 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 47468 | Bosnia and Herzegovina | 1298 | 43.58668 | 17.48789 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 |
| D60 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 47471 | Bosnia and Herzegovina | 1556 | 43.57896 | 17.54072 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 |
| D61 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 47472 | Bosnia and Herzegovina | 904 | 43.54695 | 17.27952 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 |
| D63 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 47474 | Bosnia and Herzegovina | 1197 | 43.85963 | 17.03066 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 21/06/2018 |
| D64 | 6 | 6 | *D. sylvestris* subsp. *nodosus* | ZA 47475 | Bosnia and Herzegovina | 1397 | 43.88544 | 17.05981 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 21/06/2018 |
| D65 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 47476 | Bosnia and Herzegovina | 1096 | 43.78286 | 17.16035 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 21/06/2018 |
| D66 | 6 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 47477 | Bosnia and Herzegovina | 716 | 44.34866 | 16.35956 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 22/06/2018 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| D80 | 8 | 9 | *D. sylvestris* subsp. *nodosus* | ZA 48607 | Croatia | 740 | 44.01427 | 16.41704 | S. Bogdanović, I. Ljubičić | 30/06/2018 |
| D82 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 48608 | Croatia | 934 | 43.75119 | 16.52322 | S. Bogdanović, I. Ljubičić | 01/07/2018 |
| D85 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 48611 | Bosnia and Herzegovina | 1100 | 44.25852 | 17.62419 | S. Bogdanović, I. Ljubičić | 10/07/2018 |
| D106 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 48623 | Bosnia and Herzegovina | 915 | 42.56432 | 18.44312 | S. Bogdanović, I. Ljubičić | 17/7/2018,<br>29/6/2019 |
| D124 | 7 | - | *D. sylvestris* subsp. *nodosus* | ZA 54927 | Croatia | 48 | 42.75519 | 16.92379 | I. Rešetnik, S. Bogdanović | 04/06/2019 |
| D125 | 5 | 1 | *D. sylvestris* subsp. *nodosus* | ZA 54928 | Croatia | 126 | 42.74994 | 16.88776 | I. Rešetnik, S. Bogdanović | 04/06/2019 |
| D126 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 54929 | Croatia | 46 | 42.72382 | 16.88531 | I. Rešetnik, S. Bogdanović | 04/06/2019 |
| D127 | 7 | - | *D. sylvestris* subsp. *nodosus* | ZA 54930 | Croatia | 150 | 43.26608 | 16.69359 | I. Ljubičić, A. Terlević, M. Grgurev | 04/06/2019 |
| D128 | 5 | - | *D. sylvestris* subsp. *nodosus* | ZA 54931 | Croatia | 810 | 43.52237 | 16.61158 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević, M. Grgurev | 05/06/2019 |
| D160 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 54966 | Croatia | 160 | 42.91527 | 17.42674 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 29/06/2019 |
| D183 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 56692 | Croatia | 83 | 43.75222 | 15.87861 | L. Laca, S. Bogdanović | 17/06/2020 |
| D192 | 5 | 5 | *D. sylvestris* subsp. *nodosus* | ZA 56821 | Croatia | 1713 | 43.71589 | 16.8893 | M. Doboš | 22/07/2020 |
| D193 | 5 | - | *D. sylvestris* subsp. *nodosus* | ZA 56822 | Croatia | 906 | 43.70998 | 16.8192 | S. Bogdanović | 22/07/2020 |
| SDM10 | - | - | *D. sylvestris* subsp. *nodosus* | - | Croatia | 1501 | 44.42911 | 15.36696 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 16/06/2018 |
| SDM13 | - | - | *D. sylvestris* subsp. *nodosus* | - | Croatia | 1440 | 43.32763 | 17.05769 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 18/06/2018 |
| SDM14 | - | - | *D. sylvestris* subsp. *nodosus* | - | Bosnia and Herzegovina | 551 | 43.47327 | 17.35342 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SDM15 | - | - | *D. sylvestris* subsp. *nodosus* | - | Bosnia and Herzegovina | 734 | 43.53634 | 17.44794 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 |
| SDM22 | - | - | *D. sylvestris* subsp. *nodosus* | - | Croatia | 803 | 44.88309 | 14.97183 | S. Bogdanović, I. Ljubičić | 06/09/2018 |
| SDM29 | - | - | *D. sylvestris* subsp. *nodosus* | - | Bosnia and Herzegovina | 937 | 42.71159 | 18.55117 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 29/06/2019 |
| SDM30 | - | - | *D. sylvestris* subsp. *nodosus* | - | Bosnia and Herzegovina | 500 | 42.88024 | 17.94704 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 29/06/2019 |
| SDM31 | - | - | *D. sylvestris* subsp. *nodosus* | - | Croatia | 400 | 42.87273 | 17.85456 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević | 29/06/2019 |
| D12 | 6 | 2 | *D. sylvestris* subsp. *sylvestris* | ZA 46416 | Croatia | 240 | 45.12395 | 14.20468 | S. Bogdanović, I. Ljubičić | 19/5/2018, 13/7/2019 |
| D17 | 4 | 5 | *D. sylvestris* subsp. *sylvestris* | ZA 47417 | Italy | 300 | 45.64227 | 13.8311 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 |
| D19-1 | 2 | - | *D. sylvestris* subsp. *sylvestris* | ZA 47419 | Croatia | 660 | 45.47917 | 14.04971 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 |
| D19-2 | 8 | 5 | *D. sylvestris* subsp. *sylvestris* | ZA 47419 | Croatia | 660 | 45.47917 | 14.04971 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/6/2018, 13/7/2019 |
| D20 | 5 | 5 | *D. sylvestris* subsp. *sylvestris* | ZA 47420 | Croatia | 941 | 45.45721 | 14.01632 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/6/2018, 19/7/2020 |
| D21 | 5 | 9 | *D. sylvestris* subsp. *sylvestris* | ZA 47421 | Croatia | 553 | 45.4234 | 14.01213 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/6/2018, 19/7/2020 |
| D22 | 5 | 5 | *D. sylvestris* subsp. *sylvestris* | ZA 47422 | Croatia | 525 | 45.31681 | 14.17734 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 |
| D23 | 5 | 5 | *D. sylvestris* subsp. *sylvestris* | ZA 47423 | Croatia | 326 | 45.37468 | 14.5247 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 14/06/2018 |
| D24 | 5 | 5 | *D. sylvestris* subsp. *sylvestris* | ZA 47424 | Croatia | 862 | 45.36157 | 14.61724 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 14/06/2018 |
| D34 | 5 | - | *D. sylvestris* subsp. *sylvestris* | ZA 47434 | Croatia | 1458 | 44.4293 | 15.3643 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 16/06/2018 |
| D37 | 7 | 3 | *D. sylvestris* subsp. *sylvestris* | ZA 47437 | Croatia | 1131 | 44.36475 | 15.46012 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 17/06/2018 |
| D38 | 5 | 1 | *D. sylvestris* subsp. *sylvestris* | ZA 47438 | Croatia | 1368 | 44.37109 | 15.46567 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 17/06/2018 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| D39 | 2 | 2 | *D. sylvestris* subsp. *sylvestris* | ZA 47439 | Croatia | 1388 | 44.37557 | 15.46572 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 17/06/2018 |
| D58 | 5 | - | *D. sylvestris* subsp. *sylvestris* | ZA 47469 | Bosnia and Herzegovina | 1928 | 43.59503 | 17.54076 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 |
| D59 | 6 | 6 | *D. sylvestris* subsp. *sylvestris* | ZA 47470 | Bosnia and Herzegovina | 1732 | 43.58652 | 17.53378 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 20/06/2018 |
| D81 | 4 | 5 | *D. sylvestris* subsp. *sylvestris* | ZA 48606 | Croatia | 1418 | 44.03867 | 16.39343 | S. Bogdanović, I. Ljubičić | 30/06/2018 |
| D83 | 5 | 1 | *D. sylvestris* subsp. *sylvestris* | ZA 48609 | Bosnia and Herzegovina | 207 | 44.68547 | 17.1797 | S. Bogdanović, I. Ljubičić | 10/07/2018 |
| D84 | 4 | - | *D. sylvestris* subsp. *sylvestris* | ZA 48610 | Bosnia and Herzegovina | 380 | 44.3468 | 17.24253 | S. Bogdanović, I. Ljubičić | 10/07/2018 |
| D130 | 5 | 5 | *D. sylvestris* subsp. *sylvestris* | ZA 54933 | Croatia | 345 | 44.03398 | 16.29247 | S. Bogdanović, I. Ljubičić | 14/06/2019 |
| D162 | 5 | 5 | *D. sylvestris* subsp. *sylvestris* | ZA 54968 | Croatia | 740 | 45.76377 | 15.6486 | A. Terlević, M. Grgurev | 05/07/2019 |
| D164 | 6 | - | *D. sylvestris* subsp. *sylvestris* | ZA 54970 | Slovenia | 1954 | 46.43132 | 13.73659 | A. Terlević, I. Ljubičić | 09/07/2019 |
| D165 | 5 | - | *D. sylvestris* subsp. *sylvestris* | ZA 54971 | Slovenia | 295 | 46.08895 | 14.8873 | A. Terlević, I. Ljubičić | 09/07/2019 |
| D166 | 5 | 5 | *D. sylvestris* subsp. *sylvestris* | ZA 54972 | Slovenia | 900 | 46.08407 | 14.33474 | A. Terlević, I. Ljubičić | 10/07/2019 |
| D184 | 5 | 5 | *D. sylvestris* subsp. *sylvestris* | ZA 56693 | Croatia | 136 | 45.13314 | 13.73433 | A. Terlević | 21/06/2020 |
| D196 | 5 | - | *D. sylvestris* subsp. *sylvestris* | ZA 56825 | Slovenia | 625 | 45.50694 | 14.16806 | B. Frajman | 15/08/2020 |
| D201 | 5 | - | *D. sylvestris* subsp. *sylvestris* | ZA 57144 | Croatia | 340 | 45.40548 | 14.47115 | B. Frajman | 18/10/2020 |
| SDM1 | - | - | *D. sylvestris* subsp. *sylvestris* | - | Slovenia | 351 | 45.70294 | 13.94686 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 |
| SDM5 | - | - | *D. sylvestris* subsp. *sylvestris* | - | Croatia | 639 | 45.50666 | 13.97424 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SDM6 | - | - | *D. sylvestris* subsp. *sylvestris* | - | Croatia | 567 | 45.42499 | 14.00793 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 |
| SDM7 | - | - | *D. sylvestris* subsp. *sylvestris* | - | Croatia | 330 | 45.37942 | 14.54419 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 14/06/2018 |
| SDM9 | - | - | *D. sylvestris* subsp. *sylvestris* | - | Croatia | 30 | 44.63657 | 14.96594 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 15/06/2018 |
| SDM23 | - | - | *D. sylvestris* subsp. *sylvestris* | - | Croatia | 1411 | 43.78716 | 16.49008 | M. Temunović | 07/01/2018 |
| SDM24 | - | - | *D. sylvestris* subsp. *sylvestris* | - | Croatia | 1462 | 43.7891 | 16.485 | M. Temunović | 07/01/2018 |
| SDM25 | - | - | *D. sylvestris* subsp. *sylvestris* | - | Croatia | 1090 | 43.7781 | 16.51126 | M. Temunović | 07/01/2018 |
| SDM32 | - | - | *D. sylvestris* subsp. *sylvestris* | - | Slovenia | 758 | 46.08555 | 14.34639 | A. Terlević, I. Ljubičić | 10/07/2019 |
| SDM33 | - | - | *D. sylvestris* subsp. *sylvestris* | - | Croatia | 1029 | 45.26035 | 15.14543 | D. Šincek | 02/08/2020 |
| SDM34 | - | - | *D. sylvestris* subsp. *sylvestris* | - | Croatia | 1104 | 45.26018 | 15.14457 | D. Šincek | 02/08/2020 |
| D1 | 5 | 5 | *D. sylvestris* subsp. *tergestinus* | ZA 46276 | Croatia | 80 | 42.94874 | 17.56451 | I. Rešetnik, S. Bogdanović | 09/05/2018 |
| D4 | 5 | 5 | *D. sylvestris* subsp. *tergestinus* | ZA 46279 | Croatia | 279 | 42.64521 | 18.12021 | I. Rešetnik, S. Bogdanović | 12/05/2018 |
| D7 | 5 | 4 | *D. sylvestris* subsp. *tergestinus* | ZA 46282 | Croatia | 351 | 42.81084 | 17.83079 | I. Rešetnik, S. Bogdanović | 13/05/2018 |
| D9 | 5 | 5 | *D. sylvestris* subsp. *tergestinus* | ZA 46284 | Croatia | 602 | 43.58147 | 16.32579 | I. Rešetnik, S. Bogdanović | 13/05/2018 |
| D11 | 6 | 6 | *D. sylvestris* subsp. *tergestinus* | ZA 46415 | Croatia | 55 | 44.81097 | 13.90088 | S. Bogdanović, I. Ljubičić | 17/05/2018 |
| D13 | 5 | 5 | *D. sylvestris* subsp. *tergestinus* | ZA 46417 | Croatia | 513 | 45.2676 | 14.59441 | S. Bogdanović, I. Ljubičić | 19/05/2018 |
| D16 | 5 | 5 | *D. sylvestris* subsp. *tergestinus* | ZA 47416 | Italy | 344 | 45.70153 | 13.83729 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 |
| D18 | 5 | 5 | *D. sylvestris* subsp. *tergestinus* | ZA 47418 | Slovenia | 422 | 45.57284 | 13.90681 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 |
| D25 | 5 | - | *D. sylvestris* subsp. *tergestinus* | ZA 47425 | Croatia | 8 | 45.02484 | 14.88473 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 14/06/2018 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| D29 | 5 | - | *D. sylvestris* subsp. *tergestinus* | ZA 47429 | Croatia | 274 | 44.71196 | 14.9225 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 15/06/2018 |
| D32 | 5 | - | *D. sylvestris* subsp. *tergestinus* | ZA 47432 | Croatia | 12 | 44.53338 | 15.06519 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 15/06/2018 |
| D36 | 5 | 2 | *D. sylvestris* subsp. *tergestinus* | ZA 47436 | Croatia | 397 | 44.31347 | 15.45328 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 17/06/2018 |
| D43 | 5 | - | *D. sylvestris* subsp. *tergestinus* | ZA 47453 | Croatia | 35 | 43.86523 | 15.6423 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 18/06/2018 |
| D50 | 5 | - | *D. sylvestris* subsp. *tergestinus* | ZA 47461 | Bosnia and Herzegovina | 250 | 43.32542 | 17.79292 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 19/06/2018 |
| D53 | 5 | - | *D. sylvestris* subsp. *tergestinus* | ZA 47464 | Bosnia and Herzegovina | 355 | 43.32588 | 17.80257 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 19/06/2018 |
| D62 | 5 | 5 | *D. sylvestris* subsp. *tergestinus* | ZA 47473 | Bosnia and Herzegovina | 821 | 43.84762 | 16.9733 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 21/06/2018 |
| D111 | 5 | - | *D. sylvestris* subsp. *tergestinus* | ZA 48628 | Croatia | 40 | 44.97721 | 14.445 | S. Bogdanović, S. Cambria | 21/10/2018 |
| D129 | 5 | 5 | *D. sylvestris* subsp. *tergestinus* | ZA 54932 | Croatia | 186 | 43.77399 | 15.95608 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević, M. Grgurev | 05/06/2019 |
| D185 | 5 | - | *D. sylvestris* subsp. *tergestinus* | ZA 56694 | Croatia | 220 | 45.02389 | 14.64694 | I. Rešetnik | 14/06/2020 |
| SDM2 | - | - | *D. sylvestris* subsp. *tergestinus* | - | Italy | 491 | 45.62724 | 13.90065 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 |
| SDM3 | - | - | *D. sylvestris* subsp. *tergestinus* | - | Slovenia | 472 | 45.60195 | 13.93049 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 |
| SDM4 | - | - | *D. sylvestris* subsp. *tergestinus* | - | Slovenia | 411 | 45.5605 | 13.89178 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 13/06/2018 |
| SDM8 | - | - | *D. sylvestris* subsp. *tergestinus* | - | Croatia | 25 | 45.17663 | 14.69306 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 14/06/2018 |
| SDM11 | - | - | *D. sylvestris* subsp. *tergestinus* | - | Croatia | 513 | 44.33887 | 15.44411 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 17/06/2018 |
| SDM12 | - | - | *D. sylvestris* subsp. *tergestinus* | - | Croatia | 30 | 43.76309 | 15.84544 | I. Rešetnik, S. Bogdanović, I. Ljubičić | 18/06/2018 |
| SDM26 | - | - | *D. sylvestris* subsp. *tergestinus* | - | Croatia | 251 | 43.56302 | 16.40964 | I. Ljubičić, A. Terlević, M. Grgurev | 04/06/2019 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SDM27 | - | - | *D. sylvestris* subsp. *tergestinus* | - | Croatia | 100 | 43.5176 | 16.57563 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević, M. Grgurev | 05/06/2019 |
| SDM28 | - | - | *D. sylvestris* subsp. *tergestinus* | - | Croatia | 743 | 44.25694 | 16.06509 | S. Bogdanović, I. Ljubičić, I. Rešetnik, A. Terlević, M. Grgurev | 06/06/2019 |

**Table S2.** Chi-square test between each of the 15 pairs of *Dianthus sylvestris* subspecies, for each qualitative morphological character. Significant differences ($p < 0.01$) are shown in bold. Abbreviated labels: *alb* - subsp. *alboroseus*, *bert* - subsp. *bertisceus*, *nod* - subsp. *nodosus, s.l.* - *D. sylvestris sensu lato*, sylv - subsp. *sylvestris, terg* - subsp. *tergestinus.*

| | **Woody part of the stem below basal leaves (WST)** | | **Lower part of the stem indumentum (IND)** | | **Petal denticulation (DEN)** | | **Arrangement of epicalyx scales (EPI)** | |
|---|---|---|---|---|---|---|---|---|
| Subspecies pairs | *Chi-square* test | *p*-value | *Chi-square* test | *p*-value | *Chi-square* test | *p*-value | *Chi-square* test | *p*-value |
| *alb - s.l.* | 2.30 | 0.1609 | 1.33 | 0.2624 | 3.30 | 0.0925 | 3.75 | 0.0725 |
| *alb - bert* | 0.17 | 0.6882 | 12.66 | **0.0020** | 5.52 | 0.0235 | 64.13 | **0.0005** |
| *alb - nod* | 3.43 | 0.0800 | 7.29 | **0.0045** | 11.35 | **0.0020** | 13.26 | **0.0020** |
| *alb - sylv* | 2.46 | 0.1304 | 16.71 | **0.0010** | 8.28 | 0.0050 | 26.80 | **0.0005** |
| *alb - terg* | 2.07 | 0.1654 | 0.49 | 0.5497 | 56.81 | **0.0005** | 19.99 | **0.0005** |
| *bert - s.l.* | 5.10 | 0.0280 | 6.65 | 0.0125 | 1.02 | 0.3193 | 50.15 | **0.0005** |
| *bert - nod* | 8.57 | **0.0030** | 52.60 | **0.0005** | 4.16 | 0.0605 | 189.81 | **0.0005** |
| *bert - sylv* | 6.17 | 0.0220 | 0.06 | 1.0000 | 1.07 | 0.4123 | 177.81 | **0.0005** |
| *bert - terg* | 4.97 | 0.0390 | 10.18 | **0.0015** | 63.55 | **0.0005** | 147.30 | **0.0005** |
| *nod - s.l.* | 0.01 | 1.0000 | 21.78 | **0.0005** | 7.99 | 0.0045 | 46.78 | **0.0005** |
| *nod - sylv* | 0.07 | 0.8621 | 67.94 | **0.0005** | 0.79 | 0.4163 | 5.29 | 0.0445 |
| *nod - terg* | 0.09 | 0.8541 | 19.18 | **0.0005** | 48.86 | **0.0005** | 3.91 | 0.1039 |
| *sylv - s.l.* | 0.01 | 1.0000 | 9.29 | **0.0025** | 3.69 | 0.0620 | 54.14 | **0.0005** |
| *sylv - terg* | 0.00 | 1.0000 | 13.99 | **0.0005** | 47.97 | **0.0005** | 4.88 | 0.0370 |
| *terg - s.l.* | 0.02 | 1.0000 | 0.31 | 0.7091 | 66.80 | **0.0005** | 41.20 | **0.0005** |

**Table S3**. Quantitative morphological characters studied in *Dianthus sylvestris* from the Balkan Peninsula. Morphological differences along 21 quantitative morphological characters and seven ratios between the *D. sylvestris* subspecies as revealed by Kruskal Wallis test. Significant differences ($p < 0.01$) are shown in bold.

| **Morphological character** | **Abbreviation** | **Kruskal Wallis rank sum statistic** | ***P*- value** |
|---|---:|---:|---:|
| Length of the woody part of the stem below basal leaves | BL | 5.27 | 0.383762 |
| Basal leaf length | BLL | 26.27 | **< 0.01** |
| Calyx length | CL | 32.09 | **< 0.01** |
| Length of the cauline leaf from the second stem node | CLL | 35.34 | **< 0.01** |
| Calyx teeth length | CTL | 45.10 | **< 0.01** |
| Number of epicalyx scales | ESN | 41.11 | **< 0.01** |
| Height of first branching | FBH | 21.72 | **< 0.01** |
| Number of flowers per stem | FPS | 5.22 | 0.389483 |
| Inflorescence length | IL | 8.61 | 0.125703 |
| Inner epicalyx scale apex length | ISAL | 59.96 | **< 0.01** |
| Inner epicalyx scale length | ISL | 26.95 | **< 0.01** |
| Inner epicalyx scale width | ISW | 53.41 | **< 0.01** |
| Length of the first stem internode | LIL | 23.15 | **< 0.01** |
| Plant height | PH | 16.08 | 0.006624 |
| Petal length | PL | 19.75 | **< 0.01** |
| Petal limb length | PLL | 26.43 | **< 0.01** |
| Petal limb width | PLW | 22.22 | **< 0.01** |
| Petal teeth length | PTL | 46.49 | **< 0.01** |
| Maximum number of petal teeth | PTMAX | 31.16 | **< 0.01** |
| Minimum number of petal teeth | PTMIN | 33.51 | **< 0.01** |
| Ratio Calyx teeth length / Calyx length | RCALYX | 49.65 | **< 0.01** |
| Ratio Petal teeth length / Petal limb length | RDENT | 54.74 | **< 0.01** |
| Ratio Calyx length / Claw length | RFL | 21.83 | **< 0.01** |
| Ratio Inner epicalyx scale length without apex / Inner epicalyx scale width | RIS | 63.16 | **< 0.01** |
| Ratio Inner epicalyx scale apex length / Inner epicalyx scale length | RISA | 61.09 | **< 0.01** |
| Ratio Petal limb length / Petal limb width | RLIMB | 26.23 | **< 0.01** |
| Ratio Petal limb length / Petal length | RPETAL | 42.67 | **< 0.01** |
| Length of the last stem internode below branching | UIL | 11.20 | 0.047556 |

**Table S4.** Environmental variables used for characterizing environmental variability of *Dianthus sylvestris* populations using principal component analysis (PCA) and for RDA analysis, with their abbreviated and full names/definitions, units and types. Shown are also factor loadings of PCA of environmental data related to *D. sylvestris* populations.

| **Abbreviation** | **Full name** | **Unit** | **Variable type** | **PC1** | **PC2** |
|---|---|---|---|---:|---:|
| bio04 | temperature seasonality | °C/10 | climate | 0.099 | 0.254 |
| bio08 | mean daily mean air temperatures of the wettest quarter | °C/10 | climate | -0.257 | 0.223 |
| bio09 | mean daily mean air temperatures of the driest quarter | °C/10 | climate | -0.184 | -0.407 |
| bio15 | precipitation seasonality | kg $m^{-2}$ | climate | -0.096 | -0.470 |
| bio19 | mean monthly precipitation amount of the coldest quarter | kg $m^{-2}$ | climate | 0.131 | -0.348 |
| fcf | frost change frequency | No days | extended climate | 0.317 | 0.093 |
| sdays | number of snow days | No days | extended climate | 0.345 | -0.024 |
| shc | Selyaninov's Hydrothermic Coefficient | (kg $m^{-2}$/10)/°C | extended climate | 0.151 | 0.023 |
| srad | surface solar radiation downwards | kJ $m^{-2}$ | extended climate | -0.328 | -0.170 |
| dtm_slope | slope | ° | topographic | 0.213 | -0.300 |
| dtm_east | eastness | index | topographic | 0.095 | 0.038 |
| dtm_north | northness | index | topographic | -0.037 | 0.073 |
| soil_org_carb | soil organic carbon content | 5 x g $kg^{-1}$ | soil properties | 0.264 | 0.059 |
| soil_bulk | soil bulk density | 10 x kg $m^{-3}$ | soil properties | -0.329 | -0.049 |
| soil_sand | sand content | % | soil properties | 0.201 | -0.150 |
| soil_ph_h2o | soil pH x 10 in H2O | pH | soil properties | -0.304 | -0.109 |
| soil_water | soil water content at 33kPa | | soil properties | -0.263 | -0.058 |
| dtm_con | convergence | index | topographic | -0.065 | 0.198 |
| dtm_cti | compound topographic index | index | topographic | -0.173 | 0.351 |
| dtm_vrm | vector ruggedness measure | index | topographic | 0.200 | -0.180 |

**Table S5.** Results of a forward selection procedure with double stopping criterion (*P*-value and $R^2adj$), on the RDAfull model.

| **No** | **variables** | **order** | $R^2$ | $R^2Cum$ | $R^2_{adj}Cum$ | ***F*** | ***P*-value** |
|---|---|---|---|---|---|---|---|
| 1 | sdays | 10 | 0.263248 | 0.263248 | 0.255492 | 33.94425 | 0.001 |
| 2 | longitude | 21 | 0.103145 | 0.366392 | 0.352911 | 15.3022 | 0.001 |
| 3 | bio04 | 11 | 0.035619 | 0.402012 | 0.382722 | 5.539577 | 0.002 |
| 4 | latitude | 22 | 0.039582 | 0.441594 | 0.417315 | 6.521354 | 0.002 |
| 5 | shc | 19 | 0.036414 | 0.478008 | 0.449327 | 6.348182 | 0.002 |
| 6 | srad | 9 | 0.022237 | 0.500246 | 0.466929 | 4.004706 | 0.006 |
| 7 | bio19 | 15 | 0.014966 | 0.515211 | 0.477082 | 2.747505 | 0.034 |

# Publication V

# Genomic divergence of *Dianthus sylvestris* Wulfen s.l. on the Balkan Peninsula

Martina Temunović[1], Ana Terlević[2], Simone Fior[3], Hirzi Luqman[3], Ivana Rešetnik[2], Alex Widmer[3]

[1] Faculty of Forestry and Wood Technology, Department of Forest Genetics, Dendrology and Botany, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia.

[2] Faculty of Science, Department of Biology, University of Zagreb, Trg Marka Marulića 20/II, 10000 Zagreb, Croatia.

[3] Institute for Integrative Biology, Department of Environmental System Science, ETH Zürich, Universitätstrasse 16, 8092 Zürich, Switzerland

Contemporary patterns of genomic variation in plant species often reflect past demographic processes linked to climate events such as Pleistocene glaciations. *Dianthus sylvestris* s.l. comprises a number of taxa distributed along a wide altitudinal range from sea level up to alpine peaks across southern and south-eastern Europe. We here used RAD-seq genome-wide SNP data to unravel genomic variation patterns and evolutionary history of populations collected across the Balkan and Apennine Peninsulas, as well as the Alps, including all six currently described subspecies distributed exclusively in the Balkan Peninsula. Our phylogenetic and genetic structure analyses support the existence of three main geographically structured evolutionary lineages within *D. sylvestris* (Alpine, Apennine and the Balkan), and the divergence of *D. sylvestris* subsp. *tergestinus* from the rest of the Balkan populations. Further in-depth analyses of the Balkan lineage suggested hierarchically nested phylogeographic structure within the Balkan populations. Two main genetic groups of populations were revealed based on Bayesian clustering (a more homogeneous north-western (NW) group, and a more divergent south-eastern (SE) group), or five clusters (two in the NW and three in the SE) based on alternative model-free approach. We detected significant isolation-by-distance within our populations, while geographical patterns of genetic diversity showed that southernmost populations have reduced genetic diversity. Additional genetic connectivity and migration analyses confirmed a sharp break between the two main genetic groups. Species distribution modelling (SDM) combined with the majority of our genetic analyses' results suggest that contemporary *D. sylvestris* populations in the Balkans derive from two distinct evolutionary lineages which may survived the Pleistocene climatic oscillations in two main glacial refugia located in the NW and SE of the eastern Adriatic, with possible secondary contact of the two lineages in the intermediate area of Montenegro where admixed populations occur. In addition, we cannot exclude the existence of multiple smaller microrefugia for the more divergent SE group, where certain high-elevation populations may have persisted in situ over several glaciation cycles until today and may have diverged independently from the two main Balkan groups.

**Keywords:** genetic variation, phylogeography, migrations, RAD-seq, refugia, species distribution models

## Introduction

Pleistocene climatic oscillations have largely shaped the contemporary patterns of genetic variation on Earth by shifting species distribution ranges (Hewitt 2000, 2004). European taxa survived the unfavourable climatic conditions during Pleistocene in various glacial refugia, of which thee major ones (macrorefugia *sensu* Rull 2009) correspond to the Balkan, Apennine and Iberian Peninsulas (Hewitt 1999). Plant species on the Balkan Peninsula experienced complex histories over the repeated cycles of Pleistocene climatic fluctuations (Španiel and Rešetnik 2022), which supported the emergence of a plant diversity greater than in any other comparable area in Europe (Hewitt 2011). The Balkan Peninsula has more than 60% of land above 1000 m a.s.l., resulting in highly heterogeneous topography, but also heterogeneous climate, due to large scale altitudinal variations in both temperature and precipitation (Willis 1994). Due to the high degree of habitat heterogeneity with barriers such as mountains, river canyons and valleys that triggered allopatric speciation, the Balkan Peninsula did not function as a single macrorefugium during Pleistocene glacial cycles. Instead, multiple microrefugia existed within the Peninsula (Rull 2009; Médail and Diadema 2009), known as the 'refugia-within-refugia hypothesis' (Gómez and Lunt 2007) proposed for some of the investigated taxa occurring on the Balkans (Surina *et al.* 2011; Jug-Dujaković *et al.* 2020; Reich *et al.* 2022). Eight putative microrefugia were proposed for the Balkans by Médail and Diadema (2009) – four along the eastern Adriatic coast (northern Istria, Velebit and Biokovo mountains and Montenegro) and four in Greece (Olympe and Pindos mountains, Peloponnese and Chalkidiki peninsulas). Geographical barriers contributed to the compartmentalization of the region and the creation of climatically suitable enclaves, and thus fostered the differentiation of genetic lineages both in animal (Previšić *et al.* 2009; Pavlek *et al.* 2022) and plant species (Kutnjak *et al.* 2014; Jug-Dujaković *et al.* 2020). The Dinaric Mountains have several deep and narrow canyons formed by karstic rivers which likely acted as geographical barriers to gene flow, and species distributed along the Dinaric Mountains commonly show a pattern of differentiation into two or three genetic groups (Španiel and Rešetnik 2022). For example, evidence for such allopatric diversification has been found between populations separated by the Zrmanja canyon in the Northern Dinaric Mountains (e.g., *Tanacetum cinerariifolium*; Grdiša *et al.* 2014), Neretva river valley in the Central Dinaric Mountains (e.g., *Alyssum austrodalmaticum*, Španiel *et al.* 2017; Zozomová-Lihová *et al.* 2020; *Cerastium*, Đurović *et al.* 2021), and karst rivers Drina and Sutjeska in the Southern Dinaric Mountains (e.g., *Heliosperma pusillum* group; Frajman and Oxelman 2007).

Climate fluctuations during the Pleistocene are closely related to migration dynamics and distribution shifts of plant species, which responded individually according to their environmental/habitat requirements (Stewart *et al.* 2010). For example, cold-adapted species probably shifted their distribution along altitudes, whereas thermophilic species shifted along latitudes (Surina *et al.* 2011). During cold stages (glacials) cold-adapted species likely had more continuous distribution sufficient for gene flow between different mountain ranges, and more fragmented distribution during subsequent warmer stages (i.e., interglacial contraction hypotheses, Stewart *et al.* 2010; Theodoridis *et al.* 2017). This pattern seems to be predominant for cold-adapted species and was for example observed in Dinaric high-mountain species *Cerastium dinaricum* (Kutnjak *et al.* 2014; Đurović *et al.* 2021) and *Edraianthus graminifolius* (Surina *et al.* 2011). On the other hand, thermophilous and temperate species mostly experienced range contraction and fragmentation during glacials and range expansions during interglacials (including the Holocene) which may have triggered their genetic divergence and allopatric speciation during isolation in different refugial areas (Hewitt 1996; Hardion *et al.* 2014; Zozomová-Lihová *et al.* 2020). However, recently, the pattern of interglacial expansion was also demonstrated for the first time in cold-adapted *Primula farinosa* across Europe (Theodoridis *et al.* 2017), confirming that for species with broad ecological preferences it may be difficult to assume *a-priori* their response to climatic fluctuations (Stewart *et al.* 2010).

Pleistocene ice ages left specific footprints on species genetic diversity patterns. Postglacial latitudinal recolonizations of thermophilous and temperate taxa out of the main southern refugia often resulted in a pattern of higher genetic diversity and demographic stability of southern populations and a reduction in genetic diversity towards the north (leading edge hypothesis; Hewitt 2000). Such pattern was observed for example in thermophilous *Edraianthus tenuifolius* (Surina *et al.* 2011) and *Euphorbia myrsinites* (Falch *et al.* 2019), but also in some species with a wider elevational tolerance and wider geographical distribution, like *Silene saxifraga* (Đurović *et al.* 2017). In addition, stability of southern populations which may represent relict populations that persisted in situ during Pleistocene climatic oscillations, may have resulted in their reduced within-population genetic diversity and increased divergence (rear-edge hypothesis, Hampe and Petit 2005). However, equivalent genetic diversity was observed among the populations of some thermophilic species, like *Viola suavis* s.l. (Mereďa *et al.* 2011), *Helichrysum italicum* (Ninčević *et al.* 2021) and *Salvia officinalis* (Jug-Dujaković *et al.* 2020), suggesting that they may have persisted in the Balkan macrorefugium during the cold stages in both northerly and southerly located microrefugia along the eastern Adriatic coast. Finally, increased genetic diversity of populations has been also observed to be a result of the secondary contact of previously isolated divergent lineages (Petit *et al.* 2003; Sakaguchi *et al.* 2011; Havrdová *et al.* 2015). Overall, various evolutionary and phylogeographic patterns in different species groups from the Balkan Peninsula and adjacent regions have been revealed, confirming the pattern of multiple glacial microrefugia often corresponding to major phylogeographic breaks within the Peninsula (Španiel and Rešetnik 2022).

The knowledge on demographic processes of species occupying habitats spanning wide altitudinal ranges from coastal Mediterranean environments to high alpine environments is still lacking (but see Kolář *et al.* 2016). One such ecologically divergent species is *Dianthus sylvestris* Wulfen, distributed across the Alpine, Apennine and Dinaric mountains, from Mediterranean coast to alpine peaks (Tutin and Walters 1993; Bacchetta *et al.* 2010; Gammella 2016; Brullo and Guarino 2019). It thus represents an excellent system to study the influence of habitat heterogeneity and climatic oscillations on population genetic variation, in order to increase the general knowledge about evolutionary history of ecologically divergent plant groups in response to climatic changes. *Dianthus sylvestris* is a still inconsistently treated taxonomic entity, currently in focus of several research groups who are investigating both its taxonomy and ecology (Bacchetta *et al.* 2010; Gammella 2016; Domina, Astuti, Barone, *et al.* 2021; Domina, Astuti, Bacchetta, *et al.* 2021; Terlević, Temunović, *et al.* 2022; Terlević, Bogdanović, *et al.* 2022; Luqman *et al.* 2022). Our morphometric study (Terlević, Temunović, *et al.* 2022) is focused on populations from the Balkan Peninsula and considered *D. sylvestris* as a species with six currently described subspecies (*D. sylvestris* subsp. *alboroseus* F. K. Mey, *D. sylvestris* subsp. *bertisceus* Rech. f., *D. sylvestris* subsp. *kozjakensis* Micevski, *D. sylvestris* subsp. *nodosus* (Tausch) Hayek, *D. sylvestris* subsp. *sylvestris* and *D. sylvestris* subsp. *tergestinus* (Rchb.) Hayek). Our results revealed only two morphologically distinct entities of Balkan populations with the split between the two groups corresponding to the split between allopatric north-western (NW) Balkan populations and south-eastern (SE) Balkan populations approximately along the Neretva River valley (Terlević, Temunović, *et al.* 2022), a major biogeographical border within the Balkans (Španiel and Rešetnik 2022). In addition, Luqman *et al.* (2022) recently showed that post-glacial range expansion of this species is related to spatial sorting of adaptive alleles where warm-associated genotypes increased their frequency in the emerging low-elevation habitats after the LGM and ancestral cold-associated genotypes remained at high-elevation habitats to which they are pre-adapted.

Due to the combination of both low- and high-elevation populations which are usually characterised by opposite migration dynamics, the demographic history of *D. sylvestris* s.l. is likely complex. We thus hypothesize that more thermophilous populations survived the glacial periods in several microrefugial locations with favourable conditions along the western Balkans, while the interglacials facilitated their expansion from multiple microrefugia and subsequent admixture. On the other hand, cold-adapted populations of *D. sylvestris* s.l. may have experienced decreased climatic suitability during interglacials and may be undergoing allopatric speciation from the end of the last glaciation onward due to their retreat to higher elevations in the Balkan Mountains. Knowledge of genetic diversification and population structure of *D. sylvestris* distributed across the Balkan Peninsula is a prerequisite for disentangling its evolutionary history and optimizing taxonomy. Hence, in this study we used restriction-associated DNA sequencing (RAD-seq) to 1) resolve (phylogenetic) relationships among *D. sylvestris* populations 2) explore the genetic variation of the Balkan *D. sylvestris* populations, and 3) assess putative migration patterns on the Balkan Peninsula in relation to Pleistocene climate fluctuations which may have shaped the contemporary patterns of genetic variation. Finally, we combined the genomic data with species distribution models (SDMs) generated for the present and the past (Last Glacial Maximum; LGM) period to identify putative glacial refugia and infer locations of potential ancestral Balkan populations.

## Materials and methods

### *Study area and population sampling*

We collected a total of 165 populations (992 individuals) of *D. sylvestris* s.l. across its distribution range for our genomic analyses: 132 populations from the Balkan Peninsula, and 33 populations across the European Alps and Apennine Peninsula. Voucher data are presented in Table S1, and the geographic origin of sampled populations is shown in Fig. S1 and Table S1. Leaf material of up to 10 individuals per population was sampled and immediately stored in silica gel for further DNA analyses.

### *DNA extraction and ddRAD-seq library production*

Total cellular DNA was extracted from the dried leaf tissue of each individual plant using NucleoSpin® 8 Plant II kit (Macherey-Nagel) according to the manufacturer's instructions. Silica-dried leaf material was cut into small pieces, frozen, and ground at 30 Hz for 30 s in a mixer mill (MM301; Retsch GmbH & Co.) using three ceramic beads. The protocol according to (Westergaard *et al.* 2019) was used to prepare the ddRAD-seq library. The sequencing of the library was carried out by Novogene UK.

### *Genotyping*

Raw data were demultiplexed using the internal barcode, using stacks v.2.41 (Catchen *et al.* 2013) with the default settings. Raw reads were mapped to the reference genome of *Dianthus carthusianorum* L. (Fior *et al.* unpublished) using the default settings of BWA mem v0.7.17 (Li and Durbin 2010) and reads with low mapping quality (Q20) were removed. We generated two datasets to first assign the Balkan populations to evolutionary lineages of *Dianthus sylvestris* (*sensu* Luqman *et al.* 2022) and subsequently to perform in-depth analyses on their genetic structure.

For the first dataset, hereafter “all-lineage dataset”, we produced a SNP panel using 2 randomly chosen individuals for each of the 165 populations. We preformed SNP calling using freebayes v1.3.1 (Garrison and Marth 2012); parameters: F 0.1, min-repeat-entropy 1, use-best-n-alleles 4, E -1) and variants were subsequently filtered following the ddocent pipeline (Puritz *et al.* 2014) using vcflib v.1.0.1 (Garrison *et al.* 2021) and vcftools v0.1.16 (Danecek *et al.* 2011). Briefly, we retained SNPs with minor allele count of 3, a mapping quality of 20, mean depth of 10, missingness across individuals of 0.9 and minor allele frequency of 0.01. Complex SNPs containing indels were removed and we retained 1 SNPs per 1000 bases to account for potential linkage disequilibrium. The final SNP panel included 7,021 high-quality SNPs that were then used to genotype all individuals using the same settings of freebayes. The new VCF was filtered applying the same settings used to produce the SNP panel, except missingness across individuals of 0.95 and minor allele frequency of 0.05. This resulted in a final VCF including 853 SNPs for 322 individuals. For the second dataset, hereafter “Balkan-cline dataset”, we produced a SNP panel using 2 randomly chosen individuals for each of the 108 populations assigned to the Balkan lineage in the all-lineage analyses. A panel of 65 843 SNP obtained using the same procedure described above was used to genotype all individuals, which resulted in a final VCF including 6240 SNPs for 587 individuals after filtering.

### *Delimitation of evolutionary lineages of Dianthus sylvestris*

The assignment of the Balkan populations to evolutionary lineages of *Dianthus sylvestris* (following Luqman *et al.* 2022) was based on the “all-lineage dataset”, which included 992 individuals from 165 populations and a set of 5644 SNPs. To detect genetically differentiated groups of populations we first applied the model-based Bayesian clustering method implemented in STRUCTURE 2.3.4. (Pritchard *et al.* 2000) available as a -st flag in structure-threader program (Pina-Martins *et al.* 2017), a software build to parallelize and automate the runs of STRUCTURE, fastSTRUCTURE, MavericK and ALStructure softwares. Structure-threader was ran on computer cluster Isabella using 28 cores (University Computing Centre, University of Zagreb), with the following parameters: admixture model and correlated allele frequencies, no location prior, 10000 MCMC (Monte Carlo Markov chain) iterations used for burn-in followed by 90000 MCMC iterations, K ranging from 1 to 20 with 10 runs for each K. Optimal number of genetic clusters (K) was identified based on the Evanno *et al.* (2005) method via STRUCTURE HARVESTER (Earl and vonHoldt 2012). STRUCTURE results were summarised using CLUMPAK server (Kopelman *et al.* 2015) and for the most likely K values visualised in ArcGIS 10.2.2. To investigate phylogenetic relationships among all individuals of the three lineages we carried out Maximum Likelihood (ML) analyses using IQ-TREE 2 (v. 2.2.0.3. pre-release, Minh *et al.* 2020). Alignment of SNPs in phylip format was generated using vcf2phylip.py script (available at: https://github.com/edgardomortiz/vcf2phylip; Ortiz 2019). Optimal model search was done with ModelFinder (Kalyaanamoorthy *et al.* 2017) with Felsenstein's ascertainment bias correction factor (Lewis 2001) using -m MFP+ASC. Afterwards, best-fit model according to AIC (Akaike Information Criterion) was chosen for phylogenetic tree reconstruction with alignment (.varsites.phy) depleted of invariant sites removed through IQTREE automatically. Tree search was done under a General Time Reversible model with uniform rate heterogeneity using -m GTR+F+ASC with 1000 ultra-fast bootstrapping replicates (Hoang *et al.* 2018) and random seed number generating 100 starting Maximum Parsimony trees. Finally, the optimum phylogenetic tree (.treefile) was visualised with FigTree v. 1.4.4. (Rambaut 2009). This approach was applied to both datasets described above. In addition, we calculated Nei's genetic distance (Nei 1972) between all individuals using 'StAMPP' package v. 1.6.1. (Pembleton *et al.* 2013) and constructed a Neighbour-joining (NJ) tree which was visualised in FigTree v1.4.4 (Rambaut 2009).

### *Population genetic structure of the Balkan lineage*

All population genetic structure analyses of the Balkan lineage were based on the "Balkan-cline dataset" which included 587 individuals from 108 populations and a set of 6240 SNPs. For exploratory purposes, we first run principal component analysis (PCA) on the SNP data in Adegenet R package 2.1.3 (Jombart *et al.* 2010). Population genetic structure was further assessed with two complementary clustering approaches. First, we applied a commonly used Bayesian approach implemented in STRUCTURE version 2.3.4, which relies on the assumptions of the population genetics models. We ran STRUCTURE within the *structure-threader* program, with the same analysis parameters as described above, except for the K values which ranged from 1 to 15. Second, we inferred the genetic clustering of the populations *de novo* using the discriminant analysis of principal components (DAPC) implemented in the R's Adegenet. Unlike STRUCTURE, DAPC is a model-free approach without any prior assumptions about underling population genetic processes and as such is recommended to be used in combination with model-based approaches (Dufresne *et al.* 2014). We first optimised the number of PCs to be retained based on the cross-validation method (built-in function *xvalDapc*) and then estimated the optimal number of genetic clusters based on K-means algorithm and the lowest associated Bayesian information criterion (BIC) value. DAPC was then built by maintaining the first 100 PCs and two discriminant functions.

### *Population genetic diversity and differentiation*

We estimated genome-wide population genetic diversity parameters for each diploid population (n = 100) and for the main genetic groups inferred by STRUCTURE (excluding admixed populations with < 70% assignment to a genetic group, see results), including the mean allelic richness across all loci (Ar), observed (Ho) and expected (He) heterozygosity, calculated using 'hierfstat' package v. 0.5-10 (Goudet 2005). As polyploids would violate some of the basic population genetic assumptions, eight tetraploid populations (identified in Terlević, Bogdanović, *et al.* 2022) were excluded from all the population genetics analyses. We estimated the genetic differentiation among all populations (overall $F_{ST}$) and between all population pairs (pairwise $F_{ST}$) based on Weir and Cockerham (1984) using the *genet.dist* function in hierfstat. The resulting pairwise $F_{ST}$ matrix was visualised using heatmaps. To test for genetic differentiation among main genetic groups inferred from STRUCTURE (see results), we carried out hierarchical analysis of molecular variance (AMOVA) in Arlequin 3.5.2.2. (Excoffier and Lischer 2010) and tested the significance with 10000 permutations. We also explored the spatial genetic structure of the Balkan populations by testing for isolation by distance (IBD; Rousset 1997) based on pairwise geographic distances (in km) and linearised pairwise $F_{ST}$ values ($F_{ST} / [1 - F_{ST}]$). IBD was tested with a Mantel test using 9999 permutations in vegan (Oksanen *et al.* 2020) and the resulting plot of genetic vs. geographic distances was visualised using the package MASS.

### *Genetic connectivity and migration patterns*

To identify putative genetic connectivity patterns among populations, diploid populations of the Balkan-cline dataset (n = 100) were considered to construct a Population Graph based on their genetic covariances (Dyer and Nason 2004) using ‘popgraph’ v. 1.5 and gstudio R packages (Dyer 2017). This population network reflects genetic connections (edges) among all sampled populations (nodes) arising from both gene flow and shared ancestry, and has been shown to be successful in capturing underlying demographic processes (Dyer *et al.* 2010). When population graph topology is plotted in geographic space and assessed in relation to IBD model, edges can be partitioned into extended (indicating long-distance gene-flow), compressed (indicating barriers to gene flow and/or restricted connectivity) and normal (in agreement with IBD expectations; Dyer *et al.* 2010). Furthermore, to assess directional relative migration rates (ranging between 0 and 1) and to detect putative asymmetries in gene flow between the genetic groups identified with DAPC, we used DivMigrate function (Sundqvist *et al.* 2016) implemented in the R package ‘diveRsity’ (Keenan *et al.* 2013). The analyses were run based on Jost’s D metric of genetic differentiation, using 1000 bootstraps, and alpha = 0.05. In addition, to identify putative number of migration events (m) and directionality of gene flow among DAPC groups, we used Treemix (Pickrell and Pritchard 2012) which was run with m ranging from 1–3, 10 iterations per m, varying ‘-k’ (number of SNPs per window) across each run – random k between 100 and 1000 in 50 SNP increments. TreeMix was ran through a pipeline based on scripts written by Vajana and Milanesi (2017) and R functions by Zecca, Labra and Grassi (2019) available on GitHub: https://github.com/carolindahms/TreeMix. Optimal m was selected using both Evanno and linear method implemented in the ‘optM’ package (Fitak 2021), and the resulting ML tree was plotted using R functions provided in the Treemix.

### *Species distribution modelling*

To predicted potential habitat suitability for *D. sylvestris* during present-day and the Last Glacial Maximum (LGM, ~21 kya) time periods in our study area we used species distribution models (SDMs). We initially obtained 306 occurrences of *D. sylvestris* from various sources including our extensive field sampling. To reduce the sampling bias, occurrences closer than 1 km were omitted using the “thin” function in the “spThin” package 0.2.0 (Aiello-Lammens *et al.* 2015). After thinning, a total of 238 occurrences of *D. sylvestris* were retained for developing SDMs (Fig. 4A, Table S1). We retrieved climate data from the Chelsa database (Karger *et al.* 2017, 2020) and an initial set of 19 bioclimatic variables was first evaluated for collinearity by calculating variance inflation factor (VIF) with the "usdm" R package (Naimi *et al.* 2014). Variables with VIF > 10 were discarded, thus the number of predictor variables was reduced, retaining those considered to be ecologically relevant for *D. sylvestris* (Table 1). After the variable selection procedure, we built SDMs with a total of six climatic predictors obtained for the current and LGM period at a spatial resolution of ∼1 km2 (Table 1). Corresponding variables for the LGM period were obtained from the Chelsa climate PMIP3 dataset for four different global circulation models (GCMs): NCAR-CCSM4, MRI-CGCM3, MPI-ESM-P, and MIROC-ESM.

**Table 1**. Six selected climatic variables used as predictors for *Dianthus sylvestris* SDMs and predicting the potential present and past (last glacial maximum – LGM) habitat suitability. Shown is also mean variable importance to the ensemble model.

| Variable abbreviation | Full name | Unit | Mean variable importance |
|---|---|---|---|
| bio4 | temperature seasonality | °C/10 | 0.33 |
| bio5 | mean daily maximum air temperture of the warmest month | °C/10 | 0.32 |
| bio8 | mean daily mean air temperatures of the wettest quarter | °C/10 | 0.17 |
| bio9 | mean daily mean air temperatures of the driest quarter | °C/10 | 0.21 |
| bio18 | mean monthyl precipitation amount of the warmest quarter | kg m-2 | 0.15 |
| bio19 | mean monthly precipitation amount of the coldest quarter | kg m$^{-2}$ | 0.27 |

We built habitat suitability models using an ensemble modelling approach implemented in the 'biomod2' package (Thuiller *et al.* 2019) with five modelling algorithms (Maximum Entropy (Maxent), Generalised Linear Model (GLM), Generalised Additive Model (GAM), Boosted Regression Trees (GBM), and Random Forest (RF)). To assess model performance, we used the approach of data partitioning with 70% of the occurrences used for model training and the remaining 30% of occurrences used for model evaluation. In addition to species occurrence data, we also generated 10 000 random "background" points within the study area for model evaluation. For each algorithm, we ran ten replicates using cross-validation and assessed the model performance using AUC parameter (area under the receiver operating characteristic curve). Models with AUC value higher than the calculated median AUC score across all models (AUC > 0.934) were included in the final ensemble model. We then projected the resulting ensemble model onto present and LGM climate conditions, to obtain potential current and past habitat suitability maps for *D. sylvestris*. The four different GCM projections for the LGM were averaged to obtain a single “ensemble” habitat suitability map for the LGM period. We also converted continuous habitat suitability projections into binary (presence/absence) using the ensemble model cut-off value (0.286) based on the maximum sensitivity and specificity threshold.

## Results

### *Population genetic structure and phylogenetic relationships among all populations*

The analysed “all-lineage dataset”, encompassed 165 sampled populations (a total of 992 individuals) across the Balkans, Apennines and Alps (Fig. 1, Fig. S1). Model-based STRUCTURE and the associated Evanno method revealed an optimal genetic clustering of populations at K=2 (Fig. S4A) which corresponded to the split between the Alpine populations in one group, and the Apennine and Balkan populations which clustered together in the second group (Fig. 1A). At K=3 a further split was suggested within the Balkan populations separating the north-western (NW) and the south-eastern (SE) group of populations (Fig. 1B). At K=6 separation of the three lineages of *D. sylvestris,* as well as separation of *D. sylvestris* subsp. *tergestinus* became apparent, whereas additional substructure was detected within the SE Balkan group (Fig. 1C). Genetic clustering at K=6 was largely congruent with the constructed unrooted NJ tree based on Nei’s genetic distances which nicely/strongly supported the separation of the Apennine, Alpine and Balkan lineage according to Luqman *et al.* (2022), but also the separation of *D. sylvestris* subsp. *tergestinus* from the rest of the Balkan populations which were further split into two main groups with some more heterogeneity and divergence in the SE group (Fig. 1D, Fig. S2).

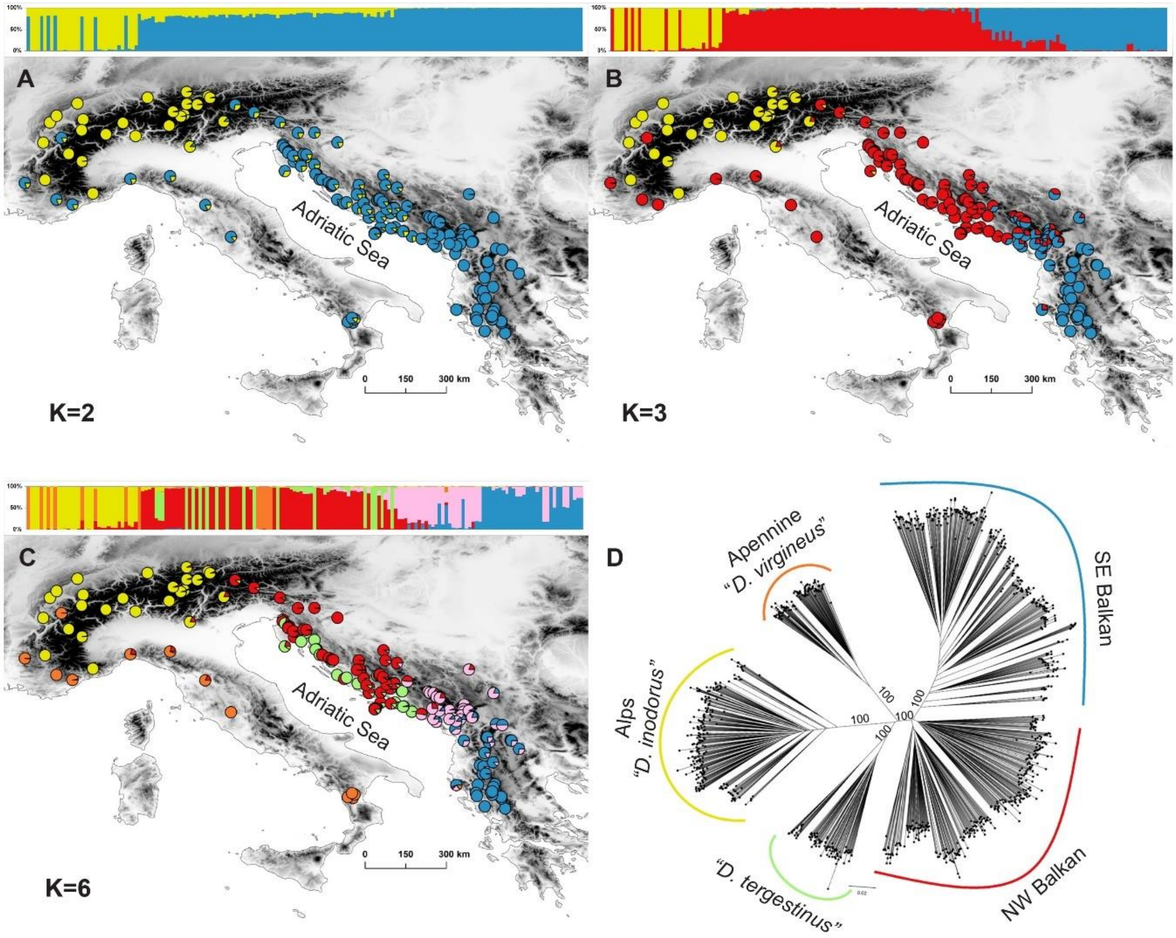


**Figure 1.** Population genetic structure of *Dianthus sylvestris* based on the "all-lineage dataset" (165 populations, 992 individuals and 5644 SNPs) as inferred from STRUCTURE at A) K=2, B) K=3 and C) K=6. Pie charts show the assignment probability to each of the clusters across populations and the barplots show individual assignments (individuals are ordered by longitude). D) Unrooted Neighbour-joining (NJ) tree based on Nei's genetic distance between all individuals. Bootstrap support as inferred for the Maximum Likelihood tree is shown above the branches (for details see Fig. S2).

### *Genetic structure of the Balkan-cline*

PCA on the SNP data showed that genetic variation pattern forms a cline with some clear discontinuities visible along the first three PC axes (Fig. 2A). STRUCTURE analysis repeated on the Balkan-cline dataset revealed again two major clusters as the optimal number of ancestral groups (Fig. S4B) corresponding to the NW and SE populations, with admixed populations occurring in the area of Montenegro (Fig. 2B). This result was well supported by the ML phylogenetic tree based on the same dataset (100% BS, Fig. 2D, Fig. S3). On the other hand, DAPC results suggested that genetic variation of Balkan populations can be optimally summarised in five clusters (K=5 had the lowest BIC value, Fig. S5), two very close groups in the NW and three more divergent in the SE (Fig. 2C).

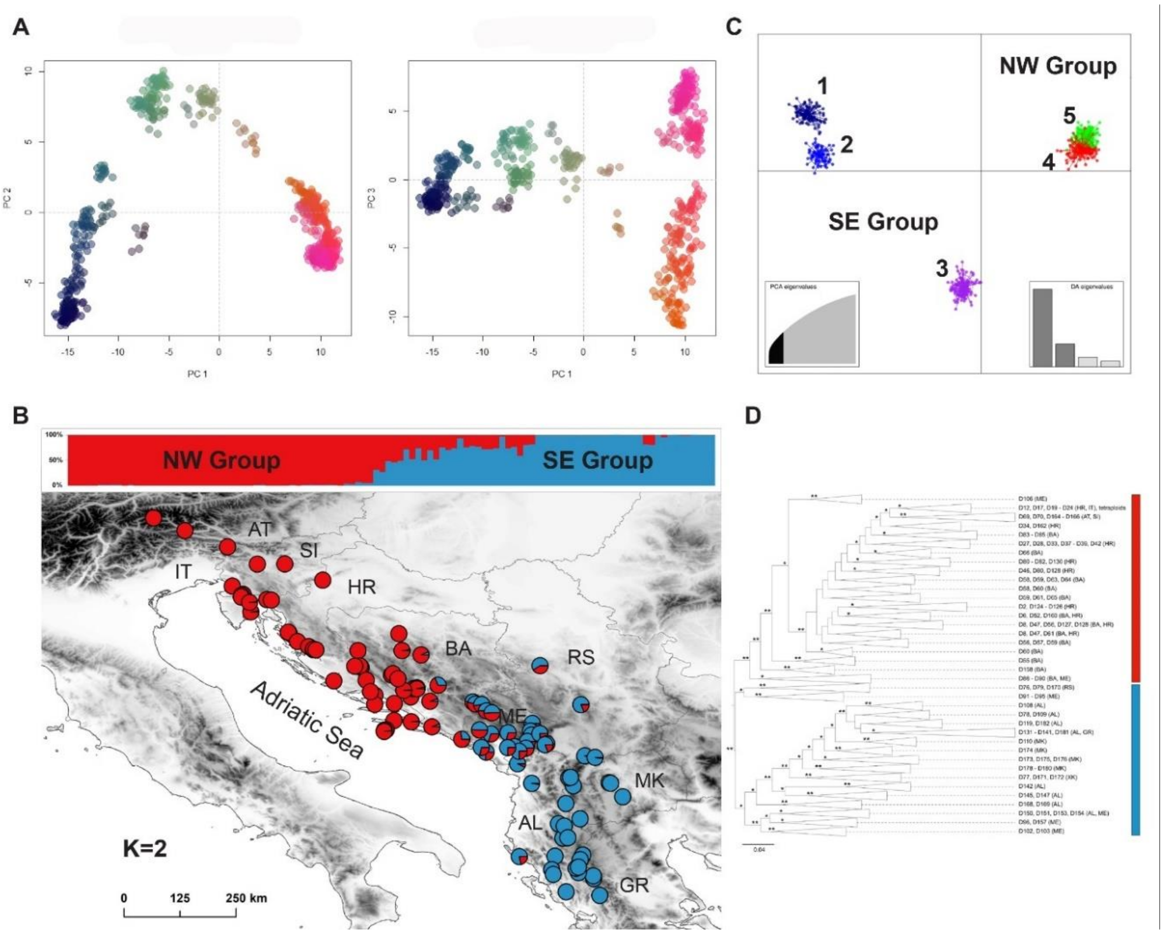


**Figure 2.** Population genetic structure of *Dianthus sylvestris* based on the "Balkan-cline dataset" (108 populations, 587 Individuals and 6240 SNPs). A) PCA scatterplot of individual genotypes on the RGB scale, where the genetic variation is represented in two complementary ways: by the distances (further away = more genetically different), and by the colours (more different colours = more genetically different). Five groups on the gradient can be seen, representing discontinuities in the cline. B) Optimal population genetic structure and individual assignments/ancestry at K=2 inferred by STRUCTURE showing two major genetic groups of *Dianthus sylvestris*: north-western (NW) group and south-eastern (SE) group. Individuals are ordered by longitude. C) Scatterplot of the five genetic clusters identified by DAPC. Bottom boxes indicate the number of retained principal components (100) and retained discriminant analysis eigenvalues. D) Phylogenetic Maximum Likelihood tree obtained via IQ-TREE showing strong support for the two main genetic groups identified with STRUCTURE (** indicates 100%, and * indicates 90–99% bootstrap support, for details see Fig. S3).

*Population genetic diversity and differentiation of the Balkan-cline*

Allelic richness per population (Ar) ranged from 1.186 to 1.277 (mean Ar = 1.218). Highest heterozygosity was observed in population D23 from Croatia (Ho = 0.359), and the lowest in population D141 from Albania (Ho = 0.189, Table S2). He was also highest in population D23 from Croatia (He = 0.266), followed by several populations from Bosnia and Herzegovina (D61, D64 and D59). Lowest He was observed in population D39 from Croatia (He = 0.168), followed by population D142 from Albania (Fig. 3A, Table S2). The observed heterozygosity (Ho) was significantly lower than expected heterozygosity (He, Fig. S6). Estimated genetic diversity parameters (Ar, Ho and He) showed very similar spatial pattern indicating a cline where southern-most populations had in general lower/reduced genetic diversity (Fig. 3A). This pattern was most pronounced for Ar which most significantly declined with latitude ($R^2 = 0.134$, $p < 0.001$, Table S3).

Genetic diversity values (Ar, Ho and He) were similar between the two genetic groups identified with STRUCTURE and had almost identical He values (He NW = 0.245; He SE = 0.243, Table 2). Overall, Balkan populations showed significant genetic differentiation based on global $F_{\mathrm{ST}} = 0.166$, $p < 0.001$) while pairwise $F_{\mathrm{ST}}$ values ranged from 0.005 (between populations D63 and D64) to 0.355 (between populations D39 and D132) (Fig. S7, Table S2). The hierarchical AMOVA results revealed significant genetic differentiation between the two main genetic groups/clusters identified with STRUCTURE ($F_{\mathrm{CT}}$ between groups = 0.13, $p < 0.0001$) as well as among populations where 13% of genetic variation was found to be among groups, 9.15% among populations within groups and 77.79% within populations (Table 3).

**Table 2.** Genetic diversity of the two main Balkan *Dianthus sylvestris* groups inferred from STRUCTURE.

| Group | Ar | Ho | He |
|---|---|---|---|
| K1 SE | 1.870 | 0.217 | 0.243 |
| K2 NW | 1.894 | 0.228 | 0.245 |

**Table 3.** Hierarchical Analysis of Molecular Variance (AMOVA) for the Balkan *Dianthus sylvestris* populations based on two genetic groups inferred from STRUCTURE.

| **Source of variation** | **Sum of squares** | **Variance components** | **% Variation** | ***F* statistics** |
|---|---|---|---|---|
| Among groups | 52591.41 | 101.330 | 13.07 | $F_{CT} = 0.131$*** |
| Among populations within two groups | 124325.06 | 70.955 | 9.15 | $F_{SC} = 0.105$*** |
| Within populations | 553827.42 | 603.298 | 77.79 | $F_{ST} = 0.222$*** |

Significance after 10000 permutations ***$p < 0.0001$

*Genetic connectivity and migration patterns*

The isolation by distance pattern was significant and revealed that genetic differences increase with geographic distance ($R^2$=0.6, p=0.001, Fig. 3B). The Population graph analysis identified two major disconnected population networks and several smaller disconnected subnetworks (Fig. 3C). Higher connectivity was apparent among the NW group of populations where several populations were connected with extended edges (Fig. 3C) connecting populations that are genetically more similar than expected based on their geographic distance, thus suggesting long-distance gene flow. The southernmost group of populations from Greece, Albania and Macedonia formed a separated population network where the majority of populations were connected with normal edges and only one compressed edge was suggested indicating barriers to gene flow (Fig. 3C). The main difference in comparison to STRUCTURE results was in the area of Montenegro where admixed populations were identified with STRUCTURE at K=2, while popgraph analysis revealed several small subnetworks consisting of 2 up to 7 populations that were entirely disconnected from the two main population networks (Fig. 3C).

Relative migration rates were estimated between five genetic clusters inferred from DAPC (Fig. 3E-F). The highest levels of relative symmetric gene flow (> 0.9) were detected between the two NW Balkan DAPC groups (group 4 and 5, Fig. 3E-F). Migration rates were half lower between the two southern-most Balkan DAPC groups (groups 1 and 2), followed by migration rates between DAPC group 2 and 3. Relatively lower migration rates ($m < 0.33$) were found between the northern and southern groups. Only one significant asymmetric migration was revealed (1000 bootstraps, $\alpha$=0.05), indicating gene flow from the southern-most DAPC group 1 (Greece and Albania) up to the area of Montenegro (DAPC group 3), but not further north (Fig. 3E-F). Treemix model uncovered relatively similar migration pattern compared to

DivMigrate analysis. Model with only one migration event (m = 1) fitted our data best according to the OptM function (accounting for > 99.99% of the variance in the data) and indicated single migration step from DAPC group 2 (Albania, North Macedonia) toward group 3 (Fig. 3E-F).

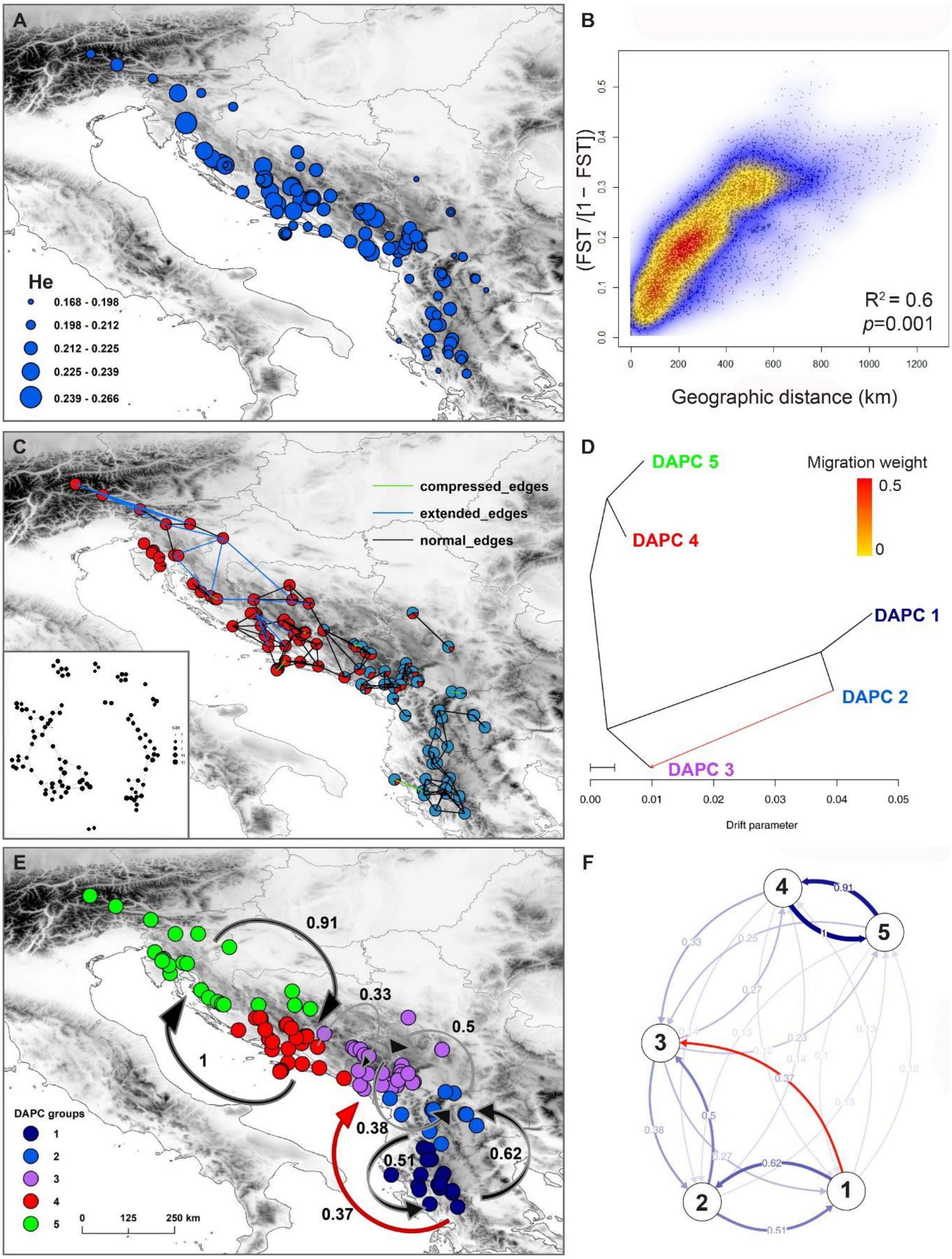


**Figure 3.** Genetic diversity, connectivity and migration patterns among *Dianthus sylvestris* populations of the Balkan-cline. A) Geographical pattern of genetic diversity depicted by Expected heterozygosity (He). B) Significant pattern of Isolation by distance (IBD) visualised with a density function. C) Population graph showing the genetic connectivity among populations based on their genetic covariances. Graph topology is mapped in geographic space and assessed in relation to the IBD model. Partitioning of the edges based on IBD: extended edges in blue indicate long-distance gene flow, compressed edges in green suggest geographical or ecological barriers to gene flow, normal edges are concordat with IBD expectations. Populations on the map are coloured according to K=2 inferred from STRUCTURE. In the inset, popgraph is visualised using the Fruchterman-Reingold algorithm where node size reflects within-population genetic variation. A-C panels are based on 100 diploid populations. D) Maximum Likelihood tree based on five genetic clusters identified with DAPC obtained with TreeMix showing one significant migration event from group DAPC 2 to DAPC 3 indicated with an arrow and coloured according to the migration weight. E-F) Directional relative migration between five genetic clusters identified with DAPC estimated by DivMigrate. Gene flow magnitude is indicated by arrow thickness. In E, DAPC clusters are plotted on the map and denoted by the same colours as in Figure 2C. Only relative migration rates above 0.33 are shown on the map indicating the highest gene flow among NW populations and as twice as lower among SW groups of populations. In F, all relative migrations rates based on Jost’s D metric are shown in the network. Only one significant asymmetric migration was detected from DAPC group 1 towards group 3 (admixed populations at K=2 inferred from STRUCTURE) indicated by the red arrow.

### *Species distribution modelling*

We used SDMs to predict suitable areas for *D. sylvestris* in our study area during present and particularly during LGM period as they could point to locations of putative glacial refugia for this species. Our ensemble prediction of current habitat suitability corresponded well to the known distribution of *D. sylvestris* in the Balkans (Fig. 4A). LGM projections indicated that habitat suitability in the study area was lower than today (up to 0.58), however potential distribution of *D. sylvestris* was relatively similar to predicted current one or may have been even wider (Fig. 4B). Climatically suitable areas for species survival were predicted along the whole eastern Adriatic coast, with the highest LGM habitat suitability predicted in the south of the Balkan peninsula (ME and AL; Fig. 4C). After converting the continuous habitat suitability map for the LGM period into suitability classes, suitable areas (those above the threshold of 0.286) were clearly divided into class of higher suitability (corresponding roughly to the SE Adriatic) and a class of lower suitability (corresponding roughly to the NW Adriatic) (Fig. 4D-E). When we overlapped this resulting map of LGM habitat suitability with the major genetic clustering inferred from Structure at K=2, the two clusters highly corresponded with the two areas of habitat suitability (Fig. 4E), suggesting that the observed genetic structure could be related to species survival in two different glacial refugia.

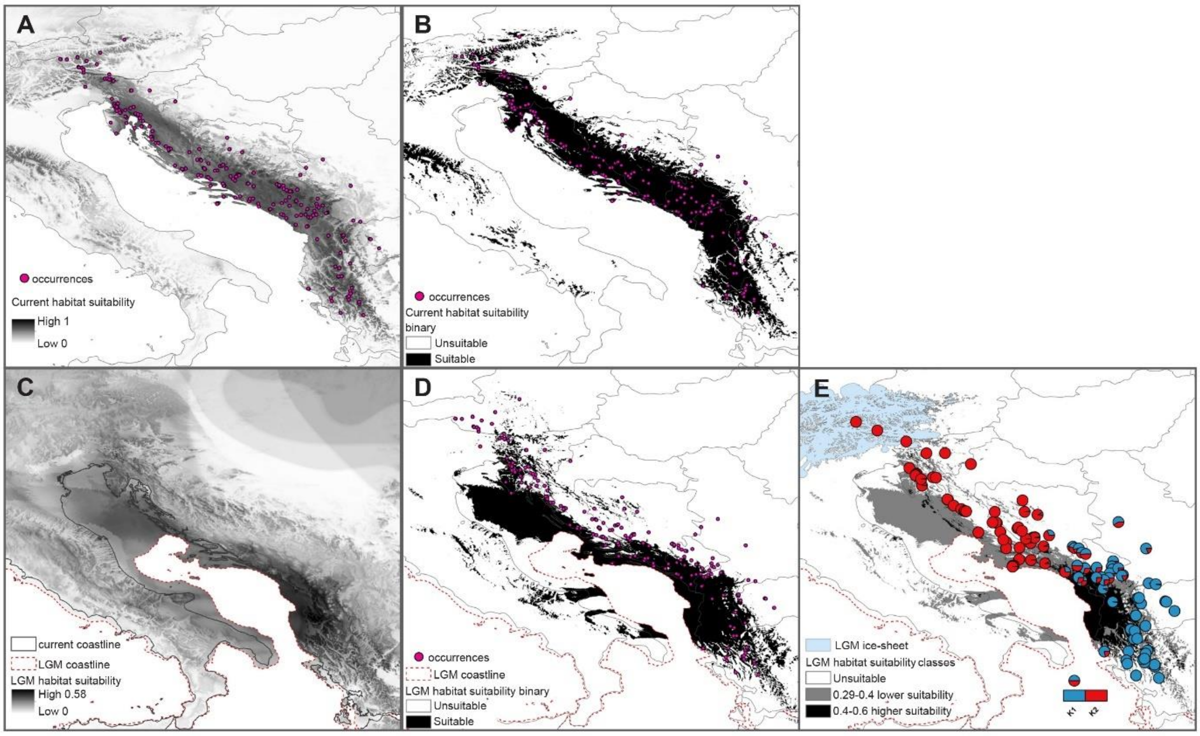


**Figure 4.** Predicted ensemble habitat suitability maps for *Dianthus sylvestris* s.l. based on 238 occurrences across the Balkan Peninsula for the current (A-B) and the Last Glacial Maximum (LGM) period (C-E). In A and C, continuous habitat suitability maps are shown with darker areas indicating higher suitability. Suitability values range from 0 to 1 for current period and from 0 to maximal suitability value (0.58) for LGM. In B and D, binary habitat suitability maps indicate only suitable or unsuitable areas based on the maximum sensitivity and specificity threshold. In E, habitat suitability classes are overlapped with the Structure output at K=2 based on the Balkan-cline dataset. Extent of the ice-sheet during LGM is also shown on the map.

## Discussion

Our phylogenetic analyses based on ML and NJ trees combined with STRUCTURE results strongly supported the presence of three main geographically structured evolutionary lineages within *D. sylvestris,* as recently revealed by Luqman *et al.* (2022). However, our more comprehensive sampling of the Balkan Peninsula revealed the divergence of *D. sylvestris* subsp. *tergestinus* from the rest of the Balkan populations. Furthermore, in-depth population genetic analyses revealed a more complex pattern of genomic variation within the Balkan lineage with hierarchical phylogeographic structure. Population genetic structure of the Balkan lineage suggested that two main genetic groups occur within the Balkan Peninsula, where the NW group seems to be more homogeneous than SE group, which was shown to be more divergent and substructured. Geographical patterns of genetic diversity showed that in general southernmost populations have reduced genetic diversity compared to the northern ones; however, the two main genetic groups seem to harbour similar levels of genetic diversity. Genetic connectivity and migration analyses indicated extensive gene flow among the populations in the NW group, and comparatively much lower gene flow among populations in the SE group, probably promoting their increased divergence. Estimates of directional migration indicated that gene flow mainly occurs from southernmost populations located in Greece, Albania and North Macedonia, approximately up to the area of Montenegro, but not further north, suggesting that SE populations were not the source for the recolonization of the NW. In addition, very limited gene flow was suggested between the populations from the NW and SE groups, indicating barriers to gene flow. Overall, our results suggest that these two groups of populations may represent two separately evolving evolutionary lineages within the Balkan lineage, particularly when combined with potential habitat suitability within the study area during LGM, providing valuable insights into putative refugial areas for *D. sylvestris* within the Balkan Peninsula. We discuss possible phylogeographic scenarios which may have driven the observed genomic variation patterns in *D. sylvestris.*

### *The three lineages and genetic divergence of Dianthus sylvestris subsp. tergestinus*

Using dated phylogeny, the results of Valente *et al.* (2010) suggested that 2.0–1.3 Mya ago an acceleration in *Dianthus* diversification took place, and an association between the climatic aridity in the Pleistocene and the shift in diversification rate of *Dianthus* has been proposed. A trichotomy in the genomic data of the corresponding study area was already revealed by Luqman *et al.* (2022), where *D. sylvestris* is split into three evolutionary lineages originating from distinct glacial refugia: Alpine, Apennine and Balkan lineage. In addition, the former study identified Adriatic Sea as one of the major genetic boundaries between the lineages. However, our STRUCTURE results revealed that at K=3, the populations from the Alps and those from the SE Balkan Peninsula form distinct genetic lineages, whereas the Apennine and the NW Balkan populations share the same genetic pool. This is for example concordant with the phylogeographical pattern revealed in *Silene saxifraga* group (Đurović *et al.* 2017). However, the separation of the three lineages was very well supported in the phylogenetic trees (Fig. 1D, Fig. S2).

Amphi-Adriatic disjunctions are known from a number of studies and have been explained by land connections and disconnections during the Messinian Salinity Crisis (Krijgsman *et al.* 1999) or Quaternary climatic changes (Gridelli 1950). However, phylogeographic connections between Balkan and Apennine populations are well documented and in most cases restricted to central and southern parts of both peninsulas (Đurović *et al.* 2017; Frajman and Schönswetter 2017; Janković *et al.* 2019; Falch *et al.* 2019; Rešetnik *et al.* 2022). Research on *Edraianthus graminifolius* (Surina *et al.* 2014), for example, revealed that the most pronounced genetic discontinuity occurs within the western Balkan Peninsula, rather than across the Adriatic Sea separating the two peninsulas, indicating that, contrary to the expectations, the Adriatic Sea did not represent the main genetic barrier for this high mountain species. Despite of our limited sampling across the Apennine Peninsula, our results do not support the existence of the amphi-Adriatic connections, therefore indicating both the Adriatic Sea and the Balkan topology as barriers to gene flow.

The area between the south-eastern Alps and northern Dinaric mountains was found to be a contact zone between lineages of, e.g., *Knautia drymeia* Heuff. (Rešetnik *et al.* 2016) and the *Heliosperma pusillum* group (Frajman and Oxelman 2007). However, the contact zone between the Alpine and Balkan lineage of *D. sylvestris* is positioned further to the northwest in the Dolomitic Alps (Fig. 1A-B). Nevertheless, all contemporary populations belonging to the Alpine lineage likely evolved independently from the Balkan lineage from an alpine-like LGM refugium located *in-situ* in the Alps from where they expanded westwards (Luqman *et al.* 2022). Consequently, ancestral *D. sylvestris* populations had genotypes closer to those of contemporary populations in current alpine habitats. Our results however imply a different evolutionary history of the Balkan lineage compared to the Alpine one (see below).

Although the STRUCTURE clustering at K=6 was not optimal according to the Evanno statistics, STRUCTURE output together with the phylogenetic trees show a clear separation of *D. sylvestris* subsp. *tergestinus,* from the rest of the Balkan populations despite their co-occurrence in sympatry. This subspecies could be distinguished from the sympatric subsp. *sylvestris* and subsp. *nodosus* by the number of epicalyx scales, but all three subspecies form a morphological gradient in other floral and vegetative traits (Terlević, Temunović, *et al.* 2022). Thus, *D. sylvestris* subsp. *tergestinus* may be an example of a cryptic and evolutionary distinct species (Bickford *et al.* 2007), which deserves an upgraded taxonomic rank. However, its taxonomic designation is out of the scope of this paper.

### *The Balkan-cline*

Majority of our results point to the main pattern of two distinct genetic groups of *D. sylvestris* in the Balkans (NW and SE), suggesting the existence of two evolutionary lineages within the Balkan-cline. This deep genetic split occurring in the vicinity of the Neretva river valley is reflected in the morphological variation of these populations, particularly in the variation of the epicalyx scales shape (Terlević, Temunović, *et al.* 2022). Moreover, the genetic split into two clusters was remarkably congruent with the pattern of habitat suitability during LGM inferred from our SDMs indicating larger area of lower suitability in the NW and smaller area of higher suitability in the SE Adriatic region (Fig. 4C-E). This pattern of habitat suitability could be put in relation with the finding of tetraploid *D. sylvestris* populations in the small area of Istria (Terlević, Bogdanović, *et al.* 2022), which gives additional support for the existence of a distinct refugium in the northern Adriatic region (Rešetnik *et al.* 2020). Finally, results obtained by Treemix and DivMigrate were largely concordant, suggesting almost no migration between the populations of the NW and SE groups, and directional migration was revealed only from the southern-most subgroups (DAPC 1 and 2) up to the area of Montenegro (DAPC group 3), where the migration likely stopped (Fig. 3E). Taken together, along with the similar levels of genetic diversity of the two groups, these results go in favour of the scenario of two distinct glacial refugia (northern and southern) for *D. sylvestris* in the Balkans. The persistence in two different refugia hosting populations with similar levels of genetic diversity was also suggested for e.g., *Viola suavis s.l.* (Mereďa *et al.* 2011) and *Arundo plinii* (Hardion *et al.* 2014).

Similar genetic breaks which coincide with the Neretva valley have been repeatedly observed in the Balkan peninsula, even for taxa of contrasting ecologies (Kučera *et al.* 2010; Đurović *et al.* 2021; Reich *et al.* 2022; Španiel and Rešetnik 2022) and are usually explained with the two main hypothesis. The hypothesis of two ecologically divergent glacial refugia with different climatic conditions along the eastern Adriatic coast during Pleistocene was proposed by Lakušić *et al.* (2013) and Kutnjak *et al.* (2014) where climatic and/or environmental differences between NW and SE Adriatic may have driven the genetic divergence of populations. A contrasting hypothesis by Đurović *et al.* (2021) implies that the Neretva river valley acted as a physical barrier to gene flow in *Cerastium* species, rather than ecological one because suitable climatic conditions for species survival were predicted on both sides of the valley during the LGM. Two distinct genetic groups of *D. sylvestris* within the Balkan lineage and very similar levels of genetic diversity observed for each group indeed indicate the presence of two main glacial refugia, one in the NW and the other in the SE of the east Adriatic coast. Although our predicted habitat suitability pattern for *D. sylvestris* gives more support for the two climatically different refugia during LGM, for the moment, and based on our results, we cannot discard any of the two in our opinion non-exclusive hypotheses described above. It could simply be that the abrupt climatic change from NW to SE of the Adriatic during LGM geographically coincides with the Neretva river valley as proposed by Lakušić *et al.* (2013), and/or with sea level fluctuations during LGM (Sikora *et al.* 2013), whereas the valley itself and the associated lowland habitats unfavourable for some taxa further reinforces the barrier to gene flow. We may assume that populations contracted and expanded to these refugial areas not only during LGM, but over multiple glacial cycles during Pleistocene, as population divergence may have been promoted by isolation on a time scale exceeding a single glaciation (Hewitt 1996). During expansions, probably related to interglacials and the Holocene for the thermophilous low-to-mid-elevation populations, the two lineages may have come into contact in the intermediate area of Montenegro where this event resulted in admixed populations observed here. Similar pattern of admixture in the intermediate area of Montenegro was also proposed for *Gentianella crispata* (Reich *et al.* 2022).

Overall, potential distribution and suitable areas of *D. sylvestris* during LGM were predicted to be very similar to current ones, only with decreased suitability and displaced to lower altitudes. This is possible due to only partially glaciated Balkan mountain tops during LGM, as opposed to Alps where Alpine lineage experienced severe range contraction during LGM and subsequent expansion during the post-glacial period (Milivojević *et al.* 2008; Žebre *et al.* 2019; Luqman *et al.* 2022). Such observed pattern in the Balkan lineage may be particularly true for the cold-adapted, high-elevation populations of *D. sylvestris* which may have followed a different migration pattern compared to their low-elevation counterparts. In such scenario, high-elevation populations would persist during Pleistocene climate oscillations more or less *in situ* shifting their distributions only along the mountain altitudinal clines to track favourable climate (Surina *et al.* 2011; Smyčka *et al.* 2022). This scenario is partly supported with our population graph results showing two main largely disconnected population networks corresponding to the NW and SE group of populations inferred from STRUCTURE, apparently giving additional weight to two distinct refugia within the Balkans. However, unlike other analyses, population graph uncovered several small population groups in the borderline of the Scardo-Pindhic and the Dinaric mountain ranges (2 to 7 populations) that were entirely disconnected from the two main population networks. This suggests, as opposed to admixture of lineages from two distinct refugia, an alternative scenario where these populations were stuck in this intermediate area within several *in situ* microrefugia during the glaciation cycles (not necessarily only since LGM) until today, and that they have diverged independently from the two main Balkan lineages.

In addition, Luqman *et al.* (2022) showed for the Alpine lineage that all contemporary *D. sylvestris* populations, both high-elevation (alpine) and low-elevation (in valleys) evolved from a common alpine-like ancestral adaptive genotype present in the LGM refugia. Moreover, low-elevation populations of the Alpine lineage were characterised by lower diversity, compared to high-elevation populations (Luqman *et al.* 2022). Thus, the genetic diversity of *D. sylvestris* in heterogeneous landscapes seems to be determined by the environmental distance between a population’s current habitat and its ancestral alpine-like habitat. If we assume that this is the case for all *Dianthus* lineages (which diverged ca. 200-115 Kya), this suggests that at least part of the Balkan populations which are alpine-like (roughly those above 2000 m altitude corresponding to the Scardo-Pindhic mountain range and Albanian Alps in the SE of the Balkan peninsula) may even had a wider distribution during the glacials than today, and have contracted to the highest mountain areas during unfavourable interglacials where they have remained until today. Consequently, habitat fragmentation during warmer stages (and currently ongoing since the LGM), accompanied by the observed reduced gene flow, may have promoted increased divergence of SE subgroups of populations and decreased genetic diversity in the southernmost areas. Such a case of higher levels of genetic diversity were observed in the northern populations with a decrease toward the southern populations was found in *Haberlea rhodopensis* (Petrova *et al.* 2015), and it was explained as a consequence of severe distribution contractions causing genetic bottlenecks during LGM. The southernmost populations also show many features of rear edge populations, according to Hampe and Petit (2005), and as such, their reduced within-population genetic diversity and increased divergence among populations may be a result of their successful long-term *in situ* persistence in the region that provided suitable conditions for their persistence under both glacials and interglacials. Accordingly, the southernmost Balkan mountains (Albania, Greece) may today, i.e. in the interglacial, represent cryptic southern refugia and/or environmental margin for the high-elevation populations of *D. sylvestris* (Stewart *et al.* 2010).

## References

**Aiello-Lammens ME, Boria RA, Radosavljevic A, Vilela B, Anderson RP**. **2015**. spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. *Ecography* **38**: 541–545.

**Bacchetta G, Brullo S, Casti M, Pietro Giusso del Galdo G**. **2010**. Taxonomic revision of the *Dianthus sylvestris* group (Caryophyllaceae) in central-southern Italy, Sicily and Sardinia. *Nordic Journal of Botany* **28**: 137–173.

**Bickford D, Lohman DJ, Sodhi NS, *et al.* 2007**. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* **22**: 148–155.

**Brullo S, Guarino R**. **2019**. *Dianthus* In: Pignatti S, ed. *Flora d'Italia. volume 4*. Milano: Edagricole, .

**Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA**. **2013**. Stacks: an analysis tool set for population genomics. *Molecular Ecology* **22**: 3124–3140.

**Danecek P, Auton A, Abecasis G, *et al.* 2011**. The variant call format and VCFtools. *Bioinformatics* **27**: 2156–2158.

**Domina G, Astuti G, Bacchetta G, *et al.* 2021**. Typification of 14 names in the *Dianthus virgineus* group (Caryophyllaceae). *PhytoKeys* **187**: 1–14.

**Domina G, Astuti G, Barone G, *et al.* 2021**. Lectotypification of the Linnaean name *Dianthus virgineus* (Caryophyllaceae) and its taxonomic consequences. *TAXON* **70**: 1096–1100.

**Dufresne F, Stift M, Vergilino R, Mable BK**. **2014**. Recent progress and challenges in population genetics of polyploid organisms: an overview of current state-of-the-art molecular and statistical tools. *Molecular Ecology* **23**: 40–69.

**Đurović S, Schönswetter P, Niketić M, Tomović G, Frajman B**. **2017**. Disentangling relationships among the members of the *Silene saxifraga* alliance (Caryophyllaceae): Phylogenetic structure is geographically rather than taxonomically segregated. *TAXON* **66**: 343–364.

**Đurović SZ, Temunović M, Niketić M, Tomović G, Schönswetter P, Frajman B**. **2021**. Impact of Quaternary climatic oscillations on phylogeographic patterns of three habitat-segregated *Cerastium* taxa endemic to the Dinaric Alps. *Journal of Biogeography* **48**: 2022–2036.

**Dyer RJ, Nason JD**. **2004**. Population Graphs: the graph theoretic shape of genetic structure. *Molecular Ecology* **13**: 1713–1727.

**Dyer RJ, Nason JD, Garrick RC**. **2010**. Landscape modelling of gene flow: improved power using conditional genetic distance derived from the topology of population networks. *Molecular Ecology* **19**: 3746–3759.

**Earl DA, vonHoldt BM**. **2012**. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**: 359–361.

**Evanno G, Regnaut S, Goudet J**. **2005**. Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology* **14**: 2611–2620.

**Excoffier L, Lischer HEL**. **2010**. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**: 564–567.

**Falch M, Schönswetter P, Frajman B**. **2019**. Both vicariance and dispersal have shaped the genetic structure of Eastern Mediterranean *Euphorbia myrsinites* (Euphorbiaceae). *Perspectives in Plant Ecology, Evolution and Systematics* **39**: 125459.

**Fitak RR**. **2021**. OptM: estimating the optimal number of migration edges on population trees using Treemix. *Biology Methods and Protocols* **6**: bpab017.

**Frajman B, Oxelman B**. **2007**. Reticulate phylogenetics and phytogeographical structure of *Heliosperma* (Sileneae, Caryophyllaceae) inferred from chloroplast and nuclear DNA sequences. *Molecular Phylogenetics and Evolution* **43**: 140–155.

**Frajman B, Schönswetter P**. **2017**. Amphi-Adriatic distributions in plants revisited: Pleistocene trans-Adriatic dispersal in the *Euphorbia barrelieri* group (Euphorbiaceae). *Botanical Journal of the Linnean Society* **185**: 240–252.

**Gammella M**. **2016**. Local adaptation and gene flow in serpentine and limestone populations of *D. sylvestris*.

**Garrison E, Kronenberg ZN, Dawson ET, Pedersen BS, Prins P**. **2021**. *Vcflib and tools for processing the VCF variant call format*. Bioinformatics.

**Garrison E, Marth G**. **2012**. Haplotype-based variant detection from short-read sequencing.

**Gómez A, Lunt D**. **2007**. Refugia within Refugia: Patterns of Phylogeographic Concordance in the Iberian Peninsula In: Weiss S, Ferrand N, eds. *Phylogeography of Southern European Refugia*. Springer, Dordrecht, The Netherlands., 155–188.

**Goudet J**. **2005**. hierfstat, a package for r to compute and test hierarchical F-statistics. *Molecular Ecology Notes* **5**: 184–186.

**Grdiša M, Liber Z, Radosavljević I, Carović-Stanko K, Kolak I, Satovic Z**. **2014**. Genetic Diversity and Structure of Dalmatian Pyrethrum (*Tanacetum cinerariifolium* Trevir. /Sch./ Bip., Asteraceae) within the Balkan Refugium. *PLOS ONE* **9**: e105265.

**Gridelli E**. **1950**. II problema delle specie a diffusione attuale transadriatica con particolare riguardo ai Coleotteri. *Bolletino di zoologia* **17**: 421–441.

**Hampe A, Petit RJ**. **2005**. Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters* **8**: 461–467.

**Hardion L, Baumel A, Verlaque R, Vila B**. **2014**. Distinct evolutionary histories of lowland biota on Italian and Balkan peninsulas revealed by the phylogeography of *Arundo plinii* (Poaceae). *Journal of Biogeography* **41**: 2150–2161.

**Havrdová A, Douda J, Krak K, *et al.* 2015**. Higher genetic diversity in recolonized areas than in refugia of *Alnus glutinosa* triggered by continent-wide lineage admixture. *Molecular Ecology* **24**: 4759–4777.

**Hewitt GM**. **1996**. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**: 247–276.

**Hewitt GM**. **1999**. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* **68**: 87–112.

**Hewitt G**. **2000**. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.

**Hewitt GM**. **2004**. Genetic consequences of climatic oscillations in the Quaternary (KJ Willis, KD Bennett, and D Walker, Eds.). *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **359**: 183–195.

**Hewitt GM**. **2011**. Mediterranean Peninsulas: The Evolution of Hotspots In: Zachos FE, Habel JC, eds. *Biodiversity Hotspots: Distribution and Protection of Conservation Priority Areas*. Berlin, Heidelberg: Springer, 123–147.

**Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS**. **2018**. UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution* **35**: 518–522.

**Janković I, Satovic Z, Liber Z, *et al.* 2019**. Genetic and morphological data reveal new insights into the taxonomy of *Campanula versicolor* s.l. (Campanulaceae). *TAXON* **68**: 340–369.

**Jombart T, Devillard S, Balloux F**. **2010**. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11**: 94.

**Jug-Dujaković M, Ninčević T, Liber Z, Grdiša M, Šatović Z**. **2020**. *Salvia officinalis* survived in situ Pleistocene glaciation in 'refugia within refugia' as inferred from AFLP markers. *Plant Systematics and Evolution* **306**: 38.

**Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS**. **2017**. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**: 587–589.

**Karger DN, Conrad O, Böhner J, *et al.* 2017**. Climatologies at high resolution for the earth's land surface areas. *Scientific Data* **4**: 170122.

**Karger DN, Schmatz DR, Dettling G, Zimmermann NE**. **2020**. High-resolution monthly precipitation and temperature time series from 2006 to 2100. *Scientific Data* **7**: 248.

**Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA**. **2013**. diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors (RB O'Hara, Ed.). *Methods in Ecology and Evolution* **4**: 782–788.

**Kolář F, Fuxová G, Záveská E, *et al.* 2016**. Northern glacial refugia and altitudinal niche divergence shape genome-wide differentiation in the emerging plant model *Arabidopsis arenosa*. *Molecular Ecology* **25**: 3929–3949.

**Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I**. **2015**. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources* **15**: 1179–1191.

**Krijgsman W, Hilgen FJ, Raffi I, Sierro FJ, Wilson DS**. **1999**. Chronology, causes and progression of the Messinian salinity crisis. *Nature* **400**: 652–655.

**Kučera J, Marhold K, Lihová J**. **2010**. *Cardamine maritima* group (Brassicaceae) in the amphi-Adriatic area: A hotspot of species diversity revealed by DNA sequences and morphological variation. *TAXON* **59**: 148–164.

**Kutnjak D, Kuttner M, Niketić M, Dullinger S, Schönswetter P, Frajman B**. **2014**. Escaping to the summits: Phylogeography and predicted range dynamics of *Cerastium dinaricum*, an endangered high mountain plant endemic to the western Balkan Peninsula. *Molecular Phylogenetics and Evolution* **78**: 365–374.

**Lakušić D, Liber Z, Nikolić T, *et al.* 2013**. Molecular phylogeny of the *Campanula pyramidalis* species complex (Campanulaceae) inferred from chloroplast and nuclear non-coding sequences and its taxonomic implications. *TAXON* **62**: 505–524.

**Lewis PO**. **2001**. A Likelihood Approach to Estimating Phylogeny from Discrete Morphological Character Data. *Systematic Biology* **50**: 913–925.

**Li H, Durbin R**. **2010**. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics (Oxford, England)* **26**: 589–595.

**Luqman H, Wegmann D, Fior S, Widmer A**. **2022**. Climate-induced range shifts drive adaptive response via spatio-temporal sorting of alleles. : 2022.06.07.495159.

**Médail F, Diadema K**. **2009**. Glacial refugia influence plant diversity patterns in the Mediterranean Basin. *Journal of Biogeography* **36**: 1333–1345.

**Mereďa P, Hodálová I, Kučera J, Zozomová-Lihová J, Letz DR, Slovák M**. **2011**. Genetic and morphological variation in *Viola suavis s.l.* (Violaceae) in the western Balkan Peninsula: two endemic subspecies revealed. *Systematics and Biodiversity* **9**: 211–231.

**Milivojević M, Menković L, Ćalić J**. **2008**. Pleistocene glacial relief of the central part of Mt. Prokletije (Albanian Alps). *Quaternary International* **190**: 112–122.

**Minh BQ, Schmidt HA, Chernomor O,** ***et al.*** **2020**. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era (E Teeling, Ed.). *Molecular Biology and Evolution* **37**: 1530–1534.

**Naimi B, Hamm NAS, Groen TA, Skidmore AK, Toxopeus AG**. **2014**. Where is positional uncertainty a problem for species distribution modelling? *Ecography* **37**: 191–203.

**Nei M**. **1972**. Genetic Distance between Populations. *The American Naturalist* **106**: 283–292.

**Ninčević T, Jug-Dujaković M, Grdiša M,** ***et al.*** **2021**. Population structure and adaptive variation of *Helichrysum italicum* (Roth) G. Don along eastern Adriatic temperature and precipitation gradient. *Scientific Reports* **11**: 24333.

**Oksanen J, Blanchet FG, Friendly M,** ***et al.*** **2020**. vegan: Community Ecology Package.

**Ortiz EM**. **2019**. vcf2phylip v2.0: convert a VCF matrix into several matrix formats for phylogenetic analysis.

**Pavlek M, Gauthier J, Tonzo V, Bilat J, Arnedo MA, Alvarez N**. **2022**. Life-history traits drive spatial genetic structuring in Dinaric cave spiders. *Frontiers in Ecology and Evolution* **10**.

**Pembleton LW, Cogan NOI, Forster JW**. **2013**. St AMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. *Molecular Ecology Resources* **13**: 946–952.

**Petit RJ, Aguinagalde I, de Beaulieu J-L,** ***et al.*** **2003**. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science (New York, N.Y.)* **300**: 1563–1565.

**Petrova G, Moyankova D, Nishii K,** ***et al.*** **2015**. The European Paleoendemic *Haberlea rhodopensis* (Gesneriaceae) Has an Oligocene Origin and a Pleistocene Diversification and Occurs in a Long-Persisting Refugial Area in Southeastern Europe. *International Journal of Plant Sciences* **176**: 499–514.

**Pickrell JK, Pritchard JK**. **2012**. Inference of Population Splits and Mixtures from Genome-Wide Allele Frequency Data (H Tang, Ed.). *PLoS Genetics* **8**: e1002967.

**Pina-Martins F, Silva DN, Fino J, Paulo OS**. **2017**. *Structure_threader* : An improved method for automation and parallelization of programs STRUCTURE , FASTSTRUCTURE and *MavericK* on multicore CPU systems. *Molecular Ecology Resources* **17**: e268–e274.

**Previšić A, Walton C, Kučinić M, Mitrikeski PT, Kerovec M**. **2009**. Pleistocene divergence of Dinaric *Drusus* endemics (Trichoptera, Limnephilidae) in multiple microrefugia within the Balkan Peninsula. *Molecular Ecology* **18**: 634–647.

**Pritchard JK, Stephens M, Donnelly P**. **2000**. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **155**: 945–959.

**Puritz JB, Hollenbeck CM, Gold JR**. **2014**. dDocent: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. *PeerJ* **2**: e431.

**Radosavljević I, Satovic Z, di Pietro R,** ***et al.*** **2022**. Phylogeographic structure of common sage (*Salvia officinalis* L.) reveals microrefugia throughout the Balkans and colonizations of the Apennines. *Scientific Reports* **12**: 15726.

**Rambaut A**. **2009**. FigTree. Tree figure drawing tool.

**Reich D, Flatscher R, Pellegrino G, *et al.* 2022**. Biogeography of amphi-adriatic *Gentianella crispata* (Gentianaceae): a northern refugium and recent trans-adriatic migration. *Plant Biosystems* **156**: 754–768.

**Rešetnik I, Frajman B, Schönswetter P**. **2016**. Heteroploid *Knautia drymeia* includes *K. gussonei* and cannot be separated into diagnosable subspecies. *American Journal of Botany* **103**: 1300–1313.

**Rešetnik I, Temunović M, Liber Z, Satovic Z, Bogdanović S**. **2020**. Phylogeography of *Campanula fenestrellata* s.l. (Campanulaceae) in the northern Adriatic. *Plant Systematics and Evolution* **306**: 42.

**Rešetnik I, Záveská E, Grgurev M, Bogdanović S, Bartolić P, Frajman B**. **2022**. Stability in the South, Turbulence Toward the North: Evolutionary History of *Aurinia saxatilis* (Brassicaceae) Revealed by Phylogenomic and Climatic Modelling Data. *Frontiers in Plant Science* **13**: 822331.

**Rousset F**. **1997**. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145**: 1219–1228.

**Rull V**. **2009**. Microrefugia. *Journal of Biogeography* **36**: 481–484.

**Sakaguchi S, Takeuchi Y, Yamasaki M, Sakurai S, Isagi Y**. **2011**. Lineage admixture during postglacial range expansion is responsible for the increased gene diversity of *Kalopanax septemlobus* in a recently colonised territory. *Heredity* **107**: 338–348.

**Sikora M, Mihanović H, Vilibić I**. **2013**. Paleo-coastline of the Central Eastern Adriatic Sea, and Paleo-Channels of the Cetina and Neretva rivers during the last glacial maximum. *Acta Adriatica* **54**: 3–12.

**Smyčka J, Roquet C, Boleda M, *et al.* 2022**. Tempo and drivers of plant diversification in the European mountain system. *Nature Communications* **13**: 2750.

**Španiel S, Rešetnik I**. **2022**. Plant phylogeography of the Balkan Peninsula: spatiotemporal patterns and processes. *Plant Systematics and Evolution* **308**: 38.

**Španiel S, Zozomová-Lihová J, Marhold K**. **2017**. Revised taxonomic treatment of the *Alyssum montanum-A. repens* complex in the Balkans: a multivariate morphometric analysis. *Plant Systematics and Evolution* **303**: 1413–1442.

**Stewart JR, Lister AM, Barnes I, Dalén L**. **2010**. Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences* **277**: 661–671.

**Sundqvist L, Keenan K, Zackrisson M, Prodöhl P, Kleinhans D**. **2016**. Directional genetic differentiation and relative migration. *Ecology and Evolution* **6**: 3461–3475.

**Surina B, Schneeweiss GM, Glasnović P, Schönswetter P**. **2014**. Testing the efficiency of nested barriers to dispersal in the Mediterranean high mountain plant *Edraianthus graminifolius* (Campanulaceae). *Molecular Ecology* **23**: 2861–2875.

**Surina B, Schönswetter P, Schneeweiss GM**. **2011**. Quaternary range dynamics of ecologically divergent species (*Edraianthus serpyllifolius* and *E. tenuifolius*, Campanulaceae) within the Balkan refugium: Quaternary range dynamics within the Balkan refugium. *Journal of Biogeography* **38**: 1381–1393.

**Terlević A, Bogdanović S, Frajman B, Rešetnik I**. **2022**. Genome Size Variation in *Dianthus sylvestris* Wulfen sensu lato (Caryophyllaceae). *Plants* **11**: 1481.

**Terlević A, Temunović M, Bogdanović S, Grgurev M, Ljubičić I, Rešetnik I**. **2022**. Morphological and ecological variability of *Dianthus sylvestris* Wulfen (Caryophyllaceae) on the Balkan Peninsula. *Botanical Journal of the Linnean Society* **submitted**.

**Theodoridis S, Randin C, szövényi P, Boucher FC, Patsiou TS, Conti E**. **2017**. How Do Cold-Adapted Plants Respond to Climatic Cycles? Interglacial Expansion Explains Current Distribution and Genomic Diversity in *Primula farinosa* L. *Systematic Biology* **66**: 715–736.

**Thuiller W, Guéguen M, Renaud J, Karger DN, Zimmermann NE**. **2019**. Uncertainty in ensembles of global biodiversity scenarios. *Nature Communications* **10**: 1446.

**Tutin T. G., Walters SM**. **1993**. *Dianthus* L. In: Tutin Thomas Gaskell, ed. *Flora Europaea*. Cambridge; New York: Cambridge University Press, 227–246.

**Valente LM, Savolainen V, Vargas P**. **2010**. Unparalleled rates of species diversification in Europe. *Proceedings of the Royal Society B: Biological Sciences* **277**: 1489–1496.

**Weir BS, Cockerham CC**. **1984**. Estimating F-Statistics for the Analysis of Population Structure. *Evolution* **38**: 1358–1370.

**Westergaard KB, Zemp N, Bruederle LP, Stenøien HK, Widmer A, Fior S**. **2019**. Population genomic evidence for plant glacial survival in Scandinavia. *Molecular Ecology* **28**: 818–832.

**Willis KJ**. **1994**. The vegetational history of the Balkans. *Quaternary Science Reviews* **13**: 769–788.

**Žebre M, Sarıkaya MA, Stepišnik U, Yıldırım C, Çiner A**. **2019**. First 36Cl cosmogenic moraine geochronology of the Dinaric mountain karst: Velež and Crvanj Mountains of Bosnia and Herzegovina. *Quaternary Science Reviews* **208**: 54–75.

**Zozomová-Lihová J, Melichárková A, Svitok M, Španiel S**. **2020**. Pleistocene range disruption and postglacial expansion with secondary contacts explain the genetic and cytotype structure in the western Balkan endemic *Alyssum austrodalmaticum* (Brassicaceae). *Plant Systematics and Evolution* **306**: 47.

## Supplementary Figures

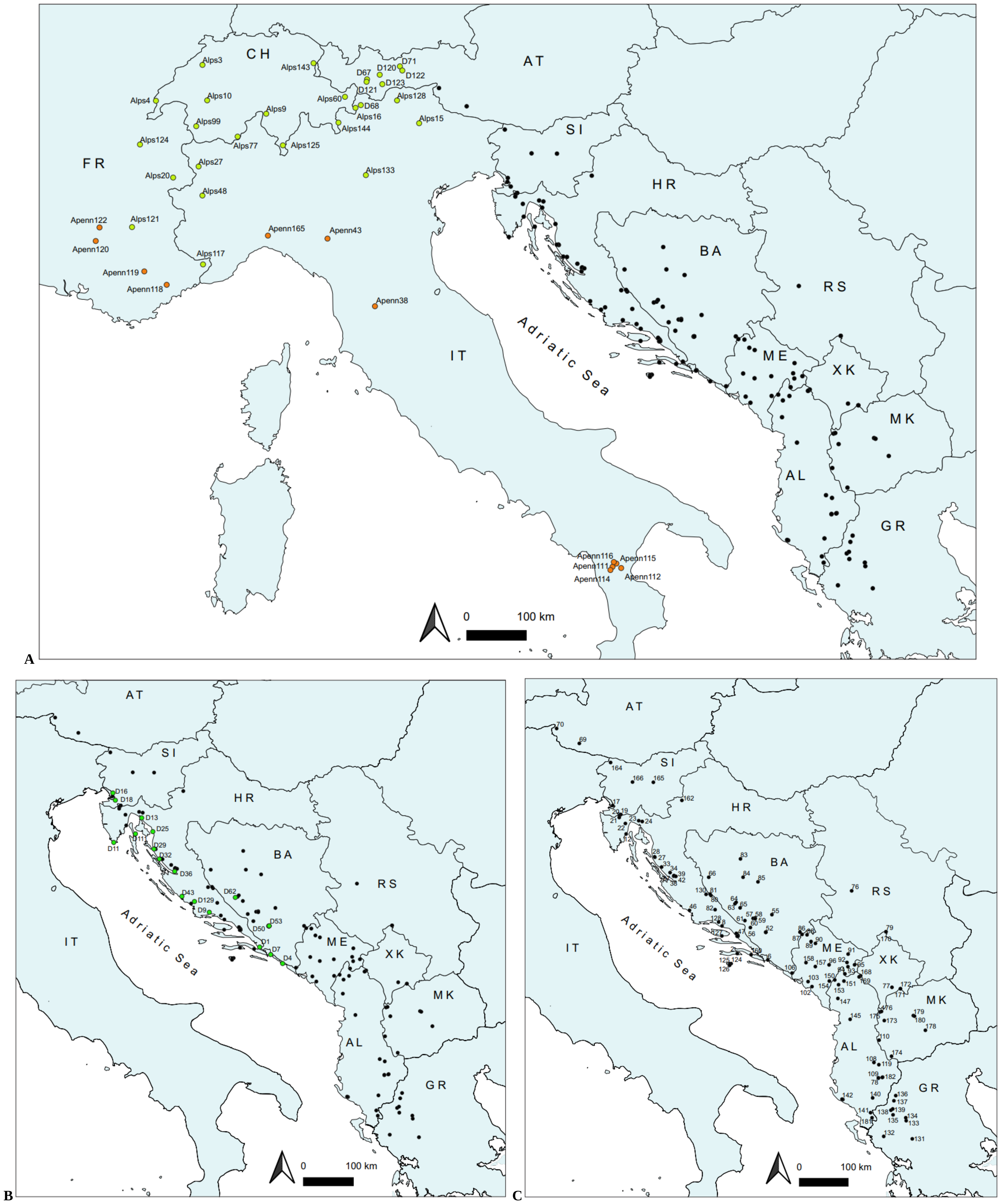


**Figure S1.** Geographical distribution of 165 sampled populations of *Dianthus sylvestris* s.l. included in the "all-lineage dataset". Labels corresponding to the A) 33 sequenced populations across the European Alps and Apennines. B) 18 sequenced population of *D. sylvestris* subsp. *tergestinus.* C) 114 sequenced populations of the Balkan cline.

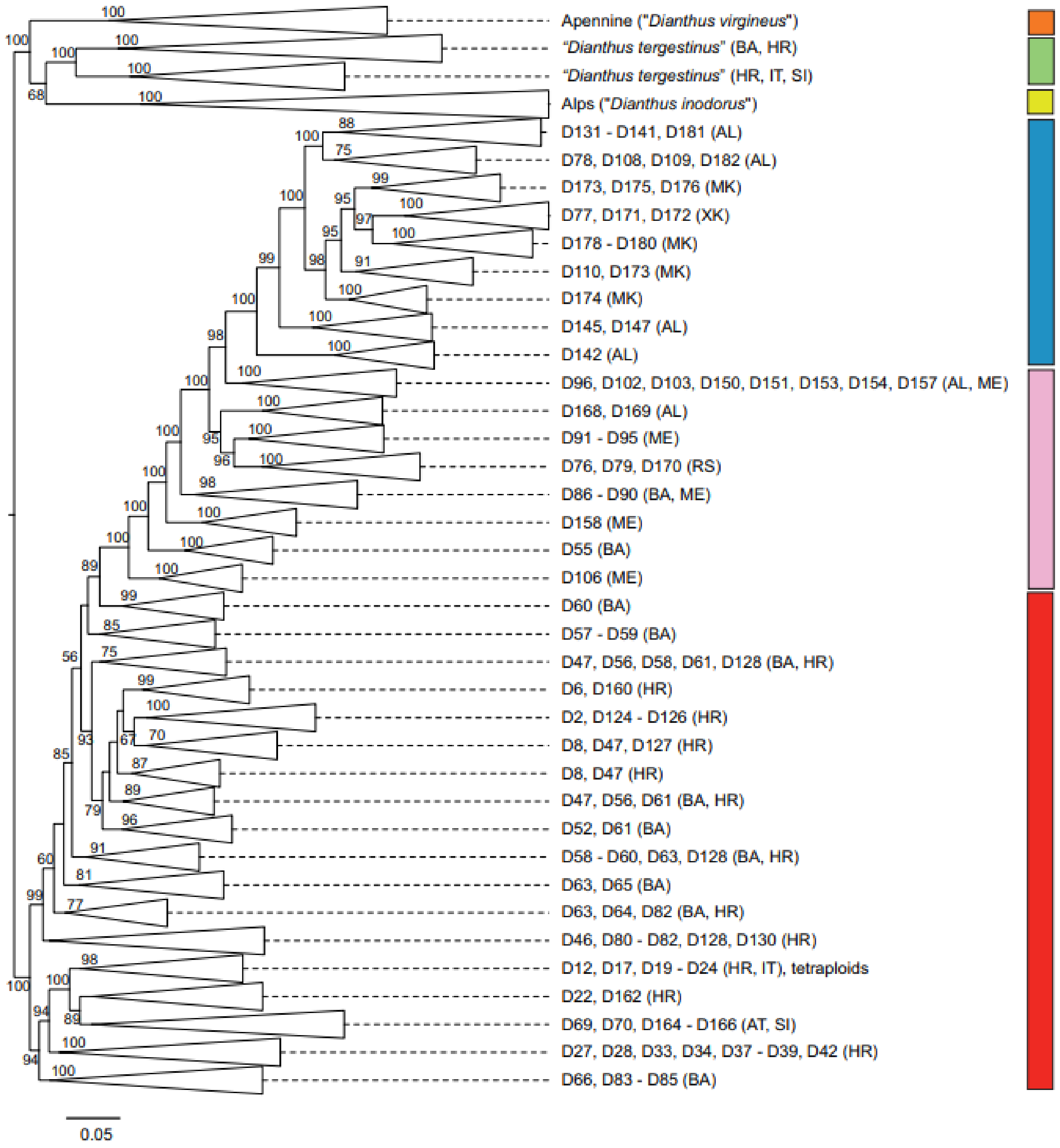


**Figure S2.** Phylogenetic Maximum Likelihood tree obtained via IQ-TREE based on the "all-lineage dataset" (165 populations, 991 individuals and 5644 SNPs) showing strong support for the three lineages of *Dianthus sylvestris* ("*Dianthus virgineus*" – Apennine lineage, and "*Dianthus inodorus*" – Alpine lineage), as well as for the separation of *D. sylvestris* subsp. *tergestinus* ("*Dianthus tergestinus*") from the rest of the Balkan populations. Colours correspond to genetic groups at K=6 (Fig. 1C). Bootstrap support as inferred for the Maximum Likelihood tree is shown above the branches.

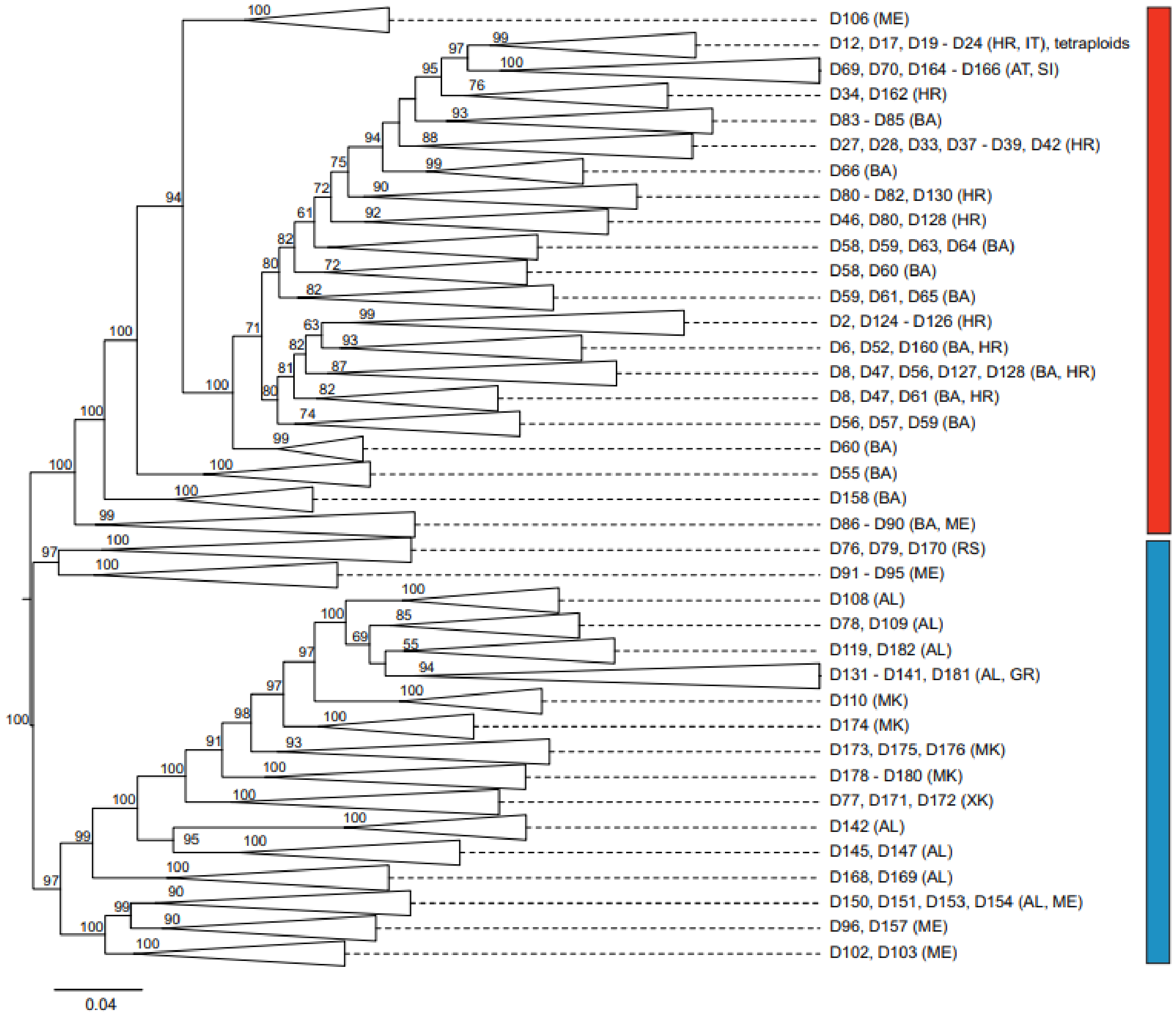


**Figure S3.** Phylogenetic Maximum Likelihood tree obtained via IQ-TREE based on the “Balkan-cline dataset” (108 populations, 587 Individuals and 6240 SNPs). Colours correspond to genetic groups at K=2 (Fig. 2B). Bootstrap support as inferred for the Maximum Likelihood tree is shown above the branches.

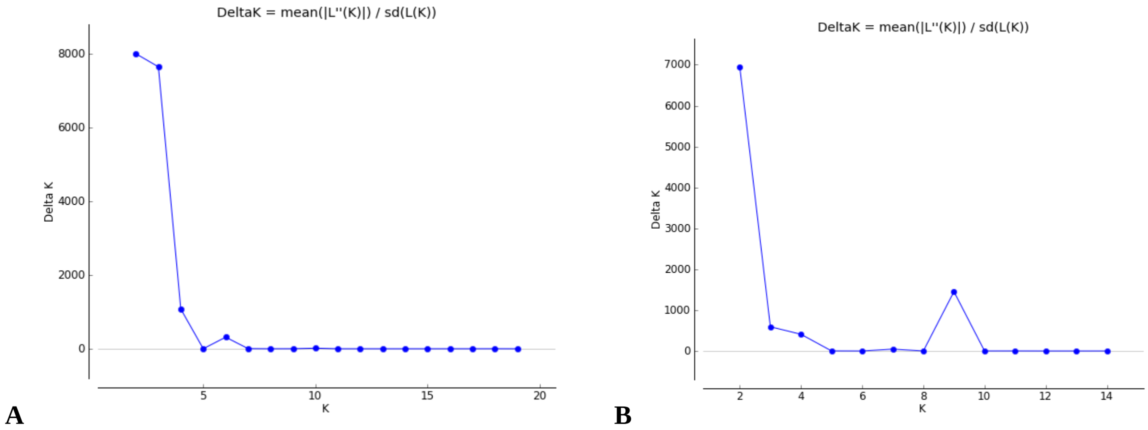


**Figure S4.** STRUCTUIRE Delta K plot according to Evanno *et al.* (2005) method for A) "all-lineage dataset" and B) "Balkan-cline dataset".

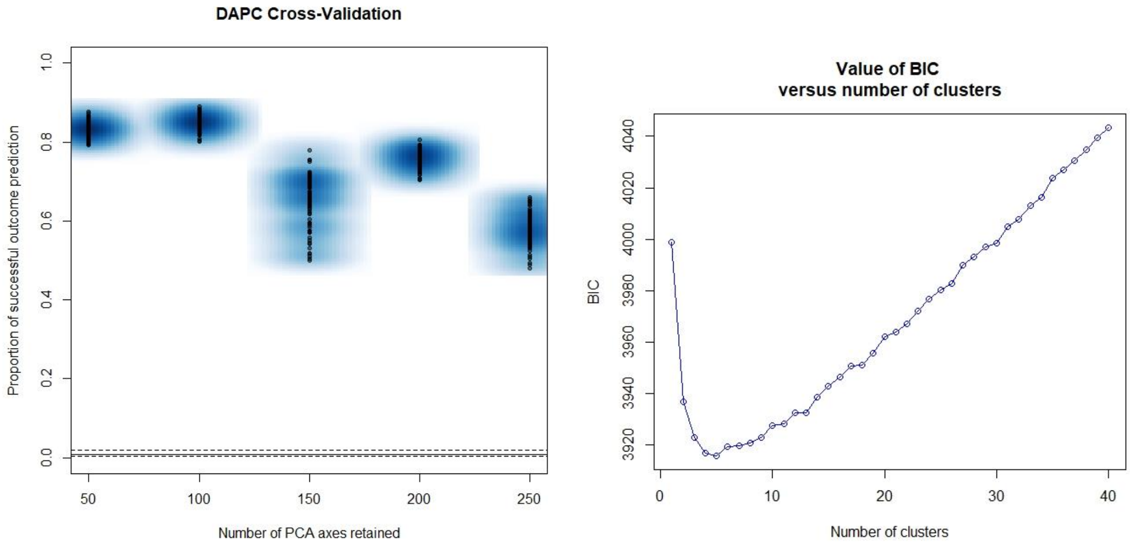


**Figure S5.** DAPC – BIC plot and Cross validation results.

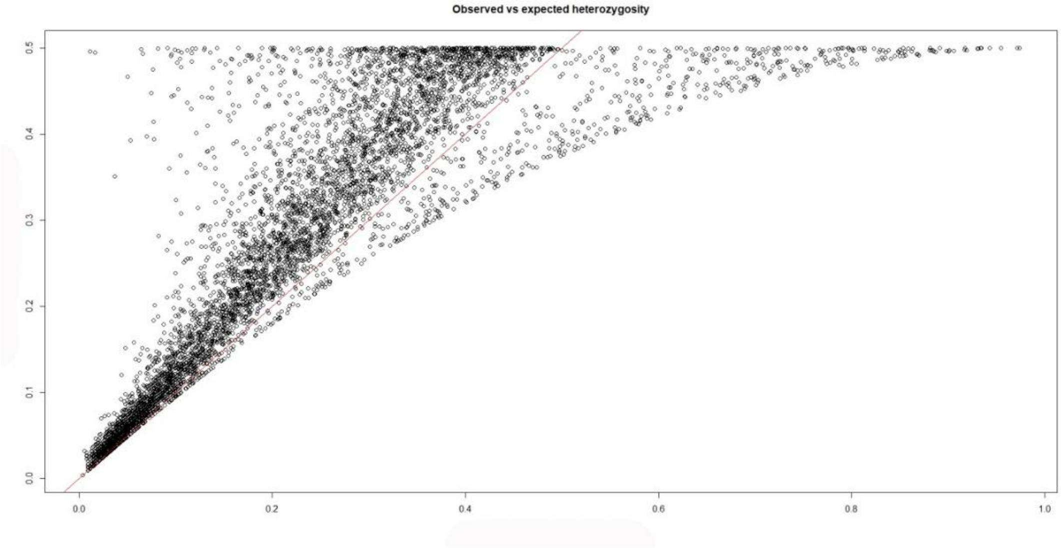


**Figure S6.** Plot of the mean observed heterozygosity (Ho), which was significantly lower than mean expected heterozygosity (He).

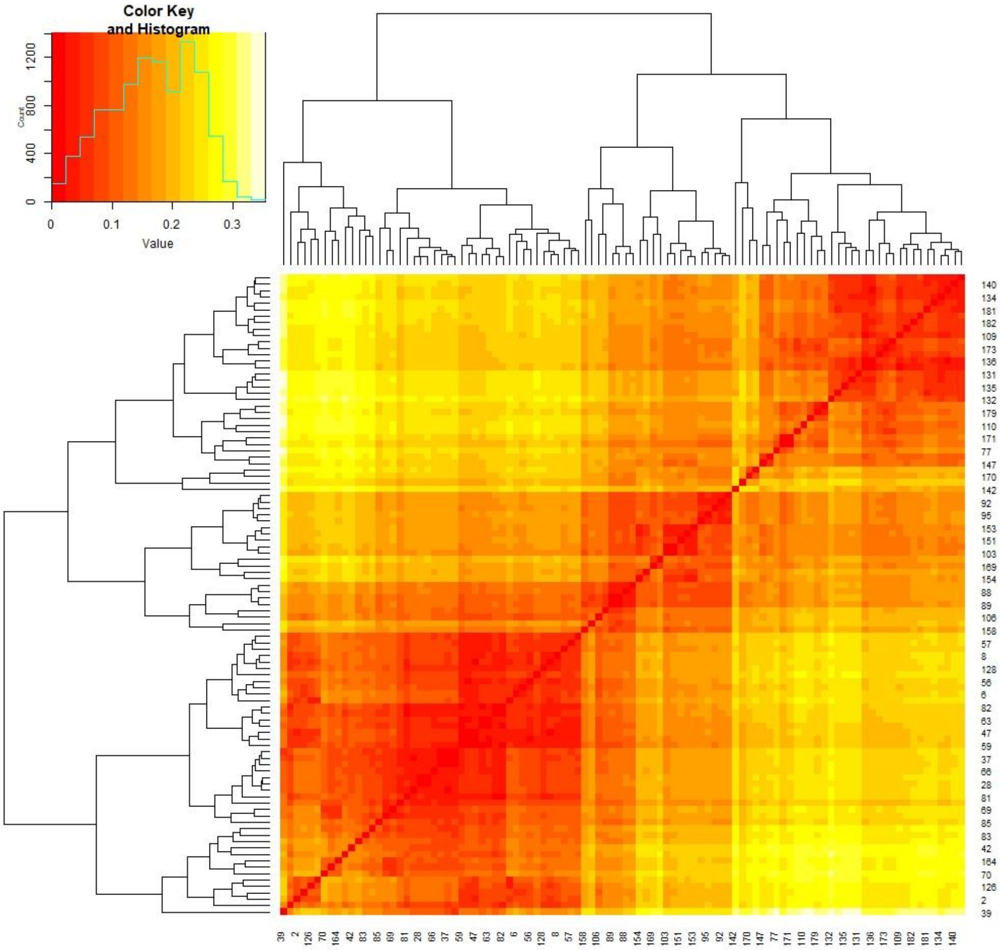


**Figure S7.** Heatmap of pairwise genetic differentiation ($F_{ST}$ values) between Balkan populations of *D. sylvestris* indicating higher genetic differentiation between populations from the NW and SE groups.

## Supplementary Tables

**Table S1.** Studied populations of the *Dianthus sylvestris* s.l., including the ID label, the number of individuals collected per population (silica No ind.), species (sp) and subspecies (ssp) names, country ISO codes, herbarium voucher ID (herbarium acronyms are according to Index Herbariorum, Thiers 2021). Collectors' names initials: AT (A. Terlević), BF (B. Frajman), DD (D. Dagnino), DG (D. Gargano), DL (D. Lakušić), DS (D. Sincek), DSh (D. Shuka), GT (G. Tomović), HL (H. Luqman), IJ (I. Janković), Ilj (I. Ljubičić), IR (I. Rešetnik), KR (K. Rohweder), LSh (L. Shuka), MD (M. Doboš), MF (M. Falch), MG (M. Grgurev), MGa (M. Gammella), MT (M. Temunović), MZ (M. Zbiljić), NK (N. Kuzmanović), PS (P. Schönswetter), SB (S. Bogdanović), SBu (S. Burg), SC (S. Cambria), SĐ (S. Đurović), SF (S. Fior). Ploidy refers to flow citometry estimations by Terlević, Bogdanović *et al.* 2022.

| No | ID | silica No ind. | sp | ssp | Country code | Locality | Habitat | Altitude (m a.s.l.) | Herbarium voucher | Collector | Collection date | Latitude | Longitude | Ploidy | morphometrics dataset | niche analysis dataset | All lineage dataset | Balkan cline dataset | treemix dataset | SDM dataset |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | D1 | 5 | sylvestris | tergestinus | HR | Klek village, mountain road toward Smrdan Grad | rocky grassland | 80 | ZA-46276 | IR, SB | 09/05/2018 | 42.948736 | 17.564511 | 2x | + | + | + |  |  | + |
| 2 | D2 | 7 | sylvestris | nodosus | HR | Korčula island, macadam in shrubland | clearings of the forest edge | 250 | ZA-46277 | IR, SB | 11/05/2018 | 42.940857 | 17.080551 | 2x | + | + | + | + | + | + |
| 3 | D4 | 5 | sylvestris | tergestinus | HR | Dubrovnik, Srđ hill, summit plateau, Križ | rocky grassland | 279 | ZA-46279 | IR, SB | 12/05/2018 | 42.645206 | 18.120210 | 2x | + | + | + |  |  | + |
| 4 | D6 | 5 | sylvestris | nodosus | HR | Mironja, near the quarry | rocky grassland along the road | 332 | ZA-46281 | IR, SB | 13/5/2018, 29/6/2019 | 42.815694 | 17.843489 | 2x | + | + | + | + |  | + |
| 5 | D7 | 5 | sylvestris | tergestinus | HR | between Kuk and Deskovice, near the quarry | rocky grassland along the road | 351 | ZA-46282 | IR, SB | 13/05/2018 | 42.810839 | 17.830790 | 2x | + | + | + |  |  | + |
| 6 | D8 | 5 | sylvestris | nodosus | HR | Omiška Dinara Mt., road near Cetina river | vertical rocks along the road | 63 | ZA-46283 | IR, SB | 13/5/2018, 29/6/2019 | 43.450246 | 16.698531 | 2x | + | + | + | + |  | + |
| 7 | D9 | 5 | sylvestris | tergestinus | HR | Kozjak Mt., close to mountain lodge Malačka | rocky grassland | 602 | ZA-46284 | IR, SB | 13/05/2018 | 43.581474 | 16.325790 | 2x | + | + | + |  |  | + |
| 8 | D11 | 6 | sylvestris | tergestinus | HR | Gornji Kamenjak, Gomila peak | rocky grassland | 55 | ZA-46415 | SB, ILj | 17/05/2018 | 44.810971 | 13.900875 | 2x | + | + | + |  |  | + |
| 9 | D12 | 6 | sylvestris | sylvestris | HR | road Zagorje-Šimuni, near Plomin | rocks along the road | 240 | ZA-46416 | SB, ILj | 19/5/2018, 13/7/2019 | 45.123950 | 14.204677 | 4x | + | + | + | + |  | + |
| 10 | D13 | 5 | sylvestris | tergestinus | HR | Križišće village, road Hreljin to Kraljevica | rocky grassland along the road | 513 | ZA-46417 | SB, ILj | 19/05/2018 | 45.267597 | 14.594413 | 2x | + | + | + |  |  | + |
| 11 | D16 | 5 | sylvestris | tergestinus | IT | Sežana, border pass | rocky grassland | 344 | ZA-47416 | IR, SB, ILj | 13/06/2018 | 45.701530 | 13.837293 | 2x | + | + | + |  |  | + |
| 12 | D17 | 4 | sylvestris | sylvestris | IT | Trieste, Monte Spacatto | vertical rocks along the road | 300 | ZA-47417 | IR, SB, ILj | 13/06/2018 | 45.642266 | 13.831103 | 4x | + | + | + | + |  | + |
| 13 | D18 | 5 | sylvestris | tergestinus | SI | Petrinje village | rocky grassland | 422 | ZA-47418 | IR, SB, ILj | 13/06/2018 | 45.572838 | 13.906808 | 2x | + | + | + |  |  | + |
| 14 | D19 | 2 | sylvestris | sylvestris | HR | Ćićarija, Vodice | rocky grassland | 660 | ZA-47419 | IR, SB, ILj | 13/06/2018 | 45.479168 | 14.049705 |  | + | + | + | + |  | + |
| 15 | D19 | 8 | sylvestris | sylvestris | HR | Ćićarija, Vodice | rocky grassland | 660 | ZA-47419 | IR, SB, ILj | 13/6/2018, 13/7/2019 | 45.479168 | 14.049705 | 4x | + | + | + | + |  | + |
| 16 | D20 | 5 | sylvestris | sylvestris | HR | Ćićarija, Žbevnica, above Brest | rocky grassland along the mountain route | 941 | ZA-47420 | IR, SB, ILj | 13/6/2018, 19/7/2020 | 45.457206 | 14.016319 | 4x | + | + | + | + |  | + |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | D21 | 5 | sylvestris | sylvestris | HR | Slum, close to village Slum | rocky grassland | 553 | ZA-47421 | IR, SB, ILj | 13/6/2018, 19/7/2020 | 45.423401 | 14.012134 | 4x | + | + | + | + | | + |
| 18 | D22 | 5 | sylvestris | sylvestris | HR | Učka Mt., close to Zrinščak, above Vela Draga | rocky grassland | 525 | ZA-47422 | IR, SB, ILj | 13/06/2018 | 45.316811 | 14.177338 | 4x | + | + | + | + | | + |
| 19 | D23 | 5 | sylvestris | sylvestris | HR | Grobnik, close to Kikovica | rocky grassland | 326 | ZA-47423 | IR, SB, ILj | 14/06/2018 | 45.374679 | 14.524703 | | + | + | + | + | | + |
| 20 | D24 | 5 | sylvestris | sylvestris | HR | Gornje Jelenje, Lazanje | vertical rocks along the road | 862 | ZA-47424 | IR, SB, ILj | 14/06/2018 | 45.361573 | 14.617238 | 4x | + | + | + | + | | + |
| 21 | D25 | 5 | sylvestris | tergestinus | HR | Bunica, close to Senj | littoral rocky coast | 8 | ZA-47425 | IR, SB, ILj | 14/06/2018 | 45.024841 | 14.884729 | 2x | + | + | + | | | + |
| 22 | D27 | 5 | sylvestris | nodosus | HR | Plješevica, Gornja Branjevina | rocky grassland | 1211 | ZA-47427 | IR, SB, ILj | 15/06/2018 | 44.707936 | 14.965827 | 2x | + | + | + | + | + | + |
| 23 | D28 | 10 | sylvestris | nodosus | HR | Butković plan, Balenske brižine | rocky grassland | 1020 | ZA-47428 | IR, SB, ILj | 15/06/2018 | 44.715302 | 14.956895 | 2x | + | + | + | + | + | + |
| 24 | D29 | 5 | sylvestris | tergestinus | HR | Donji Bileni, above Jablanac | natural shrub | 274 | ZA-47429 | IR, SB, ILj | 15/06/2018 | 44.711960 | 14.922496 | 2x | + | + | + | | | + |
| 25 | D32 | 5 | sylvestris | tergestinus | HR | Karlobag, Tatinja draga | vertical rocks along the macadam | 12 | ZA-47432 | IR, SB, ILj | 15/06/2018 | 44.533384 | 15.065192 | 2x | + | + | + | | | + |
| 26 | D33 | 5 | sylvestris | nodosus | HR | Baške Oštarije, Ura viewpoint | vertical rocks along the road | 905 | ZA-47433 | IR, SB, ILj | 15/06/2018 | 44.529482 | 15.143159 | 2x | + | + | + | + | + | + |
| 27 | D34 | 5 | sylvestris | sylvestris | HR | Visočica peak | grassland, along the mountain route | 1458 | ZA-47434 | IR, SB, ILj | 16/06/2018 | 44.429299 | 15.364303 | 2x | + | + | + | + | | + |
| 28 | D36 | 5 | sylvestris | tergestinus | HR | Paklenica, above Starigrad | vertical rocks along the road | 397 | ZA-47436 | IR, SB, ILj | 17/06/2018 | 44.313471 | 15.453283 | 2x | + | + | + | | | + |
| 29 | D37 | 7 | sylvestris | sylvestris | HR | Paklenica, Buljma | rocky stones, scrub, edge of forest | 1131 | ZA-47437 | IR, SB, ILj | 17/06/2018 | 44.364749 | 15.460115 | 2x | + | + | + | + | | + |
| 30 | D37 | 7 | sylvestris | sylvestris | HR | Paklenica, Buljma | rocky stones, scrub, edge of forest | 1213 | ZA-47437 | IR, SB, ILj | 17/06/2018 | 44.366496 | 15.462085 | 2x | + | + | + | + | | + |
| 31 | D37 | 7 | sylvestris | sylvestris | HR | Paklenica, Buljma | rocky stones, scrub, edge of forest | 1311 | ZA-47437 | IR, SB, ILj | 17/06/2018 | 44.366662 | 15.462092 | 2x | + | + | + | + | | + |
| 32 | D38 | 5 | sylvestris | sylvestris | HR | Paklenica, Struge toward Buljma | grassland | 1368 | ZA-47438 | IR, SB, ILj | 17/06/2018 | 44.371093 | 15.465668 | 2x | + | + | + | + | | + |
| 33 | D39 | 2 | sylvestris | sylvestris | HR | Paklenica, Struge | rocky grassland | 1388 | ZA-47439 | IR, SB, ILj | 17/06/2018 | 44.375566 | 15.465719 | | + | + | + | + | | + |
| 34 | D42 | 6 | sylvestris | nodosus | HR | Paklenica, Brundo, below Šegestin peak | rocky grassland | 1605 | ZA-47442 | IR, SB, ILj | 17/06/2018 | 44.361124 | 15.513128 | 2x | + | + | + | + | | + |
| 35 | D43 | 5 | sylvestris | tergestinus | HR | Vransko jezero, Banjevački stanovi | maquis and garrigue near macadam | 35 | ZA-47453 | IR, SB, ILj | 18/06/2018 | 43.865234 | 15.642296 | 2x | + | + | + | | | + |
| 36 | D46 | 6 | sylvestris | nodosus | HR | Šibenik, Martinska | maquis and garrigue near macadam | 18 | ZA-47456 | IR, SB, ILj | 18/06/2018 | 43.737357 | 15.871757 | 2x | + | + | + | + | + | + |
| 37 | D47 | 4 | sylvestris | nodosus | HR | Biokovo Mt., above Podgora, Susvid | vertilcal rocks along the road | 325 | ZA-47457 | IR, SB, ILj | 18/06/2018 | 43.259545 | 17.078427 | 2x | + | + | + | + | | + |
| 38 | D47 | 5 | sylvestris | nodosus | HR | Biokovo Mt., road to sv. Jure Peak | rocky grassland | 899 | ZA-47459 | IR, SB, ILj | 18/06/2018 | 43.261003 | 17.101612 | 2x | + | + | + | + | | + |
| 39 | D47 | 5 | sylvestris | nodosus | HR | Biokovo Mt., road to sv. Jure Peak | rocky grassland | 1018 | ZA-47459 | IR, SB, ILj | 18/06/2018 | 43.273612 | 17.100209 | 2x | + | + | + | + | | + |
| 40 | D47 | 5 | sylvestris | nodosus | HR | Biokovo Mt., road to sv. Jure Peak | rocky grassland | 1228 | ZA-47459 | IR, SB, ILj | 18/06/2018 | 43.289186 | 17.085926 | 2x | + | + | + | + | | + |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 | D47 | 5 | sylvestris | nodosus | HR | Biokovo Mt., road to sv. Jure Peak | rocky grassland | 1318 | ZA-47459 | IR, SB, ILj | 18/06/2018 | 43.312484 | 17.058714 | 2x | + | + | + | + | + |
| 42 | D47 | 2 | sylvestris | nodosus | HR | Biokovo Mt., road to Sv. Jure peak | the edge of the pine forests | 535 | ZA-47459 | IR, SB, ILj | 18/06/2018 | 43.260692 | 17.090430 | | + | + | + | + | + |
| 43 | D47 | 2 | sylvestris | nodosus | HR | Biokovo Mt., road to Sv. Jure peak | the edge of the pine forests | 516 | ZA-47459 | IR, SB, ILj | 18/06/2018 | 43.262247 | 17.086312 | | + | + | + | + | + |
| 44 | D50 | 5 | sylvestris | tergestinus | BA | Mostar, road Gornji Vukodol to Donji Vukodol | vertical rocks along the road | 250 | ZA-47461 | IR, SB, ILj | 19/06/2018 | 43.325423 | 17.792920 | 2x | + | + | + | | + |
| 45 | D52 | 7 | sylvestris | nodosus | BA | Mostar, Hum hill | rocky grassland along the road | 355 | ZA-47463 | IR, SB, ILj | 19/06/2018 | 43.325478 | 17.802829 | 2x | + | + | + | + | + |
| 46 | D53 | 5 | sylvestris | tergestinus | BA | Mostar, Hum hill | rocky grassland along the road | 355 | ZA-47464 | IR, SB, ILj | 19/06/2018 | 43.325875 | 17.802569 | 2x | + | + | + | | + |
| 47 | D52 | 7 | sylvestris | nodosus | BA | Mostar, Hum hill | rocky grassland along the road | 369 | ZA-47463 | IR, SB, ILj | 19/06/2018 | 43.328997 | 17.802182 | 2x | + | + | + | + | + |
| 48 | D53 | 5 | sylvestris | tergestinus | BA | Mostar, Hum hill | rocky grassland along the road | 372 | ZA-47464 | IR, SB, ILj | 19/06/2018 | 43.328803 | 17.802206 | 2x | + | + | + | | + |
| 49 | D52 | 7 | sylvestris | nodosus | BA | Mostar, Hum hill | rocky grassland along the road | 379 | ZA-47463 | IR, SB, ILj | 19/06/2018 | 43.327339 | 17.804562 | 2x | + | + | + | + | + |
| 50 | D53 | 5 | sylvestris | tergestinus | BA | Mostar, Hum hill | rocky grassland along the road | 389 | ZA-47464 | IR, SB, ILj | 19/06/2018 | 43.327195 | 17.804672 | 2x | + | + | + | | + |
| 51 | D52 | 7 | sylvestris | nodosus | BA | Mostar, Hum hill | rocky grassland along the road | 423 | ZA-47463 | IR, SB, ILj | 19/06/2018 | 43.328565 | 17.806905 | 2x | + | + | + | + | + |
| 52 | D53 | 5 | sylvestris | tergestinus | BA | Mostar, Hum hill | rocky grassland along the road | 423 | ZA-47464 | IR, SB, ILj | 19/06/2018 | 43.328315 | 17.806842 | 2x | + | + | + | | + |
| 53 | D55 | 7 | sylvestris | nodosus | BA | Konjic | edge of forest near macadam | 360 | ZA-47466 | IR, SB, ILj | 19/06/2018 | 43.649976 | 17.967010 | 2x | + | + | + | + | + |
| 54 | D56 | 5 | sylvestris | nodosus | BA | road Posušje to Široki Brijeg, close to village Vranić | vertical rocks along the road | 549 | ZA-47467 | IR, SB, ILj | 20/06/2018 | 43.417465 | 17.416434 | 2x | + | + | + | + | + |
| 55 | D57 | 5 | sylvestris | nodosus | BA | Blidinje, Škrge, Grgoš | rocky grassland near macadam | 1298 | ZA-47468 | IR, SB, ILj | 20/06/2018 | 43.586683 | 17.487889 | 2x | + | + | + | + | + |
| 56 | D58 | 5 | sylvestris | sylvestris | BA | Čvrsnica Mt., Široka kosa | rocky grassland near macadam | 1928 | ZA-47469 | IR, SB, ILj | 20/06/2018 | 43.595028 | 17.540758 | 2x | + | + | + | + | + |
| 57 | D59 | 6 | sylvestris | sylvestris | BA | Čvrsnica Mt., Široka kosa | rocky grassland near macadam | 1732 | ZA-47470 | IR, SB, ILj | 20/06/2018 | 43.586521 | 17.533781 | 2x | + | + | + | + | + |
| 58 | D60 | 5 | sylvestris | nodosus | BA | Čvrsnica Mt., Čavkarice | rocky grassland near macadam | 1556 | ZA-47471 | IR, SB, ILj | 20/06/2018 | 43.578959 | 17.540717 | 2x | + | + | + | + | + |
| 59 | D61 | 5 | sylvestris | nodosus | BA | road Posušje to Mesihovina, Trobukva | vertical rocks along the road | 904 | ZA-47472 | IR, SB, ILj | 20/06/2018 | 43.546950 | 17.279515 | 2x | + | + | + | + | + |
| 60 | D62 | 5 | sylvestris | tergestinus | BA | Livno, above villages Veliki Kabalići and Suhača | edge of forest near macadam | 821 | ZA-47473 | IR, SB, ILj | 21/06/2018 | 43.847624 | 16.973298 | 2x | + | + | + | | + |
| 61 | D63 | 5 | sylvestris | nodosus | BA | Krug plateau, below Cincar Mt. | rocky grassland near macadam | 1197 | ZA-47474 | IR, SB, ILj | 21/06/2018 | 43.859632 | 17.030663 | 2x | + | + | + | + | + |
| 62 | D64 | 6 | sylvestris | nodosus | BA | Cincar Mt., Begovača | rocky grassland near macadam | 1397 | ZA-47475 | IR, SB, ILj | 21/06/2018 | 43.885435 | 17.059812 | 2x | + | + | + | + | + |
| 63 | D65 | 5 | sylvestris | nodosus | BA | Between Tomislavgrad and Livno, close to village Galečić | rocky grassland | 1096 | ZA-47476 | IR, SB, ILj | 21/06/2018 | 43.782860 | 17.160352 | 2x | + | + | + | + | + |
| 64 | D66 | 6 | sylvestris | nodosus | BA | Drvar, slopes of Dejanska glav., Raduklija creek canyon | vertical rocks along the road | 716 | ZA-47477 | IR, SB, ILj | 22/06/2018 | 44.348655 | 16.359556 | 2x | + | + | + | + | + |
| 65 | D67 | 5 | sylvestris | | AT | Prutz, Ladis | dry meadow and Pinus forest | 904 | ZA-47478 | IR | 27/06/2018 | 47.075072 | 10.653611 | 2x | + | | + | | + |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 66 | D68 | 5 | sylvestris | | IT | Mals, Tartsch village, Tartscher Bichl hill | dry meadow | 1039 | ZA-47479 | IR | 27/06/2018 | 46.679892 | 10.558889 | 2x | + | | + | | | + |
| 67 | D69 | 10 | sylvestris | | AT | Gailtal Alps, Lienzer Dolomiten, toward Hochstadl peak | rocky grassland | 1971 | ZA-47480 | IR | 29/06/2018 | 46.759219 | 12.880278 | 2x | + | | + | + | + | + |
| 68 | D70 | 5 | sylvestris | | AT | Central Eastern Alps, Hohe Tauern, Clarahütte | high mountain meadow | 2030 | ZA-47481 | IR | 30/06/2018 | 47.013058 | 12.251111 | 2x | + | | + | + | + | + |
| 69 | D71 | 6 | sylvestris | | AT | Innsbruck, Nordkette Mt., Seegrube | rocky grassland | 1906 | ZA-47482 | IR | 08/07/2018 | 47.304944 | 11.358611 | 2x | + | | + | | | + |
| 70 | D76 | 5 | sylvestris | | RS | Maljen, Tometino polje | serpentinite | 640 | ZA-48631 | SĐ, GT | 15/07/2018 | 44.044240 | 20.016450 | 2x | | + | + | + | | + |
| 71 | D77 | 5 | sylvestris | | RS | Šar Planina Mts., Ostrvica | alpine rocky grasslands, Dapno-Festucetea, serpentinite | 1755 | ZA-48632 | DL, NK, IJ, MZ | 14/07/2018 | 42.233495 | 20.915371 | 2x | | + | + | + | + | + |
| 72 | D78 | 5 | sylvestris | alboroseus | AL | Maja e Ostrovicës Mts., above the village Marjan | dry rocky grounds, Festuco-Brometea | 1386 | ZA-48633 | DL, NK, IJ, MZ | 11/07/2018 | 40.574101 | 20.475512 | 2x | | + | + | + | + | + |
| 73 | D79 | 5 | sylvestris | | RS | NP Kopaonik, SE of Pančićev vrh, Nebeske stolice | ultramafiti | 1660 | ZA-48634 | SĐ, GT | 13/07/2018 | 43.261489 | 20.839731 | 2x | | + | + | + | + | + |
| 74 | D80 | 8 | sylvestris | nodosus | HR | Dinara Mt., above Glavaš village, Pekasova draga | rocky grassland along the mountain route | 740 | ZA-48607 | SB, ILj | 30/06/2018 | 44.014272 | 16.417037 | 2x | + | + | + | + | | + |
| 75 | D81 | 4 | sylvestris | sylvestris | HR | Dinara Mt., Ošljak | rocky grassland along the mountain route | 1418 | ZA-48606 | SB, ILj | 30/06/2018 | 44.038666 | 16.393428 | 2x | + | + | + | + | + | + |
| 76 | D82 | 5 | sylvestris | nodosus | HR | Svilaja Mt., road Zelovo to Gornje Ogorje | rocky grassland near macadam | 934 | ZA-48608 | SB, ILj | 01/07/2018 | 43.751194 | 16.523219 | 2x | + | + | + | + | | + |
| 77 | D83 | 5 | sylvestris | sylvestris | BA | Banja Luka, Vrbas river canyon, Tijesno | vertical rocks along the road | 207 | ZA-48609 | SB, ILj | 10/07/2018 | 44.685469 | 17.179697 | 2x | + | + | + | + | + | + |
| 78 | D84 | 4 | sylvestris | sylvestris | BA | Jajce, Pliva river bridge | under the bridge, along the dam | 380 | ZA-48610 | SB, ILj | 10/07/2018 | 44.346803 | 17.242528 | 2x | + | + | + | + | + | + |
| 79 | D85 | 5 | sylvestris | nodosus | BA | Vlašić Mt., above Babići village | vertical rocks along the road | 1100 | ZA-48611 | SB, ILj | 10/07/2018 | 44.258524 | 17.624191 | 2x | + | + | + | + | | + |
| 80 | D86 | 6 | sylvestris | bertisceus | BA | Sutjeska river canyon, close to Tjentište | rocky grassland | 638 | ZA-48662 | SB, ILj | 11/07/2018 | 43.315479 | 18.668211 | 2x | + | + | + | + | | + |
| 81 | D87 | 5 | sylvestris | bertisceus | BA | Maglić Mt. | calcareous and calcshist screes | 1580 | ZA-48663 | SB, ILj | 12/07/2018 | 43.273085 | 18.719551 | 2x | + | + | + | + | | + |
| 82 | D88 | 5 | sylvestris | bertisceus | ME | Piva river canyon, road Šćepan to Plužine | river canyon, vertical rocks along the road | 735 | ZA-48664 | SB, ILj | 12/07/2018 | 43.263261 | 18.845226 | 2x | + | + | + | + | + | + |
| 83 | D89 | 5 | sylvestris | bertisceus | ME | Pivska planina, close to Lokvice village | vertical rocks along the road | 1680 | ZA-48665 | SB, ILj | 13/07/2018 | 43.137130 | 18.940890 | 2x | + | + | + | + | + | + |
| 84 | D90 | 5 | sylvestris | bertisceus | ME | Durmitor Mt., Sedlo mountain pass | planinske rudine | 2002 | ZA-48666 | SB, ILj | 13/07/2018 | 43.100445 | 19.050844 | 2x | + | + | + | + | + | + |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 85 | D91 | 5 | sylvestris | bertisceus | ME | Lim river canyon, Tivranska klisura | river canyon, vertical rocks along the road | 711 | ZA-48612 | SB, ILj | 13/07/2018 | 42.882032 | 19.861470 | 2x | + | + | + | + | | + |
| 86 | D92 | 6 | sylvestris | bertisceus | ME | Lim river canyon, close to Andrijevica | vertical rocks along the road | 746 | ZA-48667 | SB, ILj | 13/07/2018 | 42.728919 | 19.824579 | 2x | + | + | + | + | + | + |
| 87 | D93 | 6 | sylvestris | bertisceus | ME | Zeletin, Goleš, Plana | planinske rudine | 2011 | ZA-48668 | SB, ILj | 14/07/2018 | 42.649709 | 19.844121 | 2x | + | + | + | + | + | + |
| 88 | D94 | 5 | sylvestris | bertisceus | ME | Popadija peak | rocky grassland on silicates | 1989 | ZA-48669 | SB, ILj | 14/07/2018 | 42.520644 | 19.761424 | 2x | + | + | + | + | | + |
| 89 | D95 | 5 | sylvestris | bertisceus | ME | Planinica peak | planinske rudine | 1948 | ZA-48670 | SB, ILj | 15/07/2018 | 42.679973 | 20.011363 | 2x | + | + | + | + | + | + |
| 90 | D96 | 5 | sylvestris | bertisceus | ME | Morača river canyon, N of Podgorica | river canyon, vertical rocks along the road | 278 | ZA-48613 | SB, ILj | 15/07/2018 | 42.696833 | 19.374310 | 2x | + | + | + | + | | + |
| 91 | D102 | 5 | sylvestris | | ME | Budva to Cetinje road, close to Obzovica village | rocky grassland | 931 | ZA-48619 | SB, ILj | 16/07/2018 | 42.305811 | 18.931887 | 2x | + | + | + | + | + | + |
| 92 | D103 | 5 | sylvestris | | ME | Lovčen Mt., Jezerski vrh | planinske rudine | 1681 | ZA-48620 | SB, ILj | 16/07/2018 | 42.400299 | 18.838067 | 2x | + | + | + | + | | + |
| 93 | D106 | 5 | sylvestris | nodosus | BA | Sitnica border pass | rocky stones, scrub, edge of forest | 915 | ZA-48623 | SB, ILj | 17/7/2018, 29/6/2019 | 42.564323 | 18.443123 | 2x | + | + | + | + | + | + |
| 94 | D108 | 5 | sylvestris | | AL | gorge between Kukur and Roves villages | calcareous cliffs | 969 | ZA-48625 | BF, PS | 12/07/2018 | 40.866944 | 20.384444 | 2x | | + | + | + | | + |
| 95 | D109 | 5 | sylvestris | | AL | Maja e Ostrovicës Mts., above the village Marjan | alpine meadow | 1406 | ZA-48626 | BF, PS | 13/07/2018 | 40.579722 | 20.477222 | 2x | | + | + | + | | + |
| 96 | D110 | 4 | sylvestris | | MK | Struga, Jablanica Mts, Labuniški Bačila | stabilised limestone screes | 1759 | ZA-48627 | BF, PS | 15/07/2018 | 41.272778 | 20.535000 | 2x | | + | + | + | | + |
| 97 | D111 | 5 | sylvestris | tergestinus | HR | Island of Cres, port of Merag | rocky grassland along the road, forest edge | 40 | ZA-48628 | SB, SC | 21/10/2018 | 44.977214 | 14.445000 | 2x | + | + | + | | | + |
| 98 | D119 | 4 | sylvestris | | AL | Valamara Mts., ridge above the Shkumbin river | grasslands in Pinus heldreichii subalpine zone, ophiolites | 1670 | ZA-54922 | MF | 16/07/2018 | 40.821667 | 20.501667 | 2x | + | + | + | + | + | + |
| 99 | D120 | 5 | sylvestris | | AT | Ötztal | rocky slopes | 1180 | ZA-54923 | MF | 25/07/2018 | 47.157310 | 10.925250 | | | | | | | + |
| 100 | D121 | 5 | sylvestris | | AT | Oberes Gericht | pineforest, dry and sunny, quite steep | 1160 | ZA-54924 | MF | 16/07/2018 | 47.036690 | 10.646640 | 2x | | | + | | | + |
| 101 | D122 | 5 | sylvestris | | AT | Innsbruck S | open area above a cliff, pineforest | 880 | ZA-54925 | MF | 03/09/2018 | 47.244250 | 11.418530 | 2x | | | + | | | + |
| 102 | D123 | 5 | sylvestris | | AT | Ötztal | pineforest, dry and sunny, quite steep | 1266 | ZA-54926 | MF | 25/07/2018 | 47.018720 | 10.997420 | | | | + | | | + |
| 103 | D124 | 7 | sylvestris | nodosus | HR | Island of Lastovo, Podanje | rocky grassland along the road | 48 | ZA-54927 | IR, SB | 04/06/2019 | 42.755188 | 16.923785 | 2x | + | + | + | + | | + |
| 104 | D125 | 5 | sylvestris | nodosus | HR | Island of Lastovo, Bozonji dol | rocky grassland along the road | 126 | ZA-54928 | IR, SB | 04/06/2019 | 42.749941 | 16.887758 | | + | + | + | + | + | + |
| 105 | D126 | 5 | sylvestris | nodosus | HR | Island of Lastovo, Struga Peninsula | rocky grassland above the sea | 46 | ZA-54929 | IR, SB | 04/06/2019 | 42.723823 | 16.885311 | | + | + | + | + | | + |
| 106 | D127 | 7 | sylvestris | nodosus | HR | Island of Brač, Smokovje | vertical rocks along the road | 150 | ZA-54930 | ILj, AT, MG | 04/06/2019 | 43.266081 | 16.693588 | 2x | + | + | + | + | | + |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 107 | D128 | 5 | sylvestris | nodosus | HR | Mosor Mt., above Gornje Sitno | rocky grassland along the mountain road | 810 | ZA-54931 | SB, ILj, IR, AT, MG | 05/06/2019 | 43.522366 | 16.611583 | 2x | + | + | + | + | | + |
| 108 | D129 | 5 | sylvestris | tergestinus | HR | Šibenik to Drniš road, close to Gradina village | rocky grassland along the road | 186 | ZA-54932 | SB, ILj, IR, AT, MG | 05/06/2019 | 43.773985 | 15.956080 | 2x | + | + | + | | | + |
| 109 | D130 | 5 | sylvestris | sylvestris | HR | Knin, Podinarje, Krčić creek | rocky grassland along the road | 345 | ZA-54933 | SB, ILj | 14/06/2019 | 44.033978 | 16.292465 | 2x | + | + | + | + | | + |
| 110 | D131 | 5 | sylvestris | alboroseus | GR | Pindus Mts., SE of Theodoriana village | silicates | 1023 | ZA-54934 | SB, ILj, IR, AT | 21/06/2019 | 39.426471 | 21.215909 | 2x | + | + | + | + | | + |
| 111 | D132 | 5 | sylvestris | alboroseus | GR | Gorilla Mt., Eleftherochori village | vertical rocks along the road | 664 | ZA-54935 | SB, ILj, IR, AT | 21/06/2019 | 39.497535 | 20.540515 | 2x | + | + | + | + | | + |
| 112 | D133 | 5 | sylvestris | alboroseus | GR | Lygeres, close to Chrysovitsa, Siolades village | silicates along the road | 744 | ZA-54936 | SB, ILj, IR, AT | 22/06/2019 | 39.766384 | 21.093540 | 2x | + | + | + | + | | + |
| 113 | D134 | 7 | sylvestris | alboroseus | GR | Lygeres, Aoös lake | silicates along the road | 1398 | ZA-54937 | SB, ILj, IR, AT | 22/06/2019 | 39.820819 | 21.087621 | 2x | + | + | + | + | + | + |
| 114 | D135 | 5 | sylvestris | alboroseus | GR | Kapesovo village | silicates | 1135 | ZA-54938 | SB, ILj, IR, AT | 22/06/2019 | 39.886151 | 20.786382 | 2x | + | + | + | + | + | + |
| 115 | D136 | 5 | sylvestris | alboroseus | GR | Gramos Mt., Theotokos village | silicates, edge of Quercus trojana forest | 765 | ZA-54939 | SB, ILj, IR, AT | 22/06/2019 | 40.237462 | 20.873948 | | | + | + | + | | + |
| 116 | D137 | 5 | sylvestris | alboroseus | GR | Smolikas Mt., Aoös river | silicates along the road | 631 | ZA-54940 | SB, ILj, IR, AT | 22/06/2019 | 40.144235 | 20.823990 | 2x | + | + | + | + | | + |
| 117 | D138 | 6 | sylvestris | alboroseus | GR | Astraka peak, above Mikro Papingo village | carbonates | 1296 | ZA-54941 | SB, ILj, IR, AT | 23/06/2019 | 39.972951 | 20.740756 | 2x | + | + | + | + | | + |
| 118 | D139 | 5 | sylvestris | alboroseus | GR | Pindus Mts, Tymfi Mt., Drakolimni lake | rocky limestone above sea | 2093 | ZA-54942 | SB, ILj, IR, AT | 23/06/2019 | 39.994937 | 20.785990 | 2x | | + | + | + | + | + |
| 119 | D140 | 6 | sylvestris | alboroseus | AL | Nemërçka Mts., Maja e Dhembelit Mt. | carbonates | 1523 | ZA-54943 | SB, ILj, IR, AT | 24/06/2019 | 40.215069 | 20.316456 | 2x | + | + | + | + | | + |
| 120 | D141 | 6 | sylvestris | alboroseus | AL | Gjirokastër, above Drinos river valley | carbonate clifs along the road | 400 | ZA-54944 | SB, ILj, IR, AT | 25/06/2019 | 39.945037 | 20.251086 | 2x | + | + | + | + | | + |
| 121 | D142 | 3 | sylvestris | alboroseus | AL | Ceraunian Mts., Llogara National Park, Maja Qores peak | alpine meadow | 1600 | ZA-54945 | SB, ILj, IR, AT | 25/06/2019 | 40.207207 | 19.599520 | 2x | | + | + | + | | + |
| 122 | D142 | 5 | sylvestris | alboroseus | AL | Ceraunian Mts., Llogara National Park | carbonate clifs along the road | 950 | ZA-54946 | SB, ILj, IR, AT | 25/06/2019 | 40.214363 | 19.579591 | 2x | + | + | + | + | | + |
| 123 | D145 | 5 | sylvestris | | AL | Mat river canyon, Madhesh village | silicates along the road | 145 | ZA-54947 | SB, ILj, IR, AT | 26/06/2019 | 41.679535 | 19.847393 | 2x | + | + | + | + | | + |
| 124 | D147 | 8 | sylvestris | | AL | Shkodër, close to Bardhaj village | silicates | 129 | ZA-54953 | SB, ILj, IR, AT | 27/06/2019 | 42.072188 | 19.564231 | 2x | + | + | + | + | | + |
| 125 | D150 | 6 | sylvestris | bertisceus | AL | Leqet e Hotit, Rrapsh serpentine | vertical rocks along the road, carbonate | 773 | ZA-54956 | SB, ILj, IR, AT | 27/06/2019 | 42.415086 | 19.503708 | 2x | + | + | + | + | | + |
| 126 | D151 | 7 | sylvestris | bertisceus | AL | Bjeshkët e Nemuna, Thethi, Maja Shtegut | rocky carbonate clifs and pastures | 1735 | ZA-54957 | SB, ILj, IR, AT | 28/06/2019 | 42.388436 | 19.730210 | 2x | + | + | + | + | | + |
| 127 | D153 | 5 | sylvestris | bertisceus | AL | Bjeshkët e Nemuna, Thethi | carbonate rocky clifs above road | 665 | ZA-54959 | SB, ILj, IR, AT | 28/06/2019 | 42.324863 | 19.593453 | 2x | + | + | + | + | + | + |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 128 | D154 | 5 | sylvestris | bertisceus | ME | Podgorica, Cijevna river canyon | river canyon Cijevna, carbonate rocks along the road | 139 | ZA-54960 | SB, ILj, IR, AT | 28/06/2019 | 42.398352 | 19.367112 | 2x | + | + | + | + | + | + |
| 129 | D157 | 1 | sylvestris | bertisceus | ME | Ostroška Greda Mt., Ostrog | rocky carbonate clifs along the road | 600 | ZA-54963 | SB, ILj, IR, AT | 28/06/2019 | 42.673681 | 19.030135 | | + | + | + | + | | + |
| 130 | D158 | 5 | sylvestris | bertisceus | ME | Nikšić region, Slano jezero | rocky pastures near Quercus pubescens forest | 900 | ZA-54964 | SB, ILj, IR, AT | 29/06/2019 | 42.751109 | 18.799159 | 2x | + | + | + | + | + | + |
| 131 | D160 | 5 | sylvestris | nodosus | HR | Pelješac, Popova luka village | rocky stones along the road | 160 | ZA-54966 | SB, ILj, IR, AT | 29/06/2019 | 42.915273 | 17.426740 | 2x | + | + | + | + | + | + |
| 132 | D162 | 5 | sylvestris | sylvestris | HR | Samoborsko gorje, Oštrc peak | the edge of the forests, grassland | 740 | ZA-54968 | AT, MG | 05/07/2019 | 45.763765 | 15.648604 | 2x | + | + | + | + | | + |
| 133 | D164 | 6 | sylvestris | | SI | Julian Alps, Triglav NP, close to Vršič pass | calcareous and calcshist screes | 1954 | ZA-54970 | AT, ILj | 09/07/2019 | 46.431321 | 13.736592 | 2x | + | | + | + | + | + |
| 134 | D165 | 5 | sylvestris | sylvestris | SI | Litija, Sava village | rocky stones, scrub, edge of forest | 295 | ZA-54971 | AT, ILj | 09/07/2019 | 46.088952 | 14.887297 | 2x | + | + | + | + | + | + |
| 135 | D166 | 5 | sylvestris | sylvestris | SI | Polhov Gradec, Polhograjska Grmada hill | near mountain road, carbonates | 900 | ZA-54972 | AT, ILj | 10/07/2019 | 46.084072 | 14.334739 | 2x | + | + | + | + | + | + |
| 136 | D168 | 5 | sylvestris | bertisceus | AL | Lumi i Tropojës | limestone | 1115 | ZA-54974 | DSh | 23/07/2019 | 42.473717 | 20.151667 | | + | + | + | + | | + |
| 137 | D169 | 5 | sylvestris | bertisceus | AL | Shkëlzeni Mountain | limestone | 2253 | ZA-54975, ZA-54976 | DSh | 24/07/2019 | 42.457408 | 20.122222 | 2x | + | + | + | + | | + |
| 138 | D169 | 5 | sylvestris | bertisceus | AL | Shkëlzeni Mountain | limestone | 1963 | ZA-54975, ZA-54976 | DSh | 24/07/2019 | 42.453953 | 20.111389 | 2x | + | + | + | + | | + |
| 139 | D170 | 6 | sylvestris | | RS | Kopaonik Mt., Nebeske stolice | alpine grassland | 1787 | ZA-54977 | SB, MD, MG, IR | 10/07/2019 | 43.261512 | 20.836531 | 2x | + | + | + | + | + | + |
| 140 | D171 | 5 | sylvestris | | MK | Šar planina, Ljuboten peak | alpine grassland | 2255 | ZA-54978 | SB, MD, MG, IR | 11/07/2019 | 42.199482 | 21.123851 | 2x | + | + | + | + | + | + |
| 141 | D172 | 5 | sylvestris | | MK | Šar planina, Ljuboten peak | alpine grassland | 2255 | ZA-54979 | SB, MD, MG, IR | 11/07/2019 | 42.199482 | 21.123851 | 2x | + | + | + | + | + | + |
| 142 | D173 | 6 | sylvestris | | MK | Bistra Mt. | rocky grassland along the road | 1687 | ZA-54980 | SB, MD, MG, IR | 12/07/2019 | 41.629296 | 20.684806 | 2x | + | + | + | + | | + |
| 143 | D174 | 5 | sylvestris | | MK | Galičica Mt. | rocks along the road | 1510 | ZA-54981 | SB, MD, MG, IR | 12/07/2019 | 40.965656 | 20.818794 | 2x | + | + | + | + | | + |
| 144 | D175 | 6 | sylvestris | | MK | Korab Mt., Nistrovski Korab ridge | rocky places witih alpine grassland | 2112 | ZA-54982 | SB, MD, MG, IR | 13/07/2019 | 41.791897 | 20.592906 | 2x | + | + | + | + | | + |
| 145 | D176 | 5 | sylvestris | | MK | Korab Mt., Radika river canyon | rocky blocks along the road in the canyon | 1379 | ZA-54983 | SB, MD, MG, IR | 13/07/2019 | 41.799585 | 20.628008 | 2x | + | + | + | + | | + |
| 146 | D178 | 6 | sylvestris | kozjakensis | MK | Kozjak Mt, above Krstec village | rocky grassland in Fagus sylvatica forest | 1292 | ZA-54985 | SB, IR | 14/07/2019 | 41.409144 | 21.678418 | 2x | + | + | + | + | + | + |
| 147 | D179 | 5 | sylvestris | | MK | Jakupica Mt., Solunska Glava | calcareous rocky alpine grassland | 2274 | ZA-54986 | SB, MG, IR | 17/07/2019 | 41.692786 | 21.403810 | 2x | + | + | + | + | + | + |
| 148 | D180 | 5 | sylvestris | | MK | Jakupica Mt., Solunska Glava | along the road, forest edge | 1235 | ZA-54987 | SB, MG, IR | 17/07/2019 | 41.680442 | 21.432421 | 2x | + | + | + | + | + | + |
| 149 | D181 | 5 | sylvestris | alboroseus | AL | Leshnica e Sipërme | limestone | 700 | ZA-54988 | DSh | 07/06/2019 | 39.849242 | 20.281111 | | + | + | + | + | | + |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 150 | D182 | 5 | sylvestris | | AL | Skorushë, Ura e Hasanit | serpentine | 1430 | ZA-54989, ZA-54990 | LSh | 27/07/2019 | 40.588833 | 20.579722 | 2x | + | + | + | + | + | + |
| 151 | D182 | 5 | sylvestris | | AL | Skorushë, Ura e Hasanit | serpentine | 1170 | ZA-54989, ZA-54990 | LSh | 27/07/2019 | 40.583694 | 20.570556 | 2x | + | + | + | + | + | + |
| 152 | Alps124 | 21 | sylvestris | | FR | | | 206 | | HL | 10/06/2017 | 45.743560 | 5.887870 | | | | + | | | |
| 153 | Apenn165 | 4 | sylvestris | | IT | | | 530 | | DD | 31/01/2018 | 44.600550 | 8.809254 | | | | + | | | |
| 154 | Alps121 | 20 | sylvestris | | FR | | | 1803 | | HL | 07/06/2017 | 44.499080 | 5.950400 | | | | + | | | |
| 155 | Apenn112 | 30 | sylvestris | | IT | | | 604 | | DG | 21/07/2015 | 39.829403 | 16.303786 | | | | + | | | |
| 156 | Alps3 | 21 | sylvestris | | CH | | | 673 | | HL | 28/07/2016 | 47.047807 | 7.012920 | | | | + | | | |
| 157 | Apenn38 | 19 | sylvestris | | IT | | | 280 | | MGa | 2011 | 43.666700 | 11.160000 | | | | + | | | |
| 158 | Alps4 | 22 | sylvestris | | CH | | | 1456 | | HL | 29/07/2016 | 46.424684 | 6.104079 | | | | + | | | |
| 159 | Alps117 | 20 | sylvestris | | FR | | | 1453 | | HL | 04/06/2017 | 44.070350 | 7.516830 | | | | + | | | |
| 160 | Alps15 | 23 | sylvestris | | IT | | | 2220 | | HL | 04/08/2016 | 46.466060 | 11.859290 | | | | + | | | |
| 161 | Alps133 | 23 | sylvestris | | IT | | | 1051 | | HL | 17/07/2017 | 45.632300 | 10.780350 | | | | + | | | |
| 162 | Alps77 | 25 | sylvestris | | CH | | | 2340 | | SF | 04/08/2016 | 46.036944 | 7.952500 | | | | + | | | |
| 163 | Apenn119 | 4 | sylvestris | | FR | | | 1705 | | HL | 06/06/2017 | 43.863010 | 6.321030 | | | | + | | | |
| 164 | Apenn43 | 21 | sylvestris | | IT | | | 590-600 | | MGa | 08/04/2011 | 44.633300 | 10.066700 | | | | + | | | |
| 165 | Alps125 | 20 | sylvestris | | CH | | | 828 | | HL | 26/06/2017 | 45.975350 | 8.944370 | | | | + | | | |
| 166 | Apenn111 | 30 | sylvestris | | IT | | | 585 | | DG | 22/07/2015 | 39.847182 | 16.133714 | | | | + | | | |
| 167 | Apenn114 | 30 | cfr. | | IT | | | 1824 | | DG | 04/08/2015 | 39.798429 | 16.092498 | | | | + | | | |
| 168 | Apenn115 | 31 | cfr. | | IT | | | 2134 | | DG | 13/08/2015 | 39.897224 | 16.210160 | | | | + | | | |
| 169 | Apenn116 | 30 | cfr. | | IT | | | 2181 | | DG | 06/08/2015 | 39.919789 | 16.158453 | | | | + | | | |
| 170 | Alps60 | 23 | sylvestris | | CH | | | 2080-2210 | | KR | 25/06/2016, 30/06/2016 | 46.785515 | 10.198479 | | | | + | | | |
| 171 | Alps16 | 18 | sylvestris | | CH | | | 1300 | | HL | 03/08/2016 | 46.632160 | 10.446993 | | | | + | | | |
| 172 | Alps27 | 14 | sylvestris | | IT | | | 2130 | | HL | 28/09/2016 | 45.525360 | 7.191370 | | | | + | | | |
| 173 | Alps144 | 7 | sylvestris | | CH | | | 1874 | | HL | 28/09/2017 | 46.390290 | 10.102070 | | | | + | | | |
| 174 | Alps20 | 23 | sylvestris | | FR | | | 1986 | | HL | 19/08/2016 | 45.314010 | 6.680270 | | | | + | | | |
| 175 | Alps10 | 16 | sylvestris | | CH | | | 1790 | | HL | 24/07/2016 | 46.527342 | 7.208130 | | | | + | | | |
| 176 | Apenn122 | 17 | sylvestris | | FR | | | 428 | | HL | 07/06/2017 | 44.428779 | 5.277236 | | | | + | | | |
| 177 | Apenn118 | 20 | sylvestris | | FR | | | 900 | | HL | 05/06/2017 | 43.703900 | 6.815060 | | | | + | | | |
| 178 | Alps9 | 10 | sylvestris | | CH | | | 1420 | | HL | 01/07/2016 | 46.424250 | 8.518020 | | | | + | | | |
| 179 | Alps143 | 20 | sylvestris | | CH | | | 1512 | | HL | 27/09/2017 | 47.250760 | 9.445180 | | | | + | | | |
| 180 | Apenn113 | 31 | sylvestris | | IT | | | 909 | | DG | 30/07/2015 | 39.806289 | 16.139899 | | | | + | | | |
| 181 | Alps99 | 20 | sylvestris | | CH | | | 900 | | SBu | 17/08/2016 | 46.121950 | 7.039067 | | | | + | | | |
| 182 | Alps48 | 7 | sylvestris | | IT | | | 880 | | MGa | 2011 | 45.098000 | 7.343000 | | | | + | | | |
| 183 | Apenn120 | 20 | sylvestris | | FR | | | 569 | | HL | 06/06/2017 | 44.219720 | 5.237900 | | | | + | | | |
| 184 | Alps128 | 17 | sylvestris | | IT | | | 1774 | | HL | 14/07/2017 | 46.789070 | 11.345210 | | | | + | | | |
| 185 | SDM1 | | sylvestris | sylvestris | SI | Sežana municipality resting place | vertilcal rocks along the road | 351 | | IR, SB, ILj | 13/06/2018 | 45.702937 | 13.946861 | | | + | | | | + |
| 186 | SDM2 | | sylvestris | tergestinus | IT | Trieste, Pesek village | rocky grassland | 491 | | IR, SB, ILj | 13/06/2018 | 45.627242 | 13.900654 | | | + | | | | + |
| 187 | SDM3 | | sylvestris | tergestinus | SI | Kozina village | rocky grassland | 472 | | IR, SB, ILj | 13/06/2018 | 45.601953 | 13.930488 | | | + | | | | + |
| 188 | SDM4 | | sylvestris | tergestinus | SI | Koper municipality | rocky grassland | 411 | | IR, SB, ILj | 13/06/2018 | 45.560501 | 13.891776 | | | + | | | | + |
| 189 | SDM5 | | sylvestris | sylvestris | HR | Ćićarija, close to Jelovice village | the edge of the forests | 639 | | IR, SB, ILj | 13/06/2018 | 45.506661 | 13.974236 | | | + | | | | + |
| 190 | SDM6 | | sylvestris | sylvestris | HR | between the villages Slum and Nugla | rocky grassland | 567 | | IR, SB, ILj | 13/06/2018 | 45.424990 | 14.007932 | | | + | | | | + |
| 191 | SDM7 | | sylvestris | sylvestris | HR | Grobnik | rocky grassland | 330 | | IR, SB, ILj | 14/06/2018 | 45.379421 | 14.544188 | | | + | | | | + |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 192 | SDM8 | sylvestris | tergestinus | HR | Crikvenica | vertilcal rocks along the road | 25 | IR, SB, ILj | 14/06/2018 | 45.176634 | 14.693055 | + | + |
| 193 | SDM9 | sylvestris | sylvestris | HR | Jadranska magistrala, close to Prizna | vertilcal rocks along the road | 30 | IR, SB, ILj | 15/06/2018 | 44.636572 | 14.965935 | + | + |
| 194 | SDM10 | sylvestris | nodosus | HR | Visočica | grassland, along the mountain route | 1501 | IR, SB, ILj | 16/06/2018 | 44.429109 | 15.366957 | + | + |
| 195 | SDM11 | sylvestris | tergestinus | HR | Paklenica, close to Veliki Vaganac | rocky grassland | 513 | IR, SB, ILj | 17/06/2018 | 44.338871 | 15.444109 | + | + |
| 196 | SDM12 | sylvestris | tergestinus | HR | Šibenik, Guberina, next to the old bridge | maquis and garig near macadam | 30 | IR, SB, ILj | 18/06/2018 | 43.763085 | 15.845444 | + | + |
| 197 | SDM13 | sylvestris | nodosus | HR | Biokovo Mt, SE of Mali Troglav | the edge of the forests | 1440 | IR, SB, ILj | 18/06/2018 | 43.327629 | 17.057692 | + | + |
| 198 | SDM14 | sylvestris | nodosus | BA | Posušje | vertilcal rocks along the road | 551 | IR, SB, ILj | 20/06/2018 | 43.473272 | 17.353415 | + | + |
| 199 | SDM15 | sylvestris | nodosus | BA | Blidinje to Posušje road, close to Rakitno village | vertilcal rocks along the road | 734 | IR, SB, ILj | 20/06/2018 | 43.536338 | 17.447936 | + | + |
| 200 | SDM16 | sylvestris | bertisceus | BA | Sutjeska river canyon, close to Tjentište | rocky grassland | 620 | SB, ILj | 11/07/2018 | 43.304243 | 18.649676 | + | + |
| 201 | SDM17 | sylvestris | bertisceus | ME | Maglić Mt., Trnovačko jezero | rocky grassland | 1526 | SB, ILj | 12/07/2018 | 43.251793 | 18.721639 | + | + |
| 202 | SDM18 | sylvestris | bertisceus | BA | Maglić Mt. | calcareous and calcshist screes | 1647 | SB, ILj | 12/07/2018 | 43.285038 | 18.719106 | + | + |
| 203 | SDM19 | sylvestris | bertisceus | ME | Pivsko jezero, serpentines on the road to Žabljak | river canyon, vertical rocks along the road | 809 | SB, ILj | 13/07/2018 | 43.172169 | 18.858826 | + | + |
| 204 | SDM20 | sylvestris | bertisceus | ME | Durmitor Mt, road Žabljak Trsa Plužine | vertical rocks along the road | 1692 | SB, ILj | 13/07/2018 | 43.092165 | 19.089705 | + | + |
| 205 | SDM21 | sylvestris | bertisceus | ME | Tara river canyon, close to Vrela village | vertical rocks along the road | 1346 | SB, ILj | 13/07/2018 | 43.152615 | 19.257936 | + | + |
| 206 | SDM22 | sylvestris | nodosus | HR | Velebit Mt., road Sveti Juraj to Krasno | the edge of the forests, rocky grassland | 803 | SB, ILj | 06/09/2018 | 44.883093 | 14.971827 | + | + |
| 207 | SDM23 | sylvestris | sylvestris | HR | top ridge between the peaks Svilaja and Vršina | rocky grassland | 1411 | MT | 07/01/2018 | 43.787163 | 16.490082 | + | + |
| 208 | SDM24 | sylvestris | sylvestris | HR | peak Svilaja | rocky grassland | 1462 | MT | 07/01/2018 | 43.789100 | 16.485000 | + | + |
| 209 | SDM25 | sylvestris | sylvestris | HR | Svilaja Mt. Umac | rocky grassland near macadam | 1090 | MT | 07/01/2018 | 43.778099 | 16.511263 | + | + |
| 210 | SDM26 | sylvestris | tergestinus | HR | Kozjak Mt., above Kaštel Gomilica | rocky grassland | 251 | ILj, AT, MG | 04/06/2019 | 43.563016 | 16.409640 | + | + |
| 211 | SDM27 | sylvestris | tergestinus | HR | Žrnovnica canyon, between Sridivica hill and Gradac | rocky grassland along the road | 100 | SB, ILj, IR, AT, MG | 05/06/2019 | 43.517604 | 16.575629 | + | + |
| 212 | SDM28 | sylvestris | tergestinus | HR | Lička Plješivica Mt., Poštak peak | rocky grassland along the road | 743 | SB, ILj, IR, AT, MG | 06/06/2019 | 44.256939 | 16.065089 | + | + |
| 213 | SDM29 | sylvestris | nodosus | BA | Trebinje, Klobuk border pass | edge of forest | 937 | SB, ILj, IR, AT | 29/06/2019 | 42.711594 | 18.551172 | + | + |
| 214 | SDM30 | sylvestris | nodosus | BA | Ravno, Rupni Do, Gradac | edge of forest | 500 | SB, ILj, IR, AT | 29/06/2019 | 42.880243 | 17.947038 | + | + |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 215 | SDM31 | sylvestris | nodosus | HR | Čepikuće border pass, Oblik hill slopes | | 400 | SB, ILj, IR, AT | 29/06/2019 | 42.872725 | 17.854560 | + | + |
| 216 | SDM32 | sylvestris | sylvestris | SI | Polhov Gradec, Polhograjska Grmada hill | near mountain road, carbonates | 758 | AT, ILj | 10/07/2019 | 46.085547 | 14.346393 | + | + |
| 217 | SDM33 | sylvestris | sylvestris | HR | Klek, Ogulin | Klek peak | 1029 | DS | 02/08/2020 | 45.260345 | 15.145431 | + | + |
| 218 | SDM34 | sylvestris | sylvestris | HR | Klek, Ogulin | helicopter takeoff near Klek peak | 1104 | DS | 02/08/2020 | 45.260182 | 15.144568 | + | + |
| 219 | Balkan DB 1 | | | RS | | | | SĐ | | 43.800511 | 19.519803 | | + |
| 220 | Balkan DB 3 | | | RS | | | | SĐ | | 43.586220 | 19.347322 | | + |
| 221 | Balkan DB 4 | | | MK | | | | SĐ | | 41.756200 | 20.618400 | | + |
| 222 | Balkan DB 5 | | | BA | | | | SĐ | | 43.925400 | 16.628080 | | + |
| 223 | Balkan DB 6 | | | BA | | | | SĐ | | 44.344230 | 17.253380 | | + |
| 224 | Balkan DB 7 | | | AL | | | | SĐ | | 42.054450 | 19.537890 | | + |
| 225 | Balkan DB 8 | | | AL | | | | SĐ | | 41.786988 | 20.180390 | | + |
| 226 | GBIF DSYL BALKANS 1052816295 | | | ME | | | | GBIF | | 42.825130 | 18.917570 | | + |
| 227 | GBIF DSYL BALKANS 1144595356 | | | AT | | | | GBIF | | 47.592780 | 14.140280 | | + |
| 228 | GBIF DSYL BALKANS 1264995318 | | | IT | | | | GBIF | | 46.438330 | 13.568610 | | + |
| 229 | GBIF DSYL BALKANS 1264995334 | | | AT | | | | GBIF | | 47.075560 | 12.751110 | | + |
| 230 | GBIF DSYL BALKANS 152921058 | | | SI | | | | GBIF | | 45.599720 | 13.912060 | | + |
| 231 | GBIF DSYL BALKANS 164145531 | | | AT | | | | GBIF | | 46.861820 | 13.325860 | | + |
| 232 | GBIF DSYL BALKANS 165105849 | | | SI | | | | GBIF | | 45.599740 | 14.411990 | | + |
| 233 | GBIF DSYL BALKANS 214525133 | | | AT | | | | GBIF | | 46.783330 | 12.783330 | | + |
| 234 | GBIF DSYL BALKANS 296454179 | | | AT | | | | GBIF | | 47.007440 | 12.439940 | | + |
| 235 | GBIF DSYL BALKANS 874692609 | | | IT | | | | GBIF | | 46.194720 | 12.980830 | | + |
| 236 | GBIF DSYL BALKANS 874757553 | | | IT | | | | GBIF | | 46.194720 | 12.980830 | | + |
| 237 | GBIF DSYL BALKANS 911950894 | | | AT | | | | GBIF | | 47.209980 | 13.425830 | | + |
| 238 | GBIF DSYL BALKANS 9268476 | | | AT | | | | GBIF | | 46.706100 | 12.925600 | | + |
| 239 | GBIF DSYL BALKANS 9268477 | | | AT | | | | GBIF | | 46.634700 | 12.935000 | | + |

| | | | | | | |
|---|---|---|---|---|---|---|
| 240 | Luqman Dinaric Fortress | HR | HL | 44.016560 | 16.415360 | + |
| 241 | Luqman Dolina Glinscice | IT | HL | 45.619583 | 13.873778 | + |
| 242 | Luqman Dubrava | HR | HL | 43.508210 | 16.623780 | + |
| 243 | Luqman Durmitor | ME | HL | 43.098944 | 19.051111 | + |
| 244 | Luqman Grossglockner | AT | HL | 47.065730 | 12.756470 | + |
| 245 | Luqman Kapetanovo Jezero | ME | HL | 42.816440 | 19.230430 | + |
| 246 | Luqman Komen | SI | HL | 45.841944 | 13.772181 | + |
| 247 | Luqman Konitsa | MK | HL | 40.070000 | 20.837800 | + |
| 248 | Luqman Kotor | ME | HL | 42.422230 | 18.785500 | + |
| 249 | Luqman Laserzsee | AT | HL | 46.766720 | 12.800410 | + |
| 250 | Luqman Mangart | SI | HL | 46.443540 | 13.639610 | + |
| 251 | Luqman Mostar | BA | HL | 43.247770 | 17.966450 | + |
| 252 | Luqman Movraz | SI | HL | 45.482972 | 13.913364 | + |
| 253 | Luqman Mramorje | ME | HL | 42.925167 | 19.023417 | + |
| 254 | Luqman Mt Stitan | ME | HL | 42.573817 | 19.559967 | + |
| 255 | Luqman Orjen | BA | HL | 42.572461 | 18.458978 | + |
| 256 | Luqman Parun | AL | HL | 42.322222 | 19.656944 | + |
| 257 | Luqman Rotspitze | AT | HL | 47.018970 | 12.240970 | + |
| 258 | Luqman Sneznik | SI | HL | 45.565373 | 14.318294 | + |
| 259 | Luqman Soca High | SI | HL | 46.412170 | 13.693990 | + |
| 260 | Luqman Soca Low | SI | HL | 46.404810 | 13.705320 | + |
| 261 | Luqman Ucka | HR | HL | 45.284200 | 14.202050 | + |
| 262 | Luqman Valbone | AL | HL | 42.410100 | 19.817400 | + |
| 263 | Luqman Vela Draga | HR | HL | 45.316517 | 14.169567 | + |
| 264 | Luqman Visitor | ME | HL | 42.614806 | 19.893222 | + |
| 265 | Luqman Vlaka | BA | HL | 42.736230 | 18.101060 | + |
| 266 | VHA 10 | IT | VirtualHerbariaAustria | 46.194722 | 12.980833 | + |
| 267 | VHA 12 | AT | VirtualHerbariaAustria | 46.958333 | 13.021944 | + |
| 268 | VHA 13 | ME | VirtualHerbariaAustria | 42.112778 | 19.211667 | + |
| 269 | VHA 15 | AT | VirtualHerbariaAustria | 46.710556 | 12.933056 | + |
| 270 | VHA 5 | ME | VirtualHerbariaAustria | 42.375000 | 19.238056 | + |
| 271 | VHA 7 | AT | VirtualHerbariaAustria | 47.069444 | 12.752500 | + |

**Table S2**. Genome-wide population genetic diversity summary for 100 diploid *Dianthus sylvestris* populations from the Balkan lineage. Sample size (n), Allelic richness (Ar) observed (Ho) and expected heterozygosity (He).

| POP_ID | x | y | n | Ar | Ho | He |
|---|---|---|---|---|---|---|
| 102 | 18.93189 | 42.30581 | 5 | 1.227 | 0.229 | 0.227 |
| 103 | 18.83807 | 42.4003 | 5 | 1.226 | 0.229 | 0.226 |
| 106 | 18.44312 | 42.56432 | 5 | 1.225 | 0.229 | 0.224 |
| 108 | 20.38444 | 40.86694 | 5 | 1.215 | 0.224 | 0.214 |
| 109 | 20.47722 | 40.57972 | 4 | 1.213 | 0.214 | 0.214 |
| 110 | 20.535 | 41.27278 | 4 | 1.203 | 0.212 | 0.203 |
| 119 | 20.50167 | 40.82167 | 4 | 1.211 | 0.212 | 0.211 |
| 124 | 16.92379 | 42.75519 | 7 | 1.206 | 0.208 | 0.206 |
| 125 | 16.88776 | 42.74994 | 5 | 1.219 | 0.217 | 0.220 |
| 126 | 16.88531 | 42.72382 | 5 | 1.211 | 0.222 | 0.210 |
| 127 | 16.69359 | 43.26608 | 7 | 1.224 | 0.221 | 0.224 |
| 128 | 16.61158 | 43.52237 | 5 | 1.229 | 0.227 | 0.229 |
| 130 | 16.29247 | 44.03398 | 5 | 1.208 | 0.220 | 0.207 |
| 131 | 21.21591 | 39.42647 | 5 | 1.206 | 0.214 | 0.204 |
| 132 | 20.54052 | 39.49754 | 5 | 1.197 | 0.208 | 0.195 |
| 133 | 21.09354 | 39.76638 | 5 | 1.203 | 0.208 | 0.203 |
| 134 | 21.08762 | 39.82082 | 7 | 1.216 | 0.218 | 0.216 |
| 135 | 20.78638 | 39.88615 | 5 | 1.204 | 0.207 | 0.204 |
| 136 | 20.87395 | 40.23746 | 5 | 1.219 | 0.222 | 0.219 |
| 137 | 20.82399 | 40.14424 | 5 | 1.215 | 0.218 | 0.215 |
| 138 | 20.74076 | 39.97295 | 6 | 1.210 | 0.213 | 0.210 |
| 139 | 20.78599 | 39.99494 | 5 | 1.210 | 0.221 | 0.209 |
| 140 | 20.31646 | 40.21507 | 6 | 1.216 | 0.228 | 0.215 |
| 141 | 20.25109 | 39.94504 | 6 | 1.200 | 0.189 | 0.202 |
| 142 | 19.59952 | 40.20721 | 8 | 1.192 | 0.197 | 0.192 |
| 145 | 19.84739 | 41.67954 | 5 | 1.210 | 0.224 | 0.208 |
| 147 | 19.56423 | 42.07219 | 8 | 1.209 | 0.209 | 0.208 |
| 150 | 19.50371 | 42.41509 | 6 | 1.222 | 0.225 | 0.222 |
| 151 | 19.73021 | 42.38844 | 7 | 1.222 | 0.223 | 0.222 |
| 153 | 19.59345 | 42.32486 | 5 | 1.223 | 0.220 | 0.224 |
| 154 | 19.36711 | 42.39835 | 5 | 1.215 | 0.221 | 0.214 |
| 158 | 18.79916 | 42.75111 | 4 | 1.216 | 0.218 | 0.216 |
| 160 | 17.42674 | 42.91527 | 5 | 1.206 | 0.212 | 0.206 |
| 162 | 15.6486 | 45.76377 | 5 | 1.212 | 0.212 | 0.212 |
| 164 | 13.73659 | 46.43132 | 6 | 1.209 | 0.220 | 0.208 |
| 165 | 14.8873 | 46.08895 | 5 | 1.211 | 0.215 | 0.211 |
| 166 | 14.33474 | 46.08407 | 5 | 1.226 | 0.225 | 0.226 |
| 168 | 20.15167 | 42.47372 | 5 | 1.207 | 0.213 | 0.206 |
| 169 | 20.11681 | 42.45568 | 4 | 1.226 | 0.265 | 0.220 |
| 170 | 20.83653 | 43.26151 | 6 | 1.201 | 0.211 | 0.200 |
| 171 | 21.12385 | 42.19948 | 5 | 1.206 | 0.209 | 0.206 |
| 172 | 21.12385 | 42.19948 | 5 | 1.211 | 0.213 | 0.211 |
| 173 | 20.68481 | 41.6293 | 6 | 1.213 | 0.210 | 0.214 |
| 174 | 20.81879 | 40.96566 | 5 | 1.218 | 0.222 | 0.217 |
| 175 | 20.59291 | 41.7919 | 6 | 1.214 | 0.216 | 0.214 |
| 176 | 20.62801 | 41.79959 | 5 | 1.203 | 0.200 | 0.203 |
| 178 | 21.67842 | 41.40914 | 6 | 1.200 | 0.216 | 0.198 |
| 179 | 21.40381 | 41.69279 | 5 | 1.210 | 0.220 | 0.209 |
| 180 | 21.43242 | 41.68044 | 5 | 1.206 | 0.207 | 0.206 |
| 181 | 20.28111 | 39.84924 | 5 | 1.212 | 0.210 | 0.212 |
| 182 | 20.57514 | 40.58626 | 5 | 1.222 | 0.252 | 0.218 |
| 2 | 17.08055 | 42.94086 | 7 | 1.211 | 0.218 | 0.210 |
| 23 | 14.5247 | 45.37468 | 5 | 1.277 | 0.359 | 0.266 |
| 27 | 14.96583 | 44.70794 | 5 | 1.231 | 0.232 | 0.232 |
| 28 | 14.9569 | 44.7153 | 10 | 1.230 | 0.230 | 0.230 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 33 | 15.14316 | 44.52948 | 5 | 1.232 | 0.237 | 0.232 |
| 34 | 15.3643 | 44.4293 | 5 | 1.234 | 0.231 | 0.233 |
| 37 | 15.46143 | 44.36597 | 7 | 1.231 | 0.233 | 0.231 |
| 38 | 15.46567 | 44.37109 | 5 | 1.232 | 0.231 | 0.232 |
| 39 | 15.46572 | 44.37557 | 2 | 1.186 | 0.223 | 0.168 |
| 42 | 15.51313 | 44.36112 | 6 | 1.209 | 0.230 | 0.207 |
| 46 | 15.87176 | 43.73736 | 6 | 1.220 | 0.226 | 0.220 |
| 47 | 17.08662 | 43.28407 | 11 | 1.232 | 0.232 | 0.232 |
| 52 | 17.80412 | 43.32759 | 7 | 1.225 | 0.224 | 0.224 |
| 55 | 17.96701 | 43.64998 | 6 | 1.222 | 0.224 | 0.221 |
| 56 | 17.41643 | 43.41747 | 5 | 1.225 | 0.216 | 0.225 |
| 57 | 17.48789 | 43.58668 | 5 | 1.223 | 0.232 | 0.223 |
| 58 | 17.54076 | 43.59503 | 5 | 1.220 | 0.232 | 0.218 |
| 59 | 17.53378 | 43.58652 | 6 | 1.237 | 0.233 | 0.237 |
| 60 | 17.54072 | 43.57896 | 5 | 1.225 | 0.233 | 0.224 |
| 61 | 17.27952 | 43.54695 | 5 | 1.238 | 0.235 | 0.239 |
| 6 | 17.84349 | 42.81569 | 5 | 1.222 | 0.223 | 0.221 |
| 63 | 17.03066 | 43.85963 | 5 | 1.230 | 0.230 | 0.230 |
| 64 | 17.05981 | 43.88544 | 6 | 1.238 | 0.238 | 0.238 |
| 65 | 17.16035 | 43.78286 | 5 | 1.224 | 0.219 | 0.225 |
| 66 | 16.35956 | 44.34866 | 6 | 1.232 | 0.234 | 0.232 |
| 69 | 12.88028 | 46.75922 | 9 | 1.221 | 0.219 | 0.222 |
| 70 | 12.25111 | 47.01306 | 5 | 1.201 | 0.198 | 0.202 |
| 76 | 20.01645 | 44.04424 | 5 | 1.198 | 0.209 | 0.197 |
| 77 | 20.91537 | 42.2335 | 5 | 1.199 | 0.209 | 0.198 |
| 78 | 20.47551 | 40.5741 | 5 | 1.220 | 0.219 | 0.221 |
| 79 | 20.83973 | 43.26149 | 5 | 1.197 | 0.207 | 0.197 |
| 80 | 16.69853 | 43.45025 | 8 | 1.235 | 0.235 | 0.234 |
| 8 | 16.41704 | 44.01427 | 5 | 1.220 | 0.215 | 0.220 |
| 81 | 16.39343 | 44.03867 | 4 | 1.228 | 0.231 | 0.228 |
| 82 | 16.52322 | 43.75119 | 5 | 1.236 | 0.238 | 0.236 |
| 83 | 17.1797 | 44.68547 | 5 | 1.215 | 0.215 | 0.215 |
| 84 | 17.24253 | 44.3468 | 4 | 1.206 | 0.209 | 0.205 |
| 85 | 17.62419 | 44.25852 | 5 | 1.226 | 0.236 | 0.224 |
| 86 | 18.66821 | 43.31548 | 6 | 1.210 | 0.230 | 0.209 |
| 87 | 18.71955 | 43.27309 | 5 | 1.228 | 0.225 | 0.228 |
| 88 | 18.84523 | 43.26326 | 5 | 1.228 | 0.230 | 0.227 |
| 89 | 18.94089 | 43.13713 | 5 | 1.234 | 0.230 | 0.235 |
| 90 | 19.05084 | 43.10045 | 5 | 1.229 | 0.232 | 0.228 |
| 91 | 19.86147 | 42.88203 | 5 | 1.219 | 0.219 | 0.218 |
| 92 | 19.82458 | 42.72892 | 6 | 1.222 | 0.225 | 0.222 |
| 93 | 19.84412 | 42.64971 | 6 | 1.219 | 0.226 | 0.219 |
| 94 | 19.76142 | 42.52064 | 5 | 1.220 | 0.222 | 0.220 |
| 95 | 20.01136 | 42.67997 | 5 | 1.216 | 0.217 | 0.216 |
| 96 | 19.37431 | 42.69683 | 5 | 1.207 | 0.220 | 0.206 |

**Table S3**. Geographical patterns of genetic diversity - relationship between latitude/longitude and allelic richness across all loci (Ar), observed heterozygosity (Ho) and expected heterozygosity (He).

| | **Ar** | | **Ho** | | **He** | |
|---|---|---|---|---|---|---|
| | **$R^2$** | ***p*** | **$R^2$** | ***p*** | **$R^2$** | ***p*** |
| **x (longitute)** | 0.168 | **<0.0001** | 0.096 | **0.002** | 0.148 | **<0.0001** |
| **y (latitude)** | 0.131 | **<0.001** | 0.083 | 0.074 | 0.115 | **0.001** |

# Appendix I

# Environmental variability of *Dianthus sylvestris* Wulfen (Caryophyllaceae) on the Balkan Peninsula

Ana Terlević[1], Martina Temunović[2], Sandro Bogdanović[3,4], Marin Grgurev[1], Ivica Ljubičić[3], Ivana Rešetnik[1]

[1] Faculty of Science, Department of Biology, University of Zagreb, Trg Marka Marulića 20/II, 10000 Zagreb, Croatia.
[2] Faculty of Forestry and Wood Technology, Department of Forest Genetics, Dendrology and Botany, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia.
[3] Faculty of Agriculture, Department of Agricultural Botany, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia.
[4] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska cesta 25, 10000 Zagreb, Croatia

During the review process of the manuscript "Morphological and environmental variability of *Dianthus sylvestris* Wulfen (Caryophyllaceae) on the Balkan Peninsula", criticism on performing niche analysis on the weakly supported subspecies were raised several times. In line with the reviewer's and editor's comments we decided to completely remove the niche analysis from the manuscript. Since the second research objective of this thesis was to "develop models of habitat suitability for the present and the past and analyse the ecological niches of *D. sylvestris* subspecies with the aim of investigating the relationship between their morphological, genetic and environmental divergence", this Appendix I is attached here along with publications to meet this objective. Furthermore, and according to the concept of integrative taxonomy, we think that by incorporating niche analysis in addition to morphometrics and population genomics, one can obtain more robust evidence which would elucidate the recognition of intraspecific entities.

## INTRODUCTION

In addition to morphometrics performed to capture morphological diversity and the investigation of the relationship between morphological variability and environment and geography (**Publication IV**), we performed environmental niche analyses to capture the environmental diversity between the currently accepted subspecies of *D. sylvestris* s.l. Furthermore, we compared their flowering periods to test the shift in flowering time of *D. sylvestris* subsp. *tergestinus*.

The development of niche analysis methods allowed for a quantification of species' niche and assessment of how niches vary between taxa (Broennimann *et al.* 2012). Niche analyses have been increasingly used for characterising the environmental niche of species or subspecies (Aguirre-Gutiérrez *et al.* 2015; Jaime *et al.* 2015; Janišová *et al.* 2018; Moreno-Contreras *et al.* 2020), and in integrative approaches for resolving taxonomically challenging groups (Mota-Vargas and Rojas-Soto 2016; Otero *et al.* 2019; Lin *et al.* 2021). Species' niche may be estimated by relating data on its occurrences with environmental data (climatic, edaphic and topographic) in the form of GIS layers. Based on the degree of relatedness between species, niches may be conserved (more similar than expected), constrained (divergent within a limited subset of available niches) or divergent (less similar than expected; Pyron *et al.*, 2015). Niche divergence may not always support taxon boundaries at species level, but it may be a consequence of local adaptation and reflect phenotypic diversity at intraspecific level (Dagnino *et al.* 2017). Accordingly, when distributed along an environmental gradient, such as the case of *D. sylvestris*, ecologically marginal populations may show some morphological differentiation (Alcántara-Ayala *et al.* 2020; Buitrago Aristizábal *et al.* 2020) which may lead to potentially erroneous inference on taxon boundaries. Nevertheless, as part of an integrative approach in dealing with taxonomical issues, niche quantification provides an additional line of evidence and thus may help in reaching a decision on delimitation of taxon boundaries (Dagnino *et al.* 2017; Otero *et al.* 2019).

By incorporating environmental variation, *D. sylvestris* represents a promising model to explore the population differentiation in terms of morphological variability and niche divergence/conservatism. Hence, the aim was to compare the environmental niches and test for niche conservatism/divergence between *D. sylvestris* subspecies. Therefore, a comprehensive environmental dataset was created based on populations from across the geographical range of these subspecies on the Balkan Peninsula.

## MATERIAL AND METHODS

### ENVIRONMENTAL NICHE COMPARISON BETWEEN THE SUBSPECIES

We initially obtained 182 occurrences of *D. sylvestris* for environmental niche analysis, which included 97 populations used for the morphometric and RDA analyses (**Publication IV**). To reduce the sampling bias in environmental niche analysis, occurrences with pair-distances < 1 km were excluded using the "thin" function in the "spThin" R package version 0.2.0 (Aiello-Lammens *et al.* 2015). After thinning, a total of 162 populations of *D. sylvestris* were used for the environmental niche analysis (Fig. 1).

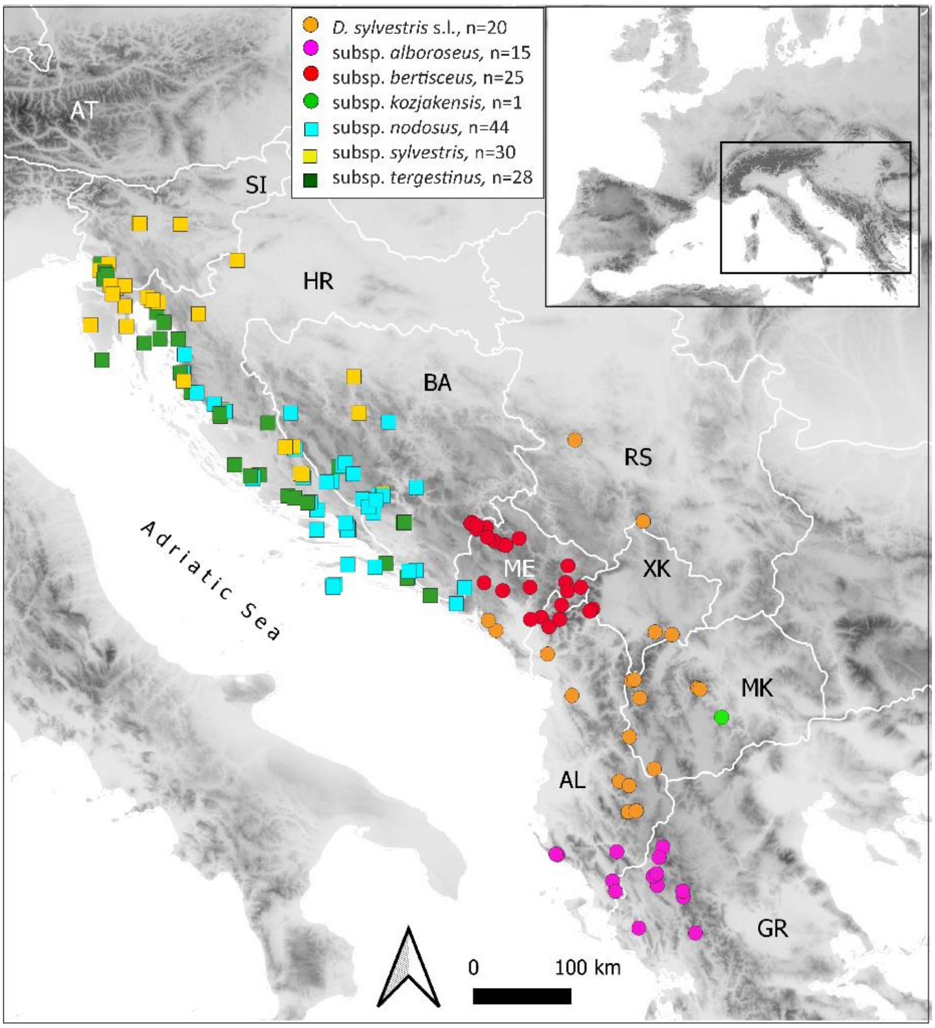


**Figure 1**. Localities of sampled populations of *Dianthus sylvestris* on the Balkan Peninsula used for environmental niche analyses (n = 182 populations; for details see Table S1 in **Publication IV**).

To characterise the environmental niche for each subspecies, we used environmental data from three databases: climate data from the Chelsa database (Karger *et al.* 2017, 2020), soil properties from the SoilGrids (Hengl *et al.* 2017) and topographic variables from the EarthEnv database (Amatulli *et al.* 2018). An initial set of 43 environmental (predictor) variables were evaluated for collinearity with the "vifstep" function of the "usdm" R package, and therefore the number of variables was reduced to 20 (Table 1). The "vifstep" function calculates Variance Inflation Factor (VIF) for all variables, excludes one with the highest VIF (greater than the threshold set at 10), and repeats the procedure until no variables with VIF greater than threshold remain. First, we tested and visualised the niche differences in univariate environmental space between the subspecies using Kruskal Wallis test, Dunn's post-hoc tests and boxplots in R. Then, niche comparison and niche overlap in multivariate environmental space between the subspecies was performed using PCA-env approach (Broennimann *et al.* 2012) as implemented in the "ecospat" R package version 3.0 (Di Cola *et al.* 2017). The obtained environmental space was divided into a 1000 × 1000 grid. We used Schoener's D metric as a summary statistic for niche overlap, which ranges from 0 (no overlap) to 1 (complete overlap).

The extent of niche differences was further evaluated using tests for niche equivalency and niche similarity for all pairs of subspecies with 1000 random permutations to evaluate the significance ($p$ = 0.05; Warren, Glor, & Turelli, 2008; Broennimann *et al.*, 2012). Niche equivalency test determines if the two observed niches are identical, where a statistically significant result indicates that the two niches are not identical. Niche similarity test determines whether the niches are more similar than would be expected at random, given the niches available across the geographic range of the species (Warren *et al.* 2008). We tested for niche conservatism, i.e. whether the niches are more similar than expected at random (alternative = "greater"), as well as for niche divergence, i.e. whether the niches are less similar than expected at random (alternative = "lower"; Broennimann *et al.*, 2012; Di Cola *et al.*, 2017). Niche similarity test includes specification of a background area, such that niches are similar relative to that area (Warren *et al.* 2008). Background area is an area in geographical space that has been accessible to the species through dispersal over relevant time periods (Barve *et al.* 2011). We applied an approach of estimating the available background according to Barve *et al.* (2011) in which we considered historical environmental changes on the landscape of interest. Thus, we depicted the background area by extracting environmental data from 10000 random points across the environment available to all of *D. sylvestris* subspecies given their putative geographic range.

## PHENOLOGY

Data on flowering periods were accessed from herbarium records of the collected populations. Most national floras and identification keys define flowering periods using a range of months, for example June-August. Thus, each collected population was assigned to the months when the plants were flowering. We used “wilcox.test” R function for performing the non-parametric Mann-Whitney U Test, to see if there is statistically significant difference in flowering period between each of the taxa pairs. The response variable is ordinal, i.e., months labelled as numbers from 4 to 8 (April to August).

## RESULTS

## ENVIRONMENTAL NICHE VARIABILITY BETWEEN THE SUBSPECIES

In the environmental PCA (Fig. 2A) PC1 explained 26.09% of the variance and mainly reflected soil organic carbon content, soil bulk density, soil pH and terrain slope in the study area. PC2 explained 16.75% of the variance corresponding to the mean daily air temperatures of the driest (bio09) and wettest (bio08) quarter, mean monthly precipitation of the coldest quarter (bio19) and temperature seasonality (bio04). Environmental niche space for each of the *D. sylvestris* subspecies is visualised in Fig. S1. Environmental niche overlap quantified with the *D* metric (Fig. 2B) varied between the subspecies, ranging from no or very limited overlap (0-0.2) to moderate overlap (0.4-0.6) following the classification provided by Rödder & Engler (2011). *Dianthus sylvestris* subsp. *bertisceus* and subsp. *tergestinus* showed the lowest overlap of 0.01, whereas subsp. *alboroseus* and subsp. *nodosus* showed the highest overlap of 0.62 (Fig. 2B).

The niche equivalency test showed that the niches were not equivalent in the pairwise comparison for seven pairs (Fig. 2B, red asterisk), but also not divergent according to the niche similarity test. Furthermore, four pairs had equivalent and conserved niches (Fig. 2B, black asterisk), and four pairs had equivalent but not conserved niches (Fig. 2B, without an asterisk). Not a single pair showed a statistically significant result when testing for niche divergence in a similarity test.

Kruskal Wallis test (Table 1) showed significant differences in 16 out of 20 environmental variables, as depicted also in the boxplots ($p < 0.01$; Fig. S2). Climate variables had the greatest statistical significance in delimiting between the subspecies, followed by soil variables.

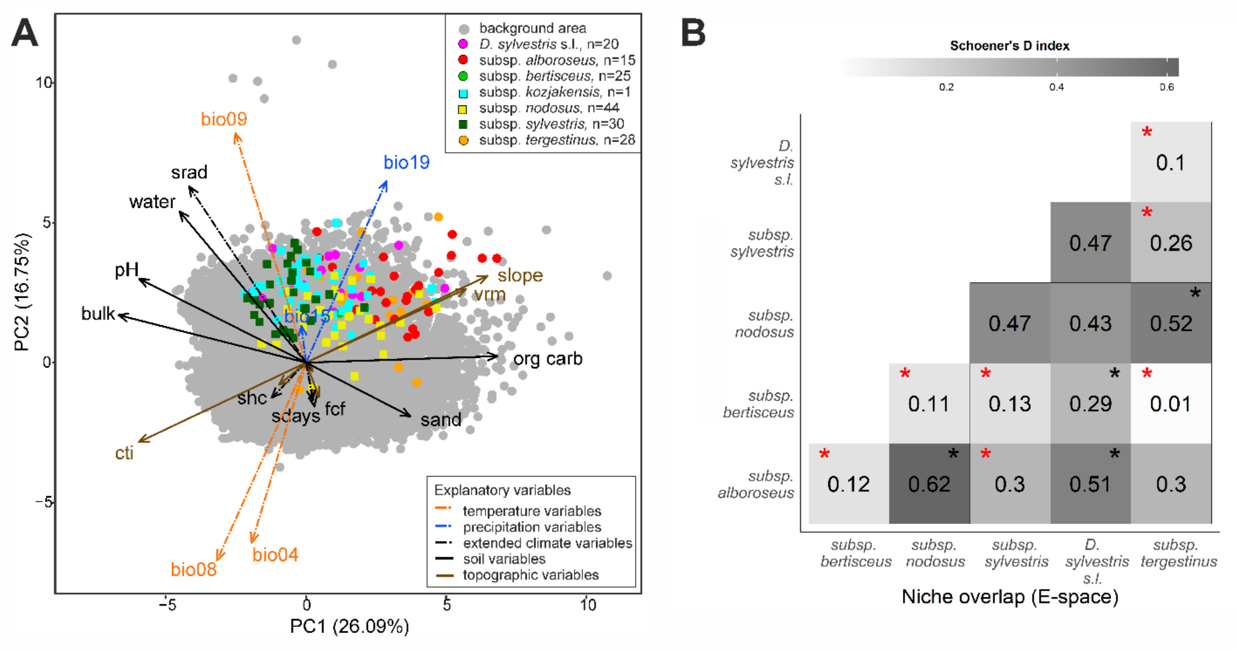


**Figure 2. A**, Environmental niche space along the first two axes of the environmental PCA based on 20 environmental variables. Coloured points: populations, grey points: available background. **B**, Summarised pair-comparison between the observed values of niche overlap (*Schoener's* D index) in the environmental niche space (E-space) and the simulated overlap from the 1000 pseudo-replicated data sets. Significant values for the similarity test (alternative = "greater") are depicted as black *, whereas significant values for the equivalency test are depicted as red *, $p < 0.05$.

## PHENOLOGY

Mann-Whitney U test was performed for each pair of taxa and on two datasets, one including all sampled populations spanning from 8 to 2274 m altitude, and one including a subset of populations from 8 to 1023 m altitude. The results showed significant difference in the flowering period of *D. sylvestris* subsp. *tergestinus* in both datasets, and of *D. sylvestris* subsp. *bertisceus* only in the "all populations" dataset ($p < 0.01$, Table 2, Fig. 3).

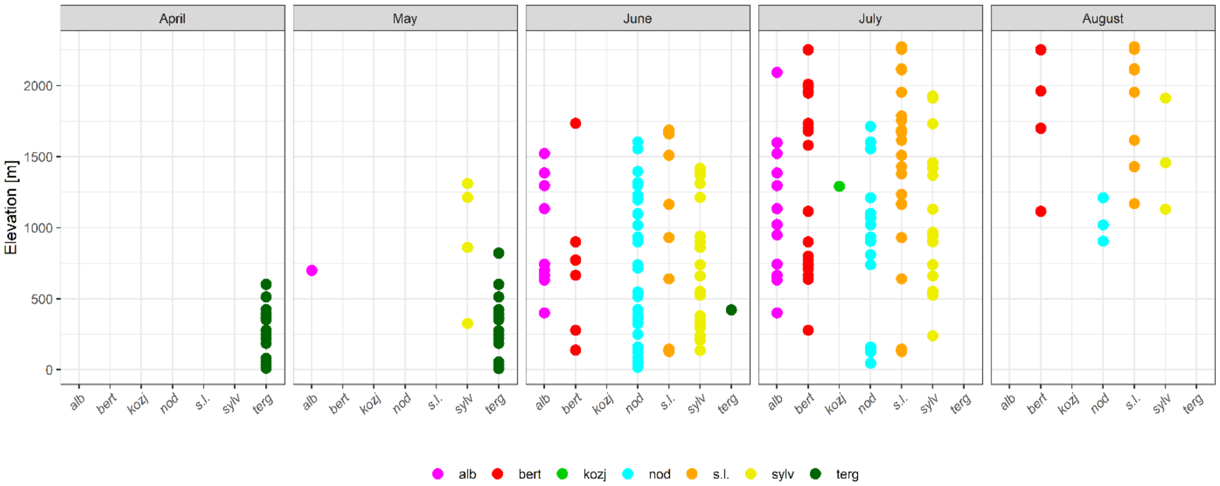


**Figure 3.** Scatterplot of populations according to their altitude and flowering period.

## DISCUSSION

### NOT EQUIVALENT, BUT STILL SIMILAR ENVIRONMENTAL NICHES

While Bacchetta *et al.* (2010) gave notes on ecology for species/populations belonging to *D. sylvestris* in central and southern Italy, Sicily and Sardinia, there is no such data describing ecological preferences of subspecies/populations on the Balkan Peninsula. To fill this knowledge gap, we characterised and compared their environmental niches. A lack of taxonomic confidence for the subspecies may lead to uncertainties regarding ecological meaning (Mota-Vargas and Rojas-Soto 2016), and consequently the taxonomic ambiguity could be reflected in the modelled niche (Smith *et al.* 2019). Therefore, our criteria for assigning populations to subspecies were based on morphological information, geographic distribution and current taxonomy of *D. sylvestris.*

The ecological niche differentiation between subsp. *bertisceus* and subsp. *tergestinus* corresponded well to their morphological differentiation. Non-equivalent environmental niches and almost no overlap observed between the two subspecies (*D* = 0.01, Fig. 2B, Fig. S1) could be explained by their geographical distance and different altitudinal affinities. Significant climatic niche differentiation may help to clarify species boundaries between highly morphologically similar species (Lin *et al.* 2021). Our study showed it to be true for the non-equivalent niches of subsp. *sylvestris* and subsp. *tergestinus,* which share parts of their geographic distributions and partially overlap in morphological space. Aside from occupying the narrowest environmental niche of all subspecies, *D. sylvestris* subsp. *tergestinus* was tested regarding the “temporal niche”, since we observed during fieldwork that this subspecies flowered first in April–May (Fig. 3). Earlier flowering time of this subspecies may be an adaptation to habitats with higher temperatures and lower soil moisture in order to complete reproduction prior to the onset of unfavourable growing conditions, or it may be constrained by the temporal niche of its pollinator (Levin 2006). Therefore, temporal isolation could be an important barrier to gene flow between subsp. *tergestinus* and the two sympatric subsp. *nodosus* and subsp. *sylvestris.* On the other hand, the significant difference revealed for *D. sylvestris* subsp. *bertisceus* (Table 2) can be explained by the wide altitudinal range this subspecies inhabits (Fig. 3), and the fact that at higher altitude the flowering season starts later.

Non-equivalent niches of subsp. *bertisceus* and subsp. *alboroseus* may be an example of how morphological extremes along environmental gradients can mistakenly be described as subspecies (Caković *et al.* 2015; Buitrago Aristizábal *et al.* 2020) and form an artificial taxonomy. In some cases, the equivalency test may be prone to rejecting the null hypothesis of niche equivalency when such is not the case, i.e. it may be prone to over-represent niche differentiation (Peterson 2011). For example, in the ecological niche comparisons of the Mexican white pines by Aguirre-Gutiérrez *et al.* (2015), some pairs of species had non-equivalent niches according to the equivalency test, while more similar niches than expected at random according to the similarity test. The investigated populations with geographic distribution and morphology between subsp. *bertisceus* and subsp. *alboroseus*, were placed in *D. sylvestris* s.l. Although niche overlap between subsp. *bertisceus* and *D. sylvestris* s.l. is low (0.2-0.4), similarity test indicated a certain degree of similarity within the environmental space, which may suggest an ongoing differentiation and a certain degree of retention of ancestral environmental characteristics of these two groups over time (Peterson *et al.* 1999). Moreover, the niche is multidimensional, and in any group some aspects may be conserved while others diversify (Wiens *et al.* 2010).

Although the niche similarity test suggested niche conservatism, subsp. *alboroseus* and subsp. *nodosus* were differentiated by quantitative morphometrics (Fig. 2A) and had moderate niche overlap ($D$ = 0.62, Fig. 2B), which could suggest that their morphological distinctiveness is not determined by environmental factors. Phenotypic variability can result from environmental plasticity or genetic differentiation (Halbritter *et al.* 2018; Hardion *et al.* 2020). For example, Hardion *et al.* (2020) in their revision of *D. superbus* subspecies provided a mixed case with some subspecies supported by phylogenetic markers, and others representing environmental plasticity, while Gammella (2016) suggested that the difference in flowering time found between *D. sylvestris* populations growing on limestone vs. serpentine soils have genetic basis. In general, our findings indicate that the niche similarity is more common, since there was not a single case of niche divergence among the subspecies.

## CONCLUSION

Here, 162 populations of *D. sylvestris* were evaluated regarding the variability of 20 environmental variables. By quantitatively assessing the niche overlap, equivalency and similarity of *D. sylvestris* subspecies, niche similarity was more common than divergence. Environment, rather than geography, plays a potentially more important role in shaping the morphological variability of this taxon. Additionally, the earlier flowering time of *D. sylvestris* subsp. *tergestinus* (April–May) is an indication of its divergence from the sympatric *D. sylvestris* subsp. *sylvestris* and subsp. *nodosus* which flower through June and July.

## ACKNOWLEDGEMENTS

The authors are grateful to Israel Moreno-Contreras for providing the R script for Fig. 2B.

## REFERENCES

**Aguirre-Gutiérrez J, Serna-Chavez HM, Villalobos-Arambula AR, Pérez de la Rosa JA, Raes N**. **2015**. Similar but not equivalent: ecological niche comparison across closely related Mexican white pines. *Diversity and Distributions* **21**: 245–257.

**Aiello-Lammens ME, Boria RA, Radosavljevic A, Vilela B, Anderson RP**. **2015**. spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. *Ecography* **38**: 541–545.

**Alcántara-Ayala O, Oyama K, Ríos-Muñoz CA, Rivas G, Ramirez-Barahona S, Luna-Vega I**. **2020**. Morphological variation of leaf traits in the *Ternstroemia lineata* species complex (Ericales: Penthaphylacaceae) in response to geographic and climatic variation. *PeerJ* **8**: e8307.

**Amatulli G, Domisch S, Tuanmu M-N, *et al.* 2018**. A suite of global, cross-scale topographic variables for environmental and biodiversity modeling. *Scientific Data* **5**: 180040.

**Barve N, Barve V, Jiménez-Valverde A, *et al.* 2011**. The crucial role of the accessible area in ecological niche modeling and species distribution modeling. *Ecological Modelling* **222**: 1810–1819.

**Broennimann O, Fitzpatrick MC, Pearman PB, *et al.* 2012**. Measuring ecological niche overlap from occurrence and spatial environmental data: Measuring niche overlap. *Global Ecology and Biogeography* **21**: 481–497.

**Buitrago Aristizábal MA, Oliveira Gouvêa De Figueiredo F, André T**. **2020**. Accommodating trait overlap and individual variability in species diagnosis of *Ischnosiphon* (Marantaceae). *Botanical Journal of the Linnean Society* **194**: 469–479.

**Caković D, Stešević D, Schönswetter P, Frajman B**. **2015**. How many taxa? Spatiotemporal evolution and taxonomy of *Amphoricarpos* (Asteraceae, Carduoideae) on the Balkan Peninsula. *Organisms Diversity & Evolution* **15**: 429–445.

**Dagnino D, Minuto L, Casazza G**. **2017**. Divergence is not enough: the use of ecological niche models for the validation of taxon boundaries. *Plant Biology* **19**: 1003–1011.

**Di Cola V, Broennimann O, Petitpierre B, *et al.* 2017**. ecospat: an R package to support spatial analyses and modeling of species niches and distributions. *Ecography* **40**: 774–787.

**Halbritter AH, Fior S, Keller I, *et al.* 2018**. Trait differentiation and adaptation of plants along elevation gradients. *Journal of Evolutionary Biology* **31**: 784–800.

**Hardion L, Perrier A, Martinez M, *et al.* 2020**. Integrative revision of *Dianthus superbus* subspecies reveals different degrees of differentiation, from plasticity to species distinction. *Systematics and Biodiversity* **18**: 255–268.

**Hengl T, Jesus JM de, Heuvelink GBM, *et al.* 2017**. SoilGrids250m: Global gridded soil information based on machine learning. *PLOS ONE* **12**: e0169748.

**Jaime R, Alcántara JM, Bastida JM, Rey PJ**. **2015**. Complex patterns of environmental niche evolution in Iberian columbines (genus *Aquilegia*, Ranunculaceae). *Journal of Plant Ecology* **8**: 457–467.

**Janišová M, Skokanová K, Hlásny T**. **2018**. Ecological differentiation, speciation, and rarity: How do they match in *Tephroseris longifolia* agg. (Asteraceae)? *Ecology and Evolution* **8**: 2453–2470.

**Karger DN, Conrad O, Böhner J, *et al.* 2017**. Climatologies at high resolution for the earth’s land surface areas. *Scientific Data* **4**: 170122.

**Karger DN, Schmatz DR, Dettling G, Zimmermann NE**. **2020**. High-resolution monthly precipitation and temperature time series from 2006 to 2100. *Scientific Data* **7**: 248.

**Levin DA**. **2006**. Flowering Phenology in Relation to Adaptive Radiation. *Systematic Botany* **31**: 239–246.

**Lin H, Gu K, Li W, Zhao Y**. **2021**. Integrating coalescent-based species delimitation with ecological niche modeling delimited two species within the *Stewartia sinensis* complex (Theaceae). *Journal of Systematics and Evolution*: jse.12732.

**Moreno-Contreras I, Sánchez-González LA, Arizmendi M del C, Prieto-Torres DA, Navarro-Sigüenza AG**. **2020**. Climatic Niche Evolution in the *Arremon brunneinucha* Complex (Aves: Passerellidae) in a Mesoamerican Landscape. *Evolutionary Biology* **47**: 123–132.

**Mota-Vargas C, Rojas-Soto OR**. **2016**. Taxonomy and ecological niche modeling: Implications for the conservation of wood partridges (genus *Dendrortyx*). *Journal for Nature Conservation* **29**: 1–13.

**Otero A, Vargas P, Valcárcel V, Fernández-Mazuecos M, Jiménez-Mejías P, Hipp AL**. **2019**. A snapshot of progenitor-derivative speciation in action in *Iberodes* (Boraginaceae). *bioRxiv*.

**Peterson AT**. **2011**. Ecological niche conservatism: a time-structured review of evidence: Ecological niche conservatism. *Journal of Biogeography* **38**: 817–827.

**Peterson AT, Soberón J J, Sánchez-Cordero V**. **1999**. Conservatism of ecological niches in evolutionary time. *Science (New York, N.Y.)* **285**: 1265–1267.

**Pyron RA, Costa GC, Patten MA, Burbrink FT**. **2015**. Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. *Biological Reviews* **90**: 1248–1262.

**Rödder D, Engler JO**. **2011**. Quantitative metrics of overlaps in Grinnellian niches: advances and possible drawbacks: Quantitative metrics of niche overlap. *Global Ecology and Biogeography* **20**: 915–927.

**Smith AB, Godsoe W, Rodríguez-Sánchez F, Wang H-H, Warren D**. **2019**. Niche Estimation Above and Below the Species Level. *Trends in Ecology & Evolution* **34**: 260–273.

**Warren DL, Glor RE, Turelli M**. **2008**. Environmental Niche Equivalency versus Conservatism: Quantitative Approaches to Niche Evolution. *Evolution* **62**: 2868–2883.

**Wiens JJ, Ackerly DD, Allen AP, *et al.* 2010**. Niche conservatism as an emerging principle in ecology and conservation biology: Niche conservatism, ecology, and conservation. *Ecology Letters* **13**: 1310–1324.

## TABLES AND SUPPLEMENTARY FIGURES

**Table 1.** Environmental variables used for environmental niche comparisons and RDA analysis, with their abbreviated and full names/definitions, units and types. Significant environmental niche differences along 20 variables between the *Dianthus sylvestris* subspecies as revealed by Kruskal Wallis test ($p < 0.01$) are shown in bold.

| **Abbreviation** | **Full name** | **Unit** | **Variable type** | **Kruskal Wallis rank sum statistic** | ***P*-value** |
|---|---|---|---|---:|---:|
| bio04 | temperature seasonality | °C/10 | climate | 43.270955 | **< 0.01** |
| bio08 | mean daily mean air temperatures of the wettest quarter | °C/10 | climate | 60.168164 | **< 0.01** |
| bio09 | mean daily mean air temperatures of the driest quarter | °C/10 | climate | 45.394996 | **< 0.01** |
| bio15 | precipitation seasonality | kg $m^{-2}$ | climate | 70.492235 | **< 0.01** |
| bio19 | mean monthly precipitation amount of the coldest quarter | kg $m^{-2}$ | climate | 42.245848 | **< 0.01** |
| fcf | frost change frequency | No days | extended climate | 55.416456 | **< 0.01** |
| sdays | number of snow days | No days | extended climate | 53.952482 | **< 0.01** |
| shc | Selyaninov's Hydrothermic Coefficient | (kg $m^{-2}$/10)/°C | extended climate | 37.439972 | **< 0.01** |
| srad | surface solar radiation downwards | kJ $m^{-2}$ | extended climate | 44.888935 | **< 0.01** |
| dtm_slope | slope | ° | topographic | 43.184477 | **< 0.01** |
| dtm_east | eastness | index | topographic | 12.865335 | 0.024674 |
| dtm_north | northness | index | topographic | 11.065501 | 0.050097 |
| soil_org_carb | soil organic carbon content | 5 x g $kg^{-1}$ | soil properties | 34.50189 | **< 0.01** |
| soil_bulk | soil bulk density | 10 x kg $m^{-3}$ | soil properties | 50.816375 | **< 0.01** |
| soil_sand | sand content | % | soil properties | 58.20695 | **< 0.01** |
| soil_ph_h2o | soil pH x 10 in H2O | pH | soil properties | 39.250477 | **< 0.01** |
| soil_water | soil water content at 33kPa | | soil properties | 29.883328 | **< 0.01** |
| dtm_con | convergence | index | topographic | 4.8926719 | 0.429119 |
| dtm_cti | compound topographic index | index | topographic | 8.3510321 | 0.137921 |
| dtm_vrm | vector ruggedness measure | index | topographic | 32.938803 | **< 0.01** |

**Table 2.** Results from the Mann-Whitney U Test. Differences in flowering periods between subspecies pairs of *Dianthus sylvestris* were tested on two datasets: 1) dataset including all sampled populations spanning from 8 to 2274 m altitude, 2) subset of populations from 8 to 1023 m altitude to account for the altitudinally driven later start of flowering. Statistically significant differences at $p < 0.01$ are indicated in bold.

| | Balkan pops **8-2274 m alt** | | Balkan pops **8-1023 m alt** | | | Balkan pops **8-2274 m alt** | Balkan pops **8-1023 m alt** |
|---|---|---|---|---|---|---|---|
| Subspecies pairs | *Wilcoxon test statistic* | *p*-value | *Wilcoxon test statistic* | *p*-value | Subspecies | No of populations | No of populations |
| *alb - s.l.* | 583 | 0.01941 | 50 | 0.8961 | *alboroseus* | 13 | 7 |
| *alb - bert* | 219 | 0.017 | 69.5 | 0.4064 | *bertisceus* | 21 | 10 |
| *alb - nod* | 685.5 | 0.3806 | 257 | 0.6784 | *nodosus* | 42 | 30 |
| *alb - sylv* | 512 | 0.5582 | 177.5 | 0.4589 | *sylvestris* | 26 | 16 |
| *alb - terg* | 777.5 | **2.224e-10** | 419.5 | **3.004e-07** | *tergestinus* | 20 | 20 |
| *bert - s.l.* | 606 | 0.9406 | 48 | 0.5482 | *sensu lato* | 23 | 4 |
| *bert - nod* | 1203 | **0.00025** | 353 | 0.09894 | | | |
| *bert - sylv* | 890 | **0.002684** | 241 | 0.06104 | | | |
| *bert - terg* | 1077 | **8.652e-13** | 501.5 | **1.607e-08** | | | |
| *nod - s.l.* | 1579 | **0.0001731** | 180 | 0.5247 | | | |
| *nod - sylv* | 1190.5 | 0.9177 | 557 | 0.5746 | | | |
| *nod - terg* | 1998.5 | **2.2e-16** | 1427 | **2.273e-14** | | | |
| *sylv - s.l.* | 1176 | **0.001868** | 124 | 0.367 | | | |
| *sylv - terg* | 1498 | **2.134e-13** | 908.5 | **6.874e-11** | | | |
| *terg - s.l.* | 1435 | **2.219e-14** | 286 | **4.014e-06** | | | |

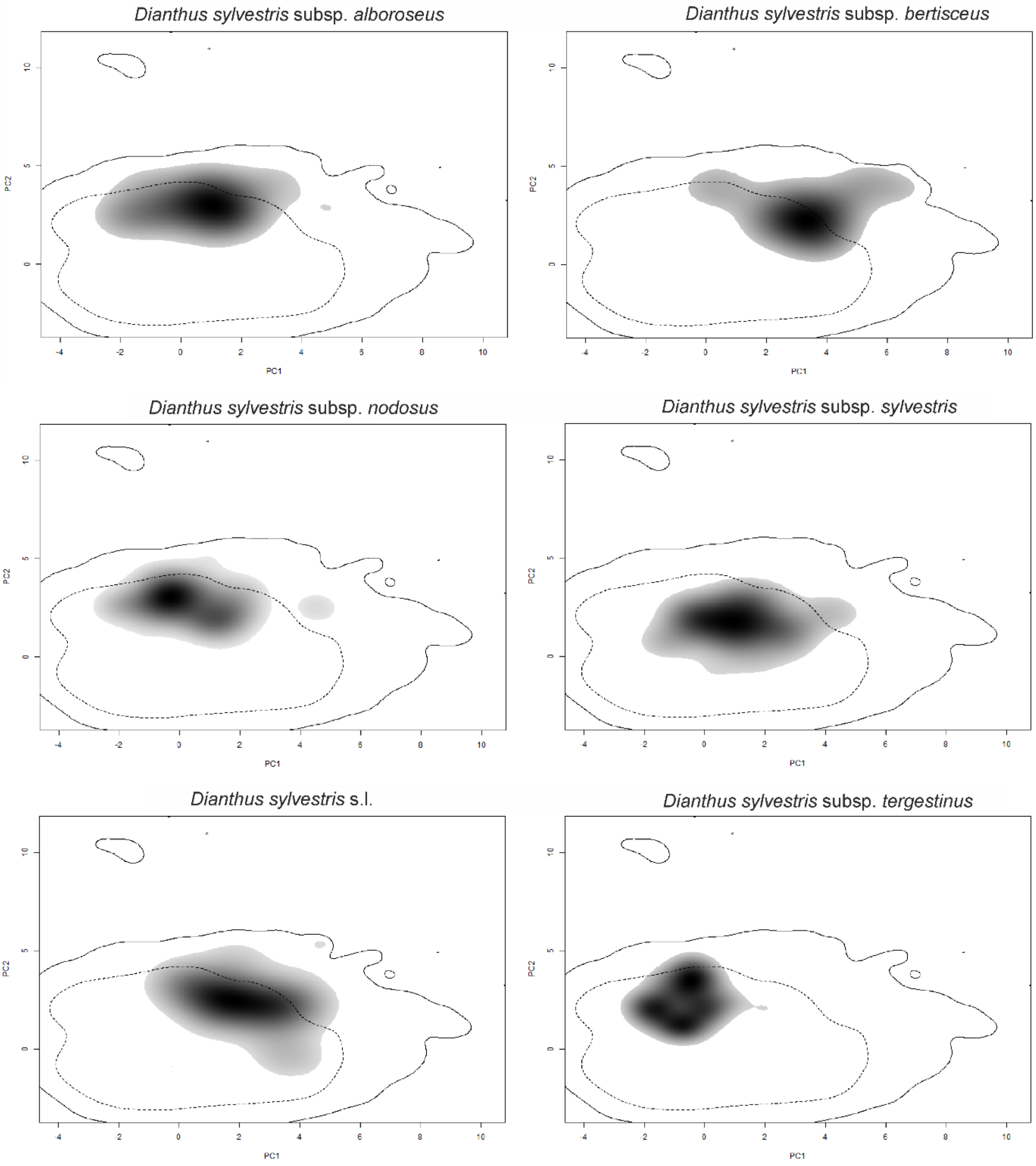


**Figure S1.** Occurrence density of *Dianthus sylvestris* subspecies along environmental space depicted by the first two axes of an environmental principal component analysis (see also Figure 2). Solid grey line indicates 100% of the available environmental niche space, and dashed line indicates 50% of the available environmental space. The available environmental niche space was obtained by extracting environmental data from 10000 random points across the environment available to all of *D. sylvestris* subspecies given their putative geographic range.

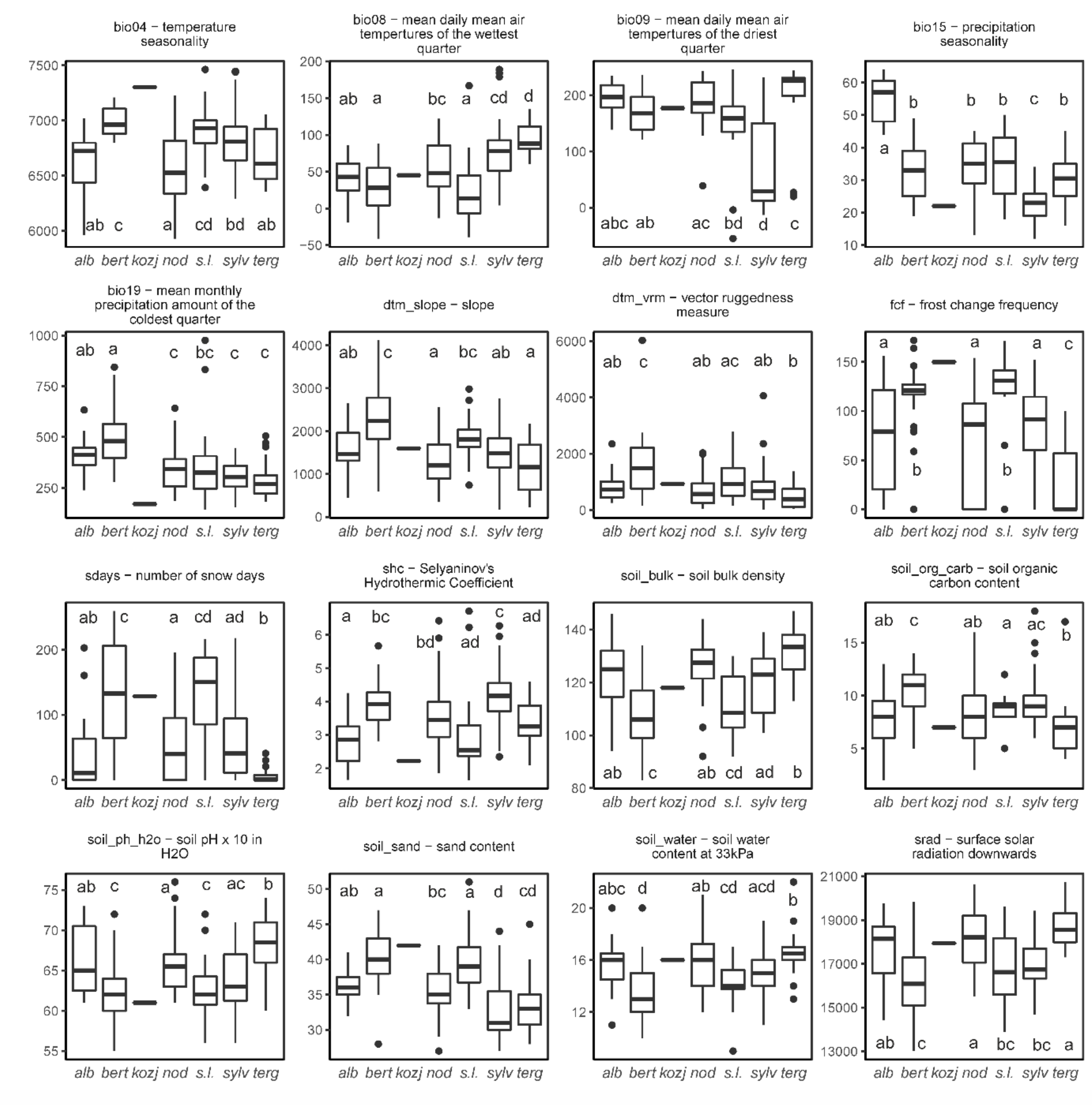


**Figure S2.** Boxplots showing environmental niche differences along the 16 environmental variables, revealed by Kruskal Wallis test as significantly different between *Dianthus sylvestris* subspecies (Table 1). Abbreviated labels: *alb* - subsp. *alboroseus*, *bert* - subsp. *bertisceus*, *kozj* - subsp. *kozjakensis*, *nod* - subsp. *nodosus*, *s.l.* - *D. sylvestris sensu lato*, *sylv* - subsp. *sylvestris*, *terg* - subsp. *tergestinus*. Means not significantly different at $p < 0.01$, according to Dunn's post-hoc test, are indicated by the same letter.

# Appendix II

# Taxonomic treatment of *Dianthus sylvestris* Wulfen (Caryophyllaceae) on the Balkan Peninsula

Ana Terlević[1], Sandro Bogdanović[2,3], Ivana Rešetnik[1]

[1] Faculty of Science, Department of Biology, University of Zagreb, Trg Marka Marulića 20/II, 10000 Zagreb, Croatia.

[2] Faculty of Agriculture, Department of Agricultural Botany, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia.

[3] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska cesta 25, 10000 Zagreb, Croatia

Our proposed taxonomic treatment is based on results obtained from an extensive population sampling across the Balkan Peninsula: morphometric analysis (461 individuals from 97 populations, **Publication IV**), phylogenomic analysis and analyses of genetic structure and divergence (587 individuals from 108 populations, including 6240 SNPs, **Publication V**), and environmental niche analysis and phenology (162 populations, **Appendix I**). STRUCTURE results together with the Neighbour joining tree (992 individuals from 165 populations the – "All-lineage-dataset", **Publication V**) showed clear divergence of *D. sylvestris* subsp. *tergestinus,* whereas the rest of the Balkan populations were split into two groups. Considering its genetic divergence, the shift in flowering time (**Appendix I**) and its morphological characteristics, this taxon is circumscribed here at the species level, *Dianthus tergestinus.* The RAD-seq SNP data including only the "Balkan-cline-dataset" (587 individuals from 108 populations), revealed two genetic groups, the south-eastern and the north-western group, which were also distinguished according to the results of morphometric analysis. These two distinct entities are circumscribed here at the subspecies level. An identification key for *D. sylvestris* taxa on the Balkan Peninsula is also provided.

***Dianthus sylvestris*** Wulfen **subsp. *sylvestris*** in Collectanea 1: 237. 1786.
*Type*: "in montibus illis prope Ponewitsch Baronis Wolkensberg in Carniolia, tum in M. Utocsek prope Pillichgraz; in iis. Vallis Rablensis; denique & in iis Vallis Canalensis &c."; lectotype designated by Domina *et al.* (2021)
*Synonyms:* see Fassou *et al.* (2022)
*Distribution*: Bosnia and Herzegovina, Croatia, Italy and Slovenia.

*Habitat and ecology*: Sub-Mediterranean to alpine carbonate or dolomite rocky places, grasslands, rocky crevices in canyons, forest fringes, from 20 up to 2000 m alt., mesophilous to alpine.

***Dianthus sylvestris* subsp. *bertisceus*** Rech. f. in Repert. Spec. Nov. Regni Veg. 38: 150. 1935.
*Type*: Fuß des Peklen (319); Schlucht bei Pec, Felsritzen (98); Schlucht der Susica (444); Lumbardska Planina, ca. 1800 m (578); Djaravica, Kalk (1232); Greben, ca. 1100 m (1540).

By examining herbarium specimens, six syntypes were found in G (G00418895!, G00418896!, G00418897!, G00418898!, G00418899!, G00418900!) and three syntypes in K belonging to the same collection (K000725353!, K000725354!, K000725355!). A lectotype was chosen and the rest were designated as isolectotypes.
*Lectotype* designated here: KOSOVO: BERTISCUS (Alpes boreales albanicae): In fauce fluvii Pećska Bistrica prope oppidum Peć (Ipek). In fissuris rupium. alt. 600–750 m s.m., substr. calc., 3.VII.1933., Rechinger fil. et Scheffer No. 98 (G00418898!, Fig. 1).

*Isolectotypes* designated here: KOSOVO: BERTISCUS (Alpes boreales albanicae): In fauce fluvii Sušica prope oppidum Peć (Ipek). In saxosis calc. alt. 750–950 m s.m., VII.1933., Rechinger fil. et Scheffer No. 444 (G00418895!); In monte Djaravica (Derviš Kom) prope Dečani. alt. 2000–2400 m s.m., substr. calc., 18.VII.1933., Rechinger fil. et Scheffer No. 1232 (G00418896!); In monte Lumbardska Planina prope oppidum Peć (Ipek), ca. 800 m s.m., substr. calc., 8.–9.VII.1933., Rechinger fil. et Scheffer No. 578 (G00418899!); In collibus circa oppidum Peć (Ipek) versus montem Peklen, alt. 500–700 m s.m., 5.VII.1933., Rechinger fil. et Scheffer No. 319 (G00418900!). MONTENEGRO: In monte Greben prope Gusinje, alt. ca. 1100 m s.m., substr. calc., 25.VII.1933., Rechinger fil. et Scheffer No. 1540 (G00418897!).

*Synonyms:*
= *Dianthus sylvestris* subsp. *alboroseus* F.K.Meyer, in Haussknechtia, Beih. 15: 53. 2011.
= *Dianthus sylvestris* subsp. *kozjakensis* Micevski in Prilozi Oddel. Biol. Med. Nauki Makedonska Akad. Nauk. Umet. 8: 43. 1990.
≡ *Dianthus bertisceus* (Rech.f.) E.Mayer and Trpin in Biol. Vestn. 13: 57. 1965
≡ *Dianthus bertisceus* (Rech.f.) Trinajstić in Suppl. Fl. Anal. Jug. 6: 7. 1979
*Distribution:* Albania, Bosnia and Herzegovina, Greece, Kosovo, Montenegro, North Macedonia and Serbia.

*Habitat and ecology*: Sub-Mediterranean to alpine carbonate and silicate rocky crevices, fissures, alpine grasslands, from 200 up to 2300 m alt., mesophilous to alpine.

Figure 1. Lectotype of the name *Dianthus sylvestris* subsp. *bertisceus* Rech. f. (G00418898!)

***Dianthus tergestinus*** (Rchb.) A.Kern., Sched. Fl. Exs. Austro-Hung.: no. 545. 1882
*Basionym: Dianthus virgineus* var. *tergestinus* (Rchb., Icon. Fl. Germ. Helv. 6: 47, t. 5049b. 1844
*Type*: none [but Trieste, Italy, can be easily inferred from the epithet "*tergestinus*" that means "from Trieste"]; lectotype designated by Domina et al. (2021).
*Synonyms:* see Domina et al. (2021) and Fassou et al. (2022)
*Habitat and ecology*: Eu-Mediterranean to sub-Mediterranean dry rocky grasslands and garrigues, carbonate rocks from sea level up to 600(–800) m alt., thermophilous.
*Distribution*: Triestine karst (Italy), Slovenian karts, along the north-eastern Adriatic coast (Albania, Bosnia and Herzegovina, Croatia and Montenegro).

## Identification key to *Dianthus sylvestris* taxa from the Balkan Peninsula

1. Spring flowering plants (April to May); petal limb entire or slightly eroded; epicalyx scales 2(4), appressed, with mucro (0.3–)0.5–0.8(–0.9) mm long …..………………... ***D. tergestinus***

1. Summer flowering plants (late May, June to August); petal limb denticulate, rarely entire; epicalyx scales 4(6), appressed to divaricated, with mucro (0.4–)0.5–1.6(–3) mm long........... **2**

2. Calyx teeth (2.8–)3.6–5.2(–8.3) mm long, incised ¼ to 1/3 calyx; inner scales ovate to subrounded, (3.8–)4.6–5.6(–7) mm wide, with mucro (0.7–)1.1–1.6(–3) mm long, divaricated ……………………………………………………………… ***D. sylvestris*** **subsp. *bertisceus***

2. Calyx teeth (2.5–)3–3.9(–4.5) mm long, incised less than ¼; inner scales transversally elliptical, (3.8–)6.4–7.4(–8.7) mm wide, with mucro (0.4–)0.5–0.8(–1.1) mm long, appressed ……………………………………………………………...***D. sylvestris*** **subsp. *sylvestris***

## Acknowledgements

Many thanks to the curators of the Herbarium G (Conservatoire et Jardin botaniques de la Ville de Genève) for sending scans of the herbarium specimens from their relevant collection.

## References

**Domina G, Astuti G, Bacchetta G, *et al.* 2021**. Typification of 14 names in the *Dianthus virgineus* group (Caryophyllaceae). *PhytoKeys* **187**: 1–14.

**Fassou G, Korotkova N, Nersesyan A, Koch MA, 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.

**Kerner A**. **1882**. *Schedae ad Floram exsiccatam Austro-hungaricam.* Vindobonae: Prostat apud Guilielmum Frick. C. R. Librarium aulicum (E Typographia Caesarea Regia Aulica et Imperiali).

**Mayer E, Trpin D**. **1965**. *Dianthus sylvestris* – kompleks v Jugoslaviji. *Biološki Vestnik* **13**: 53–59.

**Meyer FK**. **2011**. *Beiträge zur Flora von Albanien*. Thüringische Botanische Gesellschaft e.V.

**Micevski K**. **1990**. Novitäten in der Gattung *Dianthus* L. (Caryophyllaceae) in der Flora der SR Makedonien. *Macedonian Academy of Sciences and Arts, Section of Biological and Medical Sciences. Macedonian Academy of Sciences and Arts.* **8**: 31–46.

**Rechinger fil. KH**. **1935**. *Ergebnisse einer botanischen Reise in den Bertiscus (Nordalbanische Alpen).* Berlin-Dahlem: Selbstverlag des Herausgebers, Fabeckstrasse 49.

**Reichenbach HGL**. **1844**. *Icones florae Germanicae et Helveticae, Vol. 6.* Lipsiae : apud Friedericum Hofmeister (Dresdae, Typis Caroli Rammingii).

**Trinajstić I**. **1979**. Taxa nova et combinationes novae in flora Jugoslaviae VI. In: *Supplementum ad Floram analyticam Jugoslaviae*. Zagreb: Editio Universitatis Zagrebiensis - Liber, 7.

**Wulfen FX**. **1786**. Plantae Rariores Carinthiacae In: Jacquin NJ, ed. *Collectanea*. Vindobonae: Ex Officina Wappleriana., 237–239.

# DISCUSSION

The results of studies featured in this thesis present an integrative approach to elucidate the patterns and processes that were paramount in shaping the current diversity of *Dianthus sylvestris* populations on the Balkan Peninsula. All the aims and questions within this thesis were addressed and answered through four published scientific publications (**I – IV**), one scientific paper in preparation (**Publication V**), and two unpublished results (**Appendix I – II**) enclosed.

### *Phylogeographic patterns of Dianthus sylvestris on the Balkans*

Deciphering the evolutionary relationships among certain groups has been persistently difficult due to poor resolution within phylogenies as a result of rapid diversification. Such case was reported for genus *Dianthus* which was inferred to be the prime example of fast non-adaptive allopatric diversification (Valente *et al.* 2010). *Dianthus sylvestris* is one of its lineages that rapidly evolved in the central Mediterranean. Recent study by Luqman *et al.* (2022) revealed the existence of three geographically separated lineages within *D. sylvestris*, the Alpine, Apennine and Balkan lineages, that diverged ca. 200.000-115.000 years ago. Each of the three evolutionary lineages occupied distinct Pleistocene glacial refugia and each experienced independent evolutionary histories (Luqman *et al.* 2022).

With the additional comprehensive sampling through the Balkan Peninsula, in **Publication V** we confirm the genetic divergence of *D. sylvestris* populations collected across the Balkan and Apennine Peninsulas and the Alps into three lineages. In **Publication III** further evidence for independent evolutionary divergence of Alpine and Balkan lineages was observed in abruptly different relative genome sizes (RGS). The populations genetically belonging to the Alpine lineage exhibited larger RGS, whereas populations from the eastern part of the Southern Alps, genetically affiliated to the Balkan lineage, had smaller RGS that corresponds to the RGS of other Balkan populations.

The differentiation within the Balkan lineage is characterised through two main events (**Publication V**). First, the genetic structure clearly demonstrates the divergence of *D. sylvestris* subsp. *tergestinus* from the rest of the Balkan populations. Second, the remaining Balkan populations (hereafter termed Balkan cline) exhibit further hierarchical phylogeographic structure, however with more complex underlying patterns of genetic variation.

The divergence of *D. sylvestris* subsp. *tergestinus* populations was strongly supported in the Maximum Likelihood (ML) analysis and inferred from STRUCTURE analysis at K=6 (**Publication V**). Interestingly, although the *D. sylvestris* subsp. *tergestinus* populations are occurring in sympatry with *D. sylvestris* subsp. *sylvestris* and *D. sylvestris* subsp. *nodosus* along the eastern Adriatic coast, only slight admixture was observed. The possible reason for the lack of gene flow may be sought in observed noteworthy shift in flowering time of *D. sylvestris* subsp. *tergestinus,* reported in **Appendix I**. *Dianthus sylvestris* subsp. *tergestinus* flowers first in April and May, whereas *D. sylvestris* subsp. *sylvestris* and subsp. *nodosus* flower through June and July. The distinction of *D. sylvestris* subsp. *tergestinus* is further seen in morphological features such as the petal limb shape, number of epicalyx scales and the mucro length (**Appendix II**).

The differentiation of the Balkan cline was likely influenced through complex interplay between spatial and environmental drivers that have influenced the migration through Pleistocene oscillations. One of the potential evolutionary processes that could have driven the diversification of *D. sylvestris* on the Balkans is allopatric differentiation promoted primarily by heterogeneous Balkan topography (**Publication V**). A sharp genetic discontinuity in the area of Montenegro and a split into two main groups of populations, the north-western (hereafter NW Group) and the south-eastern group (hereafter SE Group), suggest two main evolutionary lineages within the Balkan cline. Moreover, the detailed exploration of morphological characters (**Publication IV**) reveals a gradual change that follows the spatial cline, clustering the populations into two groups, the north-western and the south-eastern. The congruent genetic and morphological split is positioned in the area between rivers Neretva and Sutjeska in Bosnia and Herzegovina. This area is a known geographical barrier that has been documented in various Balkan taxa (Španiel and Rešetnik 2022), e.g., in *Campanula pyramidalis - C. austroadriatica* (Lakušić *et al.* 2013), *Cerastium dinaricum* (Kutnjak *et al.* 2014) and *Gentianella crispata* (Reich *et al.* 2021), whereas in the *Heliosperma pusillum* (Frajman and Oxelman 2007) the genetic break matches the Sutjeska and Drina valleys.

Contemporary patterns of genetic variation in plants often reflect evolutionary processes linked to past climate events. Molecular clock-based estimations highlight the importance of Pleistocene climatic fluctuations across taxonomic groups (Španiel and Rešetnik 2022). For the Balkan cline, suitable habitats during the LGM were identified along the whole eastern-Adriatic coast, with higher suitability predicted in the south of the Balkans compared to the north (**Publication V**). However, the rate of migration between the two main groups was shown to be very limited, i.e., no migration from the NW Group to the SE Group was observed (**Publication V**). Moreover, there were no connectivity patterns identified among two main groups (**Publication V**). Together with the pronounced genetic separation into two groups, the presented data indicate the presence of two main glacial refugia, comparable to examples seen in *Viola suavis* s.l. (Mereďa *et al.* 2011), *Edraianthus graminifolius* (Surina *et al.* 2014) and

*Gentianella crispata* (Reich *et al.* 2021). Within each of these refugia, populations could have expanded and contracted over multiple cycles of climatic oscillations, resulting in distinct patterns.

The refugial area of the NW Group is postulated to have been in the NW part of the east Adriatic coast with possible extension in the nowadays northern Adriatic Sea (**Publication V**). Northern Dinaric glacial refugia were suggested for a number of trees and understory herbs and for some it presented a source for their postglacial colonisation of more northerly regions (see Gömöry *et al.* 2020; Španiel and Rešetnik 2022). The finding of *D. sylvestris* tetraploid populations ($2n = 4x = 60$) in the small area of Istria (**Publication III**) gives further evidence for the existence of northern Dinaric refugium where the species persisted during glacial stages. Up to this discovery, it was assumed that only diploids are present on the Balkan Peninsula. The only tetraploids within all three *D. sylvestris* lineages were known from the geographically distant Maritime Alps in France (Carolin 1957; Moore 1982), thus our results are adding evidence of a multiple independent origin of polyploids (Bardy *et al.* 2010). As the tetraploids evolved only within the NW Group, we may speculate that the more pronounced climatic oscillations in the northern Balkan Peninsula, compared to the more stable conditions in the south (Tzedakis 2004), were probably a driving force for polyploidization. Possibly, the postglacial recolonization of this area by previously isolated populations resulted in their secondary contact and hybridization which led to the formation of polyploids. Such scenario of postglacial expansion with secondary contacts that resulted in the formation of polyploids was inferred for example in *Alyssum austrodalmaticum* (Zozomová-Lihová *et al.* 2020). Since the tetraploids in Istria do not differ morphologically from their geographically close diploids (**Publication III**), and show high genetic similarity with them (**Publication V**), an autopolyploid origin has been suggested (Levin 2002), such as in *Euphorbia spinosa* (Stevanoski *et al.* 2020) and *Veronica chamaedrys* (Bardy *et al.* 2010).

The southernmost part of the Adriatic coast and adjacent eastern area was indicated as a highly suitable LGM refugial region of the SE Group (**Publication V**). This area was also found as suitable for e.g., *Edraianthus graminifolius* (Surina *et al.* 2014), *E. tenuifolius* (Glasnović *et al.* 2018) and *Salvia officinalis* (Rešetnik, Baričevič, *et al.* 2016). The populations of the SE Group exhibited more noticeable diversification and lower gene flow supported also through lower rates of directional migration. Additionally, the southernmost populations have reduced genetic diversity. Interestingly, the southernmost populations exhibited highest RGS among the Balkan taxa, and the more pronounced RGS variation in this geographic region compared to the NW Group was discovered (**Publication III**). Similar more pronounced genetic differentiation of southern compared to northern populations has been observed in several plant groups (e.g., *Silene saxifraga,* Đurović *et al.* 2017; *Euphorbia myrsinites,* Falch *et al.* 2019) and is in this case further detected in higher variability of morphological characters (**Publication IV**). The most interesting are the populations growing in the area of Montenegro. At the STRUCTURE K=2 these populations show highly admixed pattern comprising large fractions of two main gene pools (**Publication V**). Such specific structure could be a consequence of the admixture of divergent lineages through secondary contact after postglacial colonisation, as seen in *Veronica chamaedrys* (Bardy *et al.* 2010)*, Campanula fenestrellata* (Rešetnik *et al.* 2020) and *Gentianella crispata* (Reich *et al.* 2021). However, we consider this scenario of between-lineage admixture to be a false inference (Luqman *et al.* 2022). Instead, more likely these populations persisted in situ and experienced small-scale differentiation that is visible in the isolation by distance pattern which reveals the existence of small groups consisted of 2 to 7 populations across the borderline area of the Dinaric and Scardo-Pindhic mountain ranges (**Publication V**). Moreover, in **Publication III** pronounced genome size variation at small geographic distances on the same area is revealed, likely indicating at allopatric diversification.

### *Environmental variability of Dianthus sylvestris subspecies on the Balkan Peninsula*

The great variation in RGS (**Publication III**), morphology (**Publication IV**) and genetic diversity (**Publication V**) among the Balkan populations of *D. sylvestris* is demonstrated to have a limited correlation with its traditional taxonomy. The observed pattern of geographical, rather than taxonomical segregation of phylogeographical structure has been previously inferred for other species groups with high taxonomic diversity on the Balkan Peninsula, such as *Heliosperma pusillum* (Frajman and Oxelman 2007) and *Silene saxifraga* (Đurović *et al.* 2017). These groups have a similar diversification pattern, where regional morphological diversification likely resulted from adaptation to different ecological pressures.

In the wide-range species, such as *D. sylvestris* that occupy broad elevational gradients, an array of environmental variables can enforce spatial changes connected to ecological adaptations and environmental niche shifts. For example, the tightly-linked interplay of migration and adaptation was established between high- and low-elevational populations of *D. sylvestris* in the Alpine lineage (Luqman *et al.* 2022). Within the continuous distributions, the division in populations that locally adapt to different environmental niches may be reflected in their morphological variability. In **Publication IV** we investigated the relationships between morphological variability of the Balkan populations and environmental conditions, i.e., whether morphological characters are constrained by a set of environmental variables. As the distribution of *D. sylvestris* encompasses a wide elevational range across the Balkan Peninsula, it is shown that the selection operating on vegetative characters is environment-mediated, thus these characters are not taxonomically indicative. When looking at reproductive morphological characters, the shape of epicalyx scales and calyx teeth incision show an association with geography, i.e., a gradual change in northwest – southeast direction. However, the overall morphological variability of this taxon is primarily shaped by environment, rather than geography. Regardless of the elevation, the common feature of *D. sylvestris* habitats is xerothermity. Water deficit is the type of stress to which *D. sylvestris* is adapted, while some other environmental stressors cause differences in plant size, habit, phenology, etc., among populations (**Publication IV**).

Furthermore, the comparison of the environmental niches and test for niche conservatism/divergence between *D. sylvestris* subspecies was done (**Appendix I**). The narrowest environmental niche was found in *D. sylvestris* subsp. *tergestinus*, in addition to the observed non-equivalent niche with the sympatric *D. sylvestris* subsp. *sylvestris*. The existence of climatic niche differentiation may represent an additional line of evidence needed to clarify species boundaries between highly morphologically similar species (Lin *et al.* 2021). A very limited niche overlap between *D. sylvestris* subsp. *tergestinus* and *D. sylvestris* subsp. *bertisceus* likely represents adaptation to two environmental extremes of the available niche of *D. sylvestris* (constrained niches, Pyron *et al.* 2015). When looking at the geographical distributions of these two subspecies, the difference in their niches reflect the transition from the coastal Mediterranean environment to the alpine environment. It may also represent an example of a process through time where a species tracks favourable ecological niche, slowly diverging from the ancestral niche (Ackerly 2003), as evidenced for high- and low-elevational populations of *D. sylvestris* in the Alpine lineage (Luqman *et al.* 2022).

Apart from niche divergence, populations may exhibit niche conservatism as well, which is possible due to the tendency for populations to retain ancestral ecological characteristics over time (phylogenetic niche conservatism, Peterson *et al.* 1999; Pyron *et al.* 2015). We observed niche conservatism between *D. sylvestris* subsp. *nodosus* and *D. sylvestris* subsp. *alboroseus*, two geographically distant and morphologically distinct subspecies belonging to separate genetic groups. At this point we may hypothesize that the type of environment where *D. sylvestris* subsp. *nodosus* and *D. sylvestris* subsp. *alboroseus* grow is particularly suitable for *D. sylvestris* in general, and thus selection for that niche may have persisted for a relatively long period of evolutionary time, leading to niche conservatism (Pyron *et al.* 2015). Overall, niche analyses suggested niche similarity to be more common, since there was not a single significant case of niche divergence.

Although we have not investigated the pollinators, we can speculate that the observed cline in floral characters (**Publication IV**), whose direction follows that of the genetic diversity cline, is pollinator-mediated. Pollinators are key dispersal agents, and as such, they may drive the distribution of neutral genetic variation of populations within plant species (Burgin and Hopkins 2022). In **Publication V** it was found that some sampled populations are more genetically similar than predicted by geographic distance (i.e., IBD), namely the populations from the Central Dinaric Mountains up to the Carnic Alps in south Austria. One of the possible explanations for this phenomenon is that pollinator movement between spatially distant populations maintains population connectivity (Skogen *et al.* 2019). Indeed, long-tongued Lepidopteran pollinators which are able to reach the nectar at the bottom of the calyx tube (Bloch 2009), can cover greater flight distances than other insects, and therefore are able to sustain a higher effective population size (Schmitt 1980). This assumption that pollinators affect the distribution of genetic variation (beyond IBD) may be quantitatively tested using pollinator movement patterns as an explanatory variable for the plant genetic structure (Burgin and Hopkins 2022). However, the interaction between *D. sylvestris* and its pollinator, and whether its population structure is pollinator driven, remains unexplored.

### *Taxonomic implications*

Intuitive recognition of intraspecific entities may be sometimes successful in yielding a classification. However, fragmentating diversity into smaller and smaller units throughout the history of systematic botany, botanists have often created complex taxonomic situations at specific and especially intraspecific levels. A group of closely related taxa displaying continuity in morphological variability and a wide ecological preference, is hard to classify. *Dianthus sylvestris* represents an example where botanists over centuries forced this continuity into discrete units (intraspecific taxa), creating an impractical taxonomical clutter. The species is still inconsistently treated across its distribution area and the formation of a stable taxonomy for *D. sylvestris* presents a challenging task. For example, the observed tendency of botanists investigating Tuscan populations in Italy has been to assign the plants from higher altitudes to *D. sylvestris*, and the more thermophilous ones to *D. longicaulis* Ten. (Astuti *et al.* 2020). Afterwards, Domina *et al.* (2021) applied the name *D. virgineus* L. to the Apennine populations so far named *D. longicaulis*, being the oldest available name referring to plants that fall within the morphological variability of the *D. sylvestris* species complex (Bacchetta *et al.* 2010).

Moreover, the three main genetic lineages, as revealed both in Luqman *et al.* (2022) and in **Publication V**, can be distinguished morphologically (Peruzzi and Franzoni, unpublished) and suggest the possibility of their treatment as separate species. The name *Dianthus sylvestris* s.str. would then apply to populations from the Balkan genetic lineage, *D. virgineus* to those from the central Mediterranean genetic lineage, and finally, the name *D. inodorus* (L.) Gaertn. would apply to populations from the Alpine genetic lineage.

Our herbarium and literature investigation of the names applied to plants on the Balkan Peninsula revealed the inconsistent nomenclatural treatment of populations from the same locality by numerous collectors and botanists (**Publication I**). This inconsistency also led to the recognition of more than one putative subspecies co-occurring in the same locality, e.g., subsp. *sylvestris* and subsp. *nodosus,* where the subspecies rank lacks a clear geographical and/or ecological structure. Therefore, the classical subspecies concept stating that subspecies are geographically isolated populations belonging to the same species (Wilson and Brown 1953) is difficult to apply in such case. **Publication II** contributes to strengthening the applications of names lacking type material, so that the assignment of natural populations to subspecies could be facilitated by comparing with the designated types.

**Publications III-V and Appendix I** contribute to the morphological, ecological and genetic characterisation of Balkan populations, in order to offer a sensible taxonomy. Morphological delimitation and the revealed pattern of genetic divergence correlated mostly with the taxonomic classification of *D. sylvestris* subsp. *tergestinus*. **Publication IV** highlights the morphological distinctiveness of only two groups of Balkan populations, where the split between them corresponds to the split between allopatric NW populations and SE populations. The most evident morphological difference between the two groups is the variation in shape of epicalyx scales, from transversally elliptical in NW populations to ovate in SE populations. The likely persistence of the species in at least two glacial refugia within the Balkans (**Publication V**) may have triggered this morphological divergence of epicalyx scales shape.

Here, the gathered evidence from morphological, environmental and molecular patterns for each of the traditionally recognised subspecies of *D. sylvestris* on the Balkan Peninsula is summarised, and a revised taxonomic treatment is proposed.

The typical subspecies, *D. sylvestris* subsp. *sylvestris* is reported for the entire Balkan area: Slovenia, Croatia, Montenegro, Albania, North Macedonia and Greece (Rohlena 1942; Trinajstić 1979; Micevski 1990; Tutin and Walters 1993; Martinčič 2007; Dimopoulos *et al.* 2013; Stešević and Caković 2013; Raab-Straube and Raus 2013; Vangjeli 2015; Barina *et al.* 2018; Nikolić 2020). However, the description of morphological variability from the Wulfen’s protologue (1786) and the illustration in Jacquin (1781–1787, Tav. 82) do not morphologically correspond to any of the plants from south-eastern Balkan countries. Instead, evidence suggest that the typical *D. sylvestris* populations are those belonging to the NW morphological group (**Publication IV**) and genetic NW Group (**Publication V**).

*Dianthus sylvestris* subsp. *nodosus* is the only name for which the original material is unknown, since the original herbarium collection of Tausch was not found within the extensive herbaria survey in the course of the Thesis. It was described as *D. nodosus* by Tausch (1828) from the Croatian Dalmatia, and it has been recorded in the flora of Croatia, Bosnia and Herzegovina, Montenegro and Albania (Rohlena 1942; Trinajstić 1979; Stešević and Caković 2013; Vangjeli 2015; Barina *et al.* 2018; Nikolić 2020). Hayek (1924) placed *D. sylvestris* subsp. *nodosus* to a subspecific level and mentions that the stem in a typical variety of subsp. *nodosus* is puberulous, and he cites Visiani's *D. caryophyllus* L. var. *pubescens* Vis. (Visiani 1852) from the island of Hvar under its synonyms. Since Beck-Mannagetta (1909) and Hayek (1924) reported this trait, it appears in floras and identification keys as diagnostic for this taxon (e.g., stems usually puberulent below, in Tutin and Walters 1993), although there is no mention of indumentum description in the protologue. Moreover, in **Publication IV**, we showed the intrapopulation variability of indumentum coverage and consequently its questionable taxonomic importance. Adding the results of population genetic analyses which also do not support the recognition of this taxon (**Publication V**), we suggest here that there is no basis for distinction of *D. sylvestris* subsp. *nodosus* from *D. sylvestris* subsp. *sylvestris* and, therefore, it does not merit formal taxonomic recognition. Thus, we suggest treating *D. sylvestris* subsp. *nodosus* as a synonym of *D. sylvestris* subsp. *sylvestris*.

*Dianthus sylvestris* subsp. *tergestinus* was described as *D. virgineus* var. *tergestinus* by Reichenbach (1844), and this basionym was lectotypified in **Publication II**. It has been recorded for the flora of Slovenia, Croatia, Bosnia and Herzegovina, Montenegro and Albania (Trinajstić 1979; Tutin and Walters 1993; Martinčič 2007; Stešević and Caković 2013; Vangjeli 2015; Barina *et al.* 2018; Nikolić 2020). According to the illustration by Reichenbach (Tab. CCLXVI, 5049. β, 1844), the taxon has one pair of epicalyx scales, and this trait is reported in floras and keys as diagnostic. Along with pale pink colour of petals and entire petal limbs, these characters distinguish it from other subspecies of *D. sylvestris* as confirmed in **Publication IV**. Moreover, we found flowering period to be an indicative character discriminating this subspecies (**Appendix I**) and its distinction was validated with genomic data (**Publication V**). **Publications IV and V** together show that this subspecies occurs only along the eastern Adriatic coast with its southernmost locality on Srđ hill near Dubrovnik in

Croatia. However, according to the floras of Montenegro and Albania, the distribution of *D. sylvestris* subsp. *tergestinus* also includes these countries. Possibly we have not recorded it there because our fieldwork across these countries was conducted in late June and July when the plants are in late drying fruiting stage and thus inconspicuous. Considering together all the gathered evidence so far, we regard *D. sylvestris* subsp. *tergestinus* at the specific level as *D. tergestinus* (Rchb.) Kern. (**Appendix II**).

Specimens from the Albanian Alps (Prokletije Mt., Mons Bertiscus) were named by Rechinger fil. (1935) as *D. sylvestris* subsp. *bertisceus*, and N. Fumeaux (G) designated them in 2018 as syntypes. This subspecies has been recorded for the flora of Montenegro, North Macedonia, Serbia and Albania (Rohlena 1942; Trinajstić 1979; Micevski 1990; Tutin and Walters 1993; Stešević and Caković 2013; Vangjeli 2015; Barina *et al.* 2018). Rechinger fil. (1935) noted the morphological similarity between the one-flowered small plants from Prokletije Mts. and alpine *D. sylvestris* from the European Alps. In **Publication III** we reported significantly different genome size estimates between these two groups, and in **Publication V** we report that these two groups of populations belong to two different genetic groups. Moreover, populations of *D. sylvestris* subsp. *bertisceus* display the most complex genetic structure within the Balkan cline. The farther from the type localities, the less typical its morphological diagnostic characters are, and plants gradually display morphologies more typical to geographically adjacent *D. sylvestris* subsp. *kozjakensis* and/or *D. sylvestris* subsp. *alboroseus* (**Publication IV**). Thus, defining the geographical and morphological extents for this subspecies is ambiguous.

*Dianthus sylvestris* subsp. *kozjakensis* was described from Mt. Kozjak in North Macedonia (Micevski 1990) and it is the only known locality so far. *Dianthus sylvestris* subsp. *alboroseus* is a pale pink to white flowering form from south Albania, described by Meyer (2011). Since it is a relatively new subspecies, it has been reported only for the flora of Albania by Vangjeli (2015). According to Meyer (2011), petal denticulation is generally much more pronounced than in other subspecies, and petal limbs are shorter as well. Its often-divaricated epicalyx scales are similar to those of *D. sylvestris* subsp. *bertisceus.* The holotypes of *D. sylvestris* subsp. *alboroseus* and *D. sylvestris* subsp. *kozjakensis* are very much alike and the results of morphometric and genetic analyses group these two subspecies together (**Publication IV and V**). All the three aforementioned subspecies approximate in some morphological characteristics one another, mainly in the epicalyx scales shape, and thus we suggest treating them as a single subspecies, namely *D. sylvestris* subsp. *bertisceus* (**Appendix II**). Since *D. sylvestris* subsp. *bertisceus* was described prior to both *D. sylvestris* subsp. *alboroseus* and subsp. *kozjakensis*, and these three names are available for the populations from northern Greece, Albania, Montenegro and North Macedonia, the older validly published name is accepted and used (Turland *et al.* 2018).

# CONCLUSIONS

The ***First hypothesis*** could not be rejected since we demonstrate the association between genetic variability and the existence of microrefugia on the Balkan Peninsula in **Publications III-V**. The ***Second hypothesis*** could not be rejected when considering *D. sylvestris* subsp. *tergestinus*, as well as the two main Balkan genetic groups, the NW and the SE, for they can be identified according to their morphological diagnostic characters.

The most important conclusions that have arisen from this doctoral thesis are:

1. Remarkable genetic and morphological variation in studied populations of *Dianthus sylvestris* on the Balkan Peninsula showed that only the thermophilous and earlier flowering *D. sylvestris* subsp. *tergestinus* forms a genetic group separated from the rest of the Balkan cline.
2. Both morphological and genetic diversity of populations of the Balkan cline follow a geographic structure in the northwest – southeast direction. This gradient showed one major discontinuity around the Neretva River valley, thus revealing two main groups of Balkan populations, the NW and the SE group, morphologically distinct mainly in the shape of epicalyx scales, calyx teeth incision and petal denticulation.
3. Patterns of genetic variation together with species distribution models suggested that *D. sylvestris* on the Balkan Peninsula persisted during the Pleistocene glaciations in two separate glacial refugia located along the eastern-Adriatic coast. The NW group was more homogeneous with higher gene flow and connectivity, whereas the SE group was more divergent with less gene flow and lower migration rates. Moreover, very limited gene flow was suggested between the populations from the NW and SE groups, indicating barriers to gene flow.
4. Tetraploid populations were found within the NW group and they are distributed only regionally in Istria. Within tetraploids, genome downsizing was observed and they significantly differ from their diploid counterparts in an array of morphological and environmental characteristics. A deep RGS divergence between Alpine and Balkan lineages was observed with Alpine lineage exhibiting larger RGS. Moreover, the increase of RGS towards the southernmost distribution margins of the Balkan lineages was observed.
5. Based on our integrative approach considering multiple lines of evidence, populations traditionally referred to *D. sylvestris* subsp. *nodosus* and one population of *D. sylvestris* subsp. *kozjakensis* are not recognised as distinct intraspecific entities. Furthermore, *D.*

*sylvestris* subsp. *bertisceus* and *D. sylvestris* subsp. *alboroseus* approximate in some flower traits one another, and thus their taxonomic treatment as one morphologically variable subspecies is suggested. Finally, a new taxonomic treatment of *D. sylvestris* on the Balkan Peninsula is proposed: *D. sylvestris* is a genetically, morphologically and ecologically variable species with two subspecies, *D. sylvestris* subsp. *sylvestris* including the NW Balkan populations, and *D. sylvestris* subsp. *bertisceus* including the SE Balkan populations, whereas *D. tergestinus* is regarded as a separate species.

## LITERATURE

**Abbott RJ**. **2017**. Plant speciation across environmental gradients and the occurrence and nature of hybrid zones. *Journal of Systematics and Evolution* **55**: 238–258.

**Abdusalam A, Li Q**. **2018**. Morphological plasticity and adaptation level of distylous *Primula nivalis* in a heterogeneous alpine environment. *Plant Diversity* **40**: 284–291.

**Ackerly DD**. **2003**. Community assembly, niche conservatism, and adaptive evolution in changing environments. *International Journal of Plant Sciences* **164**: S165–S184.

**Aguirre-Gutiérrez J, Serna-Chavez HM, Villalobos-Arambula AR, Pérez de la Rosa JA, Raes N**. **2015**. Similar but not equivalent: ecological niche comparison across closely related Mexican white pines. *Diversity and Distributions* **21**: 245–257.

**Alcántara-Ayala O, Oyama K, Ríos-Muñoz CA, Rivas G, Ramirez-Barahona S, Luna-Vega I**. **2020**. Morphological variation of leaf traits in the *Ternstroemia lineata* species complex (Ericales: Penthaphylacaceae) in response to geographic and climatic variation. *PeerJ* **8**: e8307.

**Amatulli G, Domisch S, Tuanmu M-N, *et al.* 2018**. A suite of global, cross-scale topographic variables for environmental and biodiversity modeling. *Scientific Data* **5**: 180040.

**Astuti G, Franzoni J, Carta A, Peruzzi L**. **2020**. Indagini morfometriche su popolazioni toscane del gruppo di *Dianthus sylvestris* (Caryophyllaceae). *Notiziario della Società Botanica Italiana* **4**: 1–2.

**Avise JC**. **2007**. Twenty-five key evolutionary insights from the phylogeographic revolution in population genetics In: Weiss S, Ferrand N, eds. *Phylogeography of southern European refugia*. Springer, Dordrecht, 7–21.

**Avise JC, Arnold J, Ball RM, *et al.* 1987**. Intraspecific phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. *Annual Review of Ecology and Systematics* **18**: 489–522.

**Bacchetta G, Brullo S, Casti M, Pietro Giusso del Galdo G**. **2010**. Taxonomic revision of the *Dianthus sylvestris* group (Caryophyllaceae) in central-southern Italy, Sicily and Sardinia. *Nordic Journal of Botany* **28**: 137–173.

**Balao F, Casimiro-Soriguer R, Talavera M, Herrera J, Talavera S**. **2009**. Distribution and diversity of cytotypes in *Dianthus broteri* as evidenced by genome size variations. *Annals of Botany* **104**: 965–973.

**Bardy KE, Albach DC, Schneeweiss GM, Fischer MA, Schönswetter P**. **2010**. Disentangling phylogeography, polyploid evolution and taxonomy of a woodland herb (*Veronica chamaedrys* group, Plantaginaceae s.l.) in southeastern Europe. *Molecular Phylogenetics and Evolution* **57**: 771–786.

**Barina Z, Somogyi G, Pifkó D, Rakaj M**. **2018**. Checklist of vascular plants of Albania. *Phytotaxa* **378**: 1–339.

**Barve N, Barve V, Jiménez-Valverde A, *et al.* 2011**. The crucial role of the accessible area in ecological niche modeling and species distribution modeling. *Ecological Modelling* **222**: 1810–1819.

**Bateman RM**. **2021**. Phenotypic versus genotypic disparity in the Eurasian orchid genus *Gymnadenia*: exploring the limits of phylogeny reconstruction. *Systematics and Biodiversity* **19**: 400–422.

**Beck-Mannagetta G**. **1909**. *Flora Bosne, Hercegovine i Novopazarskog Sandžaka.* Glasnik Zemaljskog muzeja u Bosni i Hercegovini, Sarajevo.

**Beentje H**. **2016**. *The Kew Plant Glossary: An Illustrated Dictionary of Plant Terms*. Kew Publishing.

**Behroozian M, Vaezi J, Joharchi MR**. **2012**. A karyological study of some *Dianthus* L. species (Caryophyllaceae) in Northeast of Iran. *Feddes Repertorium* **123**: 265–272.

**Bertel C, Rešetnik I, Frajman B, Erschbamer B, Hülber K, Schönswetter P**. **2018**. Natural selection drives parallel divergence in the mountain plant *Heliosperma pusillum* s.l. *Oikos* **127**: 1355–1367.

**Binks RM, Steane DA, Byrne M**. **2021**. Genomic divergence in sympatry indicates strong reproductive barriers and cryptic species within *Eucalyptus salubris*. *Ecology and Evolution* **11**: 5096–5110.

**Bloch D**. **2009**. Butterflies and moths - agents of pollinator-mediated selection and species separation in the two closely related carnations *Dianthus carthusianorum* and *D. sylvestris*.

**Bogdanović S, Brullo C, Brullo S, Ljubičić I, Giusso del Galdo G**. **2016**. *Bituminaria plumosa* (Fabaceae), a critical species of the Croatian flora. *Plant Ecology and Evolution* **149**: 347–355.

**Bombonato JR, do Amaral DT, Silva GAR, *et al.*** **2020**. The potential of genome-wide RAD sequences for resolving rapid radiations: a case study in Cactaceae. *Molecular Phylogenetics and Evolution* **151**: 106896.

**Borbás V**. **1876**. *Symbolae ad "Caryophylleas" et "Melanthaceas" florae Croaticae*. Tisak Dioničke Tiskare.

**Bourgeois YXC, Warren BH**. **2021**. An overview of current population genomics methods for the analysis of whole-genome resequencing data in eukaryotes. *Molecular Ecology* **30**: 6036–6071.

**Bravo GA, Antonelli A, Bacon CD, *et al.*** **2019**. Embracing heterogeneity: coalescing the Tree of Life and the future of phylogenomics. *PeerJ* **7**: e6399.

**Broennimann O, Fitzpatrick MC, Pearman PB, *et al.*** **2012**. Measuring ecological niche overlap from occurrence and spatial environmental data: Measuring niche overlap. *Global Ecology and Biogeography* **21**: 481–497.

**Brullo S, Brullo C, Colombo P, Galdo GGD, Ilardi V, Perrone R**. **2015**. *Dianthus borbonicus* (Caryophyllaceae), a new species from Sicily. *Phytotaxa* **233**: 49.

**Burgin G, Hopkins R**. **2022**. A missing link: Connecting plant and pollinator population structure. *American Journal of Botany*.

**Buzurović U, Tomović G, Niketić M, Bogdanović S, Aleksić JM**. **2020**. Phylogeographic and taxonomic considerations on *Goniolimon tataricum* (Plumbaginaceae) and its relatives from south-eastern Europe and the Apennine Peninsula. *Plant Systematics and Evolution* **306**: 29.

**Caković D, Stešević D, Schönswetter P, Frajman B**. **2015**. How many taxa? Spatiotemporal evolution and taxonomy of *Amphoricarpos* (Asteraceae, Carduoideae) on the Balkan Peninsula. *Organisms Diversity & Evolution* **15**: 429–445.

**Caković D, Stešević D, Schönswetter P, Frajman B**. **2018**. Long neglected diversity in the Accursed Mountains of northern Albania: *Cerastium hekuravense* is genetically and morphologically divergent from *C. dinaricum*. *Plant Systematics and Evolution* **304**: 57–69.

**Carnicero P**. **2017**. *Evolution, biogeography and systematics of the genus* Cymbalaria *Hill*.

**Carolin RC**. **1957**. Cytological and Hybridization Studies in the Genus *Dianthus*. *The New Phytologist* **56**: 81–97.

**Clausen J**. **1951**. *Stages in the evolution of plant species.* Ithaca, NY: Cornell University Press.

**Collart F, Hedenäs L, Broennimann O, Guisan A, Vanderpoorten A**. **2020**. Intraspecific differentiation: Implications for niche and distribution modelling. *Journal of Biogeography*: jbi.14009.

**Cozzolino S, Scopece G, Lussu M, Cortis P, Schiestl FP**. **2021**. Do floral and ecogeographic isolation allow the co-occurrence of two ecotypes of *Anacamptis papilionacea* (Orchidaceae)? *Ecology and Evolution* **11**: 9917–9931.

**Cruzan MB, Hendrickson EC**. **2020**. Landscape Genetics of Plants: Challenges and Opportunities. *Plant Communications* **1**: 100100.

**DaCosta JM, Sorenson MD**. **2014**. Amplification Biases and Consistent Recovery of Loci in a Double-Digest RAD-seq Protocol. *PLOS ONE* **9**: e106713.

**Dagnino D, Minuto L, Casazza G**. **2017**. Divergence is not enough: the use of ecological niche models for the validation of taxon boundaries. *Plant Biology* **19**: 1003–1011.

**Di Cola V, Broennimann O, Petitpierre B, *et al.* 2017**. ecospat: an R package to support spatial analyses and modeling of species niches and distributions. *Ecography* **40**: 774–787.

**Dimopoulos P, Raus T, Bergmeier E, *et al.* (Eds.)**. **2013**. *Vascular plants of Greece: an annotated checklist*. Botanic Garden and Botanical Museum Berlin-Dahlem, Berlin and Hellenic botanical society, Athens.

**Domina G, Astuti G, Barone G, *et al.* 2021**. Lectotypification of the Linnaean name *Dianthus virgineus* (Caryophyllaceae) and its taxonomic consequences. *TAXON* **70**: 1096–1100.

**Dufresne F, Stift M, Vergilino R, Mable BK**. **2014**. Recent progress and challenges in population genetics of polyploid organisms: an overview of current state-of-the-art molecular and statistical tools. *Molecular Ecology* **23**: 40–69.

**Đurović S, Schönswetter P, Niketić M, Tomović G, Frajman B**. **2017**. Disentangling relationships among the members of the *Silene saxifraga* alliance (Caryophyllaceae): Phylogenetic structure is geographically rather than taxonomically segregated. *TAXON* **66**: 343–364.

**Đurović SZ, Temunović M, Niketić M, Tomović G, Schönswetter P, Frajman B**. **2021**. Impact of Quaternary climatic oscillations on phylogeographic patterns of three habitat-segregated *Cerastium* taxa endemic to the Dinaric Alps. *Journal of Biogeography* **48**: 2022–2036.

**Đurović SZ, Tomović G, Stevanović V, Matevski V, Niketić M**. **2014**. *Silene triflora* (Bornm.) Bornm. (Caryophyllaceae), a neglected species from the Central Balkans. *Phytotaxa* **172**: 1–12.

**Dyer RJ, Nason JD, Garrick RC**. **2010**. Landscape modelling of gene flow: improved power using conditional genetic distance derived from the topology of population networks. *Molecular Ecology* **19**: 3746–3759.

**Elith J, Leathwick JR, Hastie T**. **2008**. A working guide to boosted regression trees. *Journal of Animal Ecology* **77**: 802–813.

**Endler JA**. **1977**. *Geographic Variation, Speciation and Clines.* Princeton: Princeton University Press.

**Eriksson T, Persson NL, Smedmark JEE**. **2022**. What is *Potentilla*? A phylogeny-based taxonomy for *Potentillinae* (Rosaceae). *TAXON* **71**: 493–505.

**Evans JS, Murphy MA, Holden ZA, Cushman SA**. **2011**. Modeling Species Distribution and Change Using Random Forest In: Drew CA, Wiersma YF, Huettmann F, eds. *Predictive Species and Habitat Modeling in Landscape Ecology*. New York, NY: Springer New York, 139–159.

**Falch M, Schönswetter P, Frajman B**. **2019**. Both vicariance and dispersal have shaped the genetic structure of Eastern Mediterranean *Euphorbia myrsinites* (Euphorbiaceae). *Perspectives in Plant Ecology, Evolution and Systematics* **39**: 125459.

**Fassou G, Korotkova N, Nersesyan A, Koch MA, 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.

**Frajman B, Oxelman B**. **2007**. Reticulate phylogenetics and phytogeographical structure of *Heliosperma* (Sileneae, Caryophyllaceae) inferred from chloroplast and nuclear DNA sequences. *Molecular Phylogenetics and Evolution* **43**: 140–155.

**Frajman B, Rešetnik I, Weiss-Schneeweiss H, Ehrendorfer F, Schönswetter P**. **2015**. Cytotype diversity and genome size variation in *Knautia* (Caprifoliaceae, Dipsacoideae). *BMC Evolutionary Biology* **15**: 140.

**Frajman B, Záveská E, Gamisch A, Moser T, Schönswetter P**. **2019**. Integrating phylogenomics, phylogenetics, morphometrics, relative genome size and ecological niche modelling disentangles the diversification of Eurasian *Euphorbia seguieriana* s. l. (Euphorbiaceae). *Molecular Phylogenetics and Evolution* **134**: 238–252.

**Fujita MK, Leaché AD, Burbrink FT, McGuire JA, Moritz C**. **2012**. Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology & Evolution* **27**: 480–488.

**Gammella M**. **2016**. Local adaptation and gene flow in serpentine and limestone populations of *D. sylvestris*.

**Gjurašin S**. **1933**. *Rod* Dianthus *u flori Hrvatske i Slavonije*. Prirodoslovna istraživanja Kraljevine Jugoslavije. Jugoslavenska akademija znanosti i umjetnosti.

**Glasnović P, Temunović M, Lakušić D, Rakić T, Grubar VB, Surina B**. **2018**. Understanding biogeographical patterns in the western Balkan Peninsula using environmental niche modelling and geostatistics in polymorphic *Edraianthus tenuifolius*. *AoB PLANTS*.

**Gómez A, Lunt D**. **2007**. Refugia within Refugia: Patterns of Phylogeographic Concordance in the Iberian Peninsula In: Weiss S, Ferrand N, eds. *Phylogeography of Southern European Refugia*. Springer, Dordrecht, The Netherlands., 155–188.

**Gömöry D, Zhelev P, Brus R**. **2020**. The Balkans: a genetic hotspot but not a universal colonization source for trees. *Plant Systematics and Evolution* **306**: 5.

**Grdiša M, Liber Z, Radosavljević I, Carović-Stanko K, Kolak I, Satovic Z**. **2014**. Genetic Diversity and Structure of Dalmatian Pyrethrum (*Tanacetum cinerariifolium* Trevir. /Sch./ Bip., Asteraceae) within the Balkan Refugium. *PLOS ONE* **9**: e105265.

**Greilhuber J, Doležel J, Lysák MA, Bennett MD**. **2005**. The Origin, Evolution and Proposed Stabilization of the Terms ‘Genome Size’ and ‘C-Value’ to Describe Nuclear DNA Contents. *Annals of Botany* **95**: 255–260.

**Guisan A, Edwards TC, Hastie T**. **2002**. Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecological Modelling* **157**: 89–100.

**Guisan A, Thuiller W**. **2005**. Predicting species distribution: offering more than simple habitat models. *Ecology Letters* **8**: 993–1009.

**Guisan A, Zimmermann NE**. **2000**. Predictive habitat distribution models in ecology. *Ecological Modelling* **135**: 147–186.

**Guzmán B, Lledó MD, Vargas P**. **2009**. Adaptive Radiation in Mediterranean *Cistus* (Cistaceae). *PLOS ONE* **4**: e6362.

**Halbritter AH, Fior S, Keller I, *et al.*** **2018**. Trait differentiation and adaptation of plants along elevation gradients. *Journal of Evolutionary Biology* **31**: 784–800.

**Hardion L, Perrier A, Martinez M, *et al.*** **2020**. Integrative revision of *Dianthus superbus* subspecies reveals different degrees of differentiation, from plasticity to species distinction. *Systematics and Biodiversity* **18**: 255–268.

**Hardy H, Knapp S, Allan EL, *et al.*** **2020**. SYNTHESYS+ Virtual Access - Report on the Ideas Call (October to November 2019). *Research Ideas and Outcomes* **6**: e50354.

**Harvey MG, Smith BT, Glenn TC, Faircloth BC, Brumfield RT**. **2016**. Sequence Capture versus Restriction Site Associated DNA Sequencing for Shallow Systematics. *Systematic Biology* **65**: 910–924.

**Hayek A von**. **1924**. *Prodromus florae peninsulae Balcanicae*. Dahlem bei Berlin: Verlag des Repertoriums.

**Hengl T, Jesus JM de, Heuvelink GBM, *et al.* 2017**. SoilGrids250m: Global gridded soil information based on machine learning. *PLOS ONE* **12**: e0169748.

**Hennig W**. **1966**. *Phylogenetic Systematics* (DD Davis and R Zangerl, Trans.). University of Illinois Press, Urbana.

**Herrera S, Shank TM**. **2016**. RAD sequencing enables unprecedented phylogenetic resolution and objective species delimitation in recalcitrant divergent taxa. *Molecular Phylogenetics and Evolution* **100**: 70–79.

**Hewitt G**. **2000**. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.

**Hewitt GM**. **2011**. Mediterranean Peninsulas: The Evolution of Hotspots In: Zachos FE, Habel JC, eds. *Biodiversity Hotspots: Distribution and Protection of Conservation Priority Areas*. Berlin, Heidelberg: Springer, 123–147.

**Hodálová I, Mártonfiová L, Skokanová K, Majerová M, Somlyay L, Mereďa P**. **2020**. The utility of genome size in plant identification: a case study on *Sesleria* (Poaceae) from Croatia and Slovenia. *Plant Systematics and Evolution* **306**: 87.

**Huxley JS**. **1938**. Clines: an auxiliary taxonomic principle. *Nature* **142**: 219–220.

**Jacquin NJ**. **1787**. *Icones plantarum rariorum (1781-1786).* Vindobonae: C.F. Wappler.

**Jaime R, Alcántara JM, Bastida JM, Rey PJ**. **2015**. Complex patterns of environmental niche evolution in Iberian columbines (genus *Aquilegia*, Ranunculaceae). *Journal of Plant Ecology* **8**: 457–467.

**Janišová M, Skokanová K, Hlásny T**. **2018**. Ecological differentiation, speciation, and rarity: How do they match in *Tephroseris longifolia* agg. (Asteraceae)? *Ecology and Evolution* **8**: 2453–2470.

**Jaźwa M, Jędrzejczak E, Klichowska E, Pliszko A**. **2018**. Predicting the potential distribution area of *Solidago ×niederederi* (Asteraceae). *Turkish Journal of Botany* **42**: 51–56.

**Jensen RJ, Ciofani KM, Miramontes LC**. **2002**. Lines, Outlines, and Landmarks: Morphometric Analyses of Leaves of *Acer rubrum, Acer saccharinum* (Aceraceae) and Their Hybrid. *Taxon* **51**: 475–492.

**Jombart T, Devillard S, Balloux F**. **2010**. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11**: 94.

**Jug-Dujaković M, Ninčević T, Liber Z, Grdiša M, Šatović Z**. **2020**. *Salvia officinalis* survived in situ Pleistocene glaciation in 'refugia within refugia' as inferred from AFLP markers. *Plant Systematics and Evolution* **306**: 38.

**Karger DN, Conrad O, Böhner J, *et al.* 2017**. Climatologies at high resolution for the earth's land surface areas. *Scientific Data* **4**: 170122.

**Karger DN, Schmatz DR, Dettling G, Zimmermann NE**. **2020**. High-resolution monthly precipitation and temperature time series from 2006 to 2100. *Scientific Data* **7**: 248.

**Knight CA, Ackerly DD**. **2002**. Variation in nuclear DNA content across environmental gradients: a quantile regression analysis. *Ecology Letters* **5**: 66–76.

**Kolár F, Stech M, Trávnícek P, *et al.* 2009**. Towards resolving the *Knautia arvensis* agg. (Dipsacaceae) puzzle: primary and secondary contact zones and ploidy segregation at landscape and microgeographic scales. *Annals of Botany* **103**: 963–974.

**Konečná V, Yant L, Kolář F**. **2020**. The Evolutionary Genomics of Serpentine Adaptation. *Frontiers in Plant Science* **11**: 2004.

**Koutecký P, Štěpánek J, Baďurová T**. **2012**. Differentiation between diploid and tetraploid *Centaurea phrygia*: mating barriers, morphology and geographic distribution. *Preslia* **84**.

**Krijgsman W, Hilgen FJ, Raffi I, Sierro FJ, Wilson DS**. **1999**. Chronology, causes and progression of the Messinian salinity crisis. *Nature* **400**: 652–655.

**Krstić N, Savić L, Jovanović G**. **2012**. The Neogene lakes on the Balkan land. *Geoloski anali Balkanskoga poluostrva*: 37–60.

**Kučera J, Turis P, Zozomová-Lihová J, Slovák M**. **2013**. *Cyclamen fatrense*, myth or true Western Carpathian endemic? Genetic and morphological evidence. *Preslia* **85**: 133–158.

**Kutnjak D, Kuttner M, Niketić M, Dullinger S, Schönswetter P, Frajman B**. **2014**. Escaping to the summits: Phylogeography and predicted range dynamics of *Cerastium dinaricum*, an endangered high mountain plant endemic to the western Balkan Peninsula. *Molecular Phylogenetics and Evolution* **78**: 365–374.

**Kuzmanović N, Comanescu P, Frajman B, *et al.* 2013**. Genetic, cytological and morphological differentiation within the Balkan-Carpathian <I>Sesleria rigida</I> sensu Fl. Eur. (Poaceae): A taxonomically intricate tetraploid-octoploid complex. *Taxon* **62**: 458–472.

**Lakušić D, Liber Z, Nikolić T, *et al.* 2013**. Molecular phylogeny of the *Campanula pyramidalis* species complex (Campanulaceae) inferred from chloroplast and nuclear non-coding sequences and its taxonomic implications. *TAXON* **62**: 505–524.

**Leaché AD, Oaks JR**. **2017**. The Utility of Single Nucleotide Polymorphism (SNP) Data in Phylogenetics. *Annual Review of Ecology, Evolution, and Systematics* **48**: 69–84.

**Levin DA**. **2002**. *The Role of Chromosomal Change in Plant Evolution*. New York, USA: Oxford University Press.

**Li Y, Zou D, Shrestha N, *et al.* 2020**. Spatiotemporal variation in leaf size and shape in response to climate. *Journal of Plant Ecology* **13**: 87–96.

**Liang S-Q, Zhang X-C, Wei R**. **2022**. Ecological adaptation shaped the genetic structure of homoploid ferns against strong dispersal capacity. *Molecular Ecology* **31**: 2679–2697.

**Liber Z, Surina B, Nikolić T, Škrtić D, Šatović Z**. **2020**. Spatial distribution, niche ecology and conservation genetics of *Degenia velebitica* (Brassicaceae), a narrow endemic species of the north-western Dinaric Alps. *Plant Systematics and Evolution* **306**: 64.

**Lin H, Gu K, Li W, Zhao Y**. **2021**. Integrating coalescent-based species delimitation with ecological niche modeling delimited two species within the *Stewartia sinensis* complex (Theaceae). *Journal of Systematics and Evolution*: jse.12732.

**Linhart YB, Grant MC**. **1996**. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* **27**: 237–277.

**Linné C von**. **1753**. *Species plantarum*. Holmiae: Impensis Laurentii Salvii.

**Luqman H, Wegmann D, Fior S, Widmer A**. **2022**. Climate-induced range shifts drive adaptive response via spatio-temporal sorting of alleles. : 2022.06.07.495159.

**Mace GM**. **2004**. The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **359**: 711–719.

**Marhold K**. **2011a**. Multivariate morphometrics and its application to monography at specific and infraspecific levels. In: Stuessy TF, Lack HW, eds. *Monographic plant systematics: fundamental assessment of plant biodiversity.* A. R. Gantner Verlag, Ruggell, 73–99.

**Marhold K**. **2011b**. *Caryophyllaceae. – In: Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity.* https://www.emplantbase.org/home.html. 20 Dec. 2020.

**Martinčič A**. **2007**. *Mala flora Slovenije*. Ljubljana: Tehniška založba Slovenije.

**Martínez-Sagarra G, Castro S, Mota L, Loureiro J, Devesa JA**. **2021**. Genome Size, Chromosome Number and Morphological Data Reveal Unexpected Infraspecific Variability in *Festuca* (Poaceae). *Genes* **12**: 906.

**Mayer E, Trpin D**. **1965**. *Dianthus sylvestris* – kompleks v Jugoslaviji. *Biološki Vestnik* **13**: 53–59.

**Mazzola P, Raimondo FM, Ilardi V**. **2004**. *Dianthus minae* (Caryophyllaceae), a new species from the Madonie Mountains (N-Sicily). *Bocconea* **17**: 307–312.

**McCune JL**. **2016**. Species distribution models predict rare species occurrences despite significant effects of landscape context. *Journal of Applied Ecology* **53**: 1871–1879.

**Médail F, Diadema K**. **2009**. Glacial refugia influence plant diversity patterns in the Mediterranean Basin. *Journal of Biogeography* **36**: 1333–1345.

**Mereďa P, Hodálová I, Kučera J, Zozomová-Lihová J, Letz DR, Slovák M**. **2011**. Genetic and morphological variation in *Viola suavis s.l.* (Violaceae) in the western Balkan Peninsula: two endemic subspecies revealed. *Systematics and Biodiversity* **9**: 211–231.

**Merow C, Smith MJ, Silander JA**. **2013**. A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography* **36**: 1058–1069.

**Meyer FK**. **2011**. *Beiträge zur Flora von Albanien*. Thüringische Botanische Gesellschaft e.V.

**Micevski K**. **1990**. Novitäten in der Gattung *Dianthus* L. (Caryophyllaceae) in der Flora der SR Makedonien. *Macedonian Academy of Sciences and Arts, Section of Biological and Medical Sciences. Macedonian Academy of Sciences and Arts.* **8**: 31–46.

**Michener CD, Sokal RR**. **1957**. A Quantitative Approach to a Problem in Classification. *Evolution* **11**: 130–162.

**Minh BQ, Schmidt HA, Chernomor O, *et al.*** **2020**. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era (E Teeling, Ed.). *Molecular Biology and Evolution* **37**: 1530–1534.

**Moore DM**. **1982**. *Flora Europaea check-list and chromosome index*. Cambridge [Cambridgeshire] ; New York: Cambridge University Press.

**Morales-Saldaña S, Valencia-Ávalos S, Oyama K, Tovar-Sánchez E, Hipp AL, González-Rodríguez A**. **2021**. Even more oak species in Mexico? Genetic structure and morphological differentiation support the presence of at least two specific entities within *Quercus laeta*. *Journal of Systematics and Evolution* **n/a**.

**Mosquin T**. **1967**. Evidence for Autopolyploidy in *Epilobium angustifolium* (Onagraceae). *Evolution* **21**: 713–719.

**Mota-Vargas C, Rojas-Soto OR**. **2016**. Taxonomy and ecological niche modeling: Implications for the conservation of wood partridges (genus *Dendrortyx*). *Journal for Nature Conservation* **29**: 1–13.

**Murray BG**. **2005**. When does Intraspecific C-value Variation become Taxonomically Significant? *Annals of Botany* **95**: 119–125.

**Nieto Feliner G**. **2014**. Patterns and processes in plant phylogeography in the Mediterranean Basin. A review. *Perspectives in Plant Ecology, Evolution and Systematics* **16**: 265–278.

**Nikolić T**. **2020**. *Flora Croatica Database. Prirodoslovno-matematički fakultet, Sveučilište u Zagrebu*. URL http://hirc.botanic.hr/fcd.

**Ninčević T, Jug-Dujaković M, Grdiša M, *et al.* 2021**. Population structure and adaptive variation of *Helichrysum italicum* (Roth) G. Don along eastern Adriatic temperature and precipitation gradient. *Scientific Reports* **11**: 24333.

**Nunvářová Kabátová K, Kolář F, Jarolímová V, Krak K, Chrtek J**. **2019**. Does geography, evolutionary history or ecology drive ploidy and genome size variation in the *Minuartia verna* group (Caryophyllaceae) across Europe? *Plant Systematics and Evolution* **305**: 1019–1040.

**Otero A, Vargas P, Valcárcel V, Fernández-Mazuecos M, Jiménez-Mejías P, Hipp AL**. **2019**. A snapshot of progenitor-derivative speciation in action in *Iberodes* (Boraginaceae). *bioRxiv*.

**Owens GL, Samuk K**. **2020**. Adaptive introgression during environmental change can weaken reproductive isolation. *Nature Climate Change* **10**: 58–62.

**Pante E, Schoelinck C, Puillandre N**. **2015**. From Integrative Taxonomy to Species Description: One Step Beyond. *Systematic Biology* **64**: 152–160.

**Pax F, Hoffmann K**. **1934**. Caryophyllaceae In: Engler A, Prantl K, eds. *Die natürlichen Pflanzenfamilen II, 16c*. Leipzig, Germany: Engelmann, 275–364.

**Perný M, Tribsch A, Anchev ME**. **2004**. Infraspecific differentiation in the Balkan diploid *Cardamine acris* (Brassicaceae): Molecular and morphological evidence. *Folia Geobotanica* **39**: 405–429.

**Peterson AT, Soberón J J, Sánchez-Cordero V**. **1999**. Conservatism of ecological niches in evolutionary time. *Science (New York, N.Y.)* **285**: 1265–1267.

**Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP**. **2010**. Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology & Evolution* **25**: 459–467.

**Pickrell JK, Pritchard JK**. **2012**. Inference of Population Splits and Mixtures from Genome-Wide Allele Frequency Data (H Tang, Ed.). *PLoS Genetics* **8**: e1002967.

**Piedallu C, Gégout J-C, Lebourgeois F, Seynave I**. **2016**. Soil aeration, water deficit, nitrogen availability, acidity and temperature all contribute to shaping tree species distribution in temperate forests (S Bartha, Ed.). *Journal of Vegetation Science* **27**: 387–399.

**Pinto-Carrasco D, Delgado L, Sánchez Agudo JA, Rico E, Martínez-Ortega MM**. **2022**. Phylogeography and ecological differentiation of strictly Mediterranean taxa: the case of the Iberian endemic *Odontites recordonii*. *American Journal of Botany* **109**: 166–183.

**Prata KE, Riginos C, Gutenkunst RN, *et al.* 2022**. Deep connections: Divergence histories with gene flow in mesophotic *Agaricia* corals. *Molecular Ecology* **31**: 2511–2527.

**Pritchard JK, Stephens M, Donnelly P**. **2000**. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **155**: 945–959.

**Pyron RA, Costa GC, Patten MA, Burbrink FT**. **2015**. Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. *Biological Reviews* **90**: 1248–1262.

**Raab-Straube EV, Raus T (Eds.)**. **2013**. Euro+Med-Checklist Notulae, 1. *Willdenowia* **43**: 151–164.

**Radosavljević I, Satovic Z, Jakse J, *et al.* 2012**. Development of new microsatellite markers for *Salvia officinalis* L. and its potential use in conservation-genetic studies of narrow endemic *Salvia brachyodon* Vandas. *International Journal of Molecular Sciences* **13**: 12082–12093.

**Raj A, Stephens M, Pritchard JK**. **2014**. fastSTRUCTURE: Variational Inference of Population Structure in Large SNP Data Sets. *Genetics* **197**: 573–589.

**Rechinger fil. KH**. **1935**. *Ergebnisse einer botanischen Reise in den Bertiscus (Nordalbanische Alpen).* Berlin-Dahlem: Selbstverlag des Herausgebers, Fabeckstrasse 49.

**Reich D, Flatscher R, Pellegrino G, *et al.* 2021**. Biogeography of amphi-adriatic *Gentianella crispata* (Gentianaceae): a northern refugium and recent trans-adriatic migration. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology* **0**: 1–15.

**Reichenbach HGL**. **1844**. *Icones florae Germanicae et Helveticae, Vol. 6.* Lipsiae : apud Friedericum Hofmeister (Dresdae, Typis Caroli Rammingii).

**Rešetnik I, Baričevič D, Batîr Rusu D, *et al.* 2016**. Genetic Diversity and Demographic History of Wild and Cultivated/Naturalised Plant Populations: Evidence from Dalmatian Sage (*Salvia officinalis* L., Lamiaceae) (KM Olsen, Ed.). *PLOS ONE* **11**: e0159545.

**Rešetnik I, Frajman B, Schönswetter P**. **2016**. Heteroploid *Knautia drymeia* includes *K. gussonei* and cannot be separated into diagnosable subspecies. *American Journal of Botany* **103**: 1300–1313.

**Rešetnik I, Temunović M, Liber Z, Satovic Z, Bogdanović S**. **2020**. Phylogeography of *Campanula fenestrellata* s.l. (Campanulaceae) in the northern Adriatic. *Plant Systematics and Evolution* **306**: 42.

**Rödder D, Engler JO**. **2011**. Quantitative metrics of overlaps in Grinnellian niches: advances and possible drawbacks: Quantitative metrics of niche overlap. *Global Ecology and Biogeography* **20**: 915–927.

**Rohlena J**. **1942**. *Conspectus florae Montenegrinae*. Prag: Preslia, XX-XXI.

**Rooks F, Jarolimova V, Záveská Drábková L, Kirschner J**. **2011**. The elusive *Juncus minutulus*: A failure to separate tetra- and hexaploid individuals of the *Juncus bufonius* complex in a morphometric comparison of cytometrically defined groups. *Preslia* **83**: 565-589.

**Schaal BA, Hayworth DA, Olsen KM, Rauscher JT, Smith WA**. **1998**. Phylogeographic studies in plants: problems and prospects. *Molecular Ecology* **7**: 465–474.

**Schlosser JK, Vukotinović Lj**. **1869**. *Flora Croatica: exhibens stirpes phanerogamas et vasculares cryptogamas quae in Croatia Slavonia et Dalmatia sponte crescunt nec non illas quae frequentissime coluntur*. apud Župan (Albrecht et Fiedler).

**Schmitt J**. **1980**. Pollinator Foraging Behavior and Gene Dispersal in *Senecio* (Compositae). *Evolution* **34**: 934–943.

**Schönswetter P, Stehlik I, Holderegger R, Tribsch A**. **2005**. Molecular evidence for glacial refugia of mountain plants in the European Alps. *Molecular Ecology* **14**: 3547–3555.

**Séguier JF**. **1745**. *Plantae Veronenses Seu stirpium Quae in agro Veronensi reperiuntur methodica synopsis*. Verona: Veronae: Typis Seminiarii.

**Shrestha M, Garcia JE, Bukovac Z, Dorin A, Dyer AG**. **2018**. Pollination in a new climate: Assessing the potential influence of flower temperature variation on insect pollinator behaviour. *PLoS ONE* **13**: e0200549.

**Sikora M, Mihanović H, Vilibić I**. **2013**. Paleo-coastline of the Central Eastern Adriatic Sea, and Paleo-Channels of the Cetina and Neretva rivers during the last glacial maximum. *Acta Adriatica* **54**: 3–12.

**Skogen KA, Overson RP, Hilpman ET, Fant JB**. **2019**. Hawkmoth Pollination Facilitates Long-distance Pollen Dispersal and Reduces Isolation Across a Gradient of Land-use Change. *Annals of the Missouri Botanical Garden* **104**: 495–511.

**Skokanová K, Hodálová I, Mereďa P, Slovák M, Kučera J**. **2019**. The *Cyanus tuberosus* group (Asteraceae) in the Balkans: biological entities require correct names. *Plant Systematics and Evolution* **305**: 569–596.

**Šlenker M, Kantor A, Marhold K, *et al.* 2021**. Allele Sorting as a Novel Approach to Resolving the Origin of Allotetraploids Using Hyb-Seq Data: A Case Study of the Balkan Mountain Endemic *Cardamine barbaraeoides*. *Frontiers in Plant Science* **12**.

**Slovák M, Kučera J, Turis P, Zozomová-Lihová J**. **2012**. Multiple glacial refugia and postglacial colonization routes inferred for a woodland geophyte, *Cyclamen purpurascens*: patterns concordant with the Pleistocene history of broadleaved and coniferous tree species. *Biological Journal of the Linnean Society* **105**: 741–760.

**Slovák M, Vít P, Urfus T, Suda J**. **2009**. Complex pattern of genome size variation in a polymorphic member of the Asteraceae. *Journal of Biogeography* **36**: 372–384.

**Šmarda P, Bureš P, Horová L, Rotreklová O**. **2008**. Intrapopulation Genome Size Dynamics in *Festuca pallens*. *Annals of Botany* **102**: 599–607.

**Soberón J**. **2007**. Grinnellian and Eltonian niches and geographic distributions of species. *Ecology Letters* **10**: 1115–1123.

**Soltis PS, Soltis DE**. **2009**. The role of hybridization in plant speciation. *Annual Review of Plant Biology* **60**: 561–588.

**Soltis DE, Soltis PS, Rieseberg LH**. **1993**. Molecular Data and the Dynamic Nature of Polyploidy. *Critical Reviews in Plant Sciences* **12**: 243–273.

**Soltis DE, Soltis PS, Schemske DW, *et al.* 2007**. Autopolyploidy in angiosperms: have we grossly underestimated the number of species? *TAXON* **56**: 13–30.

**Souza G, Costa L, Guignard MS, *et al.* 2019**. Do tropical plants have smaller genomes? Correlation between genome size and climatic variables in the *Caesalpinia* Group (Caesalpinioideae, Leguminosae). *Perspectives in Plant Ecology, Evolution and Systematics* **38**: 13–23.

**Španiel S, Marhold K, Filová B, Zozomová-Lihová J**. **2011**. Genetic and morphological variation in the diploid–polyploid *Alyssum montanum* in Central Europe: taxonomic and evolutionary considerations. *Plant Systematics and Evolution* **294**: 1.

**Španiel S, Marhold K, Zozomová-Lihová J**. **2017**. The polyploid *Alyssum montanum-A. repens* complex in the Balkans: a hotspot of species and genetic diversity. *Plant Systematics and Evolution* **303**: 1443–1465.

**Španiel S, Rešetnik I**. **2022**. Plant phylogeography of the Balkan Peninsula: spatiotemporal patterns and processes. *Plant Systematics and Evolution* **308**: 38.

**Španiel S, Zozomová-Lihová J, Marhold K**. **2017**. Revised taxonomic treatment of the *Alyssum montanum-A. repens* complex in the Balkans: a multivariate morphometric analysis. *Plant Systematics and Evolution* **303**: 1413–1442.

**Stephens RE, Sauquet H, Guerin GR, Jiang M, Falster D, Gallagher RV**. **2022**. Climate shapes community flowering periods across biomes. *Journal of Biogeography* **49**: 1205–1218.

**Stešević D, Caković D**. **2013**. *Katalog vaskularne flore Crne Gore*. Crnogorska akademija nauka i umjetnosti, Podgorica.

**Stevanoski I, Kuzmanović N, Dolenc Koce J, Schönswetter P, Frajman B**. **2020**. Disentangling relationships between the amphi-Adriatic *Euphorbia spinosa* and Balkan endemic *E. glabriflora* (Euphorbiaceae). *Botanical Journal of the Linnean Society* **194**: 358–374.

**Stuessy TF**. **2009**. *Plant taxonomy: the systematic evaluation of comparative data*. New York: Columbia University Press.

**Stuessy TF, Hörandl E**. **2014**. Evolutionary systematics and paraphyly: Introduction. *Annals of the Missouri Botanical Garden* **100**: 2–5.

**Suda J, Krahulcová A, Trávníček P, Rosenbaumová R, Peckert T, Krahulec F**. **2007**. Genome Size Variation and Species Relationships in *Hieracium* Sub-genus *Pilosella* (Asteraceae) as Inferred by Flow Cytometry. *Annals of Botany* **100**: 1323–1335.

**Suda J, Kron P, Husband BC, Trávníček P**. **2007**. Flow Cytometry and Ploidy: Applications in Plant Systematics, Ecology and Evolutionary Biology In: Doležel J, Greilhuber J, Suda J, eds. *Flow Cytometry with Plant Cells*. John Wiley & Sons, Ltd, 103–130.

**Suda J, Trávníček P**. **2006**. Estimation of Relative Nuclear DNA Content in Dehydrated Plant Tissues by Flow Cytometry. *Current Protocols in Cytometry* **38**: 7.30.1-7.30.14.

**Sukumaran J, Knowles LL**. **2017**. Multispecies coalescent delimits structure, not species. *Proceedings of the National Academy of Sciences* **114**: 1607–1612.

**Sundqvist L, Keenan K, Zackrisson M, Prodöhl P, Kleinhans D**. **2016**. Directional genetic differentiation and relative migration. *Ecology and Evolution* **6**: 3461–3475.

**Surina B, Schneeweiss GM, Glasnović P, Schönswetter P**. **2014**. Testing the efficiency of nested barriers to dispersal in the Mediterranean high mountain plant *Edraianthus graminifolius* (Campanulaceae). *Molecular Ecology* **23**: 2861–2875.

**Surina B, Schönswetter P, Schneeweiss GM**. **2011**. Quaternary range dynamics of ecologically divergent species (*Edraianthus serpyllifolius* and *E. tenuifolius*, Campanulaceae) within the Balkan refugium: Quaternary range dynamics within the Balkan refugium. *Journal of Biogeography* **38**: 1381–1393.

**Tausch IF**. **1828**. *Sylloge plantarum novarum itemque minus cognitarum*. Ratisbonae: Typis viduae C.E. Brenck.

**Temunović M, Franjić J, Satovic Z, Grgurev M, Frascaria-Lacoste N, Fernández-Manjarrés JF**. **2012**. Environmental Heterogeneity Explains the Genetic Structure of Continental and Mediterranean Populations of *Fraxinus angustifolia* Vahl. *PLOS ONE* **7**: e42764.

**Theodoridis S, Nogués-Bravo D, Conti E**. **2019**. The role of cryptic diversity and its environmental correlates in global conservation status assessments: Insights from the threatened bird's-eye primrose (*Primula farinosa* L.). *Diversity and Distributions* **25**: 1457–1471.

**Thiers B**. **2021**. *Index Herbariorum. A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium*. http://sweetgum.nybg.org/science/ih. 20 Feb. 2021.

**Trigas P, Kougioumoutzis K, Ermidou A, Kalpoutzakis E**. **2018**. Multivariate morphometric analysis of *Petrorhagia* subsect. *Saxifragae* (Caryophyllaceae) in Greece, with a new species from SE Peloponnisos: *P. laconica*. *Willdenowia* **48**: 137–146.

**Trinajstić I**. **1979**. *Analitička flora Jugoslavije*. Institut za botaniku Sveučilišta u Zagrebu.

**Trucchi E, Frajman B, Haverkamp THA, Schönswetter P, Paun O**. **2017**. Genomic analyses suggest parallel ecological divergence in *Heliosperma pusillum* (Caryophyllaceae). *New Phytologist* **216**: 267–278.

**Turelli M, Barton NH, Coyne JA**. **2001**. Theory and speciation. *Trends in Ecology & Evolution* **16**: 330–343.

**Turesson G**. **1925**. The Plant Species in Relation to Habitat and Climate. *Hereditas* **6**: 147–236.

**Turland N, Wiersema J, Barrie F, *et al.* (Eds.)**. **2018**. *International Code of Nomenclature for algae, fungi, and plants*. Koeltz Botanical Books.

**Tutin TG, Walters SM**. **1993**. *Dianthus* L. In: Tutin Thomas Gaskell, ed. *Flora Europaea*. Cambridge; New York: Cambridge University Press, 227–246.

**Tzedakis PC**. **2004**. The Balkans as Prime Glacial Refugial Territory of European Temperate Trees In: Griffiths HI, Kryštufek B, Reed JM, eds. *Balkan Biodiversity*. Dordrecht: Springer Netherlands, 49–68.

**Valente LM, Savolainen V, Vargas P**. **2010**. Unparalleled rates of species diversification in Europe. *Proceedings of the Royal Society B: Biological Sciences* **277**: 1489–1496.

**Vangjeli J**. **2015**. *Excursion Flora of Albania*. Oberreifenberg: Koeltz Scientific Books.

**Vargas P, Fernández-Mazuecos M, Heleno R**. **2018**. Phylogenetic evidence for a Miocene origin of Mediterranean lineages: species diversity, reproductive traits and geographical isolation. *Plant Biology (Stuttgart, Germany)* **20 Suppl 1**: 157–165.

**Visiani RD**. **1852**. *Flora Dalmatica: sive enumeratio stirpium vascularium quas hactenus in Dalmatia lectas et sibi digessit*. Hofmeister.

**Warren DL, Glor RE, Turelli M**. **2008**. Environmental Niche Equivalency versus Conservatism: Quantitative Approaches to Niche Evolution. *Evolution* **62**: 2868–2883.

**Wendel JF, Doyle JJ**. **1998**. Phylogenetic Incongruence: Window into Genome History and Molecular Evolution In: Soltis DE, Soltis PS, Doyle JJ, eds. *Molecular Systematics of Plants II*. Boston, MA: Springer US, 265–296.

**Willis KJ**. **1994**. The vegetational history of the Balkans. *Quaternary Science Reviews* **13**: 769–788.

**Wilson EO, Brown WL Jr**. **1953**. The Subspecies Concept and Its Taxonomic Application. *Systematic Biology* **2**: 97–111.

**Wulfen FX**. **1787**. Plantae Rariores Carinthiacae In: Jacquin NJ, ed. *Collectanea*. Vindobonae: Ex Officina Wappleriana., 237–239.

**Yesson C, Culham A**. **2006**. Phyloclimatic Modeling: Combining Phylogenetics and Bioclimatic Modeling. *Systematic Biology* **55**: 785–802.

**Záveská E, Kirschner P, Frajman B, *et al.* 2021**. Evidence for Glacial Refugia of the Forest Understorey Species *Helleborus niger* (Ranunculaceae) in the Southern as Well as in the Northern Limestone Alps. *Frontiers in Plant Science* **12**.

**Zozomová-Lihová J, Melichárková A, Svitok M, Španiel S**. **2020**. Pleistocene range disruption and postglacial expansion with secondary contacts explain the genetic and cytotype structure in the western Balkan endemic *Alyssum austrodalmaticum* (Brassicaceae). *Plant Systematics and Evolution* **306**: 47.

## CURRICULUM VITAE

Ana Terlević was born in Pula, Croatia, 12 August 1993. She finished elementary and high school in Pula and continued education at the University of Zagreb, Faculty of Science, Department of Biology. She finished undergraduate study of biology in 2015, and graduate study of experimental biology, module botany, in 2018. Prior to starting her doctorate, Ana spent three months at the Jagiellonian University in Krakow in the frame of Erasmus+ traineeship, working on habitat suitability modelling of invasive plant species. Her employment as research assistant and her PhD programme at the University of Zagreb, Faculty of Science, Department of Biology, started in October 2018 and November 2018, respectively. During her PhD, she was working in the frame of the Croatian Science Foundation project "Phylogeography and evolution of three ecologically divergent groups of amphi-Adriatic plants" (Amphi-Adri-Plant). Ana published 3 short communications and 5 scientific articles, she has 13 conference proceedings with 9 active participations and received the best oral presentation award at the 8th Balkan Botanical Congress. Additionally, she participated in educational work as a teaching assistant on two undergraduate courses, Systematic Botany and Statistics. She has also received training in the Research Group Evolutionary Systematic lab, Department of Botany at the University of Innsbruck, Austria. Together with colleagues from Switzerland, Italy, and Germany working on *Dianthus*, Ana initiated a proposal of herbarium collections digitalization including several natural history museums in European cities, and the proposal received funds within the SYNTHESYS+ Virtual Access project. Ana held two popular scientific lectures for the Croatian Botanical Society and one presentation of her investigations at the PhD Café meeting of PhD students at the University of Zagreb, in the organization of the Croatian Science Foundation.

**SCIENTIFIC ID in Croatian Science Bibliography: 372461**

## ORIGINAL SCIENTIFIC PAPERS:

**Terlević A,** Temunović M, Bogdanović S, Grgurev M, Ljubičić I, Rešetnik I. 2022. Morphological and environmental variability of *Dianthus sylvestris* Wulfen (Caryophyllaceae) on the Balkan Peninsula. Botanical Journal of the Linnean Society. *In press*. doi: 10.1093/botlinnean/boac058.

**Terlević A**, Bogdanović S, Frajman B, Rešetnik I (2022): Genome Size Variation in *Dianthus sylvestris* Wulfen sensu lato (Caryophyllaceae). Plants, 11, 1481. doi:10.3390/plants11111481.

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

Nobis M, Klichowska E, **Terlević A**, Wróbel A, Erst A, Hrivnák R, Ebel AL, et al. (2019): Contribution to the flora of Asian and European countries: new national and regional vascular plant records, 8. Botany Letters, 166, 163–188. doi:10.1080/23818107.2019.1600165.

Budisavljević A, **Terlević A**, Mihelić P, Špadina B, Papković D, Nikolić T, Šegota V (2017): Vaskularna flora šume Dotrščina (Zagreb, Hrvatska). Glasnik Hrvatskog botaničkog društva, 5, 4–19.

## SHORT COMMUNICATIONS:

**Terlević A**, Koopman J, Więcław H, Rešetnik I, Bogdanović S (2021): *Carex phyllostachys* (Cyperaceae), a new species in Croatia. Acta botanica Croatica, 80, 106–111. doi:10.37427/botcro-2021-002.

**Terlević A**, Rešetnik I (2020): Inventory of the historical *Dianthus sylvestris* herbarium materials from Herbarium Croaticum and Herbarium Ivo and Marija Horvat. Glasnik Hrvatskog botaničkog društva, 8, 8–14. doi:10.46232/glashbod.8.1.2.

**Terlević A**, Nobis M (2018): A new data to distribution of *Epilobium pseudorubescens* A.K. Skvortsov (Onagraceae) in Poland. Acta Musei Silesiae, Scientiae Naturales, 67, 63–66. doi:10.2478/cszma-2018-0004.

## CONFERENCE ABSTRACTS:

Mucko M, **Terlević A**, Temunović M, Doboš M, Ljubičić I, Bogdanović S, Rešetnik I. (2022) Morphometry of *Festuca bosniaca* Kumm. et Sendtn. (Poaceae) and related species. Book of Abstracts, 7th Croatian Botanical Society.

**Terlević A**, Temunović M, Bogdanović S, Rešetnik I. (2022) The challenge of subspecies delimitation in *Dianthus sylvestris* Wulfen s.l. on the Balkan Peninsula: Integrative approach to taxonomy. Book of abstract, 7th Croatian Botanical Symposium.

Tokić P, **Terlević A**, Ljubičić I, Rešetnik I, Bogdanović, S. (2022) Morphological variability of the *Dianthus ciliatus* complex (Caryophyllaceae) in the Adriatic region. Book of abstract, 7th Croatian Botanical Symposium.

**Terlević A**, Temunović M, Bogdanović S, Grgurev M. Ljubičić I, Rešetnik I. (2022) Morphological and ecological variability of the *Dianthus sylvestris* complex (Caryophyllaceae) on the Balkan Peninsula. Apstrakti, 14. Simpozijum o flori jugoistočne Srbije i susednih regiona.

Temunović M, **Terlević A**, Luqman, Hirzi; Bogdanović S, Widmer A, Fior S, Rešetnik I. (2022) Unravelling patterns of genomic variation of *Dianthus sylvestris* Wulfen s.l. in the Balkan Peninsula. Book of Abstracts, 8th Balkan Botanical Congress.

**Terlević A**, Temunović M, Bogdanović S, Frajman B, Rešetnik I. (2022) Complex pattern of morphological and genome size variation in *Dianthus sylvestris* Wulfen s.l. (Caryophyllaceae). Book of Abstracts, 8th Balkan Botanical Congress.

**Terlević A**, Bogdanović S, Temunović M, Fior S, Luqman H, Widmer A, Frajman B, Rešetnik I. (2022) Disentangling the diversification of *Dianthus sylvestris* Wulfen s.l. on the Balkan Peninsula using an integrative approach. Book of Abstracts, 6th Faculty of Science PhD Student Symposium.

**Terlević A**, Bogdanović S, Temunović M, Fior S, Luqman H, Widmer A, Frajman B, Rešetnik I. (2022) Disentangling the diversification of the *Dianthus sylvestris* complex on the Balkan Peninsula using an integrative approach: taxonomic implications within a dynamic system. Book of Abstract, Phylogenetics and taxonomy of Caryophyllaceae.

**Terlević A**, Bogdanović S, Rešetnik I. (2021) Morphological variability in *Dianthus sylvestris* Wulfen s.l. on the Balkan Peninsula. Book of Abstracts, 5th PhD Student Symposium.

**Terlević A**, Rešetnik I. (2020) Genome size variation in *Dianthus sylvestris* group (Caryophyllaceae) across the Balkan Peninsula. Book of Abstracts, 4th PhD Student Symposium.

**Terlević A**, Rešetnik I. (2019) Inventory of the historical *Dianthus sylvestris* herbarium material from ZA and ZAHO. Book of abstracts, 6th Croatian Botanical Symposium.

**Terlević** A, Jelaska, S. (2019) Chainsaw and axe still has the highest impact on temperate forest flora in Medvednica Mt., Croatia, over a 20-year period. Book of abstracts, 6th Croatian Botanical Symposium

Budisavljević A, Mihelić P, Papković D, Špadina B, **Terlević A**, Šegota, V. (2016) Vascular flora inventarisation and mapping in Dotrščina memorial park. Book of abstracts, 5th Croatian Botanical Symposium.

## SCIENTIFIC PROJECTS:

(2018 – 2022) Research assistant on UIP-2017-05-2882 (AmphiAdriPlant - Phylogeography and evolution of three ecologically divergent groups of amphi-Adriatic plants)

(2020 – 2022) Collaborator on Croatia-Austria Bilateral project (Genome size evolution and polyploidisation in amphi-Adriatic *Aurinia* (Brassicaceae) and the *Cerastium tomentosum* and *Dianthus sylvestris* species groups (Caryophyllaceae))

## WORKSHOPS:

(2022) Introduction to Bayesian Inference in Practice, Transmitting Science, online

(2022) Programming in Python, University Computing Centre SRCE, University of Zagreb

(2021) Introduction to Python programming, University Computing Centre SRCE, University of Zagreb

(2021) Environmental variables: how to obtain and process them with R, Transmitting Science, online

(2021) How to use Isabella Computer Cluster, University Computing Centre SRCE, University of Zagreb

(2021) Introduction to Bioinformatics for Biosystematics, ForBio – Research School in Biosystematics, The Arctic University Museum of Norway

(2020) Tidy data in R, University Computing Centre SRCE, University of Zagreb

(2020) Introduction to QGIS, Exatto d.o.o.

(2020) Basic programming in R, University Computing Centre SRCE, University of Zagreb

(2019) Introduction to R syntax and its application in basic statistical and graphical data analysis, University Computing Centre SRCE, University of Zagreb

(2019) 4th Int'l Summer School on Data Science, Faculty of Electrical Engineering, Mechanical Engineering and Naval Architecture, University of Split

**SCIENTIFIC TRAINING:**

(2021) Scientific research in the Evolutionary Systematics laboratory, University of Innsbruck, Institute of Botany

(2020) Visit of the Plant Ecological Genetics research group at ETH Zürich, Institute of Integrative Biology, Department of Environmental Systems Science, ETH Zürich

(2019) Scientific research in the Evolutionary Systematics laboratory, University of Innsbruck, Institute of Botany

**ORGANIZING EXPERIENCE:**

(2022) Member of technical support at 7th Croatian botanical symposium, Zagreb, Croatia

(2019) Member of technical support at 6th Croatian botanical symposium, Zagreb, Croatia

(2017) Research and educational camp "Insula Tilagus" in organisation of Biology student association (BIUS)

(2016) Member of technical support at 2nd Croatian symposium on invasive species, Zagreb, Croatia

(2016) Research and educational camp "Mura – Drava" in organisation of Biology student association (BIUS)

(2015-2016) Head of Ornithology section in Biology student association (BIUS)

(2015) Research and educational camp "Papuk" in organisation of Biology student association (BIUS)

(2013) Research and educational camp "Apsyrtides" in organisation of Biology student association (BIUS)

(2013-2018) Popular scientific manifestation ″Biology Night″ at University of Zagreb, Faculty of Science, Biology Department