Intergeneric hybrid origin of the serious invasive tetraploid Cirsium vulgare

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ABSTRACT

Premise: The invasive tetraploid *Cirsium vulgare* hybridizes with both *Cirsium* and *Lophiolepis*. Its conflicting position in molecular phylogenies, and its peculiar combination of morphological, anatomical, and genomic features that are alternatively shared with representatives of *Cirsium* or *Lophiolepis*, strongly suggest its intergeneric hybrid origin.

Methods: Genetic relationships of *C. vulgare* (8 samples) with genus *Lophiolepis* (11 species) and other representatives of genus *Cirsium* (12 species) were evaluated using restriction site-associated DNA sequencing (RADseq) and examined using analytical or imaging approaches such as NeighborNet, Heatmap, and STRUCTURE to identify genomic nuclear genome admixture. Estimation of the intensity of spontaneous hybridization within and between *Cirsium* and *Lophiolepis* was based on herbarium revisions and a recherche of all reported hybrids pertinent to taxa currently included in *Cirsium* or *Lophiolepis*.

Key results: The genome of any examined *Cirsium* species is more similar to *C. vulgare* than to any *Lophiolepis* species, and vice versa. The nuclear genome of tetraploid *C. vulgare* is composed of two equivalent parts, each attributable either to *Lophiolepis* or to *Cirsium*; the organellar RADseq data clustered *C. vulgare* with the genus *Cirsium*. Spontaneous hybridization between *Cirsium* and *Lophiolepis* is significantly less intensive than within these genera.

Conclusions: Our analyses provide compelling evidence that the invasive species *C. vulgare* arose by an allotetraploid intergeneric origin with the maternal parent from *Cirsium* and the paternal from *Lophiolepis*. For the purpose of delimiting monophyletic genera, we propose to keep *Lophiolepis* separated from *Cirsium* and to segregate *C. vulgare* into the hybridogenous genus *Ascalea*.

INTRODUCTION

Recent phylogenetic investigations by Ackerfield *et al.* (2020), Del Guacchio *et al.* (2022), Bureš *et al.* (2023), and Moreyra *et al.* (2023) have unveiled the polyphyletic nature of the broad taxonomic concept of the genus *Cirsium* Mill., especially if some traditionally recognized natural genera – *Carduus* L., *Galactites* Moench, *Lamyropsis* (Kharadze) Dittrich, *Nothobasis* (Cass.) Cass., *Picnomon* Adans., *Silybum* Adans., and *Tyrimnus* (Cass.) Bosc – are to be retained. Consequently, Del Guacchio *et al.* (2022) initially proposed the separation of two smaller genera from the large genus *Cirsium*: *Lophiolepis* Cass., with 104 Eurasian and North African species, and *Epitrachys* (DC. ex Duby) K.Koch, comprising a single Mediterranean species. Furthermore, Moreyra *et al.* (2023) later suggested the separation of two Central-African genera, *Afrocirsium* Calleja, Garcia-Jacas, Moreyra & Susanna (three species) and *Nuriaea* Susanna, Calleja & Moreyra (two species). This taxonomic restructuring is supported phylogenetically (Del Guacchio *et al.* 2022; Moreyra *et al.* 2023), morphologically (Del Guacchio *et al.* 2022; Bureš *et al.* 2023; Moreyra *et al.* 2023), and by genomic features (Bureš *et al.* 2023).

However, the morphological distinction between *Lophiolepis* and *Cirsium* encounters complications due to the occurrence of the somewhat intermediate *Cirsium vulgare* (Savi) Ten., a Palearctic species naturalized worldwide (POWO 2023). On one hand, it shows rigid setae on the upper leaf surface (Fig. 1) and the 3D arrangement of involucral spines reminiscent of the lances of a Macedonian phalanx (Fig. 2): two features typical of *Lophiolepis*. On the other hand, *C. vulgare* differs from all *Lophiolepis* species in some morphological features, the most evident of which are the decurrent leaves (Fig. 3). This latter feature is indeed shared with ca. 10 % taxa of *Cirsium* s. str. (Bureš, pers. obs.), namely the Old World species *C. alatum* (S.G.Gmel.) Bobrov, *C. brachycephalum* Juratzka, *C. canum* (L.) All., *C. chrysacanthum* (Ball) Jahand., *C. creticum* (Lam.) d'Urv., *C. ducellieri* Maire, *C. dyris* Jahand. & Maire, *C. elbrusense* Sommier & Levier, *C. elodes* M.Bieb., *C. gaditanum* Talavera & Valdés, *C. glaberrimum* (Petr.) Petr., *C. hygrophilum* Boiss., *C. komarovii* Schischk., *C. leucopsis* DC., *C. libanoticum* DC., *C. monspessulanum* (L.) Hill, *C. palustre* (L.) Scop., *C. pectinellum* A.Gray, *C. pseudocreticum* (P.H.Davis & Parris) Yıldız, Dirmenci & Arabacı, *C. pyrenaicum* (Jacq.) All., *C.*

rhabdotolepis Petr., *Cirsium sidi-guinii* Pau & Font Quer, *C. subinerme* Fisch. & C.A.Mey., *C. svaneticum* Sommier & Levier, *C. uliginosum* (M.Bieb.) Fisch., and *C. valdespinulosum* (Sennen) Sennen or the New World species *C. coahuilense* Ownbey & Pinkava, *C. inamoenum* (Greene) D.J.Keil, *C. jorullense* Spreng., *C. lomatolepis* Petr., *C. macvaughii* G.L.Nesom, *C. magdalenense* G.L.Nesom, *C. mexicanum* DC., *C. pacificum* G.L.Nesom, *C. rhaphilepis* Petr., and *C. wrightii* A.Gray.

In recent molecular phyllogenies, *C. vulgare* was unequivocally embedded within the paraphyletic *Cirsium* lineages but never in the monophyletic clade corresponding to genus *Lophiolepis* (Ackerfield *et al.* 2020: "*Cirsium* sect. *Eriolepis*"; Del Guacchio *et al.* 2022; Bureš *et al.* 2023). More recently, a phylogenetic reconstruction obtained with a nuclear HybSeq dataset by Moreyra *et al.* (2023) consistently indicated *C. vulgare* as sister taxon to a clade corresponding to *Lophiolepis*, while the clade *Lophiolepis* + *C. vulgare* is in turn sister to *Cirsium* s. str.; nevertheless, in the phylogenetic tree based on the chloroplast dataset, *C. vulgare* resulted as nested in *Cirsium* s.str. (Supplementary material by Moreyra *et al.* 2023). Notably, genomic parameters like monoploid genome size, genomic GC content, and average chromosome size of *C. vulgare* fall in between the typical values for Eurasian *Cirsium* species and those characterizing *Lophiolepis* (see Figs. 3A, B in Bureš *et al.* 2023). Similarly, the achene weight of *C. vulgare* exhibits a clear intermediate position between the values typical of representatives of *Cirsium* and *Lophiolepis* (see Fig. 3E in Bureš *et al.* 2023). Finally, it is to be noted that *C. vulgare* is tetraploid (2n = 4x = 68), while most taxa of *Cirsium* s. str. are diploids (2n = 34) as well as almost all *Lophiolepis* taxa (Watanabe 2008; Rice *et al.* 2015; Bureš *et al.* 2004, 2023.

As a consequence, we have hypothesized that *C. vulgare* is a hybridogenous species, likely derived from hybridization between *Lophiolepis* and *Cirsium* (Bureš *et al.* 2023). This hypothesis was later echoed by Moreyra *et al.* (2023), basing on incongruences between the positions of *C. vulgare* in nuclear and chloroplast phylogenies. The possible intergeneric hybrid origin of *C. vulgare* can further be deduced from its ability to hybridize with both genera *Lophiolepis* and *Cirsium*, whereas other

hybrids between these genera very rare or poorly known (Del Guacchio *et al.* 2022; Bureš *et al.* 2023).

Cirsium vulgare, commonly known as bank thistle, bull thistle, common thistle, scotch thistle, and spear thistle, is a monocarpic species with a biennial to shortly perennial (or rarely annual) life cycle, typically flowering from late summer to autumn (Klinkhamer & De Jong 1993). Remarkably, it can produce up to 50,000 seeds per individual (Michaux 1989). Thriving in open disturbed environments such as arable field margins, forest clear-cuts, pastures, and roadsides (Klinkhamer & De Jong 1993; Keil 2006), its native distribution spans Europe, western Asia, and North Africa (Meusel *et al.* 1992; GBIF 2023; POWO 2023). Due to its affinity for human-altered habitats*, C. vulgare* has successfully naturalized and invaded regions across cool-temperate, warm-temperate, and subtropical zones on all continents except Antarctica. Consequently, it has earned a reputation as one of the most pernicious and widespread invasive plant globally (Julien & Griffiths 1998; Parsons & Cuthbertson 2001; Sagliocco *et al.* 2012; Pyšek *et al.* 2017; Román *et al.* 2021).

In this study, we analyzed the enigmatic origin of the noxious weed *Cirsium vulgare*, employing RADseq techniques. Additionally, employing herbarium revision, we investigated the ability of *C. vulgare* to form interspecific natural hybrids with representatives of *Cirsium* and *Lophiolepis* in a broader context of the intensity of spontaneous hybridization within these two genera and between them in Central Europe, the British Isles, and across the World. Finally, we taxonomically interpreted our results.

MATERIAL AND METHODS

Taxonomy

As a general rule, we follow the taxonomic circumscription and nomenclature of POWO (2023), disregarding any rank below subspecies. However, also according to Del Guacchio *et al.* (2022) and references herein, we do not recognize any infraspecific taxon within *C. vulgare*. The name *Cirsium acaulis* (POWO 2023) is amended to *C. acaulon*, retaining the Greek adjectival epithet of the

basionym *Carduus acaulos* chosen by the original author (Linnaeus, Sp. pl.: 1199. 1753), and transformed into "*acaule*" by Scopoli, Annus Hist.-Nat. 2: 62. 1769, when he transferred the species to *Cirsium* (Art. 23.5, Ex. 9) (cf. also Greuter, 2006+).

Sampling

We examined eight tetraploid individuals of *Cirsium vulgare*, each from a different population, together with11 individuals, each representing one of the diploid European, Mediterranean or West Asian species of the genus *Lophiolepis*, i.e., *L. aggregata* (Ledeb.) Bureš, Del Guacchio, Iamonico & P.Caputo, *L. bulgarica* (DC.) Del Guacchio, Bureš, Iamonico & P.Caputo, *L. caucasica* (Adams) Bureš, Del Guacchio, Iamonico & P.Caputo, *L. lobelii* (Ten.) Del Guacchio, Bureš, Iamonico & P.Caputo, *L. macrobotrys* (K.Koch) Bureš, Del Guacchio, Iamonico & P.Caputo, *Lophiolepis morisiana* (Rchb.f.) Del Guacchio, Bureš, Iamonico & P.Caputo, *L. rigida* (DC.) Bureš, Del Guacchio, Iamonico & P.Caputo, *L. scabra* (Poir.) Del Guacchio, Bureš, Iamonico & P.Caputo, *L. sommieri* (Petr.) Del Guacchio, Bureš, Iamonico & P.Caputo, *L. tenoreana* (Petr.) Del Guacchio, Bureš, Iamonico & P.Caputo, *L. trachylepis* (Boiss.) Del Guacchio, Bureš, Iamonico & P.Caputo, and 12 samples of the diploid Eurasian species of the genus *Cirsium*, i.e., *C. acaulon* (L.) Scop. subsp. *acaulon*, *C. alsophilum* (Pollini) Soldano, *C. arvense* (L.) Scop., *C. canum* (L.) All., *C. carniolicum* Scop. subsp. *carniolicum*, *C. erisithales*, *C. heterophyllum* (L.) Hill, *C. monspessulanum* (L.) Hill, *C. oleraceum* (L.) Scop., *C. palustre* (L.) Scop., *C. pannonicum* (L.) Link, and *C. spinosissimum* (L.) Scop. (Supplementary Table S1; DNA-ploidy level of the individuals has been cytometrically tested by Bureš *et al.* 2023).

DNA isolation, RADseq library preparation, and sequencing

Genomic DNA was extracted from leaves using NucleoSpin Plant II Genomic DNA Purification kit (Macherey-Nagel, Germany) following the manufacturer's instructions. The tissue was homogenized in liquid nitrogen and lysed in PL2 buffer. DNA quality was checked on 1% agarose gel, and DNA concentration was measured using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, USA). To prepare a library, we used 200 ng of DNA per diploid sample and 300 ng for tetraploid *C. vulgare*.

RADseq library was assembled following Etter *et al.* (2011) protocol with minor modifications. To cut the DNA, we used PstI restriction enzyme (New England Biolabs, USA). Single-end sequencing with dual barcoding and a read length of 99 bp was conducted using the Illumina NovaSeq 6000 (Genomics Core Facility, CEITEC, Masaryk University, CZ).

Raw sequence data treatment

The raw reads were demultiplexed and freed from restriction sites in Stacks v.2.62 (Catchen *et al.* 2013). The quality of the resulting single-end 94 bp-long reads was checked using FastQC v.0.11.5 (Andrews *et al.* 2010). To sort the reads to nuclear, chloroplast, and mitochondrial, we blasted them against complete chloroplast (*Cirsium arvense*: NC_036965.1, *C. vulgare*: NC_036967.1, and *Lophiolepis eriophora*: NC_036966.1) and mitochondrial (*Saussurea costus*: NC_059793.1, *S. inversa*: NC_066407.1) genomes in Geneious Prime software v.2023.0.1 (Biomatters Ltd, New Zealand) with medium sensitivity settings. Reads not identified as chloroplast or mitochondrial were treated as nuclear.

Sorting of sequence data into individual loci

To sort and align the reads into individual loci, we employed ipyrad v.0.9.85 (Eaton & Overcast 2020), which can handle larger phylogenetic scales (two genera: *Lophiolepis*, *Cirsium*; Eaton *et al.* 2014) and respect ploidy levels (tetraploid *C. vulgare* among diploid species; Wagner 2020). In particular, diploid and tetraploid entries were analyzed separately and, at the end of the ipyrad analysis, merged into the final outputs. To avoid grouping of paralogous sequences in nuclear reads, we performed an optimization procedure to select appropriate intra-sample and between-sample clustering thresholds (Ilut *et al.* 2014; McCartney-Melstad *et al.* 2019; Hühn *et al.* 2022). These outputs are subsequently used for further analyses as inputs. The suitable intra-sample threshold was 0.95 for both diploids and tetraploids, while the between-sample threshold was 0.93. Initial sorting of organellar reads was performed only with the optimized between-sample threshold 0.86 and then followed by manual exclusion of loci that were not uniform across *C. vulgare* samples, loci

whose samples had two different alleles, and loci containing sequences with more than two N bases. These rules led to the identification and removal of paralogs.

Loci and SNPs datasets preparation

For subsequent NeighborNet, Heatmap, and STRUCTURE analyses (see below), we prepared two datasets based on nuclear and organellar RADseq data. For the dataset based on nuclear loci, we filtered the loci to reduce the percentage of missing sequences in the concatenated dataset below 10%. This was achieved when we removed all the loci that were not shared by at least 25 of 31 samples. The final nuclear SNPs dataset based on 7,253 loci contained 27,944 parsimony informative sites with 9.07 % of missing data. We allowed a higher missing data threshold for the dataset based on organellar RADseq data because the overall percentage of missing sequences in organellar loci was high. This resulted in an organellar SNPs dataset based on 15 loci having 20.46 % missing sites and 32 parsimony informative sites.

For the quantification of shared and clade-specific nuclear loci in *Lophiolepis* and *Cirsium* genera and *C. vulgare*, we kept all the loci shared by at least eight samples, resulting in the third dataset based on 51,256 loci containing 119,029 parsimony informative sites with 50.67 % of missing data.

Assessment of genetic relationships of Cirsium vulgare *with* Lophiolepis *and* Cirsium

To assess the hybrid origin of *C. vulgare,* we applied several approaches. First, based on nuclear and organellar supermatrices, we constructed NeighborNet trees in SplitsTree5 ver.5.3.0 (Huson & Bryant 2006) employing distances calculated using the “dist.p” function in the R package phangorn v.2.8.1 (Schliep 2011). Second, using the “phylo.heatmap” function in the R package Phytools v1.0.1 (Revell 2012), we constructed a heatmap based on the nuclear supermatrix employing the same distance matrix as the NeigborNet tree. Third, we analyzed the nuclear SNP matrix in STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000). We tested K-values ranging from 1 to 4 without prior population information under the admixture model, assuming independent allele frequencies. Ten runs with a burn-in 100,000 followed by 100,000 MCMC iterations were performed for each K-value. The method of

Evanno *et al.* (2005) was applied to determine the most likely genetic clusters using the pophelper v.2.3.1 R package (Francis 2017). Fourth, based on the presence/absence of sequences representing *Lophiolepis*, *Cirsium,* or *C. vulgare* samples, each locus from the dataset of 51,256 loci was classified as shared by two or three groups or group-specific into seven categories.

Estimation of willingness to hybridize spontaneously

Herbarium specimens of *Cirsium*- and *Lophiolepis*-hybrids were identified during our revision of 23,924 *Cirsium*-specimens in herbaria BP, BRA, BRNM, BRNU, CB, CHOM, GJO, GM, GZU, HLO, HMP, HNTS, HOMP, HR, KO, LI, LIM, LIT, LTM, MJ, MMI, MP, NI, NJM, OL, OLM, OP, OSM, PL, PMK, PR, ROZ, SAV, SLO, SMBB, SOKO, SUM, TM, TNP, VM, VYM, W, WU, and ZMT (Blizňáková, 2010; Vavrinec, 2020; herbarium codes according to Thiers, 2023). These herbaria are representative of distribution and hybridization of 15 species of *Cirsium* (incl. *C. vulgare*) and three species of *Lophiolepis* in Austria, Hungary, Czech Republic, Slovakia, Slovenia, and part of Northern Italy (historical territory of South Tyrol), where hybrids were intensively documented from the 19th century. The locations of the hybrid specimens have been georeferenced and integrated into the quadrants of Central European flora mapping (quadrant = 5 × 3 minutes in the west-east or north-south direction, respectively; the total number of quadrants covering the above mentioned studied area was 10,203; Vavrinec 2020). For each species-pair (= potential interspecific hybrid within *Cirsium* or *Lophiolepis* genera or between them), distribution overlap was calculated as the number of quadrants occupied by both species. For each overlapping pair of species A and B, the willingness to hybridize spontaneously (W_H) was estimated as the percentage of quadrants occupied by the hybrid A×B from all quadrants in which both parental species A and B co-occurred according to the following formula:

$$W_H(\mathrm{SpA, B}) = 100 \times \frac{number\ of\ map\ quadrants\ occupied\ by\ hybrid\ between\ species\ A\ and\ B}{number\ of\ map\ quadrants\ in\ which\ species\ A\ and\ B\ cooccur}\ [in\ \%]$$

For overlapping (= non-disjunctive) species pairs that do not hybridize, W_H = 0. For comparison among intensities of hybridization (i) between *C. vulgare* and *Lophiolepis*, (ii) between *C. vulgare* and *Cirsium*, (iii) within *Cirsium* (excl. *C. vulgare*), (iv) within *Lophiolepis*, and (v) between *Lophiolepis* and

Cirsium (excl. *C. vulgare*), the overall willingness was calculated as a weighted average of all W_H values within respective category (i–v; weighted by the numbers of quadrants shared by respective parental species). Disjunctive species pairs were excluded from the analyses because they cannot hybridize spontaneously.

The numbers of the quadrants where species co-occur were taken from the distribution maps of *Cirsium* and *Lophiolepis* species in the Czech Republic (Bliznáková 2010), Hungary (Bartha *et al.* 2015), Slovakia (Vavrinec 2015), Slovenia (Jogan *et al.* 2001), and South Tyrol (Wilhalm *et al.* 2014). For Austria, the unpublished distribution maps of *Cirsium* species were kindly provided by Prof. H. Niklfeld (see also Vavrinec 2020). Summary data for all Central European hybrids are listed in Supplementary Table S2. The analogous analysis of the hybrid willingness in the British Isles was conducted with the respective data taken from Preston *et al.* (2002), Stace *et al.* (2015), and Supplementary Table S3.

In addition to regional analyses, we made a global-scale analysis, in which we recorded all species pairs within *Lophiolepis* and *Cirsium* (incl. *C. vulgare*) for which hybridization is reported. The parental affiliations of hybrids were taken from the protologues whose citations/links were obtained from IPNI (2023), standard floras, or nomenclatural surveys. For each category (i–v) mentioned above, the hybridization propensity (P_H) was estimated as the percentage of all hybridizing species pairs from all overlapping species pairs within the respective category according to formulae:

$$P_H(\mathrm{GenC}) = 100 \times \frac{number\ of\ hybridizing\ species\ pairs\ in\ the\ genus\ C}{number\ nondisjunctive\ pairs\ of\ species\ in\ the\ genus\ C}\ [in\ \%]$$

to estimate hybridization within *Cirsium* (excl. *C. vulgare*), or within *Lophiolepis*, and

$$P_H(\mathrm{GenA, B}) = 100 \times \frac{number\ of\ hybridizing\ pairs\ of\ species\ where\ one\ is\ from\ genus\ A\ and\ the\ other\ from\ genus\ B}{number\ of\ nondisjunctive\ pairs\ of\ species\ where\ one\ is\ from\ genus\ A\ and\ the\ other\ from\ genus\ B}\ [in\ \%]$$

to estimate hybridization between *C. vulgare* and *Lophiolepis*, between *C. vulgare* and other *Cirsium* species, or between *Lophiolepis* and *Cirsium* (excl. *C. vulgare*). The estimation of geographic overlap or disjunction across all 90,100 species pairs given by 103 species of *Lophiolepis* and 322 species of

Cirsium (incl. *C. vulgare*) was based on data reported in POWO (2023). For the complete dataset, see Supplementary Tables S4, S5.

RESULTS

RADseq data statistics

RADseq of 31 samples comprising 12 *Cirsium* samples/species, 11 *Lophiolepis* samples/species, and 8 samples of *C. vulgare* yielded an average of 1.90×10^6 raw reads per each of 31 analyzed individuals (SD 8.43×10^5; range: 0.73 to 3.74×10^6 raw reads). The average per base sequence quality was very high, with a Phred score of 36 for all positions in the reads in all samples. On average, we retained 1.38×10^6 raw reads of nuclear sequences per individual (SD 5.61×10^5; range: 0.57 to 2.99×10^6 raw reads) and 5.25×10^5 raw reads of organellar sequences per individual (SD 3.83×10^5; range: 0.14 to 1.46×10^6 raw reads). The mean coverages of nuclear and organellar loci were 10.4x (SD 3.1) and 659.1x (SD 263.4), respectively.

Molecular analysis of the genetic relations of Cirsium vulgare *to genera* Cirsium *and* Lophiolepis

The NeighborNet phylogenetic network of nuclear SNPs placed *C. vulgare* in between clearly separated clusters of *Cirsium* and *Lophiolepis* species (Fig. 4a). On the other hand, NeighborNet based on the organellar SNPs revealed the affiliation of *C. vulgare* samples to *Cirsium* with the closest relation to *C. palustre*, *C. monspessulanum*, and *C. alsophilum* (Fig. 4b).

Among-sample dist.p matrix based on the nuclear RADseq data visualized using the Heatmap suggested that all samples representing either *Lophiolepis* or *Cirsium* genera are more similar to *C. vulgare* than to each other (Fig. 5).

Based on the highest ΔK (Supplementary Fig. S1), the STRUCTURE analysis showed that the nuclear genome of *C. vulgare* is most likely composed of two subgenomes, each attributable to *Cirsium* or *Lophiolepis* genera (K = 2, Fig. 6a). All the analyzed *C. vulgare* individuals showed consistent

277 proportionally similar admixture of both suspected parental genomes. An alternative K = 3, under
278 which *C. vulgare* forms a separate cluster (Fig 6b), was less likely yet still acceptable (Supplementary
279 Fig. S1).

280 The numbers of loci that are clade-specific or shared by two or three clades are shown in the sectors
281 of the Venn diagram (Fig. 7). The highest proportion of the nuclear loci was shared by *C. vulgare* and
282 both suspected parental genera (18,892; 36.9 %). About one-third fewer loci were shared by *C.*
283 *vulgare* with *Cirsium* (13,439; 26.2 %) or *Lophiolepis* (12,217; 23.8 %). The number of loci absenting in
284 *C. vulgare* but shared by *Cirsium* and *Lophiolepis* samples was significantly smaller (1,460; 2.8 %) as
285 well as the numbers of loci occurring exclusively in samples of *C. vulgare* (2,173; 4.2 %), *Lophiolepis*
286 (2,039; 4.0 %) or *Cirsium* (1,036; 2.0 %; Fig 5).

287 *Spontaneous hybridization of* C. vulgare *with* Lophiolepis *and* Cirsium *in the context of hybridization*
288 *within and among these genera*

289 We identified 60 different hybrids involving 15 *Cirsium* species (including *C. vulgare*) and 3
290 *Lophiolepis* species, represented by 4865 herbarium specimens (Supplementary Table S2). Of these,
291 27 (0.55 %) specimens were hybrids of *Cirsium vulgare* with other species of *Cirsium* (namely, *C.*
292 *acaulon*, *C. erisithales*, *C. oleraceum*, and *C. palustre*) and 8 (0.16 %) specimens were hybrids
293 between *C. vulgare* and *Lophiolepis eriophora* (L.) Del Guacchio, Bureš, Iamonico & P.Caputo
294 (Supplementary Table S2).

295 The willingness to form spontaneous hybrids varied substantially among geographically overlapping
296 species pairs across *Lophiolepis* and *Cirsium* from zero (58 non-hybridizing but overlapping species
297 pairs) to 43.40 % in Central Europe (hybrids between *C. erisithales* and *C. greimleri* Bureš;
298 Supplementary Table S2). On average, the willingness of *Cirsium vulgare* to create spontaneous
299 hybrids with Central European species of *Cirsium* or *Lophiolepis* (0.10 % or 0.56 %, respectively) was
300 significantly lower than the willingness of *Cirsium* or *Lophiolepis* species to form congeneric hybrids
301 (6.95 % or 6.67 %, respectively; Table 1). On the other hand, the willingness of *C. vulgare* to

spontaneously hybridize with both genera was higher than the average willingness of both genera to create intergeneric hybrids (0.03 %; Table 1). Proportionally similar average willingness to form spontaneous hybrids of respective types was estimated for the British Isles, where only 11 hybrids were recognized between *C. vulgare*, eight species of *Cirsium*, and one of *Lophiolepis* (Table 1; Supplementary Table S3).

On a global (world) scale, 103 species of *Lophiolepis* and 321 of *Cirsium* were analyzed together with *C. vulgare* (Table 1). Among these species, 253 unique hybrids (hybridizing species pairs) were recorded, and 9,181 of all 90,100 species pairs geographically overlapped (Supplementary Table S4). The overall propensities of *C. vulgare* to hybridize with species of *Lophiolepis* or *Cirsium* were even higher (4.04 % or 16.49 %, respectively) than in the regional scales and the propensity to form intergeneric hybrids between *Lophiolepis* and *Cirsium* (0.63 %) was lower than the propensities to create congeneric hybrids within *Lophiolepis* or *Cirsium* (1.98 % or 3.15 %, respectively; Table 1, Supplementary Table S4).

DISCUSSION

Molecular analyses support the hypothesis that C. vulgare *originated as intergeneric hybrid between* Cirsium *and* Lophiolepis

Our results based on nuclear RADseq markers showed that *Cirsium vulgare* has a genomic makeup made of both *Cirsium* and *Lophiolepis* and that it is more similar to any of them than these genera are between each other (Figs 4–7). For instance, the numbers of nuclear loci that *C. vulgare* shares either with *Lophiolepis* or *Cirsium* (12,217 or 13,439, respectively) are proportionally similar and several times higher than its unique loci (2,173) and the loci shared by both genera but absent in *C. vulgare* (1,460; Fig. 7). Such a pattern indicates that (i) the nuclear genome of *C. vulgare* arose from the “fusion” of two genomes – the first belonging to *Cirsium* and the second to *Lophiolepis* – and that (ii) this “fusion” took place later than these genera diverged from their most recent common

ancestor. This is in line with the results of the STRUCTURE analysis which revealed that the nuclear genome of *C. vulgare* is most likely composed of two proportionally similar parts of *Cirsium* and *Lophiolepis* genomes (Fig. 6a). The less likely but still acceptable K = 3 in the STRUCTURE analysis makes sense (Fig. 6b), because it suggests that *C. vulgare* is an established species that has accumulated its own mutations distinguishing it from its parental species (as also indicated by *C. vulare*'s unique loci in Fig. 7). All these results are fully congruent with the hypothesized hybridogenous origin of *C. vulgare* based on intermediate genomic GC content between *Lophiolepis* and Eurasian *Cirsium* (Bureš *et al.* 2023), i.e., *Cirsium* s. str. (cf. also Moreyra *et al.* 2023).

The hybridogenous origin of *C. vulgare* is also entirely consistent with the conflicting position of *C. vulgare* in nuclear and chloroplast HybSeq-based phylogenies detected by Moreyra *et al.* (2023). These authors found *C. vulgare* as sister group to all *Lophiolepis* taxa and the clade *C. vulgare* + *Lophiolepis* sister to *Cirsium* s. str. in the nuclear phylogeny but embedded among paraphyletic lineages of *Cirsium* s. str. in the chloroplast-based phylogeny, namely nested within an Eurasiatic clade including taxa belonging to *Cirsium* sect. *Cirsium*. Suggestively, several taxa of that clade show winged stems as *C. vulgare.* This indicates that the maternal parent species of *C. vulgare* belonged to the *Cirsium* genus; this is also clear from the conflict between the positions of *C. vulgare* in our nuclear and organellar NeighborNet analyses (Fig. 4). Our results also revealed that two linked nuclear markers – ITS, ETS – previously used in the phylogenetic analyses by Ackerfield *et al.* (2020), Del Guacchio *et al.* (2022), and Bureš *et al.* (2023) likely came from the maternal parent of *C. vulgare*, in which they were likely fixed by a gene conversion (Wang *et al.* 2023), and, therefore, together with the chloroplast markers clearly indicated affiliation of *C. vulgare* to the genus *Cirsium*.

The closest maternal relatives in our organellar NeighborNet were Alpine-Dinaric species *C. alsophilum*, Western Mediterranean *C. monspessulanum*, and Eurasian *C. palustre* (the last two species share decurrent leaves with *C. vulgare*; Fig. 3). However, our dataset comprises only 12 *Cirsium* species. Interestingly, in much species-rich phylogenies by Del Guacchio *et al.* (2022) or Bureš

et al. (2023), its closest relative was *C. heterotrichum*, not included in the present study; independently, the same species was identified as the closest relative of *C. vulgare* in HybSeq-based chloroplast phylogeny by Moreyra *et al.* (2023). Although it is not very likely that the northern Balkan endemic *C. heterotrichum* could be the true mother of *C. vulgare*, it might be the closest living relative of this mother.

The intergeneric hybridogenity of *C. vulgare* is consistent with the intermediate morphology of its achene size when compared to *Lophiolepis* and Eurasian *Cirsium* species (Bureš *et al.* 2023) and the intermediarity of some leaf anatomical features such as trachea diameter or phloem height (see Table 2 in Özcan *et al.* 2015). Another feature in which *C. vulgare* is intermediate between *Cirsium* and *Lophiolepis* is its flowering period in Europe, where most *Cirsium* species start flowering earlier than *C. vulgare*; in contrast, all *Lophiolepis* species start their blossoming later when they co-occur with *C. vulgare* here.

Spontaneous hybridization of Cirsium vulgare *with* Lophiolepis *and* Cirsium *species in the context of spontaneous hybridization within or between these genera*

The ability of *Cirsium vulgare* to hybridize with both *Lophiolepis* or *Cirsium* was confirmed in two regional analyses (Central Europe, British Isles) and at a global World-scale analysis (Table 1). In all three analyses, *C. vulgare* hybridizes more willingly with each of these genera than these genera do with each other (Table 1). Although such a behavior of *C. vulgare* does not directly prove its intergeneric hybridogenous origin, it is at least the most expected consequence of such an origin.

On a global scale, the propensity to form intergeneric hybrids between *Lophiolepis* and *Cirsium* (excl. *C. vulgare*) was significantly lower than the propensity to form interspecific hybrids within these genera (Table 1). Although, on a regional scale, the estimation of willingness to hybridize within *Lophiolepis* is limited by a low number of species in the British Isles, also, on this scale, the willingness to form intergeneric hybrids is significantly lower than interspecific hybridization within *Cirsium* or *Lophiolepis* (Table 1). Therefore, the argument that the separation of the genus *Lophiolepis* from the

genus *Cirsium* is challenged by their "extremely frequent" hybridization (Moreyra *et al.* 2023) is incorrect and not supported by the evidence (Table 1; Supplementary Table S5C). Moreover, although intergeneric hybrids are also reported between *Cirsium* and *Carduus* (see Vukotinović 1877, p 206; Guétrot 1925, p. 29; Fournier 1928, p. 277, 1940, p. 1001–1004; Sennen 1931, p. 19), it was never used as an argument against the taxonomic relevance of these genera. Nevertheless, a comprehensive revision, ideally supported by molecular analyses (Segarra-Moragues *et al.* 2007; Michálková *et al.* 2018, 2023), of all herbarium specimens of putative hybrids may potentially reveal that many of them are only defect/pauperized individuals of pure species; see Table S5C, where nearly half of the cases of presumed intergeneric hybrids between *Cirsium* and *Lophiolepis*, for which specimens were accessible, their existence was challenged by morphology-based reassessment. During our revision of Central European herbaria we found 84.8% reliability of original determination of *Cirsium* and *Lophiolepis* hybrid specimens, namely 9.9 % of them we identified as pure species and 5.3 % as different hybrids; in species the reliability of original determination was higher (93.9 %).

Estimation of willingness to hybridize based on the herbarium revision revealed substantial variation across *Cirsium* and *Lophiolepis* species pairs in Central Europe as mentioned, e.g., by Wagenitz (1986), Stöhr (2006) or Bureš *et al.* (2010). The estimation follows the simple methodology used in Hybrid Flora of the British Isles (Stace *et al.* 2015) that have comparable area but lower number of *Cirsium* and *Lophiolepis* species/hybrids (10/11 in British Isles vers. 18/60 in Central Europe). Despite this difference, the overall pattern of hybridization was similar in both regions (Table 1). This well illustrates that the hybridization propensity of a given taxonomic group should be generally consistent across regions as postulated by Whitney *et al.* (2010).

In both Central Europe and British Isles, hybrids are traditionally the focus of taxonomists and regional botanists and their distribution is here representatively documented (see Table 1 in Whitney *et al.* 2010; Stace *et al.* 2015). Although it makes our estimates of hybrid willingness more reliable, the interpretation is territorially limited because of the considerably smaller size of these regions

when compared to the distribution ranges of the entire genera *Cirsium* or *Lophiolepis*. To compensate for this deficiency, we did additional global-scale analysis (using the same hybrid categories = lines in Table 1), in which all 153 hybridizing species pairs recorded across all 323 species of *Cirsium* (incl. *C. vulgare*) and 103 species of *Lophilepis* were compared to 9192 overlapping species pairs (Table 1).

Invasiveness of C. vulgare *could be a consequence of its allopolyploid hybrid origin*

Interspecific (or intergeneric) hybridization can initiate polyploidization and the emergence of transgressive phenotypes, both of which may effectively aid the expansion or colonization of niches inaccessible by parental species (Stebbins 1985; Soltis and Soltis 2000; Fawcett & Van de Peer 2010; Stelkens *et al.* 2014; Hodgins *et al.* 2018; Van de Peer *et al.* 2021). Accordingly, a tendency towards higher invasiveness (which is de facto also an ecological success of a species) has been repeatedly observed in polyploid (te Beest *et al.* 2011; Pandit *et al.* 2011; Moura *et al.* 2021) or hybridogenous species (Ellstrand and Schierenbeck 2000; Hovick & Whitney 2014; Hodgins *et al.* 2018). *Cirsium vulgare* nicely illustrates such a scenario. It is allotetraploid and, compared to *Cirsium* or *Lophiolepis* species, has a thicker cuticle and higher stomatal index (Özcan *et al.* 2015), transgressive traits that can be associated with propensities for invasiveness (Cavaleri & Sack 2010). *Cirsium vulgare* has rapidly expanded its originally Eurasian geographic range to all continents, eventually occupying an area larger than all the species of its parental genus *Lophiolepis* (Supplementary Figs. S2, S3). In addition, a recently published analysis of *Cirsium vulgare*'s current distribution, using models that considered its niche dynamics, revealed a significant potential for this species to further expand in new areas with warmer climates (Román *et al.* 2021).

From the affinity to habitats disturbed by human activity, it can be concluded that the expansion of the *C. vulgare* natural range occurred with the post-Neolithic spread of agriculture and pastoralism followed by the establishment of villages, towns, roads, and other human activities in the landscape. However, *C. vulgare* could have originated a long time before this natural range expansion. The

interglacial fossil records are sometimes reported (cf. Klinkhamer and de Jong 1993,) but these are probably based only on very approximately determined material (such as "achenes of *Cirsium vulgare* type") by Watts *et al.* (1963), who detected it in southern Ireland (Co. Limerick) in deposits dated approximately as Elsterian-Saalian interglacial (337–300 thousand years BP).

Taxonomic and phylogenetic consequences of hybridogenity of C. vulgare

The monophyletic nature of *Lophiolepis* (paternal genus of *C. vulgare*) and its separation from the rest of *Cirsium* (maternal genus of *C. vulgare*) has been confirmed in all recent phylogenetic studies (Ackerfield *et al.* 2020; Del Guacchio *et al.* 2022; Bureš *et al.* 2023; Moreyra *et al.* 2023). In a comprehensive HybSeq phylogenetic analyses, the nuclear- and chloroplast-based trees were in conflict regarding the position of *Lophiolepis* (Moreyra *et al.* 2023). Whereas in the nuclear concatenated phylogeny, *Lophiolepis* and *Cirsium* were sister clades, in the nuclear coalescent (often regarded as the most reliable among the two), *Lophiolepis* was sister to *Picnomon* and both sister in turn to *Lophiolepis*; more relevant, in the concatenated chloroplast phylogeny, *Cirsium* and *Lophiolepis* resulted much more distant because they were separated by another three genera, namely *Afrocarduus*, *Afrocirsium*, and *Silybum* (Moreyra *et al.* 2023). A coalescent plastid tree was not provided. As said above and underlined by Moreyra *et al.* (2023), *C. vulgare* belonged to *Cirsium* in the chloroplast tree but to *Lophiolepis* clade in both nuclear trees. Nevertheless, we must add that *C. vulgare* was never nested in *Lophiolepis* in these cases but rather sister to it (see Figs. 1, 2 in Moreyra *et al.* 2023), which is a very relevant point. Considering the strong evidence for the hybridogenous origin of *C. vulgare* presented in our study, it cannot be ruled out that the sister position of *Lophiolepis* and *Cirsium* in the concatenated nuclear HybSeq phylogeny by Moreyra *et al.* (2023), might simply be a consequence of the inclusion of *C. vulgare* into the dataset.

Because established hybridogenous species (or genera) cannot be recognized as nothotaxa (Art. H.3.3, Note 1 in Turland *et al.* 2018), the allotetraploid *Cirsium vulgare* cannot be ascribed to the nothogenus ×*Lophiocirsium* Del Guacchio, Bureš, Iamonico & P.Caputo comprising occasionally

originating spontaneous intergeneric hybrids between species of *Cirsium* and *Lophiolepis* genera. Hybridogenous species of more or less ancient intergeneric origin can be reliably identified only by molecular techniques, and they are still rarely recognized (Wallace & Jansen 1995; German & Frisen 2014; Díaz Lifante *et al.* 2023), more often only hypothesized (e.g., Soejima *et al.* 2008; Calvo *et al.* 2012). In addition, there is no general agreement about the taxonomic assessment of these hybridogenous taxa, and the ICN is almost silent on this matter.

To achieve stability for the classification, the most appropriate solution seems to classify the established intergeneric allotetraploid *Cirsium vulgare* as a separate monotypic genus, distinguished from *Lophiolepis* by the decurrent leaves (Fig. 3c, h vers. a, b, f, g) and from *Cirsium* by the presence of rigid setae on the upper leaf surface (Fig. 1h–p). Fortunately, the generic name *Ascalea* Hill is available for such a genus (detail taxonomical treatment is given in separate paper - Del Guacchio *et al.* in prep.).

The segregation of *C. vulgare* into separated hybridogenous genus *Ascalea* makes also clear the morphologic delimitation of *Lophiolepis* against *Cirsium*. The previous argument of Ackerfield *et al.* (2020, p. 729) that rigid setae of *Ascalea lanceolata* are actually not homologous to those of *L. eriophora* (or other *Lophiolepis*) because they emerge from veins and have enlarged bases is at odds with our anatomical observations. We found that the bases of the sclerenchymatized rigid setae in *A. lanceolata* are surrounded by neighboring cells, so the setae grow out of raised areolae or protrusions (Fig. 1a, b), but the sclerenchymatized setae itself do not differ from *L. eriophora* in base width and shape (Fig. 1c, d vers. k, l, or 2e, f, g vers. m, n. o, p). Moreover, such protrusions are not synapomorphic exclusively for *A. lanceolata* since they occur also in other *Lophiolepis* taxa such as *L. sorocephala* subsp. *congesta* (Fisch. & C.A.Mey. ex DC.) Bureš, Del Guacchio, Iamonico & P.Caputo (Fig. 1h). When we examined connection between the setae and veins in *A. lanceolata* and *L. eriophora* we found there is actually no difference: in both species the larger setae are connected with veins (see Fig. 1f, g, o, p) while smaller often not (Fig. 1e, m, n). Summarizing, the rigid setae on

upper leaf surface of *A. lanceolata* are very likely homologous to those of *Lophiolepis eriophora* and other *Lophiolepis* species.

Data Availability Statement

Raw sequencies generated during this study were submitted to NCBI on October 25th, 2023, as Sequence Read Archive (SRA) submission no. SUB13926806.

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Authors contributions

PBu and FZ conceived of the study. JŠ and EM performed the labwork behind RADseq data. JŠ and PV analyzed RADseq data. PBu, MÖ, EM, and JŠ collected samples. PBu, PBl, and MV revised the herbarium material. KV prepared microscopic photos of setae. EDG and PBu performed the nomenclature analysis. PBu, EDG, and FZ wrote the manuscript.

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Tables:

Table 1. Intensity of hybridization between *Cirsium vulgare* and *Cirsium* or *Lophiolepis*, within *Cirsium*, within *Lophiolepis*, and between these genera represented by 322 and 103 species, respectively, in this analysis.

	Central Europe [a]				Great Britain & Ireland [a]				World [b]			
	All species pairs	Overlapping species pairs	Hybridizing species pairs	**Overall willingness to hybridize**	All species pairs	Overlapping species pairs	Hybridizing species pairs	**Overall willingness to hybridize**	All species pairs	Overlapping species pairs	Hybridizing species pairs	**Hybridization propensity**
Cirsium [c] × *Cirsium* [c]	91	76	52	**6.95 %**	28	24	8	**2.38 %**	51,360	6,262	197	**3.15 %**
Cirsium [c] × *Lophiolepis*	42	23	2	**0.03 %**	8	8	0	**0 %**	33,063	1,589	10	**0.63 %**
Lophiolepis × *Lophiolepis*	3	2	1	**6.67 %**	1	na	na	**na**	5,253	962	19	**1.98 %**
C. vulgare × *Cirsium* [c]	14	14	4	**0.10 %**	– 8	8	2	**0.08 %**	321	271	11	**4.06 %**
C. vulgare × *Lophiolepis*	3	3	1	**0.56 %**	1	1	1	**2.40 %**	103	97	16	**16.49 %**

[a] Willingness = average of ratios 100 x number of map quadrants occupied by a hybrid / number of the quadrants in which parental species co-occur (in %); Overall willingness to hybridize = weighted average of the willingnesses (across all non-disjunctive species pairs within category; weighted by respective numbers of quadrants in which both species of respective pair co-occur). For primary data, see Supplementary Tables S2, S3)

[b] Hybridization propensity = percentage of hybridizing species pairs from geographically overlapping species pairs. For primary data, see Supplementary Tables S4, S5)

[c] *Cirsium* excl. *C. vulgare*

Figure captions:

Figure 1. Rigid setae on the upper leaf surface of *Cirsium vulgare* (a–g), *Lophiolepis sorocephala* subsp. *congesta* (Fisch. & C.A.Mey. ex DC.) Bureš, Del Guacchio, Iamonico & P.Caputo (h), *L. eriophora* (j–p). The setae are usually connected to veins (f, g, o, p) but not necesarilly (e, m, n). Photo by P. Bureš (a, b, h, j) and Kristýna Veselá (c–g, k–p).

Figure 2. Shape of the flower heads and involucres: *Lophiolepis caucasica* (a, b), *L. vallis-demonii* (Lojac.) Del Guacchio, Bureš, Iamonico & P.Caputo (c),), *L. ciliata* subsp. *szovitsii* (K.Koch) Bureš, Del Guacchio, Iamonico & P.Caputo (d, e), *L. bulgarica* (DC.) Del Guacchio, Bureš, Iamonico & P.Caputo (f), *L. eriophora* (g, h), *L. spathulata* (Moretti) Del Guacchio, Bureš, Iamonico & P.Caputo (i), *Cirsium vulgare* (j–l), *C. canum* (m), *C. heterophyllum* (n), *C. pubigerum* (Desf.) DC. (o), *C. palustre* (p), *C. tuberosum* (L.) All. (q), *C. pannonicum* (r), *C. erisithales* (s), *C. rivulare* (t), *C. monspessulanum* (u). Involucral bracts of *Lophiolepis* (a-i) have long terminal spines arranged as lances of the Macedonian phalanx, while in *Cirsium* (m–u), the terminal spines are absent; moreover, the whitish or purple sticky vitae are present on the outer surface of involucral bracts of *Cirsium* (m–u) but absent in *Lophiolepis* (a–i). *Cirsium vulgare* (j–l) shares these features with *Lophiolepis* (a–i). Photo by P. Bureš.

Figure 3. Shape of the leaf base: a – *Lophiolepis serrulata* (M.Bieb.) Del Guacchio, Bureš, Iamonico & P.Caputo ; b – *L. ossetica* (Adams) Del Guacchio, Bureš, Iamonico & P.Caputo; c – *Cirsium vulgare*; d – *C. alatum*; e – *C. creticum*; f – *Lophiolepis spathulata* (Moretti) Del Guacchio, Bureš, Iamonico & P.Caputo; g – *L. tenoreana*; h – *Cirsium vulgare*; i – *C. brachycephalum*. *Cirsium vulgare* (c, h) shares the decurrent leaves with some species of *Cirsium* (d, e, i, j) but differ in this feature from all taxa of *Lophiolepis* (which all have sessile leaves with semi-ampexicaul base – a, b, f, g). Photo by P. Bureš.

Figure 4. NeighborNet phylogenetic network visualizing genetic relationship among 8 samples of *Cirsium vulgare* (green: VUL1–8) and samples representing species of genera *Cirsium* (blue) and *Lophiolepis* (red) based on nuclear (a) and organellar (b) RADseq data. *Lophiolepis* species: *L. aggregata* (AGGR); *L. bulgarica* (BULG); *L. caucasica* (CAUC); *L. lobelii* (LOBE); *L. macrobotrys* (MACR); *L. morisiana* (MORI); *L. rigida* (RIGI); *L. scabra* (SCAB); *L. sommieri* (SOMM); *L. tenoreana* (TENO); *L. trachylepis* (TRAC). *Cirsium* species: *C. acaulon* (ACAU); *C. alsophilum* (ALSO); *C. arvense* (ARVE); *C. canum* (CANU); *C. carniolicum* (CARN); *C. erisithales* (ERIS); *C. heterophyllum* (HETE); *C. monspessulanum* (MONS); *C. oleraceum* (OLER); *C. palustre* (PALU); *C. pannonicum* (PANN); *C. spinosissimum* (SPIN).

Figure 5. Heatmap visualizing similarities among the samples representing *Cirsium vulgare* and both suspected parental genera *Lophilepis* and *Cirsium* derived from dist.p matrix based on nuclear RADseq data. Samples of both genera are more similar to *C. vulgare* than to each other. Darker color in the heatmap indicates greater degree of similarity.

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767 **Figure S1.** Evanno plots

768 **Figure S2.** Geographic range of *Cirsium vulgare* (a) is larger than cumulative geographic range of all
769 species belonging to its paternal genus *Lophiolepis* (b). Based on GBIF (2023).

770 **Figure S3.** Geographic range of *Cirsium vulgare* (a) is larger than cumulative geographic range of all
771 species belonging to its paternal genus *Lophiolepis* (b). Based on POWO (2023).

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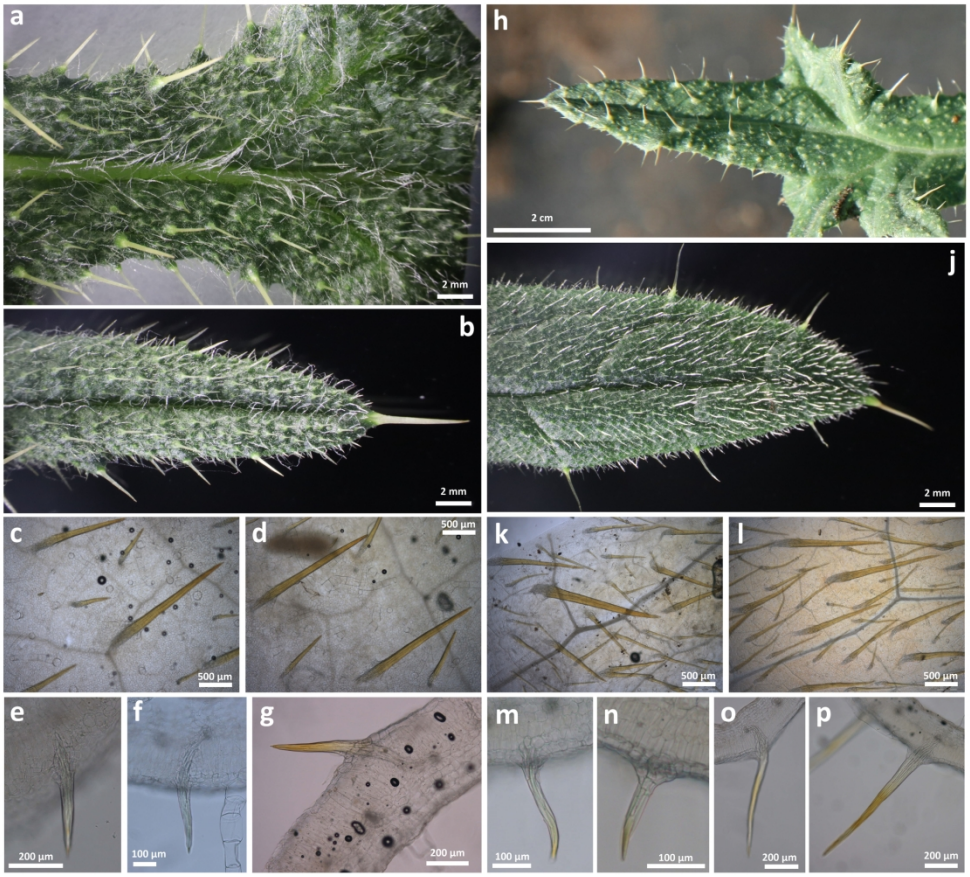

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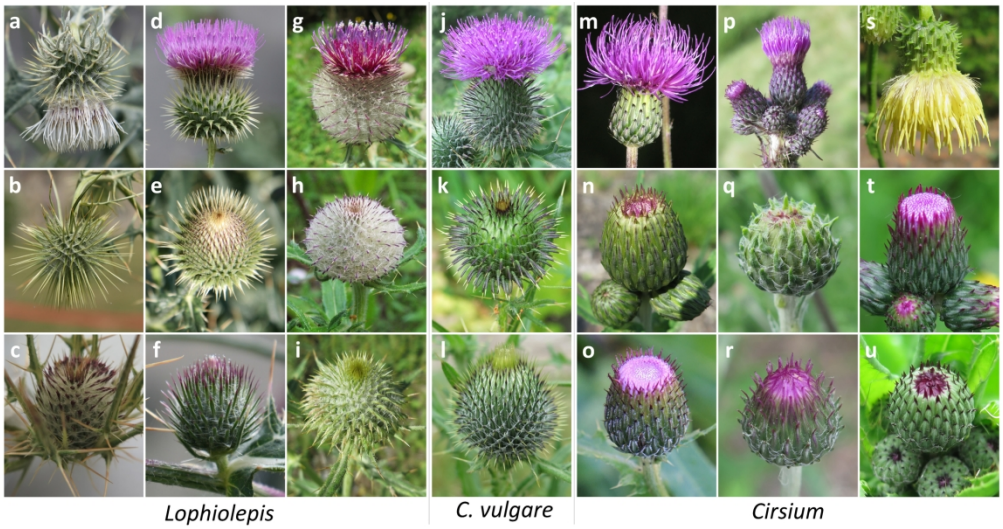

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199x142mm (300 x 300 DPI)

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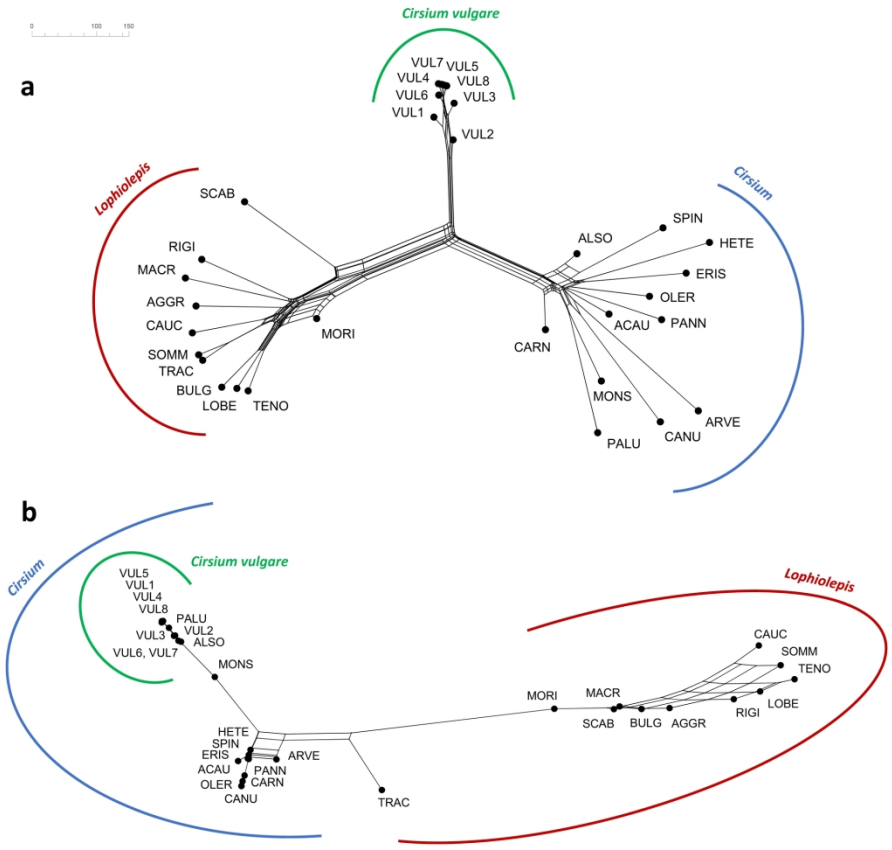

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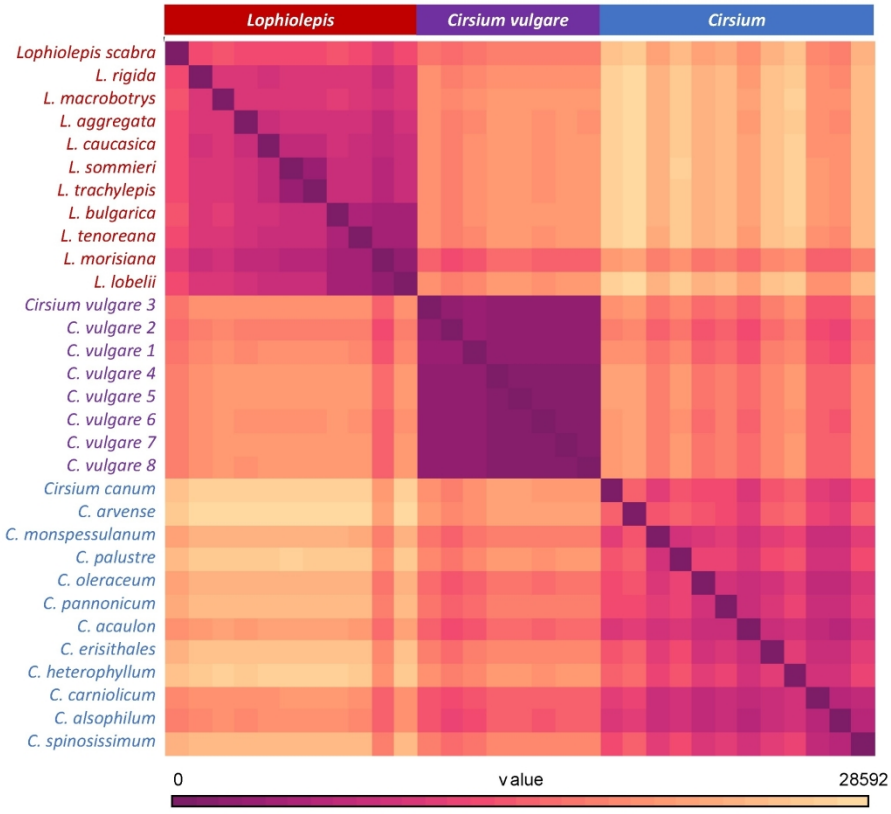

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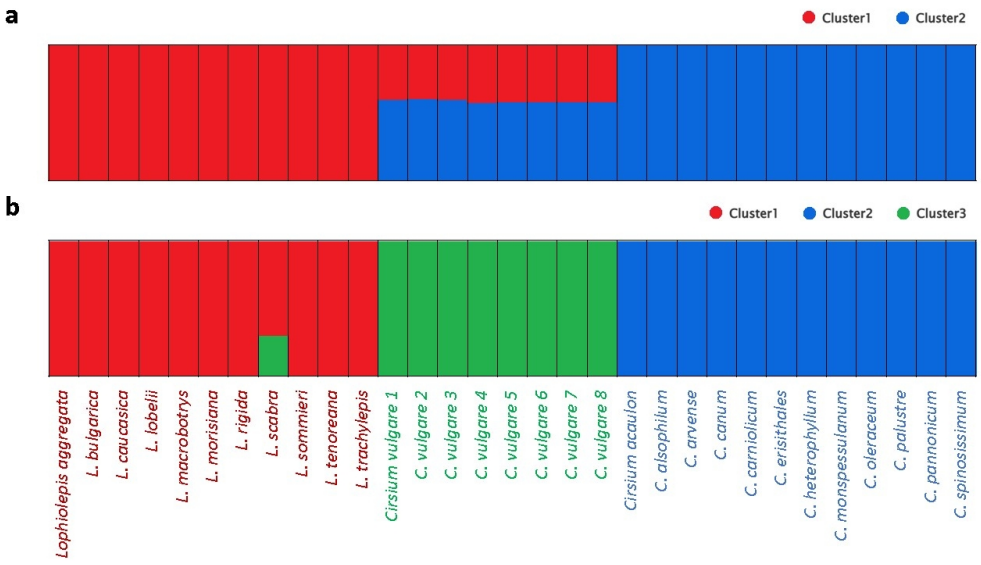

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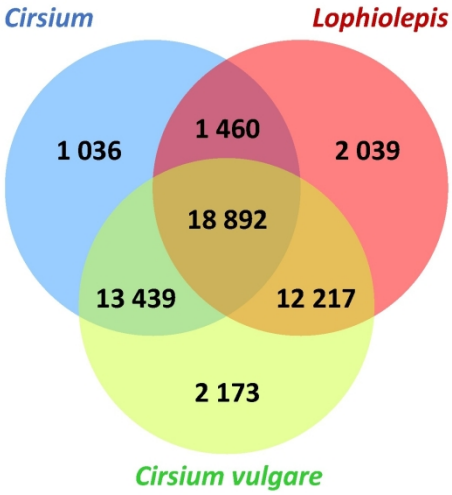

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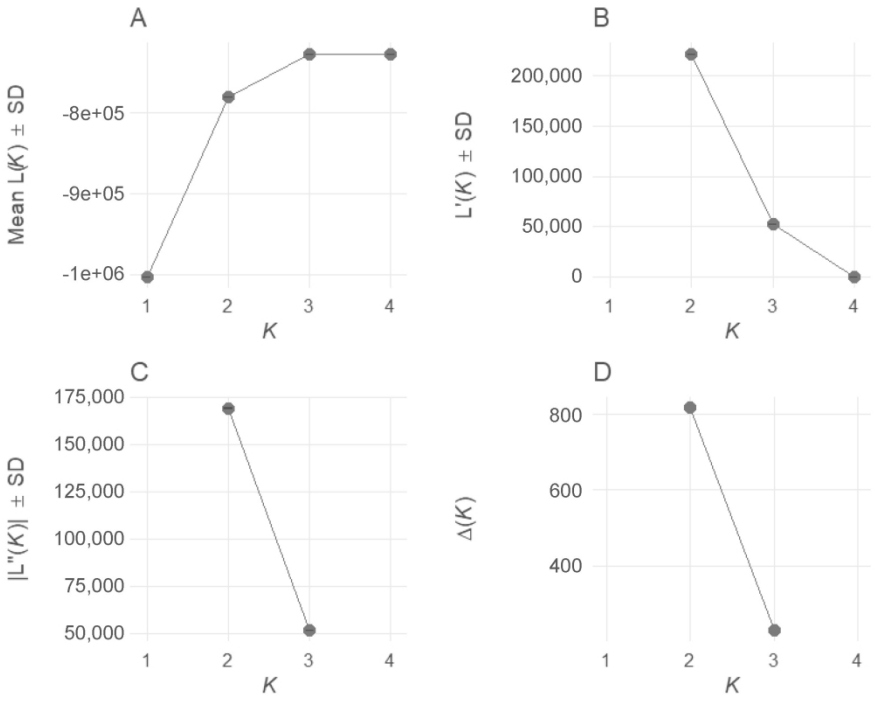

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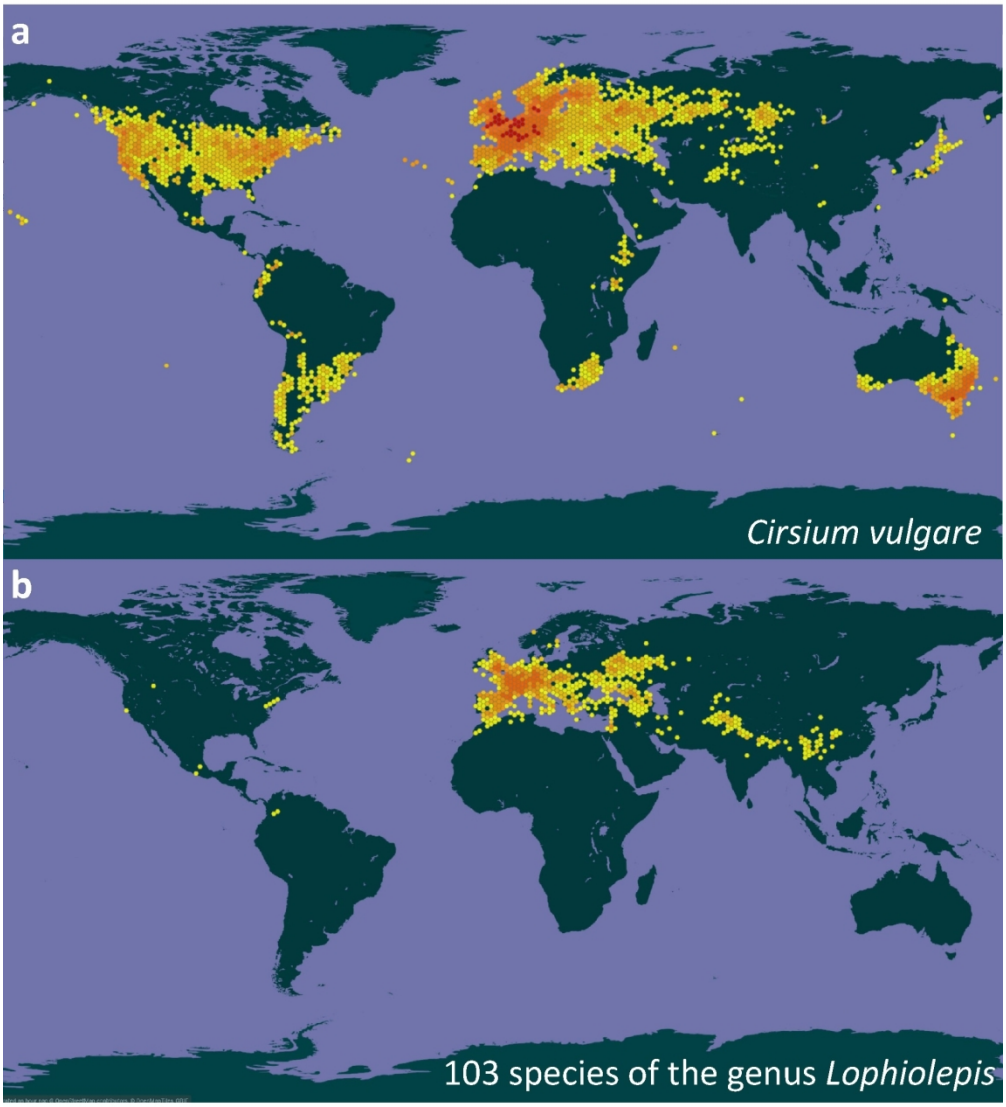

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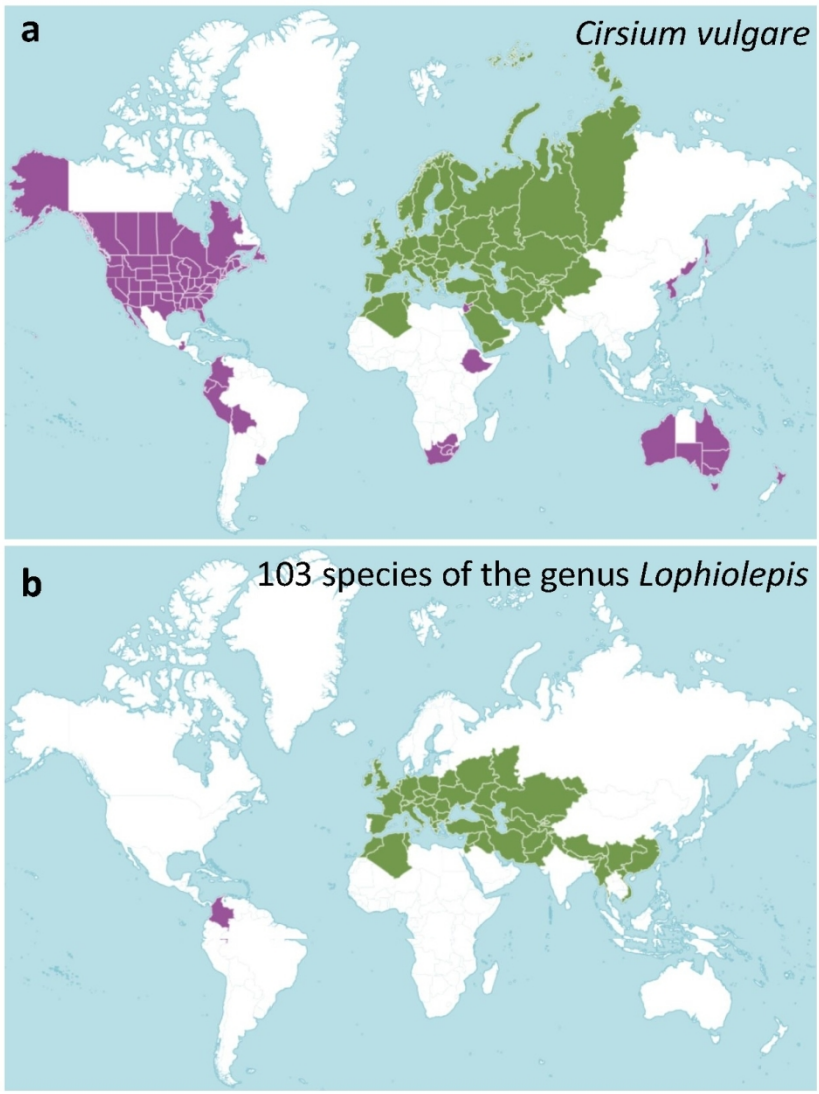


338x381mm (113 x 113 DPI)

Supplementary Table S1. Localities of the studied samples

Sample	Locality/Population	Coordinates	Collector, Date
Cirsium acaulon	Czech Republic, Bílé Karpaty Mts, Nedašov: S slopes of natural reserve Kaňoury, 2.7 E of the church in the village, 611 m s.m.	49°06'53.9"N, 18°06'18.1"E	P. Bureš & E. Lajkepová* 21/Jul/2013
C. alsophilum	Italy, Alpi dell'Adamello e della Presanella, Campo Carlo Magno: wet ditches along the road from Campo Carlo Magno to Folgarida, 800 m NW of the church in the village, 1636 m s.m.	46°15'03.3"N, 10°50'48.3"E	P. Bureš & E. Lajkepová* 2/Aug/2015
C. arvense	Czech Republic, Brno: University Campus of Masaryk Univ. in Bohunice, 276 m s.m.	49°10'45.2"N, 16°34'01.9"E	J. Šmerda 26/Sep/2020
C. canum	Czech Republic, dist. Chrudim, Chrast u Chrudimi: wet places in the meadow SE of the pond Chrašický, 266 m s.m.	49°54'11.6"N, 15°57'01.4"E	P. Bureš & J. Bureš 19/May/2011
C. carniolicum	Slovenia, Julijske Alpe, Zgornja Sorica: along the road in Bohinjsko sedlo, 1.5 km SSE of the village, 1 228 m s.m.	46°14'14.9"N, 14°01'11.0"E	P. Bureš & E. Lajkepová* 15/Jul/2016
C. erisithales	Bosnia and Herzegovina, Lunjevača Mts. Drvar: S slopes of Klekovača Massiv, 12 km ENE of the village, 1594 m s.m.	44°24'54.5"N, 16°31'37.9"E	P Bureš & M. Vavrinec 15/Jul/2017
C. heterophyllum	Austria, Zillertaler Alpen, Lanersbach: along the pathway to Grüblspitze, 2 km W of the village, 1972 m s.m.	47°09'27.0"N, 11°42'13.7"E	P. Bureš & E. Lajkepová* 4/Aug/2015
C. monspessulanum	Spain, Gerona Province, L'Armentera: Poplar wood along the brook, 1 m s.m.	42°09'38.9"N, 03°06'21.3"E	P. Bureš & M. Burešová 11/Jul/2010
C. oleraceum	Austria, Kärnten County, Karnische Alpen Mts. Nassfeld Pass: wet grassland along the road to Tröpolach, 1447 m s.m.	46°33'51.3"N, 13°16'39.3"E	P. Bureš & E. Michálková* 7/Sep/2018
C. palustre	Czech Republic, distr. Havlíčkův Brod, Libice nad Doubravou: wet places along the road NW of the village, 418 m s.m.	49°44'56.6"N, 15°41'38.6"E	Ester Lajkepová* & Petr Bureš 3/Jul/2011
C. pannonicum	Slovakia, Velká Fatra, Ružomberok: slope grassland at Malino Brdo hill, 4.5 km SW of the train station in the town, 959 m s.m.	49°03'13.2"N, 19°15'56.2"E	P. Bureš & E. Lajkepová* 3/Aug/2016
C. spinosissimum	Italy, Alpi dell'Adamello e della Presanella Mts, Campo Carlo Magno: ruderal grassland, 0.4 km NE of the church in the village, 1759 m s.m.	46°14'41.8"N, 10°50'09.2"E	P. Bureš & E. Lajkepová* 2/Aug/2015
C. vulgare 1	Czech Republic, Eastern Bohemia, Polička: ruderal grassland, 2.4 km WNW of the railway station in the town, 581 m s.m.	49°43'34.5"N, 16°13'56.5"E	P. Bureš & J. Bureš 20/May/2011
C. vulgare 2	Austria, Ötztaler Alpen, Sölden: at Ötztaler Gletscherstrasse 1.7 km WSW of the church in the village; 2025 m s.m.	46°57'49.7"N, 10°59'06.7"E	P. Bureš 4/Aug/2013
C. vulgare 3	Slovakia, Velká Fatra, Ružomberok: slopes along the pathway to Predný Čebrať hill, 1.8 km NW of the train station in the town; 923 m s.m.	49°05'38.2"N, 19°17'13.9"E	E. Lajkepová* & J. Šmerda 1/Jul/2016
C. vulgare 4	Turkey, Giresun: Yavuzkemal, roadsides, 1072 m s.m.	40°39'11.2"N, 38°20'23.0"E	M. Özcan 27/Aug/2008
C. vulgare 5	Albania, Malësia e Gjakovës, distr. Shkodër. Curraj i Epërm: forest spring along the pathway from Curraj i Epërm to Curraj i Poshtëm, 2.5 km SE of the church in the village, 900 m s.m.	42°19'59.0"N, 19°56'19.8"E	Ester Lajkepová* & Ondřej Michálek 31/Jul/2017
C. vulgare 6	Czech Republic, distr. Blansko, Lhotky: clearing in the forest E of the village, 543 m s.m.	49°17'19.6"N, 16°47'42.3"E	Ester Lajkepová* & Petr Bureš 4/Jul/2011
C. vulgare 7	Czech Republic, distr. Uherské Hradiště, Korytná: dry bushy pasture 1.5 km NNW of the church in the village, 306 m s.m.	48°57'11.6"N, 17°39'31.5"E	Ester Lajkepová* & Petr Bureš 1/Jul/2011
C. vulgare 8	Czech Republic, distr. Žďár nad Sázavou, Chlumětín: wet meadows along the road to Svratka, 645 m s.m.	49°43'11.4"N, 15°59'53.9"E	Ester Lajkepová* & Petr Bureš 6/Jul/2011
Lophiolepis aggregata	Turkey, Trabzon: Bayburt - Trabzon, Soganlı pass, Çaykara road, among grass, 2100–2150 m s.m.	40°32'37.2"N, 40°14'01.4"E	M. Özcan 9/Aug/2009
L. bulgarica	Turkey, Giresun: Yavuzkemal, roadsides, margins of spruce forest, 1122 m s.m.	40°38'21.0"N, 38°19'07.1"E	M. Özcan 27/Aug/2008
L. caucasica	Turkey, Artvin: Ardanuç, Tepedüzü Village, roadsides, 1310 m s.m.	41°05'14.0"N, 42°04'34.5"E	M. Özcan 31/Aug/2007
L. lobelii	Italy, Abruzzo Province, Gran Sasso Mts: alpine grassland at NE slope of M. della Scindarella Mt. near the road to Campo Imperatore, 1403 m s.m.	42°26'03.1"N, 13°07'49.9"E	P. Bureš & I. Burešová 20/May/2010

L. macrobotrys	Turkey, Gümüshane: Sarıçiçek road, , near cultivated areas, 2006 m s.m.	40°23'00.8"N, 39°50'45.0"E	M. Özcan 26/Aug/2007
L. morisiana	Italy, Belluno Province, Dolomiti Mts., Pieve: grasslands and pastures near hotel La Baita at the road to Passo Falzarego, 1604 m s.m.	46°29'41.5"N, 11°59'19.4"E	P. Bureš & E. Michálková* 7/Sep/2018
L. rigida	Turkey, Artvin: Ardanuç, open slopes, eroded areas, 526–555 m s.m.	41°07'32.1"N, 42°03'57.3"E	M. Özcan 20/Jun/2009
L. scabra	Morocco, Larache - achenes collection 964 Cirsium scabrum MA-0-LYJB-076121/ Index seminum 2009 Jardin Botanique de Lyon	35°12'08.1"N, 06°07'09.8"W	Jardin Botanique de Lyon
L. sommieri	Turkey, Bayburt: Kılıçkaya highplateau, roadsides, 2106 m s.m.	40°30'16.3"N, 40°15'06.1"E	M. Özcan 18/Oct/2008
L. tenoreana	Italy, Puglia Province, peninsula Gargano: pasture at S margin of Parco Naturale Foresta umbra, 647 m s.m.	41°45'19.0"N, 15°59'47.2"E	P. Bureš & I. Burešová 18/May/2010
L. trachylepis	Turkey, Trabzon: Akçaabat, Hıdırnebi high plateau, Stony pastures, roadsides, 1307 m s.m.	40°58'04.9"N, 39°26'03.1"E	M. Özcan 06/Sep/2008

* E. Lajkepová = later E. Michálková

Supplementary Table S2A. Willingness to hybridize spontaneously among **Central European** *Cirsium* and *Lophiolepis* species: **Within-*Cirsium* hybrids:**

76 overlapping from 91 parental pairs, 52 actual hybrids

Species pair / hybrid	Number of herbarium specimens	Quadrants occupied by hybrid	Quadrants occupied by both parents	Willingness to hybridize (%)
C. acaulon - C. alsophilum	0	0	18	0
C. acaulon × C. arvense	7	6	771	0.78
C. acaulon × C. canum	79	48	344	13.95
C. acaulon × C. erisithales	49	20	182	10.99
C. acaulon - C. greimleri	0	0	1	0
C. acaulon × C. heterophyllum	51	19	310	6.13
C. acaulon × C. oleraceum	273	148	643	23.02
C. acaulon - C. palustre	0	0	610	0
C. acaulon × C. pannonicum	71	32	194	16.49
C. acaulon × C. rivulare	1	1	184	0.54
C. acaulon × C. spinosissimum	17	5	184	2.72
C. alsophilum - C. arvense	0	0	32	0
C. alsophilum × C. erisithales	41	9	33	27.27
C. alsophilum × C. heterophyllum	18	2	21	9.52
C. alsophilum - C. oleraceum	0	0	14	0
C. alsophilum - C. palustre	0	0	30	0
C. alsophilum - C. pannonicum	0	0	1	0
C. alsophilum - C. spinosissimum	0	0	13	0
C. arvense × C. brachycephalum	1	1	536	0.19
C. arvense × C. canum	6	4	2534	0.16
C. arvense - C. carniolicum	0	0	58	0
C. arvense - C. erisithales	0	0	1062	0
C. arvense - C. greimleri	0	0	88	0
C. arvense - C. heterophyllum	0	0	1107	0
C. arvense × C. oleraceum	31	22	4120	0.53
C. arvense × C. palustre	7	7	3810	0.18
C. arvense - C. pannonicum	0	0	665	0
C. arvense × C. rivulare	2	1	1647	0.06
C. arvense - C. spinosissimum	0	0	621	0
C. brachycephalum × C. canum	8	7	315	2.22
C. brachycephalum - C. erisithales	0	0	1	0
C. brachycephalum × C. oleraceum	1	1	23	4.35
C. brachycephalum - C. palustre	0	0	51	0
C. brachycephalum - C. pannonicum	0	0	7	0
C. brachycephalum - C. rivulare	0	0	31	0
C. canum × C. erisithales	1	1	93	1.08
C. canum - C. heterophyllum	0	0	153	0
C. canum × C. oleraceum	667	387	1319	29.34
C. canum × C. palustre	197	126	950	13.26
C. canum × C. pannonicum	99	66	383	17.23

C. canum × *C. rivulare*	54	39	708	5.51
C. carniolicum × *C. erisithales*	37	22	57	38.60
C. carniolicum × *C. greimleri*	1	1	7	14.29
C. carniolicum - *C. heterophyllum*	0	0	9	0
C. carniolicum × *C. oleraceum*	10	3	54	5.56
C. carniolicum × *C. palustre*	5	5	54	9.26
C. carniolicum - *C. pannonicum*	0	0	9	0
C. carniolicum - *C. rivulare*	0	0	2	0
C. carniolicum × *C. spinosissimum*	5	3	22	13.64
C. erisithales × *C. greimleri*	48	23	53	43.40
C. erisithales × *C. heterophyllum*	171	76	370	20.54
C. erisithales × *C. oleraceum*	276	147	922	15.94
C. erisithales × *C. palustre*	213	120	984	12.20
C. erisithales × *C. pannonicum*	117	38	248	15.32
C. erisithales × *C. rivulare*	110	63	345	18.26
C. erisithales × *C. spinosissimum*	184	63	286	22.03
C. greimleri × *C. heterophyllum*	58	17	49	34.69
C. greimleri × *C. oleraceum*	16	10	82	12.20
C. greimleri × *C. palustre*	38	15	91	16.48
C. greimleri - *C. pannonicum*	0	0	2	0
C. greimleri × *C. rivulare*	4	4	27	14.81
C. greimleri × *C. spinosissimum*	3	3	12	25.00
C. heterophyllum × *C. oleraceum*	187	116	949	12.22
C. heterophyllum × *C. palustre*	90	62	1250	4.96
C. heterophyllum - *C. pannonicum*	0	0	57	0
C. heterophyllum × *C. rivulare*	18	15	183	8.20
C. heterophyllum × *C. spinosissimum*	94	51	466	10.94
C. oleraceum × *C. palustre*	423	313	3424	9.14
C. oleraceum × *C. pannonicum*	12	10	528	1.89
C. oleraceum × *C. rivulare*	489	284	1513	18.77
C. oleraceum × *C. spinosissimum*	28	17	469	3.62
C. palustre × *C. pannonicum*	58	21	354	5.93
C. palustre × *C. rivulare*	342	205	1395	14.70
C. palustre × *C. spinosissimum*	23	14	641	2.18
C. pannonicum × *C. rivulare*	85	42	269	15.61
C. rivulare × *C. spinosissimum*	1	1	53	1.89
			Mean willingness to hybridize =	8.26 %
			Overall willingness to hybridize (weighted average) =	**6.95 %**

Supplementary Table S2B. Willingness to hybridize spontaneously among **Central European** *Cirsium* and *Lophiolepis* species: ***Cirsium vulgare* × *Cirsium*-hybrids:**

14 overlapping from 14 parental pairs, 4 actual hybrids

Species pair / hybrid	Number of herbarium specimens	Quadrants occupied by hybrid	Quadrants occupied by both parents	Willingness to hybridize (%)
C. acaulon × *C. vulgare*	9	6	557	1.08
C. alsophilum - *C. vulgare*	0	0	26	0
C. arvense - *C. vulgare*	0	0	5965	0
C. brachycephalum - *C. vulgare*	0	0	492	0
C. canum - *C. vulgare*	0	0	2020	0
C. carniolicum - *C. vulgare*	0	0	54	0
C. erisithales × *C. vulgare*	3	2	966	0.21
C. greimleri - *C. vulgare*	0	0	84	0
C. heterophyllum - *C. vulgare*	0	0	920	0
C. oleraceum × *C. vulgare*	13	11	3465	0.32
C. palustre × *C. vulgare*	2	2	3248	0.06
C. pannonicum - *C. vulgare*	0	0	472	0
C. rivulare - *C. vulgare*	0	0	1332	0
C. spinosissimum - *C. vulgare*	0	0	579	0
			Mean willingness to hybridize =	0.12 %
			Overall willingness to hybridize (weighted average) =	**0,10 %**

Supplementary Table S2C. Willingness to hybridize spontaneously among **Central European** *Cirsium* and *Lophiolepis* species: ***Cirsium* × *Lophiolepis*-hybrids:**

23 overlapping from 42 parental pairs, 2 actual hybrids

Species pair / hybrid	Number of herbarium specimens	Quadrants occupied by hybrid	Quadrants occupied by both parents	Willingness to hybridize (%)
C. arvense - L. boujartii	0	0	69	0
L. boujartii - C. brachycephalum	0	0	22	0
L. boujartii - C. canum	0	0	67	0
L. boujartii - C. oleraceum	0	0	6	0
L. boujartii - C. palustre	0	0	1	0
L. boujartii - C. rivulare	0	0	1	0
C. acaulon - L. eriophora	0	0	328	0
C. alsophilum - L. eriophora	0	0	18	0
C. arvense - L. eriophora	0	0	1208	0
C. brachycephalum - L. eriophora	0	0	25	0
C. canum - L. eriophora	0	0	448	0
C. carniolicum - L. eriophora	0	0	40	0
L. eriophora - C. erisithales	0	0	547	0
L. eriophora - C. greimleri	0	0	40	0
L. eriophora - C. heterophyllum	0	0	309	0
L. eriophora × C. oleraceum	1	1	851	0.12
L. eriophora - C. palustre	0	0	889	0
L. eriophora - C. pannonicum	0	0	308	0
L. eriophora × C. rivulare	1	1	396	0.25
L. eriophora - C. spinosissimum	0	0	186	0
C. arvense - L. furiens	0	0	11	0
C. brachycephalum - L. furiens	0	0	9	0
C. canum - L. furiens	0	0	6	0
			Mean willingness to hybridize =	0.02 %
			Overall willingness to hybridize (weighted average) =	**0.03 %**

Supplementary Table S2D. Willingness to hybridize spontaneously among **Central European** *Cirsium* and *Lophiolepis* species: ***Cirsium vulgare* × *Lophiolepis*-hybrids:**

3 overlapping from 3 parental pairs, 1 actual hybrid Species pair / hybrid	Number of herbarium specimens	Quadrants occupied by hybrid	Quadrants occupied by both parents	Willingness to hybridize (%)
L. boujartii - C. vulgare	0	0	62	0
L. eriophora × C. vulgare	8	6	999	0.60
L. furiens - C. vulgare	0	0	9	0
			Mean willingness to hybridize =	0.20 %
			Overall willingness to hybridize (weighted average) =	**0.56 %**

Supplementary Table S2E. Willingness to hybridize spontaneously among Central European *Cirsium* and *Lophiolepis* species: **Within-*Lophiolepis* hybrids:**

2 overlapping from 3 parental pairs, no actual hybrid

Species pair / hybrid	Number of herbarium specimens	Quadrants occupied by hybrid	Quadrants occupied by both parents	Willingness to hybridize (%)
L. boujartii - L. eriophora	1	1	8	12.5
L. eriophora - L. furiens	0	0	7	0
			Mean willingness to hybridize =	6.25 %
			Overall willingness to hybridize (weighted average) =	**6.67 %**

Supplementary Table S3A. Willingness to hybridize spontaneously among **British and Irish** *Cirsium* and *Lophiolepis* species: **Within-*Cirsium* hybrids** (data taken from Stace *et al.*, 2015):

24 overlapping from 28 parental pairs, 8 actual hybrids

Hybrid / Species pair	Quadrants occupied by hybrid	Quadrants occupied by both parents	Willingness to hybridize (%)
C. acaulon × *C. arvense*	7	760	0.92
C. acaulon × *C. dissectum*	2	324	0.62
C. acaulon - *C. heterophyllum*	0	23	0
C. acaulon - *C. oleraceum*	0	9	0
C. acaulon × *C. palustre*	4	748	0.53
C. acaulon - *C. rivulare*	0	7	0
C. acaulon × *C. tuberosum*	17	23	73.91
C. arvense - *C. dissectum*	0	1092	0
C. arvense - *C. heterophyllum*	0	572	0
C. arvense - *C. oleraceum*	0	25	0
C. arvense × *C. palustre*	58	3607	1.61
C. arvense - *C. rivulare*	0	15	0
C. arvense - *C. tuberosum*	0	19	0
C. dissectum - *C. heterophyllum*	0	21	0
C. dissectum - *C. oleraceum*	0	8	0
C. dissectum × *C. palustre*	94	1101	8.54
C. dissectum - *C. rivulare*	0	8	0
C. dissectum - *C. tuberosum*	0	7	0
C. palustre × *C. tuberosum*	2	23	8.70
C. heterophyllum - *C. oleraceum*	0	11	0
C. heterophyllum × *C. palustre*	37	806	4.60
C. heterophyllum - *C. rivulare*	0	2	0
C. oleraceum - *C. palustre*	0	25	0
C. palustre - *C. rivulare*	0	18	0
C. palustre - *C. tuberosum*	0	19	0
		Mean willingness to hybridize =	3.98 %
		Overall willingness to hybridize (weighted average) =	**2.38 %**

Supplementary Table S3B. Willingness to hybridize spontaneously among **British and Irish** *Cirsium* and *Lophiolepis* species: ***Cirsium* × *Lophiolepis*-hybrids** (data taken from Stace *et al.*, 2015):

8 overlapping from 8 parental pairs, no actual hybrid

Species pair / hybrid	Quadrants occupied by hybrid	Quadrants occupied by both parents	Willingness to hybridize (%)
C. acaulon - L. eriophora	0	280	0
C. arvense - L. eriophora	0	359	0
C. dissectum - L. eriophora	0	180	0
L. eriophora - C. heterophyllum	0	22	0
L. eriophora - C. oleraceum	0	3	0
L. eriophora - C. palustre	0	350	0
L. eriophora - C. rivulare	0	4	0
L. eriophora - C. tuberosum	0	16	0
		Mean willingness to hybridize =	0.00 %
		Overall willingness to hybridize (weighted average) =	**0.00 %**

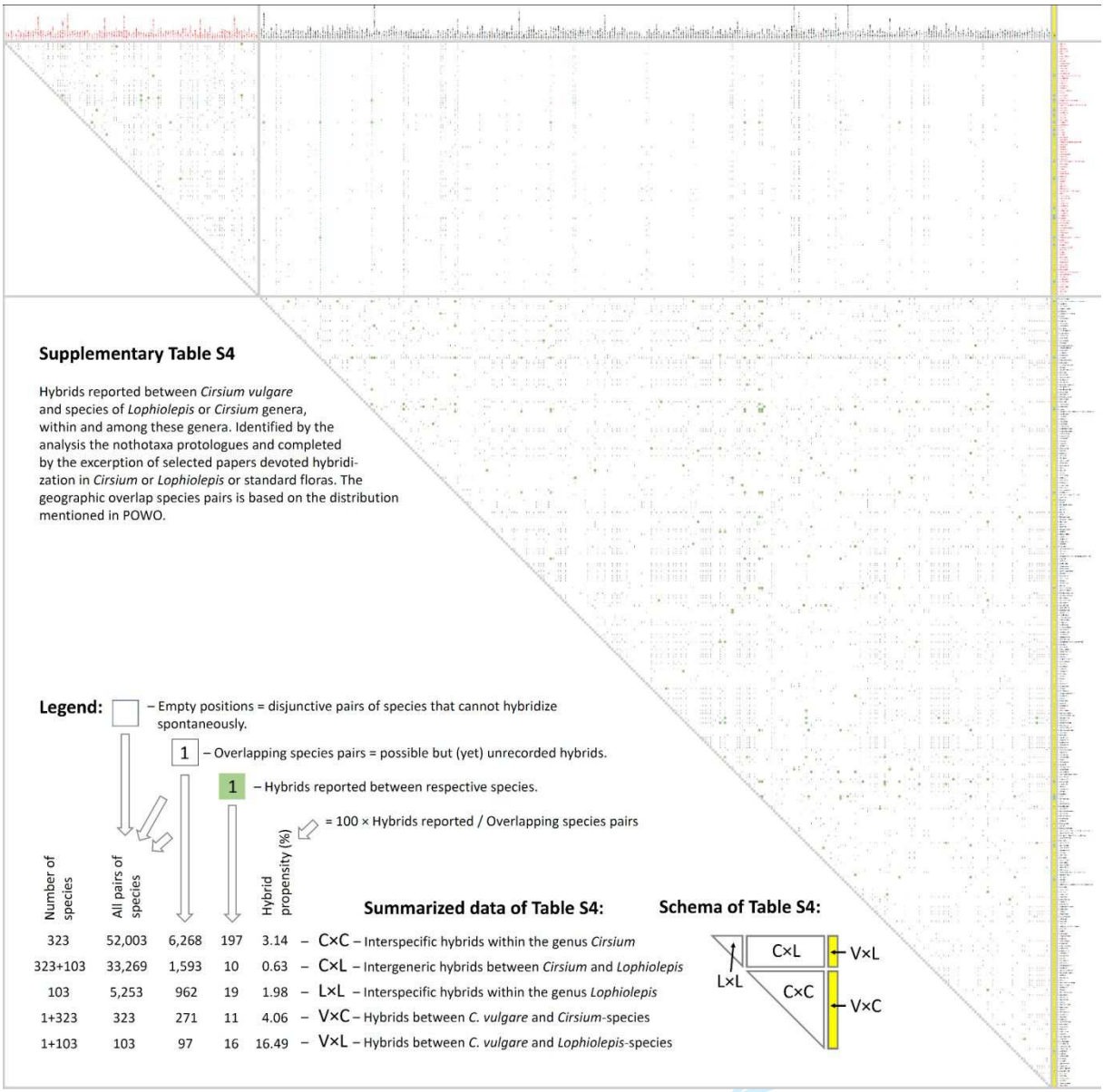
Supplementary Table S3C. Willingness to hybridize spontaneously among **British and Irish** *Cirsium* and *Lophiolepis* species: ***Cirsium vulgare* × *Cirsium*-hybrids** (data taken from Stace *et al.*, 2015):

8 overlapping from 8 parental pairs, 2 actual hybrid			
Species pair / hybrid	Quadrants occupied by hybrid	Quadrants occupied by both parents	Willingness to hybridize (%)
C. acaulon × *C. vulgare*	4	759	0.53
C. arvense - *C. vulgare*	0	3721	0
C. dissectum - *C. vulgare*	0	1087	0
C. heterophyllum - *C. vulgare*	0	684	0
C. oleraceum - *C. vulgare*	0	25	0
C. palustre × *C. vulgare*	4	3654	0.11
C. rivulare - *C. vulgare*	0	18	0
C. tuberosum - *C. vulgare*	0	19	0
		Mean willingness to hybridize =	0.08 %
		Overall willingness to hybridize (weighted average) =	

Supplementary Table S3D. Willingness to hybridize spontaneously among **British and Irish** *Cirsium* and *Lophiolepis* species: ***Cirsium vulgare* × *Lophiolepis*-hybrids** (data taken from Stace *et al.*, 2015):

One overlapping parental pair and actual hybrid			
Species pair / hybrid	Quadrants occupied by hybrid	Quadrants occupied by both parents	Willingness to hybridize (%)
C. vulgare × *L. eriophora*	11	467	2.40 %

Supplementary Table S4



Supplementary Table S5A. *Cirsium*-species reported as hybridizing with *Cirsium vulgare*.

Species	Hybrid with *Cirsium vulgare* reported by:
C. acaulon	Nägeli in Koch (1845: 997); Löhr (1852: 362); Reichenbach (1853: 69); Seehaus (1861-1862: 187); Ruhmer (1881: 240); Focke (1881: 204); Schulze (1896: 45); Tourlet (1903: 310); Sychowa (1971: 372, 378); Wagenitz (1987: 908); Bureš (2004: 409); Lippert & Meierott (2014: 89); Stace *et al.* (2015: 291).
C. arvense	Siegert (1848: 128); Kirschleger (1852: 444); Borbás (1878: 392 – later doubted by Petrak 1960: 37); Gandoger (1875: 130); Focke (1881: 204); Camus (1891a: 81); Fleischer in Dörfler (1894-1915: 3640– later doubted by Petrak, 1912: 79, 1960: 37, Wagenitz, 1987: 915, and Bureš, 2004: 419, who identified the type material as *Cirsium vulgare*); Fritsch (1930: 80); Soó (1970: 143); Sychowa (1971: 378, 383); Lambinon *et al.* (1992: 696).
C. canum	Uechtritz (1885: 230); Soó (1970: 144); Sychowa (1971: 370, 378); Wagenitz (1987: 908).
C. erisithales	Khek (1905: 41); Fritsch (1907: 405); Hayek (1911–1914: 600); Sychowa (1971: 375, 378).
C. esculentum	Charadze (2001: 201).
C. heterophyllum	Goller & Huter in Hutter (1906: 309); Wagenitz (1987: 908); Bureš (2004: 416).
C. oleraceum	Gaudin (1829: 180); Koch (1843: 294); Schultz (1842-48: 34; 1846: 250); Wimmer (1845: 58); Focke (1881: 204); Hallier (1887: 48); Karsten (1895: 697); Fugger & Kastner (1899: 208); Rechinger (1900: 59); Fritsch (1907: 407); Hayek (1911–1914: 600); Petrak (1960: 38); Soó (1970: 145); Sychowa (1971: 376, 378); Wagenitz (1987: 908); Bureš (2004: 411); Polatschek (1997: 528); Stöhr (2006: 209); Lippert & Meierott (2014: 89).
C. palustre	Nägeli in Koelliker (1839: 53); Nägeli (1840 158); Nägeli in Koch (1845: 996); Löhr (1852: 362); Reichenbach (1853: 69, Tab. DCCCXLIII); Petermann (1849: 311); Focke (1881: 204); Camus (1891b: 105); Fugger & Kastner (1899: 208); Guétrot (1928: 2); Soó (1970: 145); Sychowa (1971: 377); Wagenitz (1987: 908); Lambinon *et al.* (1992: 696); Polatschek (1997: 534); Bureš (2004: 417); Stöhr (2006: 209); Lippert & Meierott (2014: 89); Stace *et al.* (2015: 291).
C. pannonicum	Loser (1860: 286); Sychowa (1971: 378).
C. pyrenaicum	Sennen (1912: 196).
C. roseolum	Tzvelev (1991: 151); Tzvelev (1994: 246).
C. waldsteinii	Khek (1909: 54); Sychowa (1971: 373, 378); Wagenitz (1987: 908).

Supplementary Table S5B. *Lophiolepis*-species reported as hybridizing with *Cirsium vulgare*.

Species	Hybrid with *Cirsium vulgare* reported by:
L. boujartii	Dobrescu & Nyárády in Dobrescu (1959: 79); Nyárády (1964: 731).
L. ciliata	Focke (1881: 205); Arevshatyan (1995: 296).
L. decussata	Nyárády (1964: 732, 971).
L. echinata	Rouy (1900: 112).
L. eriophora	Kittel (1844: 551; doubted by Petrak, 1912: 79 who identified the specimens as *Cirsium vulgare*); Schultz F. W. (1846: 246; 1849: 228); Schultz C. H. (1849: 547; doubted by Petrak, 1912: 79 who identified the specimens as *Cirsium vulgare*); Löhr (1852: 362); Reichenbach (1853: 60); Döll (1859: 937); Gandoger (1875: 130–131); Nyman (1878–82: 406); Focke (1881: 204); Schatz (1891: 273); Rechinger (1903: 64); Gross (1904: 70); Huter (1906: 310–311); Fritsch (1907: 407); Hayek (1911–1914: 599); Petrak (1912: 80); Hepp (1956: 51); Soó (1970: 144); Sychowa (1971: 378, 379); Wagenitz (1987: 907–908); Lambinon *et al.* (1992: 696); Polatschek (1997: 513); Bureš (2004: 409); Breitfeld *et al.* (2011: 150); Lippert & Meierott (2014: 89); Stace *et al.* (2015: 290).
L. ferox	Rouy (1904: 78).
L. furiens	Nyárády (1964: 734, 735); Soó (1970: 144).
L. caucasica	Grossgeim (1934: 189).
L. chlorocoma	Charadze (2001: 76).
L. kosmelii	Arevshatyan (1995: 296).
**L. odontolepis*	Sennen (1910: 232; 1912: 196; doubted by Petrak 1912: 79 who identified the type material as *L. odontolepis*).
L. ossetica	Arevshatyan (1995: 296).
**L. richteriana*	Sennen (1902: 376; 1902-03: 8); Sennen in Pau (1907: 27); doubted by F. Petrak (1912: 79) who identified the type material as *Lophilepis richteriana*.
L. serrulata	Boissier (1875: 553).
L. turkestanica	Lazkov (2009: 225; Fig. 1).

* Later doubted hybrids.

Supplementary Table S5C. Intergeneric hybrids reported between species of genera *Cirsium* and *Lophiolepis*

Species	Hybrid reported by:
C. acaulon × *L. eriophora*	Lambert (1911: 79).
* *C. arvense* × *L. richteriana*	Sennen (1904: 426); Sennen in Pau (1905: 321); doubted by F. Petrak (1912: 79) who identified the type-material as *Lophiolepis richteriana* subsp. *costae*.
C. arvense × *L. eriophora*	Rouy (1904: 42).
C. canum × *L. ciliata*	Schur (1866: 420); Focke (1881: 204).
* *C. canum* × *L. eriophora*	Podpěra (1904: 339); doubted by Bureš (2004: 419) who identified the type-material as *Lophiolepis eriophora*.
* *C. heterophyllum* × *L. eriophora*	Handschke in Čelakovský (1885: 73); doubted by Bureš (2004: 419) who identified the specimen as *Lophiolepis eriophora*.
C. oleraceum × *L. eriophora*	Mérat (1843: 242); Fournier (1928: 278); Wagenitz (1987: 908).
* *C. palustre* × *L. eriophora*	Schulze (1903: 35); doubted by F. Petrak (1912: 79) and Bureš (2004: 419) who identified the type-material as *Cirsium vulgare*.
* *C. rivulare* × *L. eriophora*	Dörfler (1911: 494); doubted by F. Petrak (1912: 80) who identified the specimens as *Cirsium vulgare* × *Lophiolepis eriophora*.
C. spinosissimum × *L. eriophora*	Hayek (1922: 268); Wagenitz (1987: 908).

* Later doubted hybrids.

Supplementary Table S5D. Survey of congeneric hybrids within *Lophiolepis*

Species	Hybrid reported by:
L. adjarica × *L. trachylepis*	Davis & Parris (1975: 411).
L. adunca × *L. ciliata*	Petrak (1938: 299).
L. adunca × *L. haussknechtii*	Sheidai et al. (2016).
L. boujartii × *L. furiens*	Dobrescu & Nyárády in Nyárády (1964: 731, 971).
L. boujartii × *L. ligularis*	Petrak (1912: 78), Özcan 2023.
L. caucasica × *L. ciliata*	Woronow in Grossheim (1934: 189); Charadze (2001: 132).
L. caucasica × *L. kosmelii*	Charadze (2001: 107).
L. caucasica × *L. trachylepis*	Davis & Parris (1975: 411).
L. cephalotes × *L. lappacea*	Dirmenci *et al*. (2019: 369).
L. cephalotes × *L. macrobotrys*	Dirmenci *et al*. (2019: 368).
L. ciliata × *L. kosmelii*	Arevshatyan (1995: 296); Charadze (2001: 107).
L. decussata × *L. furiens*	Nyárády (1934: 71).
L. eriophora × *L. ferox*	Focke (1881: 204).
L. eriophora × *L. morisiana*	Coste (1903: 372).
L. furiens × *L. grecescui*	Petrak (1912: 77).
**L. furiens* × *L. ligularis*	Rohlena [in litt.] in Petrak (1912: 79; but according to Petrak the original material is only *L. boujartii*).
L. haussknechtii × *L. strigosa*	Petrak (1910: 436, 1979: 280).
L. kosmelii × *L. ossetica*	Charadze (2001: 108).
L. lappacea × *L. macrobotrys*	Dirmenci *et al*. (2019: 369).

* Later doubted hybrids.

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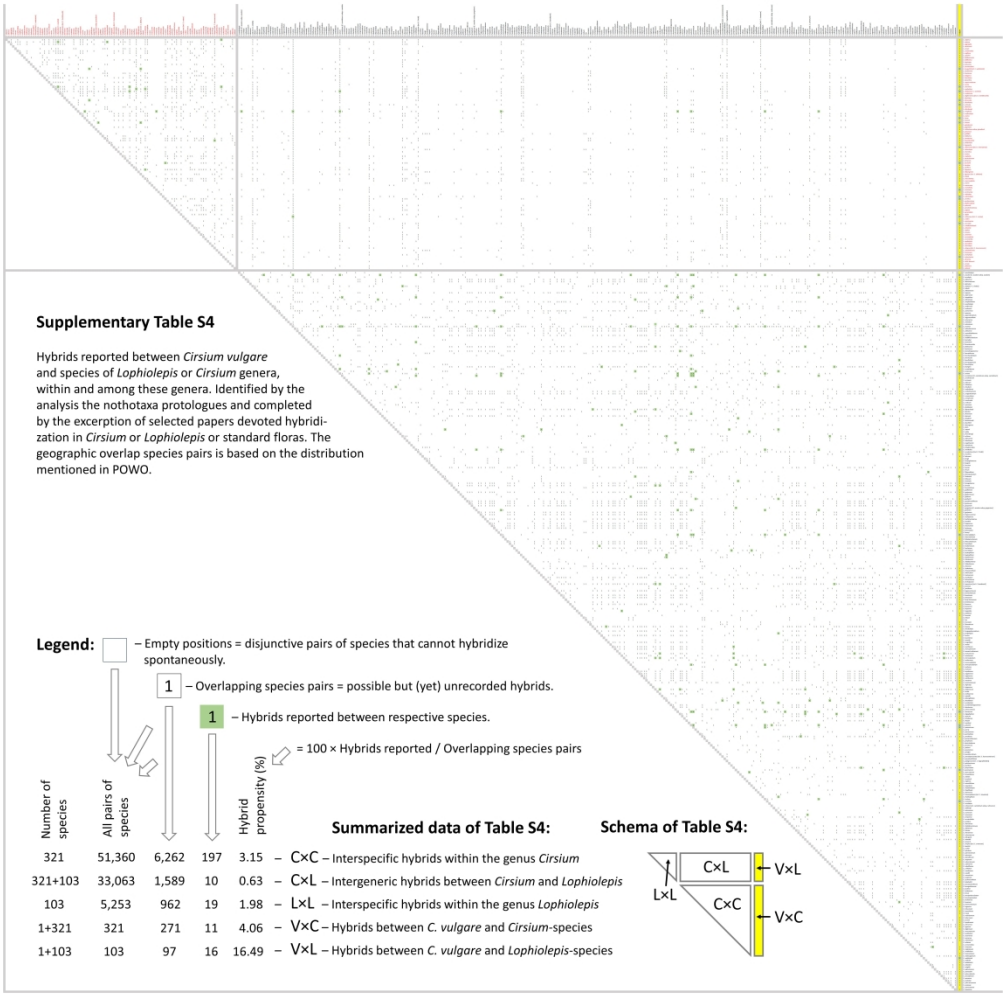

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