

## RESEARCH ARTICLE

# Hiding in plain sight: Integrative analyses uncover a cryptic *Salvia* species in Europe

Attila Mátis,<sup>1\*</sup> Tamás Malkócs,<sup>2,3\*</sup> Thomas Kuhn,<sup>1</sup> Levente Laczkó,<sup>2,4,5</sup> Ivan Moysiyenko,<sup>6</sup> Anna Szabó,<sup>1</sup> Alexandru S. Bădărău,<sup>7</sup> & Gábor Sramkó<sup>2,4,5</sup>

<sup>1</sup> Hungarian Department of Biology and Ecology, Faculty of Biology and Geology, Babeş-Bolyai University, 5–7 Clinicilor Street, 400006 Cluj-Napoca, Romania

<sup>2</sup> Department of Botany, Institute of Biology and Ecology, University of Debrecen, Egyetem tér 1, 4032 Debrecen, Hungary

<sup>3</sup> Institute of Aquatic Ecology, Centre for Ecological Research, 4026 Debrecen, Hungary

<sup>4</sup> MTA-DE “Lendület” Evolutionary Phylogenomics Research Group, Debrecen, Hungary

<sup>5</sup> ELKH-DE Conservation Biology Research Group, Debrecen, Hungary

<sup>6</sup> Department of Botany, Kherson State University, 27 Universytetska St., 73000 Kherson, Ukraine

<sup>7</sup> Faculty of Environmental Sciences and Engineering, Babeş-Bolyai University, 30 Fântânele Street, 400294 Cluj-Napoca, Romania

\* Attila Mátis and Tamás Malkócs contributed equally to this work.

Address for correspondence: Tamás Malkócs, [tamas.malkocs@gmail.com](mailto:tamas.malkocs@gmail.com)

DOI <https://doi.org/10.1002/tax.12818>

**Abstract** *Salvia* is the most species-rich genus of the family Lamiaceae, currently numbering almost 1000 species. The diagnostic feature of the genus is the unique staminal lever mechanism that allows for specific pollination modes. We encountered an unusual *Salvia* form in the field, in SE Romania, which resembles *S. austriaca* but features a radically different lever mechanism. This form proved to be geographically widespread on the Pontic steppe, never occurring in sympatry with *S. austriaca*. We used an integrative approach, employing morphometric and phylogenomic (RADseq) analyses, to study this unusual form. The taxon’s floral morphology proved to be consistently and subtly different from that of *S. austriaca*, and similarly, Bayesian species delimitation using genome-wide SNP data indicated species-level differences. Our results provide compelling evidence that points toward the discovery of an unrecognized species. This species has been overlooked for centuries, misidentified as *S. austriaca*, a closely related taxon. The new species differs from *S. austriaca* in key features of floral structure, habitat preference, and distribution. The potential range of this cryptic species, its pollination biology, ecology, and phylogeography are discussed.

**Keywords** Dobrogea; floral structure; phylogenomics; speciation; species delimitation; steppe

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

## ■ INTRODUCTION

The genus *Salvia* L., colloquially known as sages, is the most diverse genus of the Lamiaceae Martinov family and one of the largest genera of flowering plants, numbering around 980 species (Will & Claßen-Bockhoff, 2017), with new species continuously being described (e.g., Celep & al., 2015; García & Zamudio, 2015; Martínez-Gordillo & al., 2016; Martínez-Ambriz & al., 2019; Wei & al., 2019). It has a nearly worldwide distribution, with diversity hotspots on four continents, and presenting extreme diversity regarding habitat, ecology and pollination biology (Harley & al., 2004).

The diagnostic feature of the genus is the unique staminal lever mechanism (Claßen-Bockhoff & al., 2003; Claßen-Bockhoff, 2017). This lever mechanism is formed by the two asymmetric anthers present in the *Salvia* flower, each

with an elongated connective that separates the two thecae from each other. The upper connective arm (abaxial) has two fertile pollen sacs, while the lower connective arm (adaxial) is often sterile and forms structures that restrict the access to nectar (Claßen-Bockhoff & al., 2004a). The stamens are versatile, with a mobile connective that can swing around the joint formed by the thin filament tip (Troll, 1929). Pollination (by insects or birds) is achieved by pushing the lever that drives the deposition of pollen onto a specific body part of the pollinator (Claßen-Bockhoff & al., 2003, 2004b; Celep & al., 2020a).

*Salvia* flowers have been extensively studied for their unique staminal lever mechanism, a complex functional unit involved in flower–pollinator interactions (e.g., Hruba, 1962; Claßen-Bockhoff & al., 2003; Reith & al., 2007; Celep & al., 2020a). The lever mechanism provides mechanical isolation

**Article history:** Received: 20 Dec 2021 | returned for (first) revision: 3 Mar 2022 | (last) revision received: 25 Jul 2022 | accepted: 27 Jul 2022

**Associate Editor:** Michael D. Pirie | © 2022 The Authors.

TAXON published by John Wiley & Sons Ltd on behalf of International Association for Plant Taxonomy.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

in otherwise interfertile *Salvia* species, thus effectively preventing hybridization—an important feature considering that hybrids are rarely competitive in nature, as they compete for the same habitat as the parental species (Claßen-Bockhoff & al., 2004b; Celep & al., 2020a). The staminal lever has been proposed as a key innovation for the evolutionary radiation of this mega-genus and a major driver of speciation (Claßen-Bockhoff & al., 2003, 2004b) because significant species radiations are correlated with the presence of the lever mechanism. Versatile staminal lever contraptions, similar to that of *Salvia*, have evolved only rarely in angiosperms. Similar structures that have the same function and could act as a key innovation facilitating diversification are also present in several *Calceolaria* L. (Calceolariaceae Olmstead) species, a genus in which they evolved twice independently (Trapp, 1956b; Sérsic, 2004), in *Pseudosopubia obtusifolia* Engl. of Orobanchaceae Vent. (Trapp, 1956a), and in *Roscoea purpurea* Sm. of Zingiberaceae Martinov (Troll, 1929).

During the last two decades, several studies demonstrated the paraphyly of this large genus (Walker & al., 2004; Walker & Sytsma, 2007; Will & Claßen-Bockhoff, 2014, 2017; Kriebel & al., 2020, 2021; Rose & al., 2021), and that the staminal lever evolved independently three (Walker & Sytsma, 2007; Drew & Sytsma, 2012) or five times (Will & Claßen-Bockhoff, 2017), each time resulting in convergent morphology and function. As a direct consequence of these studies, there is an ongoing debate regarding the exact circumscription of the genus with two major taxonomic treatments proposed, both based on previous studies with extensive molecular and morphological analyses and sampling a large number of taxa: maintaining *Salvia* with the inclusion of additional 15 species belonging to *Dorystaechas* Boiss. & Heldr., *Meriandra* Benth., *Perovskia* Kar., *Rosmarinus* L., and *Zhumeria* Rech.f. & Wendelbo (Drew & al., 2017), or splitting up this large genus into six genera (Will & Claßen-Bockhoff, 2017). Kriebel & al. (2019) recently examined how diversification of this large genus was influenced by repeated shifts in area, biomes, and pollinators with evidence pointing to other important drivers of speciation besides the special pollination mechanism.

*Salvia* phylogenetics mostly relied on classical phylogenetic methods (i.e., Sanger-sequencing of candidate-genes from the plastid and nuclear genomes) that provided substantial phylogenetic resolution between main lineages within the genus, but often failed to effectively separate closely related species (Walker & Sytsma, 2007; Takano & Okada, 2011; Jenks & al., 2013; Li & al., 2013; Walker & al., 2015; Will & Claßen-Bockhoff, 2017; Hu & al., 2018). Genomic approaches usually provide better resolution in the latter case (Rannala & Yang, 2008; Lemmon & Lemmon, 2013; McCormack & al., 2013), as was demonstrated in the genus *Salvia* as well (Kriebel & al., 2019; Rose & al., 2021). Using different genomic approaches, Rose & al. (2021) generated congruent topologies from nuclear and plastome datasets, but they concluded that there is widespread discordance among individual gene trees possibly arising from incomplete lineage sorting

and horizontal gene flow. Reduced representation genomic library (RRL) methods are cost and labor efficient approaches in generating high-resolution genomic data of non-model organisms (Davey & al., 2011), which typically provide thousands of single nucleotide polymorphisms (SNPs) for phylogenomic inference (Leaché & Oaks, 2017). One of the most powerful methods among RRL approaches is restriction-site associated DNA sequencing (RADseq), originally described as a particular method of RRL approaches (Miller & al., 2007; Baird & al., 2008), but the name has recently been adopted in a wider sense to include all RRL methods that employ restriction enzymes to locate loci across the genome for next-generation sequencing (Andrews & al., 2016). The original version of RADseq (also called sdRAD, see Rivera-Colón & al., 2021) is a powerful and reliable method (Puritz & al., 2014; Rochette & al., 2019) that was successfully used to elucidate evolutionary relationships between recently diverged taxa (e.g., Reitzel & al., 2013; Bateman & al., 2018; Sramkó & al., 2019; Karbstein & al., 2020). Recent analytical developments further improved RADseq data analyses in several ways including the application of species delimitation (Leaché & al., 2014; Pante & al., 2015) and the estimation of divergence times from SNP data (Stange & al., 2018).

During our field work in the Dobrogea region of Romania and Bulgaria (a historical region known as “Scythia Minor” or “Lesser Scythia”, comprising present-day southeast Romania and northeast Bulgaria) characterized by extensive steppe habitats as part of the Pontic steppe zone (Kajtoch & al., 2016: fig. 1), we encountered a *Salvia* taxon similar to *S. austriaca* Jacq. in overall morphology, but displaying a radically different staminal lever system. This unusual form deposits the pollen onto the back of the pollinating insect using a dorsal anther movement (hereafter called “*Salvia* FDPD”, i.e., *Salvia* form with dorsal pollen deposition), whereas *S. austriaca* s.str. deposits the pollen onto the side of the abdomen of the insect, using an approximately lateral lever movement (i.e., lateral pollen deposition). *Salvia austriaca* shares this feature of lateral pollen deposition with its putative close relative *S. staminea* Montbret & Aucher ex Benth. (= *S. armeniaca* (Bordz.) Grossh.), a species with a native range covering the dry mountain-steppes of eastern Turkey, Iran and Transcaucasia (Pobedimova, 1954; Hedge, 1982a,b; Galstyan, 2021). According to the above sources, these two species are the only known members of the *S.* ser. *Austriacae* Pobed. (sect. *Plethiosphace* Benth., subg. *Sclarea* (Moench) Benth.). In contrast, Bentham (1848) and then Menitskiy (1987), based on different characters, included *S. austriaca* and *S. staminea* in different sections, *S.* sect. *Plethiosphace* and sect. *Aethiopsis* Benth., respectively. Given the high importance of the staminal lever mechanism in speciation of the genus *Salvia* (Claßen-Bockhoff & al., 2003, 2004b; Will & Claßen-Bockhoff, 2017), we tested the taxonomic value of *Salvia* FDPD by seeking answers for the following questions: (i) What is the geographic extent of the distribution of *Salvia* FDPD? (ii) How stable is the morphological difference in flower morphology between *S. austriaca*

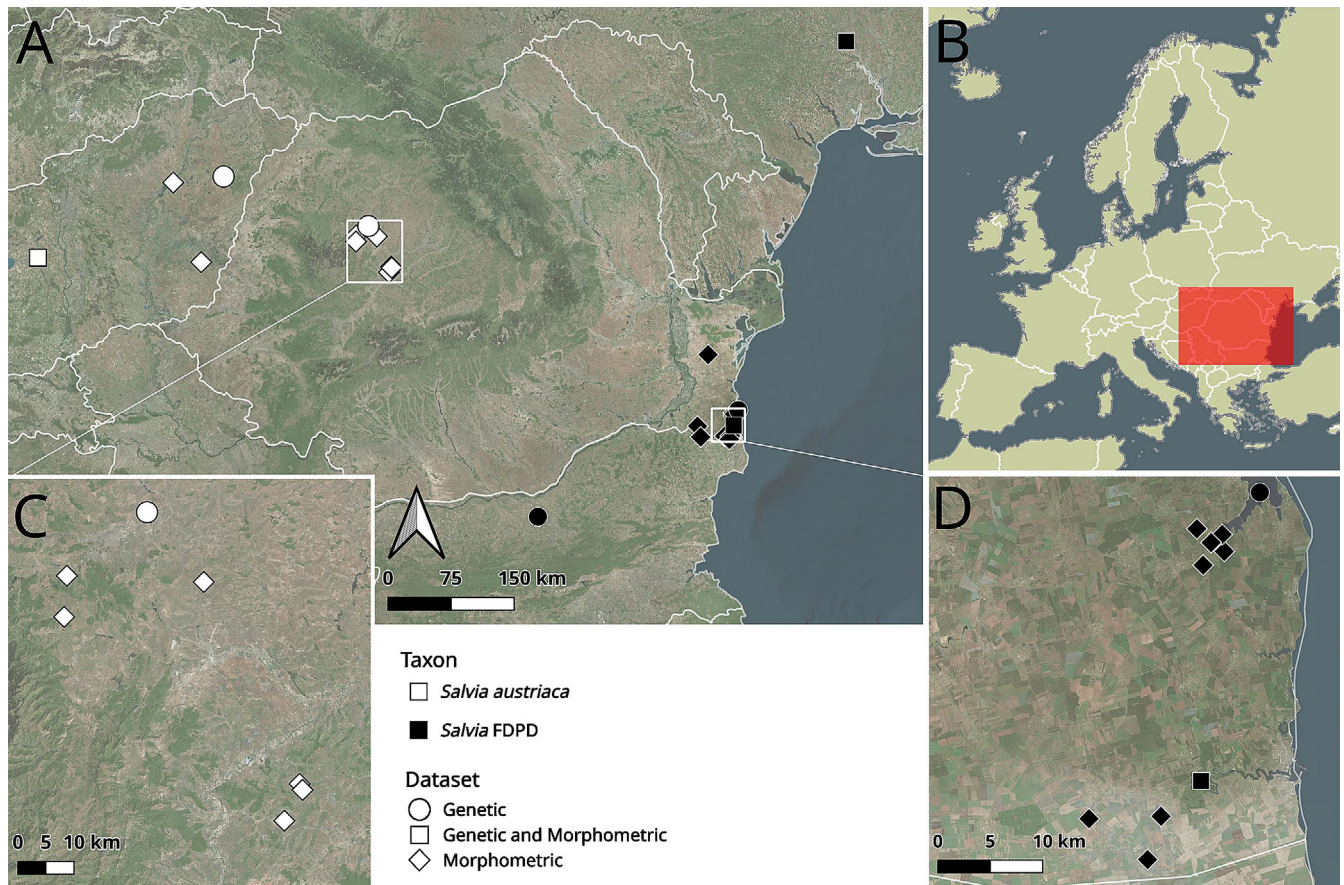
and *Salvia* FDPD? (iii) What is the genetic relationship between *S. austriaca* and *Salvia* FDPD in the rigorous analysis of species delimitation statistics based on sdRAD genomic data? We also examine the taxonomic relationship between *Salvia* FDPD and *S. austriaca* var. *perlanata* Nyár., a variety described by Nyárády (1942) from SE Romania.

## ■ MATERIALS AND METHODS

**Collection of distribution data.** — It is sometimes impossible to confidently study the morphology of fragile floral macrostructures in herbarium specimens, because spatial structures usually get damaged during pressing (Chen & al., 2018). Given the fragility of these morphological characters in *Salvia* flowers, we restricted our screening for occurrence records to public image repositories of plants with a geographic tag of the location where the picture was taken. Therefore, we screened large global ([www.inaturalist.org](http://www.inaturalist.org)) and local ([www.botanikaforum.com](http://www.botanikaforum.com); [www.naturgucker.de](http://www.naturgucker.de); [www.plantarium.ru](http://www.plantarium.ru)) image repositories. We were looking for close-up pictures on *Salvia* specimens registered as *S. austriaca* and checked the staminal lever type—whether it is in a dorsal or lateral

position. Online georeferenced photos were considered only if morphological keys regarding the position of the upper connective arms of the stamens could be unequivocally identified. The observations that could also be geolocated were collected and—whenever exact locality information was given—georeferenced and used for producing a map of observations in QGIS v.3.4 ([www.qgis.org](http://www.qgis.org)). For geographic coordinates of each observation, see suppl. Table S1. Delimitation of the steppe zone on our map follows Lavrenko & al. (1991).

**Taxon sampling for morphometrics.** — Morphological measurements were taken from a total of 64 live *Salvia* specimens: 35 specimens of *Salvia* FDPD (a total of eight populations from Romania and one from Ukraine) and 29 specimens of *S. austriaca* (a total of six populations from Romania, and three from Hungary) (Fig. 1). Populations were more than 1 km apart and separated by some kind of physical barrier, like extended forests, rivers or lakes. On each specimen, four quantitative vegetative traits were measured: stem height, number of stem-leaf pairs, length of rosette-leaf blade, and length of petiole. Furthermore, between one and six live flowers were collected from each specimen and digitized on millimeter paper on site. Following this, stamens were excised from every flower and digitized on millimeter paper. On the



**Fig. 1.** A, Spatial distribution of sampled *Salvia* L. populations; B, Study area highlighted in red on a map of Europe; C, Transylvanian sites enlarged; D, Dobrogean sites enlarged. — White, *Salvia austriaca* Jacq. s.str.; black, *Salvia* FDPD; circle, genetic samples; diamond, morphometric samples; square: both genetic and morphometric samples; white lines represent country borders.

digitized plant material we measured six quantitative floral traits using the software ImageJ v.1.53k (Schneider & al., 2012) (traits were selected based on the study of Thimm, 2008): absolute length of the abaxial lever arm, distance between joint and pollen-sacs, absolute length of the adaxial lever arm (Fig. 2), length of corolla, height of corolla, and length of calyx-tube.

**Statistical analyses of morphometric data.** — We compared the vegetative traits between *Salvia austriaca* and *Salvia* FDPD using linear models, except for the number of stem-leaf pairs, where we used a generalized linear model. In the case of floral traits, we used linear mixed-effects models to compare specimen mean values of *S. austriaca* and *Salvia* FDPD. For each trait a separate model was built: morphological traits were included as response variables, taxon identity as explanatory variable, and sampled individual as random effect. Linear mixed-effects modeling was applied using the “lmer” function from the *lme4* v.1.1-30 package (Bates & al., 2015). Model construction was followed by model validation by inspecting normality and homogeneity of residuals. In order to visualize the multi-trait based differentiation between *S. austriaca* and *Salvia* FDPD, we performed a principal component analysis (PCA) on the six floral traits of individuals where no missing data were present, followed by plotting the specimens by the first two PC axes using the *factoextra* v.1.0.7 (Kassambara & Mundt, 2019) and *FactoMineR* v.2.4 (Lê & al., 2008) packages. The PCA biplot was constructed using the package *ggplot2* v.3.3.6 (Wickham, 2016). All statistical analyses were performed in the R v.3.6.0 statistical environment (R Core Team, 2019).

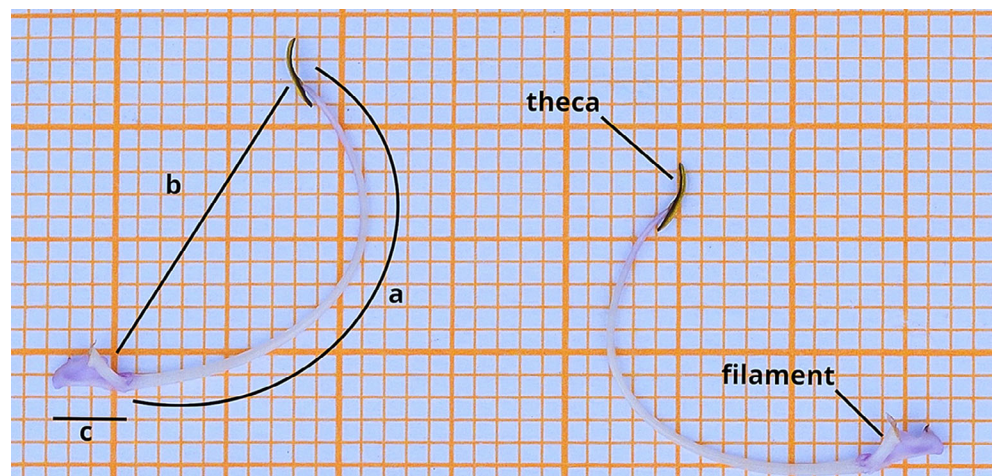
**DNA extraction, sequencing and SNP calling.** — Samples were collected for genetic analysis from four populations of *Salvia* FDPD and three populations of *S. austriaca* (Fig. 1, Appendix 1). Two populations of *Salvia nutans* L. and one population of both *Salvia nemorosa* L. and *Salvia pratensis* L. (Appendix 1) were included as outgroups and to compare the phylogenetic distance between our focal taxa and among these widely accepted species-level taxa. Similar to *S. austriaca*, these outgroup taxa are included in *S.* sect.

*Plethiosphace*, and their close phylogenetic proximity is supported by molecular evidence (Will & Claßen-Bockhoff, 2017; Kriebel & al., 2019; Rose & al., 2021). From each population, one or two individuals were sampled in the field by collecting leaf material into silica-gel. DNA was extracted from silica-gel dried samples using the E.Z.N.A. Plant DNA DS Mini Kit (Omega Bio-tek, Norcross, Georgia, U.S.A.). The quality of genomic DNA extracts was evaluated on a 1% agarose gel and quantified using a Qubit 3.0 fluorometer (ThermoFisher Scientific, Waltham, Massachusetts, U.S.A.).

We implemented a RADseq (Baird & al., 2008) protocol following Paun & al. (2016) and Bateman & al. (2018). Given the relatively small genome of *Salvia* species (Leitch & al., 2019), we used 210 ng DNA per individual in the initial restriction digest step using the relatively frequent-cutter enzyme *PstI* (New England Biolabs, Ipswich, Massachusetts, U.S.A.). The P1-ligated DNA fragments were sheared with a Bioruptor Pico (Diagenode, Seraing, Belgium) for five cycles of 30 s “on”, 30 s “off” at high power setting. The final library was sequenced at UD-GenoMed Medical Genomic Technologies (Debrecen, Hungary) on an Illumina NextSeq platform using a mid-output kit producing 150 bp single-end reads.

The library was demultiplexed and quality filtered using the “process\_radtags” pipeline from the software STACKS v.2.2 (Catchen & al., 2011). After demultiplexing and quality filtering, we retained  $1.05 \pm 0.29$  million reads per individual. The reads were then mapped to the reference genome of *Salvia splendens* Ker Gawl. (Dong & al., 2018) with BWA v.0.7.12 (Li & Durbin, 2009) using default parameters. Mapped reads were processed using the “ref\_map.pl” script, and one random SNP per locus was exported with the “populations” module in STACKS v.2.2. Finally, using VCFtools v.0.1.13 (Danecek & al., 2011), we only kept SNPs that were present in at least 80% of the individuals, yielding 2911 virtually unlinked SNPs in total.

**Phylogenomic analyses, species delimitation and divergence dating.** — We assessed the phylogenetic and taxonomic relationships between our samples using three approaches: (1) maximum-likelihood phylogenetic tree



**Fig. 2.** Quantitative floral traits measured: absolute length of the abaxial lever arm (a), distance between joint and pollen-sacs (b), absolute length of the adaxial lever arm (c). Photo: Gabriel Gigea.

reconstruction as implemented in RAxML v.8.2.12 (Stamatakis, 2006); (2) multivariate genetic structure analysis using discriminant analysis of principal components (DAPC) as implemented in the R package *adegenet* (Jombart & Ahmed, 2011); (3) Bayes factor species delimitation method (BFD) (Leaché & al., 2014) as implemented in the BEAST v.2.5.0 package (Bouckaert & al., 2014) add-on SNAPP v.1.4.2 (Bryant & al., 2012); and a dated species-tree reconstruction using coalescence as implemented in SNAPP. As genome-wide SNPs can only be used in a maximum likelihood tree search with ascertainment bias correction option, we removed invariant sites from our dataset using the *phrynomics* v.2.0 package (<https://github.com/bbanbury/phrynomics.git>; accessed: 11 Jan 2020) in R (keeping 1884 SNPs). RAxML was run using this dataset with the GTR + CAT model of molecular evolution (Stamatakis, 2006). The statistical robustness of our obtained phylogenetic tree was assessed via non-parametric bootstrap (BS) using the automatic option in RAxML (i.e., the pseudo-replication was carried on until convergence as implemented in the extended majority-rule consensus tree option of the bootstrap convergence criterion).

We used *k*-means clustering and DAPC to define a priori groups to infer genetic structure using a multivariate method and for downstream analyses. We used all PC axes for *k*-means clustering and chose four grouping schemes ( $K = 4–7$ ) with the lowest Bayesian information criterion (BIC) scores. In the DAPC analysis, we included the first four PC-s to avoid over-fitting as inferred from a-scores associated with the number of retained PC-s, then we retained the first two discriminant functions.

Next, we compared models of species delimitation within the “*Salvia austriaca* group” (i.e., *S. austriaca* s.str. and *Salvia* FDPD) using the BFD method. Our null model considered all individuals in the two morphological groups within the *Salvia austriaca* group to be the same species, whereas the alternative model split the two morphological groups into separate species. We ran four path sampling analyses in total, two independent repetitions for both the null and the alternative models, using  $\alpha = 0.3$  for 200,000 Markov chain Monte Carlo (MCMC) iterations with 20% burn-in, 10,000 pre-burn-in, and for 100 steps in each run. We calculated the Bayes factor (2lnBF) from marginal likelihood estimates (lnMLE) for the alternative models and compared them to the null models following Kass & Raftery (1995).

In order to provide a dated species-tree, we ran SNAPP by adopting the options described in Stange & al. (2018). We conducted two runs of 1,000,000 iterations sampling every 500th. To generate the SNAPP input files, we used the Ruby script “snapp\_prep.rb” of M. Matschiner ([https://github.com/mratschiner/snapp\\_prep](https://github.com/mratschiner/snapp_prep), accessed: 5 Jan 2020). We used one constraint timing the root to a mean of 3.88 mya (million years ago) with a 0.2 offset and standard deviation of 1 based on the estimated origin of the most-recent common ancestor (MRCA) of *Salvia austriaca*, *S. nutans* and *S. pratensis* estimated by Kriebel & al. (2019). Convergence in all SNAPP analyses was evaluated through effective sample size (ESS)

values and trace plots in the software Tracer v.1.6 (Rambaut & Drummond, 2013).

## ■ RESULTS

### Distribution of the taxa of the *Salvia austriaca* group. —

Our collection of occurrence data for taxa of the *Salvia austriaca* group with two different pollen deposition modes (i.e., dorsal and lateral deposition) indicates strong eco-geographical differentiation with an almost complete vicariance of the two forms (Fig. 3). The taxon with lateral pollen deposition (i.e., *S. austriaca* s.str.) occupies the forest-steppe zone of the Pannonian basin, Transylvanian basin, the Pontic steppe, the SE part of Crimea, and the Balkans, whereas *Salvia* FDPD is confined to the short-grass steppe zone (or true steppe zone) as defined by Lavrenko & al. (1991) of the Pontic steppe. The westernmost observations of *Salvia* FDPD are from northern Bulgaria (settlement: Bozhurluka), whereas the easternmost is from the city of Rostov (Russia).

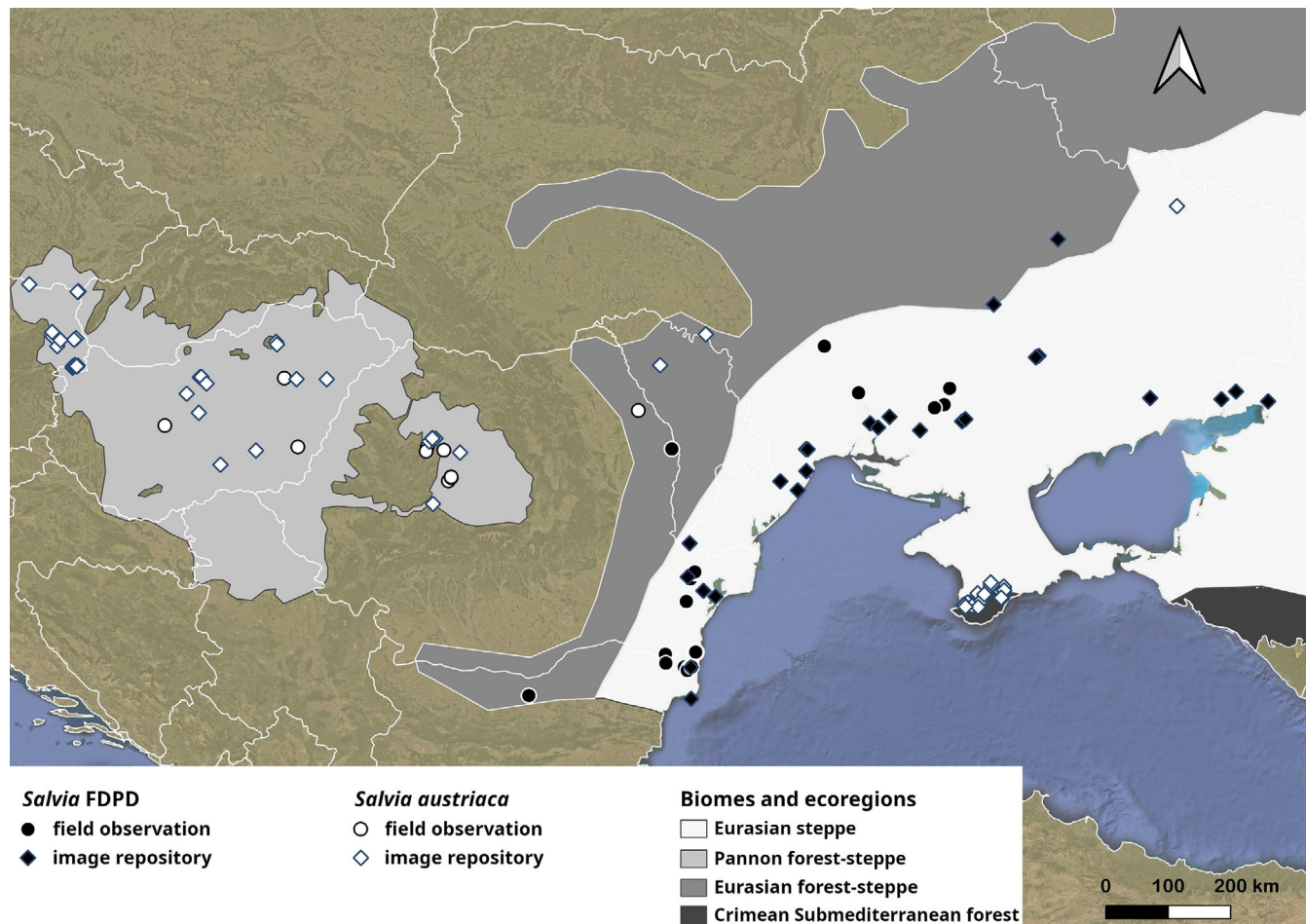
### Morphological differentiation between *Salvia austriaca* and *Salvia* FDPD. —

While processing the collected samples, we noted that several individuals exhibited reduced, shortened staminal levers, indicating that *Salvia* FDPD might be exhibiting gynodioecy, a dimorphic sexual system where populations are composed of both hermaphroditic and female individuals. Gynodioecy is known in *S. austriaca*, *S. candidissima*, *S. cyanescens* and *S. pratensis* as well (Kaul, 1988; Zhang & Claßen-Bockhoff, 2019). Measurements of quantitative floral traits were performed only on hermaphroditic flowers. Based on the results of the linear mixed-effects models, five of the six investigated floral traits differed significantly between the two taxa: calyx length ( $\chi^2 = 34.70$ ,  $p < 0.0001$ ), corolla height ( $\chi^2 = 6.18$ ,  $p = 0.0129$ ), corolla length ( $\chi^2 = 107.64$ ,  $p < 0.0001$ ), absolute length of the abaxial lever arm ( $\chi^2 = 269.67$ ,  $p < 0.0001$ ), and absolute length of the adaxial lever arm ( $\chi^2 = 10.25$ ,  $p = 0.0014$ ) had significantly greater values for *Salvia* FDPD compared to *S. austriaca* s.str. (Table 1). In contrast, we only observed a marginally significant difference between the number of stem leaf pairs ( $\chi^2 = 6.11$ ,  $p = 0.0134$ ), where the higher values also belonged to *Salvia* FDPD. There was no difference in the other vegetative traits between the two taxa (data not shown).

In the PCA, 74.6% of total variation in flower-traits was explained by the first two PC-axes (PC1 – 54.0%, PC2 – 20.6%). However, the two focal taxa were differentiated only along the first PC-axis (Fig. 4).

### Phylogenomics, species delimitation and divergence dating. —

Our maximum likelihood (ML) phylogenetic tree reconstruction based on unlinked genome-wide SNPs produced a well-resolved phylogram (Fig. 5A,B), in which samples of the same a priori classified taxa are monophyletic and strongly supported (BS = 100), but some of the tip nodes received moderate support (i.e.,  $70 \leq BS \leq 99$ ). The topology strongly supports the separation of the *Salvia austriaca* group from the rest of the samples (our a priori outgroup) along the



**Fig. 3.** Distribution of *Salvia* FDPD and *S. austriaca* Jacq. Delimitation of the steppe zone follows Lavrenko & al. (1991). Delimitation of the Crimean Submediterranean forest ecoregion follows Olson & al. (2001). For geographic coordinates of each observation, see suppl. Table S1.

**Table 1.** Biometric data (floral and vegetative) of morphological characters for *Salvia austriaca* Jacq. (N = 29) and *Salvia* FDPD (N = 35).

Variable	<i>Salvia austriaca</i>	<i>Salvia</i> FDPD
Floral traits	Values in mm	Values in mm
ch* (corolla height)	(6.24) 10.69 ± 0.55 (17)	(8.36) 12.38 ± 0.54 (21.33)
cl* (corolla length)	(14.27) 17.7 ± 0.26 (20.4)	(19) 22.75 ± 0.4 (26.34)
a* (absolute length of the abaxial lever arm)	(12.79) 14.44 ± 0.22 (17.23)	(16.04) 20.71 ± 0.31 (24)
b (distance between joint and pollen-sacs)	(11.25) 14.06 ± 0.35 (18.57)	(9.69) 13.69 ± 0.39 (18.27)
c* (absolute length of the adaxial lever arm)	(2.15) 2.72 ± 0.07 (3.54)	(2.23) 3.06 ± 0.09 (4.04)
cal* (calyx length)	(7.4) 8.22 ± 0.15 (9.86)	(8.05) 9.54 ± 0.15 (10.82)
Vegetative traits	Values in cm, except stlp	Values in cm, except stlp
sth (stem height)	(24) 56.23 ± 2.27 (79)	(8) 61.2 ± 3.65 (104)
stlp* (number of stem leaf pairs)	(1) 1.53 ± 0.14 (3)	(1) 1.69 ± 0.09 (3)
lpet (length of petiole of rosette leaf)	(1.8) 3.67 ± 0.32 (7.5)	(1) 4.46 ± 0.44 (12)
lobl (length of leaf blade of rosette leaf)	(5.2) 11.61 ± 0.53 (14.5)	(8.5) 13.83 ± 0.77 (25)

Values represent (minimum) mean ± standard error (maximum).

\* indicates significant differences between measurements ( $p < 0.05$ ).

longest branch on the tree (Fig. 5A). Within the latter clade, *S. nutans* is sister to the crown-clade formed by *S. pratensis* and *S. nemorosa*.

The species tree reconstruction based on coalescence analysis (Fig. 6) yielded a fully congruent topology to the ML-tree, in which taxa of the *Salvia austriaca* group are sister to the lineage formed by *S. nutans*, *S. pratensis* and *S. nemorosa*. The next split within this lineage is much younger and is represented by *S. nutans*, which separated from the other two species during the Middle Pleistocene. The following isolation event is marked by the separation of the two taxa of the *Salvia austriaca* group, which was followed by the split between *S. nemorosa* and *S. pratensis*. All nodes were fully supported in the species tree (posterior probability [PP] = 1.0). The divergence dating analysis indicated that *S. austriaca* s.str. and *Salvia* FDPD diverged 0.197 mya (95% confidence interval [CI]: 0.302–0.087 mya) (Fig. 6).

The DAPC analysis attained the lowest BIC scores to models with 5 and 7 clusters (suppl. Fig. S1). At  $K = 5$ , all individuals were assigned into a cluster—according to existing classification and splitting the taxa of the *Salvia austriaca* group—with full posterior probability (Fig. 5C). Interestingly, models with higher number of clusters assigned individuals from *S. nemorosa* and *S. pratensis* into separate clusters, whereas the clusters formed by samples of the taxa of the *Salvia austriaca* group were not divided (suppl. Fig. S2).

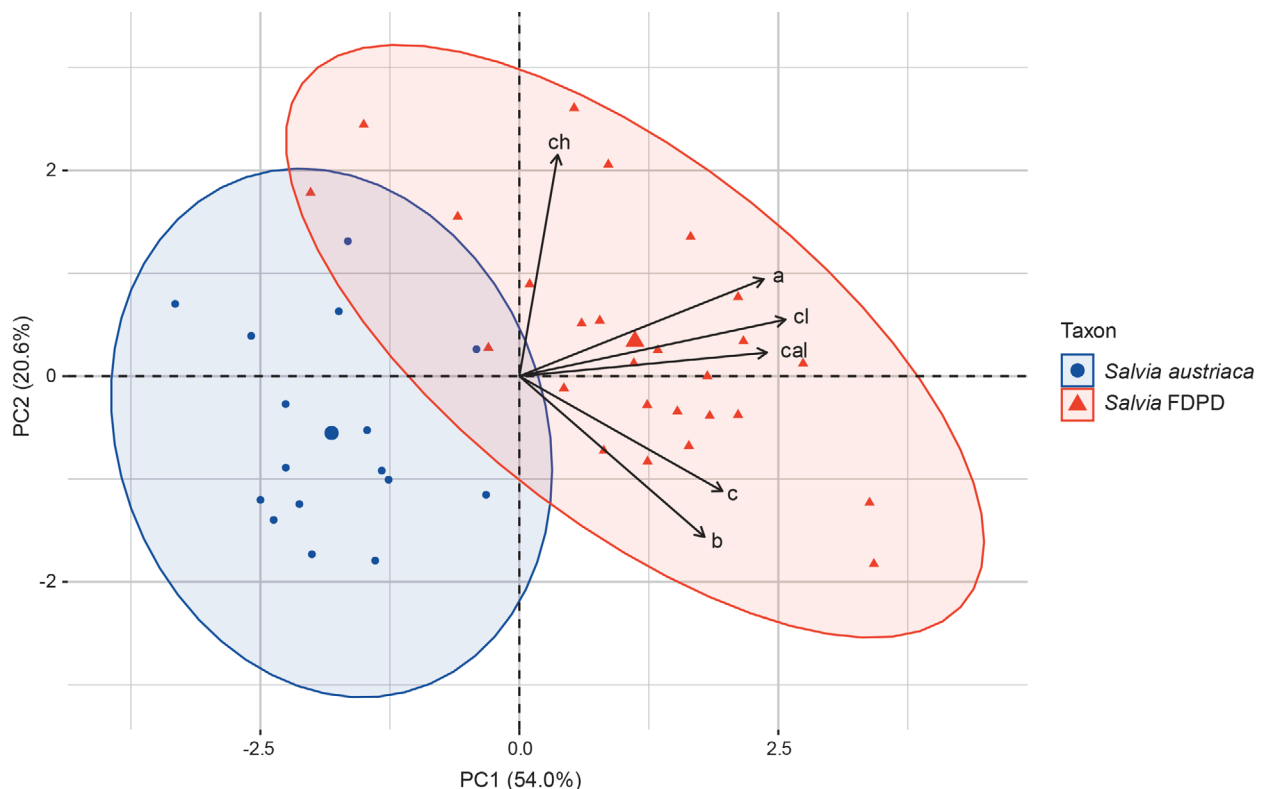
Similarly, at  $K = 4$ , *S. nemorosa* and *S. pratensis* were lumped together, whereas *S. austriaca* s.str. and *Salvia* FDPD were still conceived as separate groups (suppl. Fig. S2).

The BFD favored the alternative model, which considered *Salvia austriaca* s.str. and *Salvia* FDPD as separate entities. The null model, grouping all individuals into one species within the *Salvia austriaca* group (i.e., *S. austriaca* s.l.), performed worse in both repetitions with a mean BF value of 348.67 in favor of the alternative model (Table 2) across the two repetitions. This is considered decisive according to Kass & Raftery (1995). Mean ESS was 336.34 (standard deviation = 200.74) across both models and all steps and repetitions, implying that the chain length was adequate to reach convergence.

## DISCUSSION

### Distribution of the taxa of the *Salvia austriaca* group. —

The strong eco-geographical differentiation with an almost complete vicariance of the two forms (Fig. 3) argues for a significant and recent separation of the two studied taxa. It is very likely that these taxa have been recently isolated and strongly adapted to live in different ecological conditions. These might be determined by the different climatic conditions of the more humid forest-steppe and the dryer true steppe zone (Erdős & al., 2018). Nevertheless, we also have to note the almost



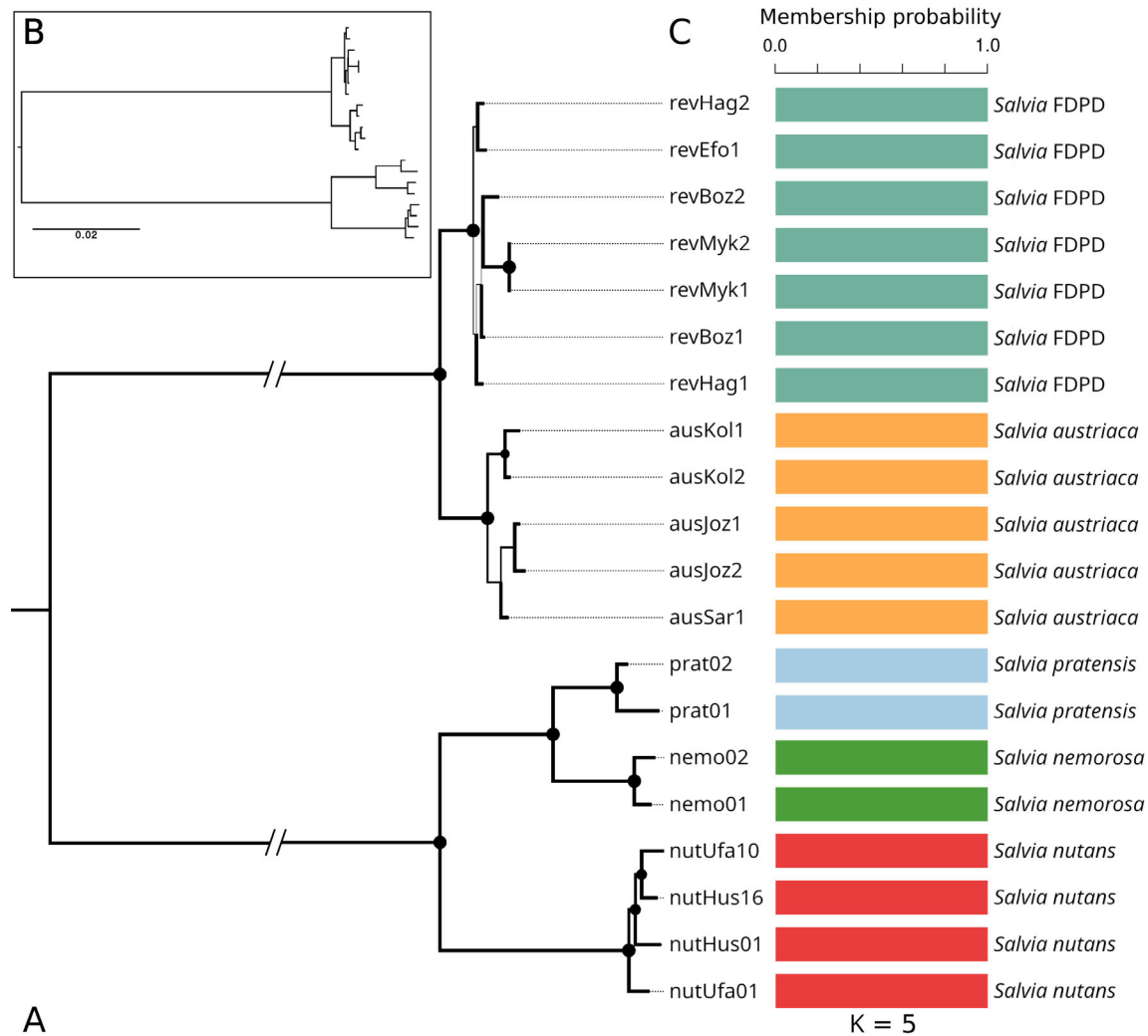
**Fig. 4.** PCA biplot of *Salvia* L. samples based on six quantitative floral characters, where the arrows represent loadings. Blue circles, *S. austriaca* Jacq. (N = 16); red triangles, *Salvia* FDPD (N = 26); ch, corolla height; cl, corolla length; cal, calyx length; a, absolute length of the abaxial lever arm; b, distance between joint and pollen-sacs; c, absolute length of the adaxial lever arm.

total absence of *Salvia pratensis*, a species with dorsal pollen deposition, from the whole true steppe region (Meusel & Jäger, 1992). Therefore, we cannot exclude the role of inter-specific competition in shaping the current distribution of the taxa examined.

Although our westernmost observation of *Salvia* FDPD (Bulgaria: Bozhurluka) lays within the forest-steppe zone, this locality is well-known to support several species characteristic of the true steppe zone (e.g., *Paeonia tenuifolia* L., *Salvia nutans*, *Adonis volgensis* Stev., etc.; pers. obs.), and, thus, can possibly be regarded as an extrazonal enclave of the true steppe zone. The easternmost observation (Russia: city of Rostov) lays well-within the true steppe zone, which expands further to the northeast. Given the availability of habitats, we

cannot fully exclude that the distribution area of *Salvia* FDPD spreads further to the east. However, the distribution—as currently known—can be described as characteristic of the true steppe zone of the Pontic steppe.

Highly interesting are the observations of *Salvia austriaca* s.str. from the southeastern part of Crimea, where a sub-Mediterranean climate and vegetation is prevailing (Cordova, 2007). This observation further argues for a strong ecogeographic differentiation between the two taxa. Having said this, we need to note the lack of genetic samples from this part of the area, which makes it necessary to question the equality of the *Salvia* form with lateral pollen deposition in Crimea to *S. austriaca* s.str. Nevertheless, floral morphology does not argue against conspecificity.

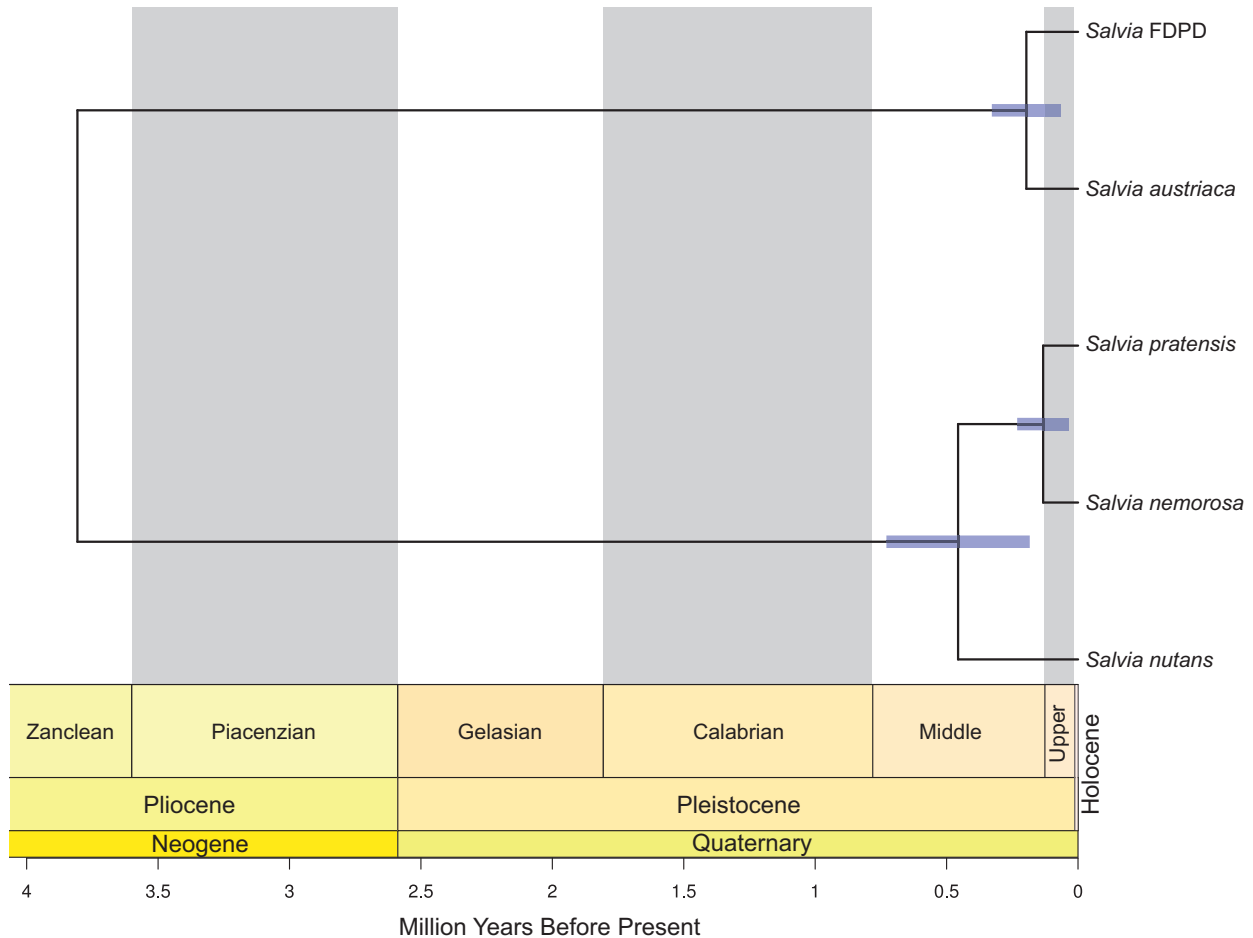


**Fig. 5.** Phylogenetic relationships between the studied taxa. **A**, Phylogenetic tree shown as a phylogram resulting from a ML search in RAxML using the ascertainment bias correction option. Circles at nodes represent maximum bootstrap support (BS = 100), thick branches represent BS  $\geq$  70, and thin branches represent BS < 70, where branch thickness increases in proportion to bootstrap support. The two main internal branches were shortened to condense the figure. **B**, Topology of the untruncated ML phylogram. **C**, Posterior membership probabilities based on the first two discriminant functions of four PCs using group assignment from *k*-means clustering at *K* = 5 in a DAPC analysis. The order of individuals follows the order of the tips of the phylogenetic tree. The beginning of an individual's ID refers to the taxon: rev, *Salvia* FDPD; aus, *S. austriaca* Jacq. s.str.; prat, *S. pratensis* L.; nemo, *S. nemorosa* L.; nut, *S. nutans* L.

If there is a striking discontinuity in the range of *Salvia austriaca* s.str., it must date back to the warmer and wetter periods of the Eemian interglacial (130,000–115,000 years ago; Neem Community Members, 2013) or to the Holocene climate optimum (7000–8000 years ago). As shown by several authors (Conea, 1970; Ghenea & Rădan, 1993; Van Andel & Tzedakis, 1996; Markova, 2000; Tomescu, 2000; Lindner & al., 2004; Magyari & al., 2010; Timar-Gabor & al., 2011; Bălescu, & al., 2020), there was a continuity of forest-steppe habitats between Crimea and central Europe during these

periods, thus implying that *S. austriaca* s.str. is currently in refugium in Crimea, possibly since 115,000 years ago.

**Morphological differentiation between *Salvia austriaca* s.str. and *Salvia* FDPD.** — The morphometric analysis of the two taxa revealed several statistically significant differences in their floral morphology based on multiple characters: calyx length, corolla height, corolla length, the absolute length of the abaxial lever arm, the absolute length of the adaxial lever arm are considerably smaller in *S. austriaca* s.str. The number of stem leaf pairs is somewhat larger in *Salvia* FDPD,



**Fig. 6.** The dated species tree of the studied *Salvia* L. taxa based on the best model of species delimitation as inferred by SNAPP. Blue bars represent 95% confidence intervals. The geological time chart below the chronogram is presented according to the International Union of Stratigraphy.

**Table 2.** Marginal likelihood estimates (lnMLE) and Bayes factors (2lnBF) for alternative delimitation hypotheses: grouping *Salvia austriaca* Jacq. and *Salvia* FDPD individuals into one species, *S. austriaca* s.l., or splitting them into two species.

Repetition	Delimitation	lnMLE	2lnBF
1	1 species: <i>nemorosa</i> , <i>pratensis</i> , <i>nutans</i> , <i>austriaca</i> s.l.	-17,754.2	–
1	2 species: <i>nemorosa</i> , <i>pratensis</i> , <i>nutans</i> , <i>austriaca</i> , FDPD	-17,686.4	135.6
2	1 species: <i>nemorosa</i> , <i>pratensis</i> , <i>nutans</i> , <i>austriaca</i> s.l.	-17,968.3	–
2	2 species: <i>nemorosa</i> , <i>pratensis</i> , <i>nutans</i> , <i>austriaca</i> , FDPD	-17,687.5	561.7

Both scenarios were run in two independent analyses (Repetitions 1 and 2).

whereas the other measured vegetative parts are not morphologically different. This hints at a strong selection on flower morphology, which may be the main driver behind the differentiation of these two taxa.

**Phylogenomics, species delimitation and divergence dating.** — Our phylogenomic results obtained by utilizing a powerful genomic approach, RADseq (Andrews & al., 2016), depict a clear picture of the relationship between the studied *Salvia* taxa. All a priori assigned taxa were fully recovered as monophyletic entities in the ML tree (Fig. 5A,B), which justifies their lumping for the species-tree estimation (Fig. 6). Furthermore, it is also a fine demonstration of how successful a genomic approach can be in untangling phylogenetic relationships between taxa with shallow divergence: whereas phylogenetic analyses based on candidate-genes (Walker & Sytsma, 2007; Takano & Okada, 2011; Jenks & al., 2013; Li & al., 2013; Walker & al., 2015; Will & Claßen-Bockhoff, 2017; Hu & al., 2018) only provided resolution between the main clades within *Salvia* with a lack of species-level resolution, we produced a well-resolved species-level phylogeny by utilizing RADseq.

The multivariate analysis of genetic structure mirrors this finding as it found the same number of groups as the a priori classification of our samples. Other plausible groupings did not find clusters within the focal taxa, which argues for their homogeneous genetic make-up. Even though using only one secondary calibration point is suboptimal for dating purposes, the dated species-tree reconstruction provides an unprecedented insight into the evolutionary history of our examined taxa. This is especially interesting, as this divergence is dated earlier than that of *Salvia nemorosa* and *S. pratensis* (0.197 mya vs. 0.133 mya), two taxa that are widely accepted as separate species. As suggested by the divergence dating analysis, our two focal taxa most likely split around 200,000 years ago, approximately at the beginning of the Penultimate Glacial Period and continued their divergent evolution during the Eemian interglacial. The expansion-contraction cycles of alternating steppe and woodland vegetation in this period (Kajtoch & al., 2016) could have provided the conditions facilitating the eco-geographical differentiation described above.

Finally, our species delimitation analysis based on the BFD fully supports the separation of the two taxa of the *Salvia austriaca* group (i.e., *S. austriaca* s.str. and *Salvia* FDPD) at the species level. Therefore, taking all above arguments, the eco-geographical, the morphological, the genetic and the explicit species delimitation results into consideration, we are confident to describe *Salvia* FDPD as a new species to science.

## ■ TAXONOMIC TREATMENT

Although Nyárady (1942) described a *Salvia* taxon from one of the locations of our new species in Dobrogea (Bulgaria), he considered it at the level of variety within *Salvia austriaca*. After careful examination of the specimen (CL

No. 193909!), we came to the conclusion that it must represent *Salvia* FDPD. However, Nyárady did not make a note on the floral morphology of his variety; he only emphasized the wooly abaxial surface of the basal leaves, the larger calyx, and the lanuginose inflorescence. Article 11.2. of the *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code)* establishes that a name has no priority outside the rank at which it is published. Given that the epithet ‘*perlanata*’ does not grasp a characteristic feature of the new taxon, and since we are not obliged to use the above epithet, we here describe *Salvia* FDPD with the following new name at species rank, but refer to Nyárady’s variety as its heterotypic synonym.

***Salvia revelata* Mátis & A.Z.Szabó, sp. nov.** – Holotype: ROMANIA. Pădurea Dumbrăveni Reserve (Constanța County, SV Dobrogea), on moderately steep (20°), southwest-facing slope, with calcareous, rocky substrate, in a grassland enclave (Ponto-Sarmatic steppe habitat), surrounded by *Carpinus orientalis* dominated forest, N 43.977070°, E 27.978860°, 77 m a.s.l., 16 May 2015, Mátis & al. (CL No. 668802).

= *Salvia austriaca* var. *perlanata* Nyár. in Acta Univ. Szeged., Sect. Sci. Nat., Pars Bot. 1: 43. 1942 – Syntypes: “Hab. Dobrogea, distr. Caliacra. 1. colline ‘Movila’ prope pagum Ghiarsuiuciu, alt. cca 110 m s. m., 14. VI. 1925 [not found]; 2. In campis litoralibus inter pagos Caiabei-kiöi et Sürtükiöi, alt. cca 40–50 m s. m., 14. VII. 1923 [CL No. 193909!]”.

For an image of the holotype, see Fig. 7.

**Diagnosis.** – *Salvia revelata* is overall very similar in habitus to *S. austriaca*, hence the persistent confusion with the latter species. The most significant distinguishing features are all concentrated in the reproductive structures (Table 1, Fig. 8). *Salvia revelata* has a significantly longer corolla and calyx, and longer upper (abaxial) and lower (adaxial) connective arms of the stamens. Even if the absolute length of the abaxial arm is longer, the distance between joint and theca on the upper connective arm is similar to that of *S. austriaca* because the arm is more curved, enveloped by the falcate upper lip. Unlike the free upper connective arms of *S. austriaca*, the upper connective arms of *S. revelata* are postgenitally fused along their thecae, and, thus, they act as a single functional unit, a feature known to enhance pollen removal (Ren & Tang, 2010). There are also significant structural differences regarding the position of the upper connective arms: in *S. austriaca* they are tilted outward relative to the main symmetry axis of the flower, and, thus, they are laterally protruding from the flower in the transversal plane, while in *S. revelata* they are held together by the upper lip that encloses them, thus they have a vertical position in the median plane of the flower. Regarding lever mechanism and pollination biology, in *S. revelata* pollen is deposited on the dorsal part of the insect by a downward movement of the upper connective arms, resulting in nototribic (dorsal) pollination, whereas in *S. austriaca* pollen is deposited on the flanks of the insect with

a pincer-like movement of the upper connective arms, resulting in plagiotribic (lateral) pollination (Fig. 9).

**Description.** – Plants perennial, herbaceous, up to 100 cm tall. Taproot ± cylindrical. Stem erect, usually simple, or branching in the upper part, quadrangular, with reddish line on the edges, internodes long. Lower part of stem covered with long, simple hairs and long-stipitate glands, the upper part (inflorescence) covered profusely with long, multicellular, stiff hairs, short hairs and many long-stipitate glands. Leaves mostly radical, forming a compact rosette, spreading over the ground, elliptical, ovate or oblong, blade 8–23 cm long, 5–10 cm broad, mostly obtuse, cordate at base, on the margin doubly crenate, toward the petiole often lobed, mid-vein broad, flattened and reddish, glabrous above, more or less tomentose below, covered with short hairs (Fig. 10B) and scattered sessile glands between the veins, petiole shorter than blade, 1–9 cm. Cauline leaves 1–2 (3) pairs, reduced, sessile and elliptical, irregularly lobate and dentate, pubescent below with sessile glands, those right below the inflorescence small and entire at margin. Bracts broadly ovate, entire at the margin, acuminate, as long as or longer than calyx, ciliate at margin, below with long, multicellular hairs and with many glands. Inflorescence simple or with 1–2 pair of branches

shorter than the main axis (Figs. 10A, 11A). Lower verticillasters distant, upper ones approximate, usually with 6 petiolate flowers. Most individuals are hermaphroditic, with flowers containing functional staminal levers (Fig. 11C), but some individuals in every population seem to be functionally females, with flowers in which the stamens are reduced (Figs. 10E, 11B), resulting in a non-functional lever mechanism (gynodioecy). Calyx 8–10 mm long, 7–8 mm broad, bilabiate, upper lip with 3 very small, acuminate teeth, lower with 2 ovate, more pronounced, acuminate teeth, with long hairs on the veins and covered with short hairs, and many glands between the veins (Fig. 10C). Corolla yellowish-white or cream-colored, 19–26 mm long, with height 8–18 mm, with floral tube only shortly exerted from the calyx. Upper lip quite falcate, flattened, on exterior covered abundantly with long-stipitate, magenta-colored glands. Lower lip with elongated, erect lateral lobes and broad, conduplicate, emarginate, unevenly and obtusely toothed, with few magenta-colored glands on the exterior (Fig. 10C). Two versatile, monothebate stamens with very short filament, highly mobile filament-connective joint, upper (abaxial) connective arms very long, 18–24 mm, with vertical position in the central axis of the flower, curved and enveloped by the upper corolla lip, but with fertile thecae long exerted and postgenitally fused (Fig. 11D); shovel-like lower (adaxial) connective arms sterile, short, flattened, 2–4 mm, postgenitally fused at a narrow line, entirely blocking the corolla throat. Style much longer than upper (abaxial) connective arms, long exerted, with unequal stigma lobes. Nutlets slightly trigonous, subglobose, 1–2 mm in diameter, brown, smooth (Fig. 10D).

**Distribution.** – The species is currently known from the Pontic steppe. Even in the forest-steppe ecoregion, it grows exclusively in open steppe patches. One single locality is known from the Balkan mixed forest ecoregion at the boundary of forest steppe: near the Bozhurluka nature reserve in Studena river site of community interest, in pannonic loess and subpannonic steppic grasslands (MEWRB, 2011–2013). Its easternmost known locality is proximate to the River Don estuary (Russia, Rostov Province); the westernmost known distribution point is located in Veliko Tarnovo Province (Bulgaria).

**Habitat and ecology.** – *Salvia revelata* grows in petrophilous steppe habitats on loess and clay, between 40 and 120 m altitude. In Dobrogea it occurs sporadically in Ponto-Sarmatic steppic grassland habitats (Fig. 10A) dominated by *Festuca callieri* (Hack.) Markgr.-Dann., *Koeleria splendens* C.Presl, *Poa angustifolia* L., with *Bothriochloa ischaemum* (L.) Keng and sometimes *Thymus zygioides* Griseb., associated with limestone rocky outcrops and xerophilous forest edges. Other forbs co-occurring with *Salvia revelata* are *Astragalus onobrychis* L., *Taraxacum serotinum* (Waldst. & Kit.) Poir., *Teucrium chamaedrys* L., *Thymus roegneri* K.Koch, but also some rare steppic species, such as *Cytisus jankae* Velen. or *Paeonia tenuifolia*, occur in these grasslands.

**Phenology and pollination.** – Flowering in May–June, fruiting in July. Pollination carried out by bumblebees, with nototribic pollen placement.



**Fig. 7.** Holotype of *Salvia revelata* Mátis & A.Z.Szabó. Photo: Attila Mátis.

**Etymology.** – The specific epithet is from the Latin *revelatus* in the nominative feminine singular, meaning “uncovered”, “revealed”, “disclosed”, referring to the fact that this taxon is no longer cryptic, but has been long hiding in spite of its relatively large distribution.

**Vernacular names.** – Pontic sage (English), понтийско какула/pontijsko kakula (Bulgarian), pontuszi zsálya (Hungarian), jaleş pontic (Romanian), понтический шалфей/ponticheskij šalfej (Russian), понтична шавлія/pontychna šavlija (Ukrainian).

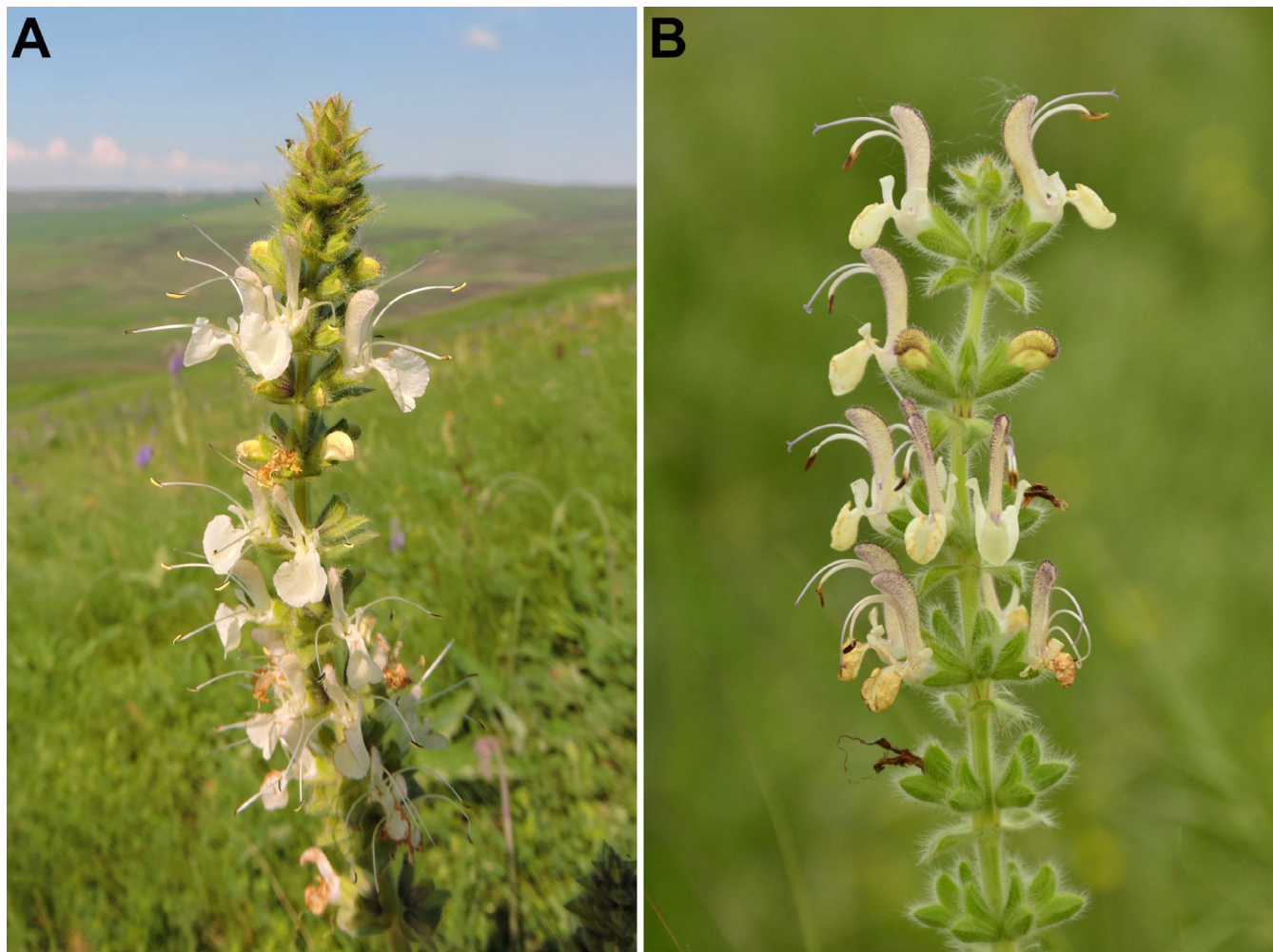
**Preliminary conservation status of IUCN.** – Not evaluated (NE). This species is distributed over a large area from north Bulgaria to Russia, Rostov Province, and is a sporadic species, thus could probably be considered as Least Concern (LC).

## ■ CONCLUSION

Our analyses above have uncovered the existence of a relatively widespread species of *Salvia* in Europe, which has not

been recognized as such for centuries. Its superficial similarity to a common forest-steppe species, *S. austriaca*, may have contributed to its elusiveness. Our analyses also revealed that these two species, *S. austriaca* and *S. revelata*, are closely related, and separated only ca. 200,000 years ago. Additionally, they have an almost complete vicarious distribution with the former species characteristic of the forest-steppe zone, and the latter to the true steppe zone. We believe it is worth analyzing the background of this pattern beyond taxonomy as it can shed some light on the nature of the speciation mechanisms in *Salvia* and give some insight into the natural history of the Pontic steppe.

The staminal lever mechanism in *Salvia* may have evolved for increased precision in pollen deposition and transfer, as variations in shape, size and orientation of the arms allow for site-specific pollen placement on different areas of the pollinator’s body (Claßen-Bockhoff & al., 2003, 2004b). This maximization of pollen-placement precision is similar to pollinia transfer of species of the Orchidaceae Juss. or the Asclepiadaceae Borkh., a feature that allows sharing of pollinators



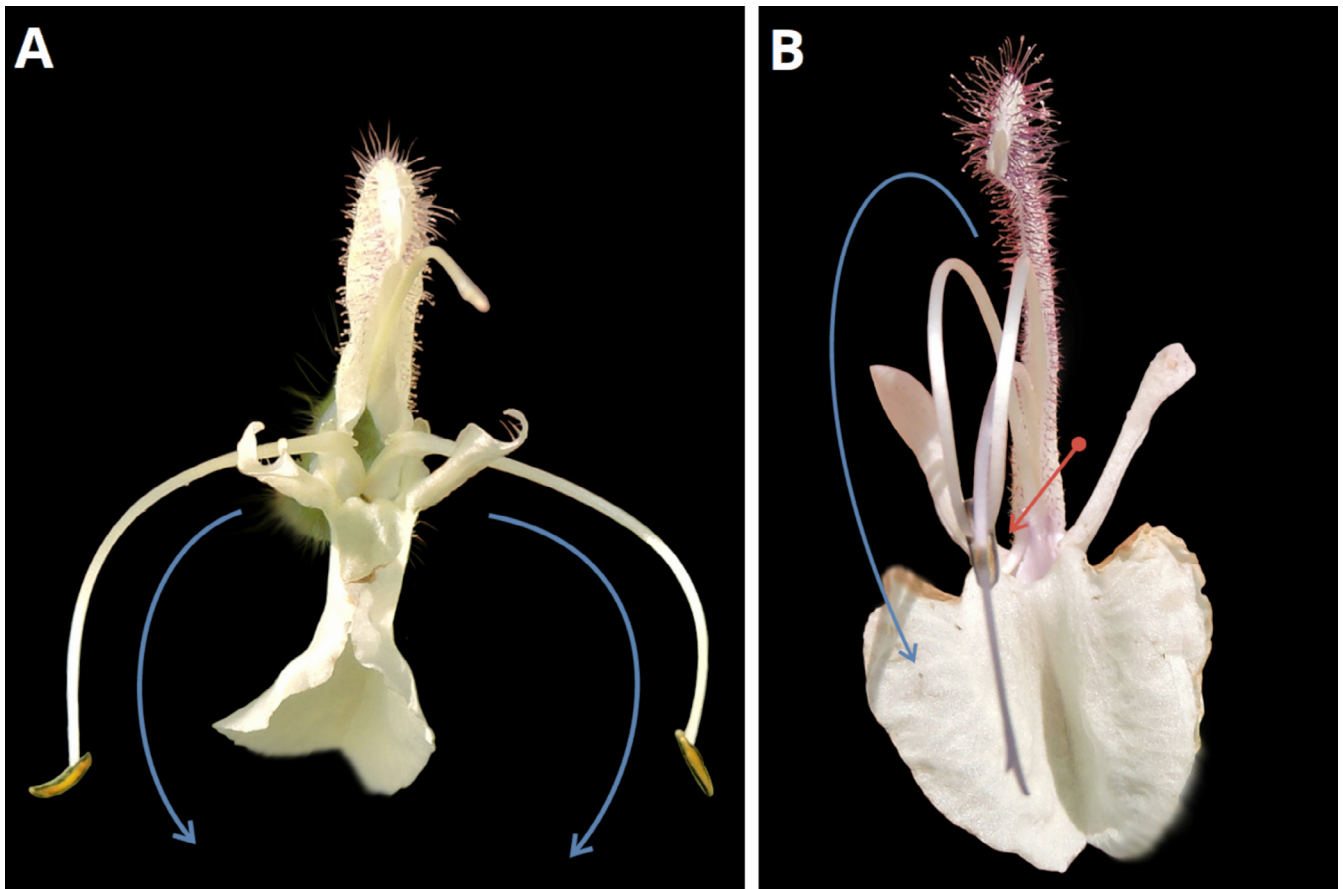
**Fig. 8.** **A**, *Salvia austriaca* Jacq. from Valea Florilor (Transylvania, Romania); **B**, *Salvia revelata* Mátis & A.Z.Szabó from Kapustyne (Mykolaiv Oblast, Ukraine). — Photos: A by Attila Mátis; B by Gábor Sramkó.

by multiple species while avoiding interbreeding or competition (Pauw, 2006), and conform to the “*Pedicularis* type” of mechanical isolation (Grant, 1994), where reproductive isolation is induced by the change in the specific region of pollen deposition on the pollinator’s body. Thus, the staminal lever can ensure mechanical prezygotic isolation between sympatric *Salvia* species (Claßen-Bockhoff & al., 2004b; Celep & al., 2020b) by allowing different co-flowering species to share the same pollinator without interbreeding.

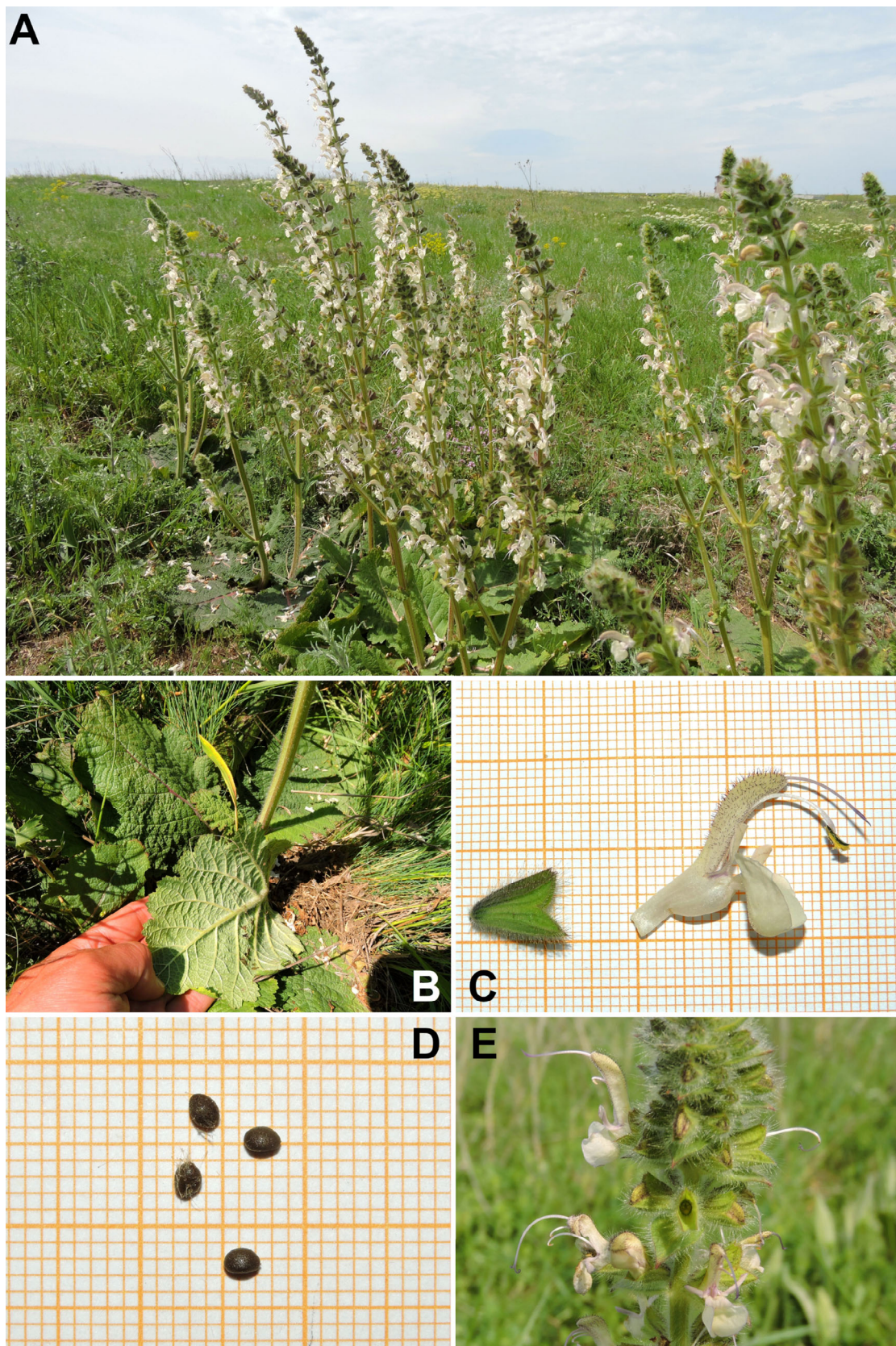
There are three alternative scenarios regarding the speciation mechanism behind the observed difference between *Salvia austriaca* and *S. revelata*. All scenarios rely on the observation that while *S. austriaca* often co-occurs with *S. pratensis* (a species with similar floral proportions and ecological preferences, but different pollen deposition), *S. revelata* does not seem to co-occur with any of the two former species (Fig. 3).

If lateral pollen deposition is the plesiomorphic state in the *Salvia austriaca* s.l. clade (i.e., the form displayed by *S. austriaca* is the ancestral one), when the ancestor came into contact with *S. pratensis*, the lateral placement was maintained to effectively prevent hybridization and allow the two species to co-occur. During a process called “ecological sorting” (e.g., Strong & al., 1979; Waser, 1983; Rummel &

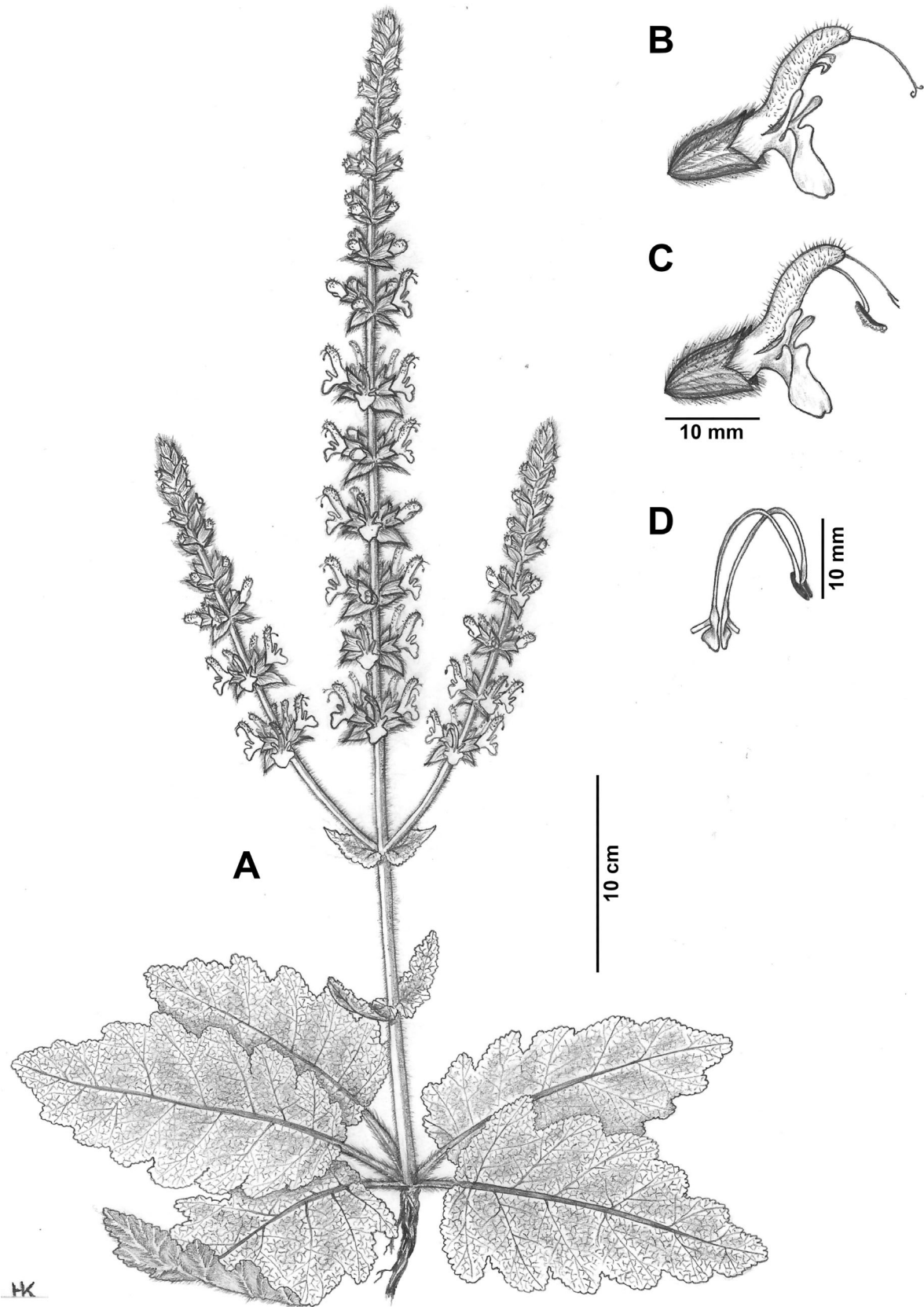
Roughgarden, 1985; Armbruster, 1986; Armbruster & al., 1994; Kraft & al., 2015), reproductive competition would eliminate one of two competing species during community assembly. In this case, the coexistence of *S. austriaca* and *S. pratensis* is possible because they were already “preadapted” (using different parts of the pollinator’s body as a form of mechanical isolation) at the time of their initial contact. It was demonstrated that interspecific competition for pollinators plays a role in ecological sorting in, e.g., *Costus* L. (Schemske, 1981), *Erica* L. (Heystek & Pauw, 2014), *Limnanthes* R.Br. (Briscoe Runquist & al., 2016), *Mimulus* L. (Briscoe Runquist & al., 2016), and *Clarkia* Pursh (Briscoe Runquist & al., 2016; Eisen & Geber, 2018). In the absence of *S. pratensis* in the more arid true steppe zone, the ancestor of *S. revelata* secondarily evolved the dorsal deposition of pollen, possibly due to the lack of evolutionary pressure coming from reproductive interference. However, in this scenario we have to assume that either there is an evolutionary disadvantage of lateral pollen deposition in *Salvia*—judged by the scarcity of this strategy, perhaps due to decreased precision or amount of deposited pollen (Van der Niet & al., 2014)—or that there had been a congeneric species with lateral staminal levers that forced this change.



**Fig. 9.** Images of staminal lever mechanism for comparison: **A**, *Salvia austriaca* Jacq.; **B**, *S. revelata* Mátis & A.Z.Szabó. — Curved blue arrows indicate plane and direction of staminal lever movement. Note postgenitally fused thecae in *S. revelata* (red arrow). Photos: Attila Mátis; post processing by István Kovács.



**Fig. 10.** Images of *Salvia revelata* Mátis & A.Z.Szabó: **A**, Several flowering plants in typical Ponto-Sarmatic steppe at Techirghiol, Dobrogea (southeast Romania); **B**, Basal leaf rosette showing the hairy underside of one leaf; **C**, Calyx and corolla of hermaphroditic flower in lateral view; **D**, Nutlets; **E**, Female individual with reduced stamens in flowers. — Photos: Attila Mátis.



**Fig. 11.** Illustration of *Salvia revelata* Mátis & A.Z.Szabó: **A**, Habit of flowering plant; **B**, Female flower with reduced stamens; **C**, Hermaphroditic flower; **D**, Stamens with fused thecae. — Drawn by Krisztina Havadtói.

In the second scenario, we assume the vertical staminal lever mechanism to be the plesiomorphic state in the *Salvia austriaca* s.l. clade (i.e., the one retained by *S. revelata*), from which the lateral staminal lever mechanism evolved. Here, the shift in floral morphology is assumed to have been induced by contact and reproductive interference with *S. pratensis* in more mesophilic habitats. This co-evolutionary process involves character displacement, where the difference in adaptive floral morphologies (with highly specific pollen placement strategies that assure prezygotic isolation) is a consequence of sympatry and interspecific competition between local species assemblages (Armbruster & Muchhala, 2009; Armbruster & al., 2014). Floral character displacement is known to have played a role in the formation of community assemblage structures in, e.g., *Dalechampia* L. (Armbruster, 1985, 1986), *Styldium* Sw. ex Willd. (Armbruster & al., 1994), *Burmeistera* H.Karst. & Triana (Muchhala & Potts, 2007) and *Leavenworthia* Torr. (Norton & al., 2015).

Both of the above scenarios consider only the pre-existence of a *Salvia* species with a dorsal pollen deposition (e.g., *S. pratensis* or its progenitor) in the sympatric area. As dorsal pollen deposition is more widespread in the genus, it is likely that such a mechanism already existed when the ancestor of *S. austriaca* appeared in the forest-steppe zone.

As a third scenario, random changes in flower morphology might have led to the reproductive isolation of the two forms while occurring in sympatry. This type of non-adaptive speciation is known to happen rarely (see, e.g., Straw, 1956; Grant, 1981; Armbruster, 1993; Armbruster & al., 1994, 2014; Cozzolino & Widmer, 2005), and is extremely unlikely in our case, considering that even more distantly related *Salvia* species—sometimes even with quite different pollen placement strategy—are known to hybridize (e.g., Kerner von Marilaun, 1891; Hihara & al., 2001; Wester & Claßen-Bockhoff, 2002; Herraiz-Peñalver & al., 2015; Nachychnko & Sosnovsky, 2020) usually producing offspring with intermediate morphology (Webb & Carlquist, 1964; Bernáth & Németh, 2000; Tychonievich & Warner, 2011; Celep & al., 2020b). Therefore, even low levels of hybridization would eliminate prezygotic isolation in two sympatric populations.

Although it is well supported by our results that *Salvia revelata* is a separate species from *S. austriaca*, our current sampling is insufficient to establish the complete evolutionary history of these two taxa, and to elucidate what evolutionary forces played a role in their divergence. In order to achieve this, more extensive sampling focusing on the northern and eastern-most parts of the distribution area of *S. revelata* is required, with an additional focus on the Crimean and northern Ukrainian populations of *S. austriaca*. A comparison of the complete phylogeographical history of the two species and some more distantly related outgroups might shed light on the origins of their current distribution. Also, a better-resolved phylogeny including *S. staminea* would aid in determining which pollination type (i.e., dorsal or lateral pollen deposition) is the more ancient in *S. ser. Austriacae*, or whether lateral pollination evolved multiple times in *S. subg. Sclarea*. We proved that the genomic

methodology applied in our study (i.e., RADseq) is a valuable tool for studying these phylogenetic relationships, highlighting its utility within species groups of *Salvia*, but might also be useful at the genus level (see Eaton & Ree, 2013; Hipp & al., 2014; Leaché & Oaks, 2017).

## ■ AUTHOR CONTRIBUTIONS

AM, AS & GS conceived the idea and designed the research. GS & TM obtained funding. AM, AS, GS, IM, TK & TM conducted field work and collected data. AS, LL, TK & TM analyzed the data. AM, AS, ASB, GS, IM, LL, TK & TM prepared the manuscript. — TM, <https://orcid.org/0000-0002-6582-1219>; TK, <https://orcid.org/0000-0001-5103-1809>; LL, <https://orcid.org/0000-0002-9379-7527>; IM, <https://orcid.org/0000-0002-0689-6392>; ASB, <https://orcid.org/0000-0001-5113-2802>; GS, <https://orcid.org/0000-0001-8588-6362>

## ■ ACKNOWLEDGMENTS

The authors would like to express their gratitude to the following people for their help on various aspects of the study: László Bartha, Gabriel Gigea, Krisztina Havadtői, István Kovács, Andrea J. Nagy, Szilárd Pólska, Dorottya Sándor, Alexander Sennikov, Kiril Vassilev, Orsolya Vincze, Andriy V. Yena, Melinda Zsoldos. The work was supported by the ÚNKP-19-3-I-DE-527 New National Excellence Program of the Hungarian Ministry for Innovation and Technology awarded to TM, and the Hungarian Ministry for Innovation and Technology via an NKFI-FK project (FK137962) to GS. TM is a student of the Pál Juhász-Nagy Doctoral School of Biology and Environmental Sciences, University of Debrecen.

## ■ LITERATURE CITED

- Andrews, K.R., Good, J.M., Miller, M.R., Luikart, G. & Hohenlohe, P.A. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. *Nat. Rev. Genet.* 17: 81–92. <https://doi.org/10.1038/nrg.2015.28>
- Armbruster, W.S. 1985. Patterns of character divergence and the evolution of reproductive ecotypes of *Dalechampia scandens* (Euphorbiaceae). *Evolution* 39: 733–752. <https://doi.org/10.1111/j.1558-5646.1985.tb00416.x>
- Armbruster, W.S. 1986. Reproductive interactions between sympatric *Dalechampia* species: Are natural assemblages random or organized? *Ecology* 67: 522–533. <https://doi.org/10.2307/1938595>
- Armbruster, W.S. 1993. Evolution of plant pollination systems: Hypotheses and tests with the Neotropical vine *Dalechampia*. *Evolution* 47: 1480–1505. <https://doi.org/10.1111/j.1558-5646.1993.tb02170.x>
- Armbruster, W.S. & Muchhala, N. 2009. Associations between floral specialization and species diversity: Cause, effect or correlation? *Evol. Ecol.* 23: 159–179. <https://doi.org/10.1007/s10682-008-9259-z>
- Armbruster, W.S., Edwards, M.E. & Debevec, E.M. 1994. Floral character displacement generates assemblage structure of western Australian Triggerplants (*Styldium*). *Ecology* 75: 315–329. <https://doi.org/10.2307/1939537>
- Armbruster, W.S., Shi, X.-Q. & Huang, S.-Q. 2014. Do specialized flowers promote reproductive isolation? Realized pollination accuracy of three sympatric *Pedicularis* species. *Ann. Bot. (Oxford)* 113: 331–340. <https://doi.org/10.1093/aob/mct187>

- Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A. & Johnson, E.A. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE* 3: e3376. <https://doi.org/10.1371/journal.pone.0003376>
- Bălescu, S., Jordanova, D., Brisson, L.F., Hardy, F., Huot, S. & Lamothe, M. 2020. Luminescence chronology of the north-eastern Bulgarian loess-paleosol sequences (Viatovo and Kaolino). *Quatern. Int.* 552: 15–24. <https://doi.org/10.1016/j.quaint.2019.04.020>
- Bateman, R.M., Sramkó, G. & Paun, O. 2018. Integrating restriction site-associated DNA sequencing (RAD-seq) with morphological cladistic analysis clarifies evolutionary relationships among major species groups of bee orchids. *Ann. Bot. (Oxford)* 121: 85–105. <https://doi.org/10.1093/aob/mcx129>
- Bates, D., Mächler, M., Bolker, B. & Walker, S. 2015 Fitting linear mixed-effects models using lme4. *J. Statist. Software* 67(1): 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Benthams, G. 1848. Labiatae: LVII. *Salvia*. Pp. 262–358 in: Candolle, A. de (ed.), *Prodromus systematis naturalis regni vegetabilis*, vol. 12. Parisiis [Paris]: sumptibus Victoris Masson. <https://doi.org/10.5962/bhl.title.286>
- Bernáth, J. & Németh, É. 2000. Genetic improvement of cultivated species of the genus *Salvia*. Pp. 130–144 in: Kintzios, S.E. (ed.), *Sage: The genus Salvia*. London: CRC Press.
- Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A. & Drummond, A.J. 2014. BEAST2: A software platform for Bayesian evolutionary analysis. *PLoS Computat. Biol.* 10(4): e1003537. <https://doi.org/10.1371/journal.pcbi.1003537>
- Briscoe Runquist, R., Grossenbacher, D., Porter, S., Kay, K. & Smith, J. 2016. Pollinator-mediated assemblage processes in California wildflowers. *J. Evol. Biol.* 29: 1045–1058. <https://doi.org/10.1111/jeb.12845>
- Bryant, D., Bouckaert, R., Felsenstein, J., Rosenberg, N.A. & RoyChoudhury, A. 2012. Inferring species trees directly from biallelic genetic markers: Bypassing gene trees in a full coalescent analysis. *Molec. Biol. Evol.* 29: 1917–1932. <https://doi.org/10.1093/molbev/mss086>
- Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W. & Postlethwait, J.H. 2011. Stacks: Building and genotyping loci de novo from short-read sequences. *G3: Genes Genomes Genet.* 1: 171–182. <https://doi.org/10.1534/g3.111.000240>
- Celep, F., Dirmenci, T. & Güner, Ö. 2015. *Salvia hasankeyfense* (Lamiaceae), a new species from Hasankeyf (Batman, South-eastern Turkey). *Phytotaxa* 227: 289–294. <https://doi.org/10.11646/phytotaxa.227.3.9>
- Celep, F., Atalay, Z., Dikmen, F., Doğan, M., Sytsma, K.J. & Claßen-Bockhoff, R. 2020a. Pollination ecology, specialization, and genetic isolation in sympatric bee-pollinated *Salvia* (Lamiaceae). *Int. J. Pl. Sci.* 181: 800–811. <https://doi.org/10.1086/710238>
- Celep, F., Raders, E. & Drew, B.T. 2020b. Two new hybrid species of *Salvia* (*S.* × *karamanensis* and *S.* × *doganii*) from Turkey: Evidence from molecular and morphological studies. *Turk. J. Bot.* 44: 647–660. <https://doi.org/10.3906/bot-2007-28>
- Chen, Y., Jabbour, F., Novikov, A., Wang, W. & Gerber, S. 2018. A study of floral shape variation in Delphinieae (Ranunculaceae) using geometric morphometrics on herbarium specimens. *Bot. Lett.* 165: 368–376. <https://doi.org/10.1080/23818107.2018.1427145>
- Claßen-Bockhoff, R. 2017. Stamen construction, development and evolution in *Salvia* s.l. *Nat. Volatiles Essential Oils* 4: 28–48.
- Claßen-Bockhoff, R., Wester, P. & Tweraser, E. 2003. The staminal lever mechanism in *Salvia* L. (Lamiaceae): A review. *Pl. Biol.* 5: 33–41. <https://doi.org/10.1055/s-2003-37973>
- Claßen-Bockhoff, R., Crone, M. & Baikova, E. 2004a. Stamen development in *Salvia* L.: Homology reinvestigated. *Int. J. Pl. Sci.* 165: 475–498. <https://doi.org/10.1086/386565>
- Claßen-Bockhoff, R., Speck, T., Tweraser, E., Wester, P., Thimm, S. & Reith, M. 2004b. The staminal lever mechanism in *Salvia* L. (Lamiaceae): A key innovation for adaptive radiation? *Organisms Diversity Evol.* 4: 189–205. <https://doi.org/10.1016/j.ode.2004.01.004>
- Conea, A. 1970. *Formațiuni cuaternare în Dobrogea: Loessuri și paleosoluri*. Bucharest: Ed. Academiei Republicii Socialiste România.
- Cordova, C.E. 2007. Holocene Mediterraneanization of the southern Crimean vegetation: Paleocological records, regional climate change, and possible non-climatic influences. Pp. 319–344 in: Yanko-Hombach, V., Gilbert, A.S., Panin, N. & Dolukhanov, P.M. (eds.), *The Black Sea flood question: Changes in coastline, climate, and human settlement*. Dordrecht: Springer. [https://doi.org/10.1007/978-1-4020-5302-3\\_13](https://doi.org/10.1007/978-1-4020-5302-3_13)
- Cozzolino, S. & Widmer, A. 2005. Orchid diversity: An evolutionary consequence of deception? *Trends Ecol. Evol.* 20: 487–494. <https://doi.org/10.1016/j.tree.2005.06.004>
- Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A. & 1000 Genomes Project Analysis Group 2011. The variant call format and VCFtools. *Bioinformatics* 27: 2156–2158. <https://doi.org/10.1093/bioinformatics/btr330>
- Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M. & Blaxter, M.L. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nat. Rev. Genet.* 12: 499–510. <https://doi.org/10.1038/nrg3012>
- Dong, A.-X., Xin, H.-B., Li, Z.-J., Liu, H., Sun, Y.-Q., Nie, S., Zhao, Z.-N., Cui, R.-F., Zhang, R.-G., Yun, Q.-Z., Wang, X.-N., Maghuly, F., Porth, I., Cong, R.-C. & Mao, J.-F. 2018. High-quality assembly of the reference genome for scarlet sage, *Salvia splendens*, an economically important ornamental plant. *Gigascience* 7(7): giy068. <https://doi.org/10.1093/gigascience/gyi068>
- Drew, B.T. & Sytsma, K.J. 2012. Phylogenetics, biogeography, and staminal evolution in the tribe Mentheae (Lamiaceae). *Amer. J. Bot.* 99: 933–953. <https://doi.org/10.3732/ajb.1100549>
- Drew, B.T., González-Gallegos, J.G., Xiang, C.L., Kriebel, R., Drummond, C.P., Walked, J.B. & Sytsma, K.J. 2017. *Salvia* united: The greatest good for the greatest number. *Taxon* 66: 133–145. <https://doi.org/10.12705/661.7>
- Eaton, D.A.R. & Ree, R.H. 2013. Inferring phylogeny and introgression using RADseq data: An example from flowering plants (*Pedicularis*: Orobanchaceae). *Syst. Biol.* 62: 689–706. <https://doi.org/10.1093/sysbio/syt032>
- Eisen, K.E. & Geber, M.A. 2018. Ecological sorting and character displacement contribute to the structure of communities of *Clarkia* species. *J. Evol. Biol.* 31: 1440–1458. <https://doi.org/10.1111/jeb.13365>
- Erdős, L., Ambarlı, D., Anenkhonov, O.A., Bátor, Z., Cserhalmi, D., Kiss, M., Kröel-Dulay, G., Liu, H., Magnes, M., Molnár, Z., Naqinezhad, A., Semenishchenkov, Y.A., Tölgyesi, C. & Török, P. 2018. The edge of two worlds: A new review and synthesis on Eurasian forest-steppes. *Appl. Veg. Sci.* 21: 345–362. <https://doi.org/10.1111/avsc.12382>
- Galstyan, T. 2021. *A field guide to the plants of Armenia*. Bath: Filbert Press.
- García, B.Y.B. & Zamudio, S. 2015. Four new species of *Salvia* (Lamiaceae) from central Mexico. *Phytotaxa* 217: 35–52. <https://doi.org/10.11646/phytotaxa.217.1.3>
- Ghenea, C. & Rădan, S.C. 1993. New data on the loess age in Dobrogea. *Romanian J. Stratigr.* 75: 133–137.
- Grant, V. 1981. *Plant speciation*. New York: Columbia University Press. <https://doi.org/10.7312/gran92318>
- Grant, V. 1994. Modes and origins of mechanical and ethological isolation in angiosperms. *Proc. Natl. Acad. Sci. U.S.A.* 91: 3–10. <https://doi.org/10.1073/pnas.91.1.3>

- Harley, R.M., Atkins, S., Budantsev, A.L., Cantino, P.D., Conn, B. J., Grayer, R., Harley, M.M., de Kok, R., Krestovskaja, T., Morales, R., Paton, A.J., Ryding, O. & Upson, T. 2004. Labiatae. Pp. 167–275 in: Kadereit, J.W. (ed.), *The families and genera of vascular plants*, vol. 7, *Flowering plants: Dicotyledons; Lamiales (except Acanthaceae including Avicenniaceae)*. Berlin & Heidelberg: Springer. [https://doi.org/10.1007/978-3-642-18617-2\\_11](https://doi.org/10.1007/978-3-642-18617-2_11)
- Hedge, I.C. 1982a. *Salvia*. P. 477 in: Rechinger, K.H. (ed.), *Flora Iranica*, vol. 150, *Labiatae*. Graz: Akademische Druck- u. Verlagsanstalt.
- Hedge, I.C. 1982b. *Salvia*. Pp. 400–461 in: Davis, P.H. (ed.), *Flora of Turkey*, vol. 7. Edinburgh: Edinburgh University Press.
- Herrera-Peñalver, D., Elguea-Culebras, O. de & G. Sánchez-Vioque, R. & Santana Méridas, O. 2015. Identification of a hybrid species of sage (*Salvia officinalis* L. × *S. lavandulifolia* subsp. *lavandulifolia*) through the study of the essential oil. *J. Essential Oil Res.* 27: 363–372. <https://doi.org/10.1080/10412905.2015.1031918>
- Heystek, A. & Pauw, A. 2014. Does competition for pollinators contribute to structuring *Erica* communities? *J. Veg. Sci.* 25: 648–656. <https://doi.org/10.1111/jvs.12127>
- Hihara, S., Iwatsubo, Y. & Naruhashi, N. 2001. A new natural hybrid of *Salvia* (Lamiaceae) from Japan, *Salvia* × *sakuensis*. *J. Phytogeogr. Taxon.* 49: 163–170.
- Hipp, A.L., Eaton, D.A.R., Cavender-Bares, J., Fitzek, E., Nipper, R. & Manos, P.S. 2014. A framework phylogeny of the American Oak clade based on sequenced RAD data. *PLoS ONE* 9: e93975. <https://doi.org/10.1371/journal.pone.0093975>
- Hruby, K. 1962. Key to the supraspecific taxa of the genus *Salvia*. *Preslia* 34: 368–373.
- Hu, G.X., Takano, A., Drew, B.T., Liu, E.D., Soltis, D.E., Soltis P.S., Peng, H. & Xiang, C.-L. 2018. Phylogeny and staminal evolution of *Salvia* (Lamiaceae, Nepetoideae) in East Asia. *Ann. Bot. (Oxford)* 122: 649–668. <https://doi.org/10.1093/aob/mcy104>
- Jenks, A.A., Walker, J.B. & Kim, S.C. 2013. Phylogeny of new world *Salvia* subgenus *Calospatha* (Lamiaceae) based on cpDNA (*psbA-trnH*) and nrDNA (ITS) sequence data. *J. Pl. Res.* 126: 483–496. <https://doi.org/10.1007/s10265-012-0543-1>
- Jombart, T. & Ahmed, I. 2011. adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics* 27: 3070–3071. <https://doi.org/10.1093/bioinformatics/btr521>
- Kajtoch, Ł., Cieślak, E., Varga, Z., Paul, W., Mazur, M.A., Sramkó, G. & Kubisz, D. 2016. Phylogeographic patterns of steppe species in eastern central Europe: A review and the implications for conservation. *Biodivers. & Conservation* 25: 2309–2339. <https://doi.org/10.1007/s10531-016-1065-2>
- Karbstein, K., Tomasello, S., Hodač, L., Dunkel, F.G., Daubert, M. & Hörandl, E. 2020. Phylogenomics supported by geometric morphometrics reveals delimitation of sexual species within the polyploid apomictic *Ranunculus auricomus* complex (Ranunculaceae). *Taxon* 69: 1191–1220. <https://doi.org/10.1002/tax.12365>
- Kass, R.E. & Raftery, A.E. 1995. Bayes factors. *J. Amer. Statist. Assoc.* 90: 773–795. <https://doi.org/10.1080/01621459.1995.10476572>
- Kassambara, A. & Mundt, F. 2019. factoextra: Extract and visualize the results of multivariate data analyses. Software and manual available at <https://cran.r-project.org/package=factoextra>
- Kaul, M.L.H. 1988. *Male sterility in higher plants*. Monographs on Theoretical and Applied Genetics 10. Berlin & Heidelberg: Springer. <https://doi.org/10.1007/978-3-642-83139-3>
- Kerner von Marilaun, A. 1891. *Pflanzenleben*, vol. 2, *Geschichte der Pflanzen*. Leipzig & Wien: Bibliographisches Institut.
- Kraft, N.J.B., Adler, P.B., Godoy, O., James, E.C., Fuller, S. & Levine, J.M. 2015. Community assembly, coexistence and the environmental filtering metaphor. *Funct. Ecol.* 29: 592–599. <https://doi.org/10.1111/1365-2435.12345>
- Kriebel, R., Drew, B.T., Drummond, C.P., González-Gallegos, J.G., Celep, F. & Mahdjoub, M.M., Rose, J.P., Xiang, C.L., Hu, G.-X., Walker, J.B., Lemmon, E.M., Lemmon, A.R. & Sytsma, K.J. 2019. Tracking temporal shifts in area, biomes, and pollinators in the radiation of *Salvia* (sages) across continents: Leveraging anchored hybrid enrichment and targeted sequence data. *Amer. J. Bot.* 106: 573–597. <https://doi.org/10.1002/ajb2.1268>
- Kriebel, R., Drew, B., González-Gallegos, J.G., Celep, F., Heeg, L., Mahdjoub, M.M. & Sytsma, K.J. 2020. Pollinator shifts, contingent evolution, and evolutionary constraint drive floral disparity in *Salvia* (Lamiaceae): Evidence from morphometrics and phylogenetic comparative methods. *Evolution (Lancaster)* 74: 1335–1355. <https://doi.org/10.1111/evo.14030>
- Kriebel, R., Drew, B.T., González-Gallegos, J.G., Celep, F., Antar, G. M., Pastore, J.F.B., Uriá, R. & Sytsma, K.J. 2021. Stigma shape shifting in sages (*Salvia*: Lamiaceae): Hummingbirds guided the evolution of New World floral features. *Bot. J. Linn. Soc.* 199: 428–448. <https://doi.org/10.1093/botlinnean/boab096>
- Lavrenko, E.M., Karamysheva, Z.V. & Nikulina, R.I. 1991. *Stepi Evrazii* [Steppes of Eurasia]. Leningrad: Nauka.
- Lê, S., Josse, J. & Husson, F. 2008. FactoMineR: An R package for multivariate analysis. *J. Statist. Software* 25: 1–18. <https://doi.org/10.18637/jss.v025.i01>
- Leaché, A.D. & Oaks, J.R. 2017. The utility of single nucleotide polymorphism (SNP) data in phylogenetics. *Annual Rev. Ecol. Evol. Syst.* 48: 69–84. <https://doi.org/10.1146/annurev-ecolsys-110316-022645>
- Leaché, A.D., Fujita, M.K., Minin, V.N. & Bouckaert, R.R. 2014. Species delimitation using genome-wide SNP data. *Syst. Biol.* 63: 534–542. <https://doi.org/10.1093/sysbio/syu018>
- Leitch, I.J., Johnston, E., Pellicer, J., Hidalgo, O. & Bennett, M.D. 2019. Plant DNA C-values database, release 7.1, Apr. 2019. <https://cvalues.science.kew.org/> (accessed 5 May 2019).
- Lemmon, E.M. & Lemmon, A.R. 2013. High-throughput genomic data in systematics and phylogenetics. *Annual Rev. Ecol. Evol. Syst.* 44: 99–121. <https://doi.org/10.1146/annurev-ecolsys-110512-135822>
- Li, H. & Durbin, R. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 25: 1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>
- Li, Q.Q., Li, M.H., Yuan, Q.J., Cui, Z.H., Huang, L.Q. & Xiao, P.G. 2013. Phylogenetic relationships of *Salvia* (Lamiaceae) in China: Evidence from DNA sequence datasets. *J. Syst. Evol.* 51: 184–195. <https://doi.org/10.1111/j.1759-6831.2012.00232.x>
- Lindner, L., Gozhik, P., Marciniak, B., Marks, L. & Yelovicheva, Y. 2004. Main climatic changes in the Quaternary of Poland, Belarus and Ukraine. *Geol. Quart.* 48: 97–114.
- Magyari, E.K., Chapman, J.C., Passmore, D.G., Allen, J.R.M., Huntley, J.P. & Huntley, B. 2010. Holocene persistence of wooded steppe in the Great Hungarian Plain. *J. Biogeogr.* 37: 915–935. <https://doi.org/10.1111/j.1365-2699.2009.02261.x>
- Markova, A.K. 2000. The Mikulino (= Eemian) mammal faunas of the Russian plain and Crimea. *Netherlands J. Geosci.* 79: 293–301. <https://doi.org/10.1017/S0016774600021776>
- Martínez-Ambroz, E., Fragozo-Martínez, I. & Martínez-Gordillo, M. 2019. A new species of *Salvia* from the Fulgentes clade (Lamiaceae), from Puebla, Mexico. *Phytotaxa* 409: 29–38. <https://doi.org/10.11646/phytotaxa.409.1.4>
- Martínez-Gordillo, M., Fragozo-Martínez, I. & Salas-Morales, S. H. 2016. *Salvia robertoana* (Lamiaceae), a new species from Oaxaca, Mexico. *Phytotaxa* 269: 271–278. <https://doi.org/10.11646/phytotaxa.269.4.2>
- McCormack, J.E., Hird, S.M., Zellmer, A.J., Carstens, B.C. & Brumfield, R.T. 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. *Molec. Phylogen. Evol.* 66: 526–538. <https://doi.org/10.1016/j.ympev.2011.12.007>

- Menithkiy, Y.** 1987. *Salvia*. Pp. 124–125 in: Takhtadjan, A.L. (ed.), *Flora Armenii* [Flora of Armenia], vol. 8. Yerevan: Izdatel'stvo Akademii Nauk Armianskoi SSR.
- Meusel, H. & Jäger, E.** 1992. *Vergleichende Chorologie der zentral-europäischen Flora*, vol. 3. Jena: Fischer.
- MEWRB [Ministry of Environment and Water of Republic of Bulgaria]** 2011–2013. Kartirane i opredelyane na prirodozashitnoto sastoyanie na prirodni mestoobitaniya i vidove - faza I [Mapping and Determining the Nature Conservation Status of Natural Habitats and Species - Phase I]. <http://natura2000.moew.government.bg/Home/ProtectedSite?code=BG0000233&siteType=HabitatDirective>
- Miller, M.R., Dunham, J.P., Amores, A., Cresko, W.A. & Johnson, E.A.** 2007. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. *Genome Res.* 17: 240–248. <https://doi.org/10.1101/gr.5681207>
- Muchhala, N. & Potts, M.D.** 2007. Character displacement among bat-pollinated flowers of the genus *Burmeistera*: Analysis of mechanism, process and pattern. *Proc. Roy. Soc. London, Ser. B., Biol. Sci.* 274: 2731–2737. <https://doi.org/10.1098/rspb.2007.0670>
- Nachychko, V.O. & Sosnovsky, Y.V.** 2020. On the Romanian endemic species of *Salvia* (Lamiaceae) and its natural hybrids: Nomenclatural and taxonomic aspects. *Phytotaxa* 434: 270–280. <https://doi.org/10.11646/phytotaxa.434.3.5>
- Neem Community Members** 2013. Eemian interglacial reconstructed from a Greenland folded ice core. *Nature* 493: 489–494. <https://doi.org/10.1038/nature11789>
- Norton, N.A., Fernando, M.T.R., Herlihy, C.R. & Busch, J.W.** 2015. Reproductive character displacement shapes a spatially structured petal color polymorphism in *Leavenworthia stylosa*. *Evolution (Lancaster)* 69: 1191–1207. <https://doi.org/10.1111/evo.12659>
- Nyárády, E.G.** 1942. Új növények a Délkeleti-Kárpátok és a Fekete-tenger vidékének flórájához. *Acta Univ. Szeged., Sect. Sci. Nat., Pars Bot.* 1: 31–45.
- Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E.C., D'amico, J.A., Itoua, I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P. & Kassem, K.R.** 2001. Terrestrial ecoregions of the world: A new map of life on Earth: A new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *Bioscience* 51: 933–938. [https://doi.org/10.1641/0006-3568\(2001\)051\[0933:TEOTWA\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2)
- Pante, E., Abdelkrim, J., Viricel, A., Gey, D., France, S.C., Boisselier, M.C. & Samadi, S.** 2015. Use of RAD sequencing for delimiting species. *Heredity* 114: 450–459. <https://doi.org/10.1038/hdy.2014.105>
- Paun, O., Turner, B., Trucchi, E., Munzinger, J., Chase, M.W. & Samuel, R.** 2016. Processes driving the adaptive radiation of a tropical tree (*Diospyros*, Ebenaceae) in New Caledonia, a biodiversity hotspot. *Syst. Biol.* 65: 212–227. <https://doi.org/10.1093/sysbio/syv076>
- Pauw, A.** 2006. Floral syndromes accurately predict pollination by a specialized oil-collecting bee (*Rediviva peringueyi*, Melittidae) in a guild of South African orchids (Coryciinae). *Amer. J. Bot.* 93: 917–926. <https://doi.org/10.3732/ajb.93.6.917>
- Pinheiro, J., Bates, D. & R Core Team** 2021. nlme: Linear and non-linear mixed effects models. R Package. Version 3.1-152. Software and manual available at <https://cran.r-project.org/package=nlme>.
- Pobedimova, Y.G.** 1954. *Salvia*. Pp. 178–260 in: Komarov, V.L. (ed.), *Flora SSSR* [Flora of the U.S.S.R.], vol. 21. Moscow & Leningrad: Izdatel'stvo Akademii Nauk SSSR.
- Puritz, J.B., Matz, M.V., Toonen, R.J., Weber, J.N., Bolnick, D.I. & Bird, C.E.** 2014. Demystifying the RAD fad. *Molec. Ecol.* 23: 5937–5942. <https://doi.org/10.1111/mec.12965>
- R Core Team** 2019. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.
- Rambaut, A. & Drummond, A.** 2013. Tracer, version 1.6. Software distributed by the author. University of Edinburgh. <https://github.com/beast-dev/tracer/releases/tag/v1.6>
- Rannala, B. & Yang, Z.** 2008. Phylogenetic inference using whole genomes. *Annual Rev. Genomics Human Genet.* 9: 217–231. <https://doi.org/10.1146/annurev.genom.9.081307.164407>
- Reith, M., Baumann, G., Claßen-Bockhoff, R. & Speck, T.** 2007. New insights into the functional morphology of the lever mechanism of *Salvia pratensis* (Lamiaceae). *Ann. Bot. (Oxford)* 100: 393–400. <https://doi.org/10.1093/aob/mcm031>
- Reitzel, A.M., Herrera, S., Layden, M.J., Martindale, M.Q. & Shank, T.M.** 2013. Going where traditional markers have not gone before: Utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. *Molec. Ecol.* 22: 2953–2970. <https://doi.org/10.1111/mec.12228>
- Ren, M. & Tang, J.** 2010. Anther fusion enhances pollen removal in *Campsis grandiflora*, a hermaphroditic flower with didynamous stamens. *Int. J. Pl. Sci.* 171: 275–282. <https://doi.org/10.1086/650157>
- Rivera-Colón, A.G., Rochette, N.C. & Catchen, J.M.** 2021. Simulation with RADinitio improves RADseq experimental design and sheds light on sources of missing data. *Molec. Ecol. Resources* 21: 363–378. <https://doi.org/10.1111/1755-0998.13163>
- Rochette, N.C., Rivera-Colón, A.G. & Catchen, J.M.** 2019. Stacks2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Molec. Ecol.* 28: 4737–4754. <https://doi.org/10.1111/mec.15253>
- Rose, J.P., Kriebel, R., Kahan, L., DiNicola, A., González-Gallegos, J.G., Celep, F., Lemmon, E.M., Lemmon, A.R., Sytsma, K.J. & Drew, B.T.** 2021. Sage insights into the phylogeny of *Salvia*: Dealing with sources of discordance within and across genomes. *Frontiers Pl. Sci. (Online journal)* 12: 767478. <https://doi.org/10.3389/fpls.2021.767478>
- Rummel, J.D. & Roughgarden, J.** 1985. A theory of faunal buildup for competition communities. *Evolution* 39: 1009–1033. <https://doi.org/10.1111/j.1558-5646.1985.tb00444.x>
- Schemske, D.W.** 1981. Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. *Ecology* 62: 946–954. <https://doi.org/10.2307/1936993>
- Schneider, C.A., Rasband, W.S. & Eliceiri, K.W.** 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature, Meth.* 9(7): 671–675. <https://doi.org/10.1038/nmeth.2089>
- Sérsic, A.** 2004. Pollination biology in the genus *Calceolaria* L. (Calceolariaceae). *Stapfia* 82: 1–121.
- Sramkó, G., Paun, O., Brandrud, M.K., Laczkó, L., Molnár, A. & Bateman, R.M.** 2019. Iterative alloamy–autogamy transitions drive actual and incipient speciation during the ongoing evolutionary radiation within the orchid genus *Epipactis* (Orchidaceae). *Ann. Bot. (Oxford)* 124: 481–497. <https://doi.org/10.1093/aob/mcz103>
- Stamatakis, A.** 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>
- Stange, M., Sánchez-Villagra, M.R., Salzburger, W. & Matschner, M.** 2018. Bayesian divergence-time estimation with genome-wide single-nucleotide polymorphism data of sea catfishes (Ariidae) supports Miocene closure of the Panamanian Isthmus. *Syst. Biol.* 67: 681–699. <https://doi.org/10.1093/sysbio/syy006>
- Straw, R.M.** 1956. Floral isolation in *Penstemon*. *Amer. Naturalist* 90: 47–53. <https://doi.org/10.1086/281906>
- Strong, D.R., Szyska, L.A. & Simberloff, D.S.** 1979. Test of community-wide character displacement against null hypotheses. *Evolution (Lancaster)* 33: 897–913. <https://doi.org/10.1111/j.1558-5646.1979.tb04743.x>

- Takano, A. & Okada, H.** 2011. Phylogenetic relationships among subgenera, species and varieties of Japanese *Salvia* L. (Lamiaceae). *J. Pl. Res.* 124: 245–252. <https://doi.org/10.1007/s10265-010-0367-9>
- Thimm, S.G.** 2008. *Pollen-placement and pollen-portioning in diverse Salvia-species*. Ph.D. thesis. Johannes Gutenberg-Universität Mainz, Germany. <http://doi.org/10.25358/openscience-2222>
- Timar-Gabor, A., Vandenberghe, D.A.G., Vasiliu, S., Panaoitu, C.E., Panaiotu, C.G., Dimofte, D. & Cosma, C.** 2011. Optical dating of Romanian loess: A comparison between silt-sized and sand-sized quartz. *Quatern. Int.* 240: 62–70. <https://doi.org/10.1016/j.quaint.2010.10.007>
- Tomescu, A.M.F.** 2000. Evaluation of Holocene pollen records from the Romanian Plain. *Rev. Palaeobot. Palynol.* 109: 219–233. [https://doi.org/10.1016/S0034-6667\(99\)00056-1](https://doi.org/10.1016/S0034-6667(99)00056-1)
- Trapp, A.** 1956a. Entwicklungsgeschichtliche Untersuchungen über die Antherengestaltung sympetaler Blüten. *Beitr. Biol. Pflanzen* 32: 279–312.
- Trapp, A.** 1956b. *Zur Morphologie und Entwicklungsgeschichte der Staubblätter sympetaler Blüten*. Jena: Fischer.
- Troll, W.** 1929. *Roscoea purpurea* SM., eine Zingiberacee mit Hebelmechanismus in den Blüten; Mit Bemerkungen über die Entfaltungsbewegungen der fertilen Staubblätter von *Salvia*. *Planta* 7: 1–28. <https://doi.org/10.1007/BF01908933>
- Tychonievich, J. & Warner, R.M.** 2011. Interspecific crossability of selected *Salvia* species and potential use for crop improvement. *J. Amer. Soc. Hort. Sci.* 136: 41–47. <https://doi.org/10.21273/JASHS.136.1.41>
- Van Andel, T. & Tzedakis, P.C.** 1996. Palaeolithic landscapes of Europe and environs, 150,000–25,000 years ago: An overview. *Quatern. Sci. Rev.* 15: 481–500. [https://doi.org/10.1016/0277-3791\(96\)00028-5](https://doi.org/10.1016/0277-3791(96)00028-5)
- Van der Niet, T., Peakall, R. & Johnson, S.D.** 2014. Pollinator-driven ecological speciation in plants: New evidence and future perspectives. *Ann. Bot. (Oxford)* 113: 199–212. <https://doi.org/10.1093/aob/mct290>
- Walker, J.B. & Sytsma, K.J.** 2007. Staminal evolution in the genus *Salvia* (Lamiaceae): Molecular phylogenetic evidence for multiple origins of the staminal lever. *Ann. Bot. (Oxford)* 100: 375–391. <https://doi.org/10.1093/aob/mcl176>
- Walker, J.B., Sytsma, K.J., Treutlein, J. & Wink, M.** 2004. *Salvia* (Lamiaceae) is not monophyletic: Implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Menthaeae. *Amer. J. Bot.* 91: 1115–1125. <https://doi.org/10.3732/ajb.91.7.1115>
- Walker, J.B., Drew, B.T. & Sytsma, K.J.** 2015. Unravelling species relationships and diversification within the iconic California Floristic Province sages (*Salvia* subgenus *Audibertia*, Lamiaceae). *Syst. Bot.* 40: 826–844. <https://doi.org/10.1600/036364415X689285>
- Waser, N.M.** 1983. Competition for pollination and floral character differences among sympatric plant species: A review of evidence. Pp. 277–293 in: Jones, C.E. & Little, R.J. (eds.), *Handbook of experimental pollination biology*. New York: Scientific and Academic Editions.
- Webb, A.A. & Carlquist, S.** 1964. Leaf anatomy as an indicator of *Salvia apiana-mellifera* introgression. *Aliso* 5: 437–449. <https://doi.org/10.5642/aliso.19640504.04>
- Wei, Y.K., Pendry, C.A., Zhang, D.G. & Huang, Y.B.** 2019. *Salvia daiguii* (Lamiaceae): A new species from west Hunan, China. *Edinburgh J. Bot.* 76: 359–368. <https://doi.org/10.1017/S096042861900009X>
- Wester, P. & Claßen-Bockhoff, R.** 2002. *Salvia haenkei* Benth. and *S. orbignaei* Benth. – Two ornithophilous species from Bolivia and their hybrids. Poster presented at Botanikertagung, 22–27 September 2002, Freiburg, Germany.
- Wickham, H.** 2016. *ggplot2: Elegant graphics for data analysis*. New York: Springer. <https://doi.org/10.1007/978-3-319-24277-4>
- Will, M. & Claßen-Bockhoff, R.** 2014. Why Africa matters: Evolution of Old World *Salvia* (Lamiaceae) in Africa. *Ann. Bot. (Oxford)* 114: 61–83. <https://doi.org/10.1093/aob/mcu081>
- Will, M. & Claßen-Bockhoff, R.** 2017. Time to split *Salvia* s.l. (Lamiaceae) – New insights from Old World *Salvia* phylogeny. *Molec. Phylogen. Evol.* 109: 33–58. <https://doi.org/10.1016/j.ympev.2016.12.041>
- Zhang, B. & Claßen-Bockhoff, R.** 2019. Sex-differential reproduction success and selection on floral traits in gynodioecious *Salvia pratensis*. *B. M. C. Pl. Biol.* 19: 375. <https://doi.org/10.1186/s12870-019-1972-y>

#### Appendix 1. Geographic locations and SRA accession numbers (BioProject accession number: PRJNA849002) of specimens included in this study.

Species, Lab ID, locality (country codes given according to the ISO 3166-1 alpha-2 standard), latitude, longitude, collector plus coll. number (herbarium code), sequence information (Sequence Read Archive [SRA; <https://www.ncbi.nlm.nih.gov/sra>] accession number).

*Salvia austriaca* Jacq.: ausJoz1, Debrecen-Józsa (HU), 47.590784, 21.585314, *Á. Lovas-Kiss & al. Soo-48344* (DE), SRS13507390; *Salvia austriaca*: ausJoz2, Debrecen-Józsa (HU), 47.590784, 21.585314, *Lovas-Kiss & al. Soo-48345* (DE), SRS13507391; *Salvia austriaca*: ausKol1, Cluj-Napoca: Valea Calda (RO), 46.808743, 23.696131, *A. Mátis & A. Szabó Soo-48356* (DE), SRS13507402; *Salvia austriaca*: ausKol2, Cluj-Napoca: Valea Calda (RO), 46.808743, 23.696131, *A. Mátis & A. Szabó Soo-48357* (DE), SRS13507403; *Salvia austriaca*: ausSar1, Sárkeresztúr (HU), 47.000761, 18.530032, *G. Sramkó Soo-48348* (DE), SRS13507404; *Salvia revelata* Mátis & A.Z.Szabó, sp. nov.: revBoz1, Bozhurluka (BG), 43.414276, 25.401062, *G. Sramkó Soo-48352* (DE), SRS13507405; *Salvia revelata*: revBoz2, Bozhurluka (BG), 43.414276, 25.401062, *G. Sramkó Soo-48353* (DE), SRS13507406; *Salvia revelata*: revEfo1, Eforie Sud (RO), 44.037856, 28.621514, *A. Mátis & A. Szabó Soo-48358* (DE), SRS13507393; *Salvia revelata*: revHag1, Hagieni reserve (RO), 43.804756, 28.472409, *A. Mátis & A. Szabó Soo-48359* (DE), SRS13507409; *Salvia revelata*: revHag2, Hagieni reserve (RO), 43.804756, 28.472409, *A. Mátis & A. Szabó Soo-48360* (DE), SRS13507392; *Salvia revelata*: revMyk1, Mykhaylivskiy steppe (UA), 47.41983, 31.6248, *T. Malkócs & al. Soo-48342* (DE) SRS13507407; *Salvia revelata*: revMyk2, Mykhaylivskiy steppe (UA), 47.41983, 31.6248, *T. Malkócs & al. Soo-48343* (DE), SRS13507408; *Salvia nemorosa* L.: nemo01, Gostilya (BG), 43.55384, 24.16401, *G. Sramkó Soo-45265* (DE), SRS13507398; *Salvia nemorosa*: nemo02, Gostilya (BG), 43.55321, 24.16134, *G. Sramkó Soo-48351* (DE), SRS13507399; *Salvia nutans* L.: nutHus01, Huși (RO), 46.695141, 28.104288, *G. Sramkó Soo-48349* (DE), SRS13507394; *Salvia nutans*: nutHus16, Huși (RO), 46.695141, 28.104288, *G. Sramkó Soo-48350* (DE), SRS13507395; *Salvia nutans*: nutUfa10, Ufa: Kipchak-Askarovo (RS), 53.9495, 55.05375, *P. Volkova Soo-48354* (DE), SRS13507396; *Salvia nutans*: nutUfa10, Ufa: Kipchak-Askarovo (RS), 53.9495, 55.05375, *P. Volkova Soo-48355* (DE), SRS13507397; *Salvia pratensis* L.: prat01, Debrecen-Józsa (HU), 47.5852, 21.5874, *G. Sramkó Soo-48346* (DE), SRS13507400; *Salvia pratensis*: prat02, Debrecen-Józsa (HU), 47.5852, 21.5874, *G. Sramkó Soo-48347* (DE), SRS13507401.