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RESEARCH ARTICLE

Intergeneric hybrid origin of the invasive tetraploid *Cirsium vulgare*

P. Bureš[1], E. Del Guacchio[2,3], J. Šmerda[1], M. Özcan[4], P. Blizňáková[1], M. Vavrinec[1], E. Michálková[1], P. Veselý[1], K. Veselá[1] & F. Zedek[1]

1 Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic
2 Department of Biology, University of Naples Federico II, Naples, Italy
3 Botanical Garden, University of Naples Federico II, Naples, Italy
4 Faculty of Forestry, Department of Forest Engineering, Artvin Coruh University, Artvin, Türkiye

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Correspondence
P. Bureš and F. Zedek, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, Brno, CZ 61137, Czech Republic.
E-mail: bures@sci.muni.cz and fzedek@gmail.com
E. Del Guacchio, Department of Biology, University of Naples Federico II, Botanical Garden, Via Foria 223, 80139 Naples, Italy.
E-mail: emanuele.delguacchio@unina.it

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ABSTRACT

- The invasive tetraploid *Cirsium vulgare* hybridizes with both *Cirsium* and *Lophiolepis*. Its conflicted 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.
- 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 and imaging approaches, such as NeighborNet, Heatmap, and STRUCTURE, to identify nuclear genomes admixture. Estimation of the intensity of spontaneous hybridization within and between *Cirsium* and *Lophiolepis* was based on herbarium revisions and published data for all reported hybrids pertinent to taxa currently included in *Cirsium* or *Lophiolepis*.
- 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 the 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.
- Our analyses provide compelling evidence that the invasive species *C. vulgare* has an allotetraploid intergeneric origin, with the maternal parent from *Cirsium* and the paternal from *Lophiolepis*. For the purpose of delimiting monophyletic genera, we propose keeping *Lophiolepis* separate from *Cirsium* and segregating *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 revealed 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 genomic features (Bureš *et al.* 2023).

However, the distinction between *Lophiolepis* and *Cirsium* encounters complications because of *Cirsium vulgare* (Savi) Ten., a Palearctic species naturalized worldwide (POWO 2023), showing intermediate morphological and genomic traits. Specifically, unlike genus *Cirsium*, *C. vulgare* has rigid setae on the upper leaf surface (compare Figs. 1 and 2) and a 3D arrangement of involucral spines reminiscent of the lances of a Macedonian phalanx (Fig. 3): 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. 4). This latter feature is indeed shared with ca. 10% of taxa of *Cirsium* s. str. (Bureš, pers. obs.), namely the Old World species *C. alatum*, *C. brachycephalum*, *C. canum*, *C. chrysacanthum* (Ball) Jahand., *C. creticum*, *C. ducellieri* Maire, *C. dyris* Jahand. & Maire, *C. elbrusense* Sommier & Levier, *C. elodes*, *C. gaditanum* Talavera & Valdés, *C. glaberrimum* (Petr.) Petr., *C. hygrophilum* Boiss., *C. komarovii* Schischk., *C. leucopsis* DC., *C. libanoticum* DC., *C. monspessulanum*,

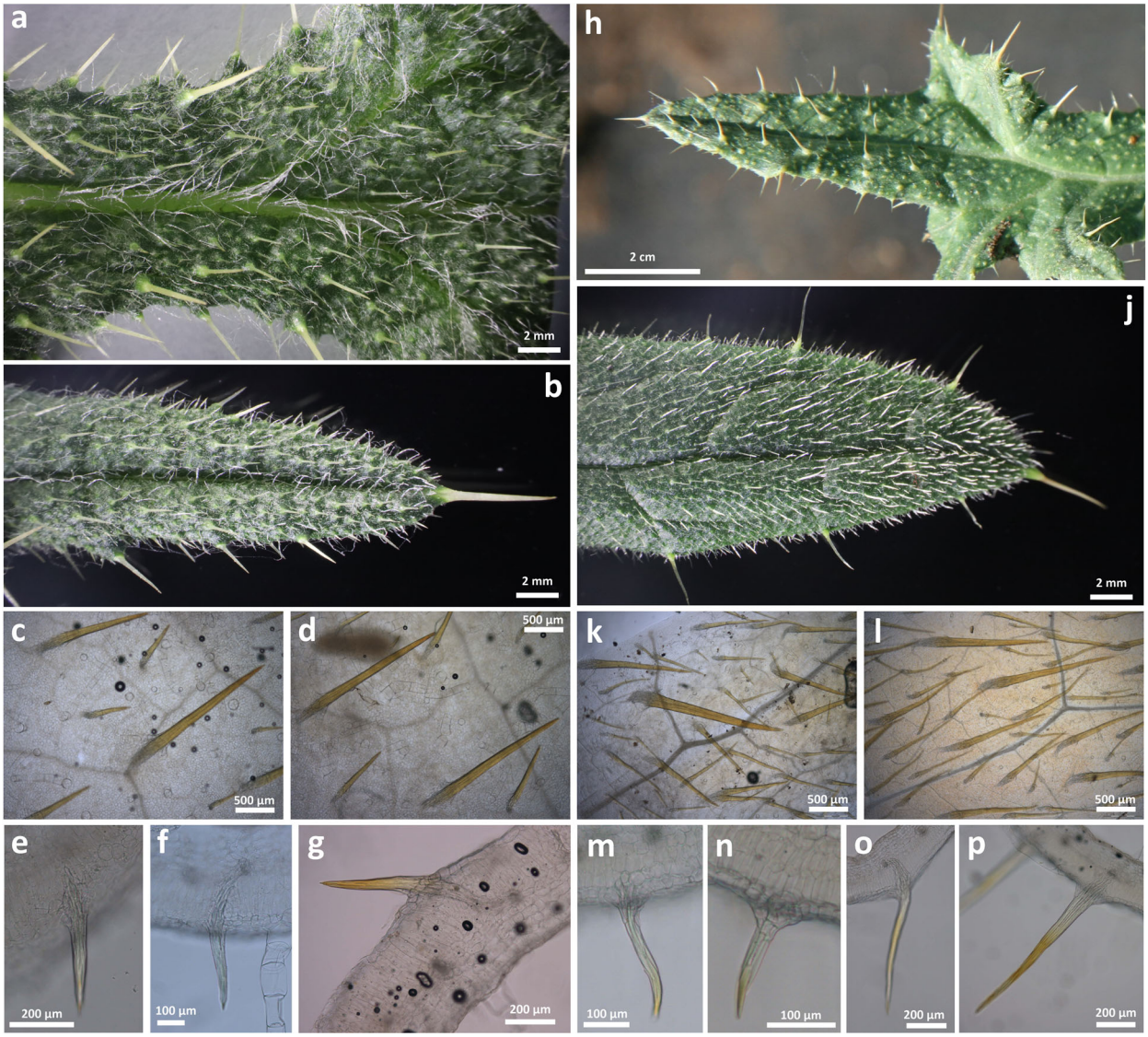

Fig. 1. Rigid setae on the upper leaf surface of *Cirsium vulgare* (a–g), *Lophiolepis sorocephala* subsp. *congesta* (Fisch. & C. A. Mey. ex DC.) Bureš, Del Guacchio, Iamonico & P. Caputo (h), *L. eriophora* (L.) Del Guacchio, Bureš, Iamonico & P. Caputo (j–p). The setae are usually connected to veins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

C. palustre, *C. pectinellum* A. Grey, *C. pseudocreticum* (P. H. Davis & Parris) Yıldız, Dirmenci & Arabacı, *C. pyrenaicum*, *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, and 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. Grey. Also, the achene weight of *C. vulgare* exhibits an intermediate position between the values typical of representatives of *Cirsium* and *Lophiolepis* (see Fig. 3E in Bureš *et al.* 2023). Finally, 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 Fig. 3A,B in Bureš *et al.* 2023). It is noteworthy that *C. vulgare* is tetraploid (2n = 4x = 68), while most taxa of *Cirsium* s. str. are diploid (2n = 34), as well as almost all *Lophiolepis* taxa (Bureš *et al.* 2004, 2023; Watanabe 2008; Rice *et al.* 2015).

As a consequence, we have hypothesized that *C. vulgare* is an allopolyploid species, likely derived from hybridization between *Lophiolepis* and *Cirsium* (Bureš *et al.* 2023), which is at odds with recent molecular phylogenies based on few markers, in which *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, however, a HybSeq phylogenetic reconstruction based on hundreds of markers showed conflict between nuclear (*C. vulgare* grouped with *Lophiolepis*) and

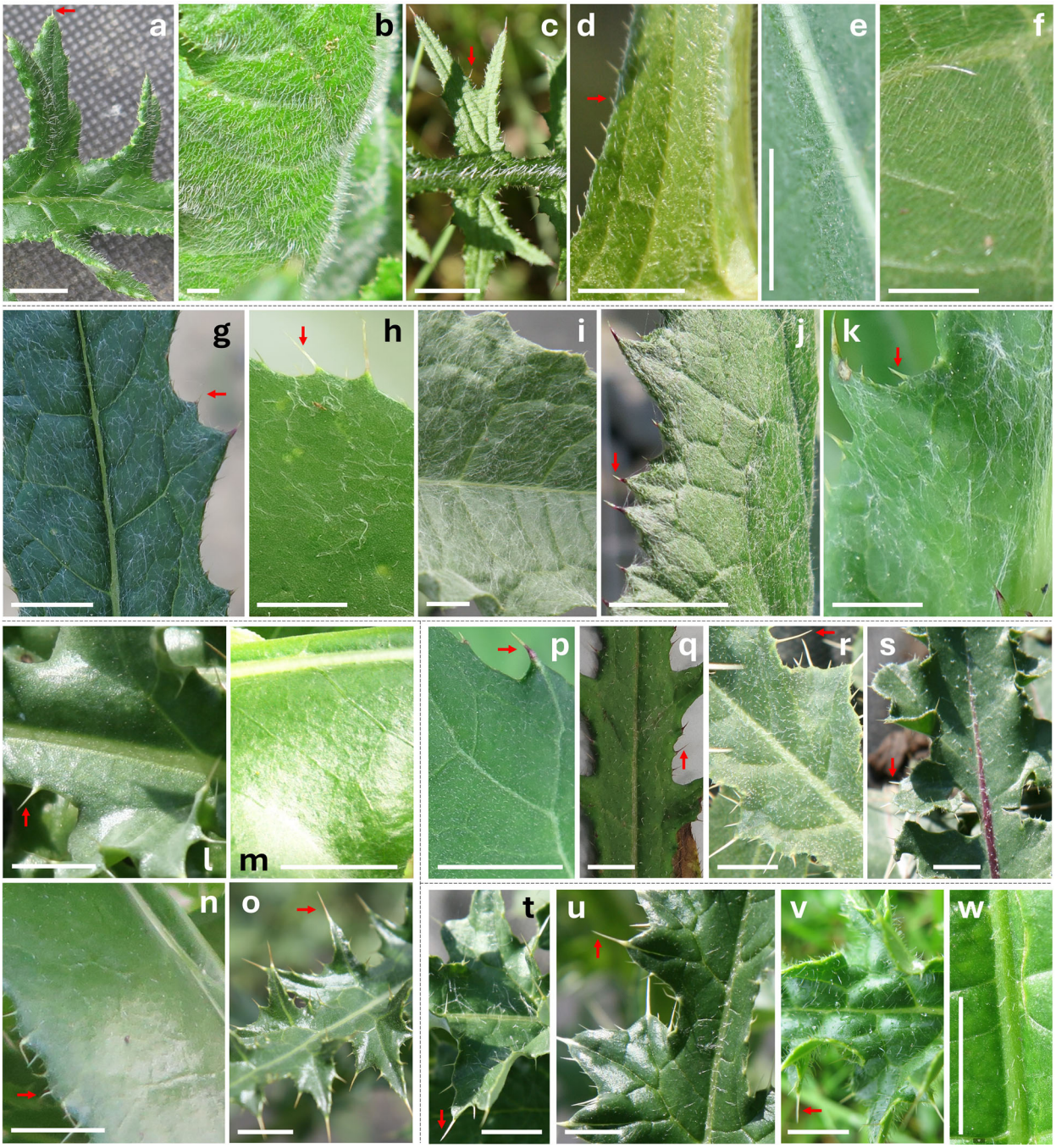

Fig. 2. Indumentum of upper leaf surface in the genus *Cirsium* is typically composed of unbranched, elongated, uniseriate multicellular weak trichomes, never rigid setae or spines, which occur exclusively at leaf margin in this genus (red arrows). (a–f) Hairy pubescent to pilose indumentum formed by more-or-less narrow hairs covering the whole leaf surface or the area around the central leaf vein in the leaves of (a) *C. tuberosum* (L.) All., (b) *C. waldsteinii* Rouy, younger leaf, (c) *C. palustre* (L.) Scop., younger leaf, (d) *C. erisithales* Scop., leaf lobe, (e) *C. pannonicum* (L.f.) Link, area around the central leaf vein, and (f) *C. carniolicum* Scop. subsp. *carniolicum*, area around the central leaf vein. (g–k) Arachnoid indumentum formed by sparse, long and loosely entangled hairs in the leaves of (g) *C. waldsteinii*, (h) *C. canum* (L.) All., (i) *C. elodes* M. Bieb., (j) *C. greimleri* Bureš, (k) *C. heterophyllum* (L.) Hill. (l–o) Glabrous leaf surface without or almost without hairs in (l) *C. candelabrum* Griseb., (m) *C. canum*, (n) *C. monspessulanum* (L.) Hill, and (o) *C. pubigerum* (Desf.) DC. (p–s) Sparsely puberulous indumentum formed by short, curly hairs in the leaves of (p) *C. alsophilum* (Pollini) Soldano, leaf lobe, (q) *C. palustre*, older leaf, (r) *C. pyrenaicum* (Jacq.) All., and (s) *C. rhizocephalum* C. A. Mey. (t–w) Sparsely pilose indumentum formed by longer, slightly curled hairs restricted to the area around the central leaf vein in the leaves of (t) *C. echinus* (M. Bieb.) Hand.-Mazz., (u) *C. obvallatum* (M. Bieb.) M. Bieb., (v) *C. spinosissimum* (L.) Scop., and (w) *C. rivulare* (Jacq.) All. Many species vary in indumentum type, mainly depending on the habitat or development stage of the leaf (e.g., b *versus* g, c *versus* q, or h *versus* m). Bar = 1 cm. Photo by P. Bureš.

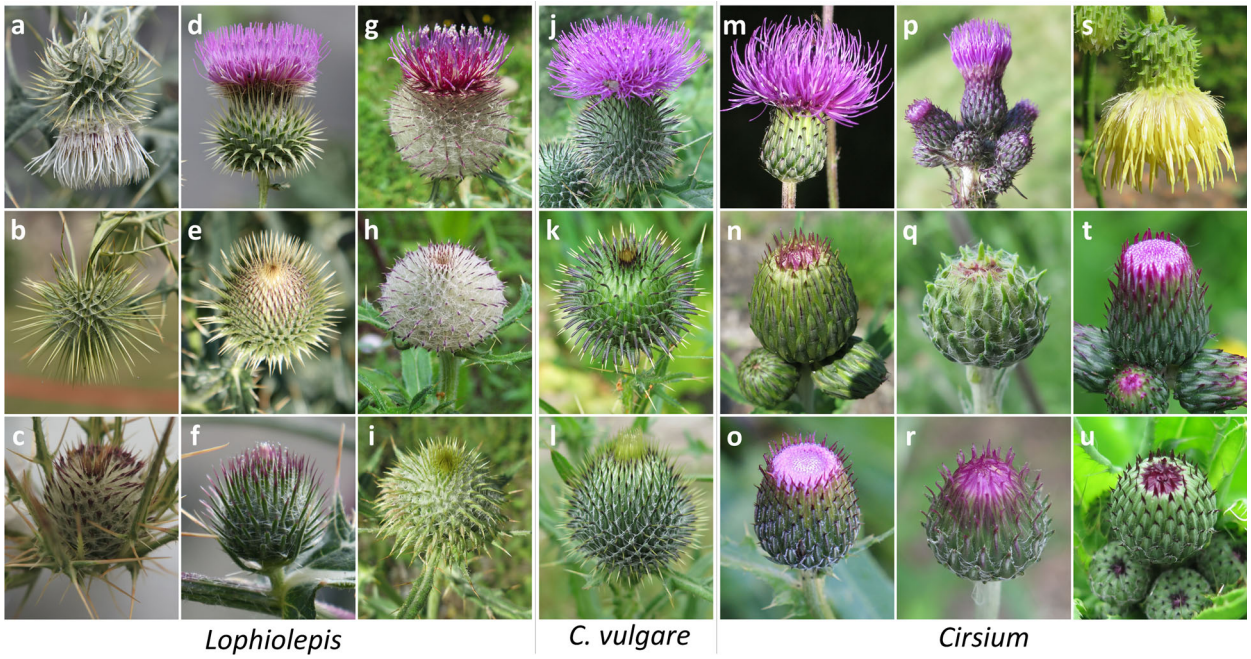

Fig. 3. Shape of the flower heads and involucres: (a, b) *Lophiolepis caucasica* (Adams) Bureš, Del Guacchio, Iamonico & P. Caputo, (c) *L. vallis-demonii* (Lojac.) Del Guacchio, Bureš, Iamonico & P. Caputo, (d, e) *L. ciliata* subsp. *szovitsii* (K. Koch) Bureš, Del Guacchio, Iamonico & P. Caputo, (f) *L. bulgarica* (DC.) Del Guacchio, Bureš, Iamonico & P. Caputo, (g, h) *L. eriophora*, (i) *L. spathulata* (Moretti) Del Guacchio, Bureš, Iamonico & P. Caputo, (j–l) *Cirsium vulgare*, (m) *C. canum*, (n) *C. heterophyllum*, (o) *C. pubigerum*, (p) *C. palustre*, (q) *C. tuberosum* (L.) All., (r) *C. pannonicum*, (s) *C. erisithales*, (t) *C. rivulare*, (u) *C. monspessulanum*. 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). Photos by P. Bureš.

plastid (*C. vulgare* nested within *Cirsium*) phylogenies, supporting the hypothesized hybridogenous origin of *C. vulgare* (Moreyra *et al.* 2023).

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 are 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 short 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; Bureš 2004; Keil 2006). Its native distribution spans Europe, western Asia, and North Africa (Meusel & Jäger 1992; POWO 2023). Due to its affinity with 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 plants globally (Julien & Griffiths 1998; Parsons & Cuthbertson 2001; Sagliocco *et al.* 2012; Pyšek *et al.* 2017; Román *et al.* 2021).

In this study, we analysed the enigmatic origin of the noxious weed *C. 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* and compared this with the intensity of spontaneous hybridization within and between these two genera 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, following Del Guacchio *et al.* (2022) and references herein, we do not recognize any infraspecific taxon within *C. vulgare*.

Sampling

We examined eight tetraploid individuals of *C. vulgare*, each from a different population, together with 11 individuals, each representing one of the diploid European, Mediterranean, or West Asian species of the genus *Lophiolepis* – namely, *L. aggregata* (Ledeb.) Bureš, Del Guacchio, Iamonico & P. Caputo, *L. bulgarica* (DC.) Del Guacchio, Bureš, Iamonico & P. Caputo, *L. caucasica*, *L. lobelii* (Ten.) Del Guacchio, Bureš, Iamonico & P. Caputo, *L. macrobotrys* (K. Koch) Bureš, Del Guacchio, Iamonico & P. Caputo, *L. morisiana* (Rchb.f.) Del Guacchio, Bureš, Iamonico & P. Caputo, *L. rigida* (DC.) Bureš, Del Guacchio, Iamonico & P. Caputo, *L. scabra* (Poir.) Del Guacchio, Bureš,

Fig. 4. Shape of the leaf base: (a) *Lophiolepis serrulata* (M. Bieb.) Del Guacchio (a), Bureš, Iamonico & P. Caputo; (b) *L. ossetica* (Adams) Del Guacchio, Bureš, Iamonico & P. Caputo; (c) *Cirsium vulgare*; (d) *C. alatum* (S. G. Gmel.) Bobrov; (e) *C. creticum* (Lam.) d'Urv.; (f) *Lophiolepis spathulata* (Moretti) Del Guacchio, Bureš, Iamonico & P. Caputo; (g) *L. tenoreana* (Petr.) Del Guacchio, Bureš, Iamonico & P. Caputo; (h) *Cirsium vulgare*; (i) *C. brachycephalum* Juratzka. *Cirsium vulgare* (c, h) shares the decurrent leaves with some species of *Cirsium* (d, e, i, j) but differs in this feature from all taxa of *Lophiolepis* (which all have sessile leaves with semi-ampexicaul base – a, b, f, g). Photos by P. Bureš.

Iamonico & P. Caputo, *L. sommieri* (Petr.) Del Guacchio, Bureš, Iamonico & P. Caputo, *L. tenoreana*, and *L. trachylepis* (Boiss.) Del Guacchio, Bureš, Iamonico & P. Caputo – and 12 samples of the diploid Eurasian species of the genus *Cirsium* – namely, *C. acaulon* subsp. *acaulon*, *C. alsophilum*, *C. arvense* (L.) Scop., *C. canum*, *C. carniolicum* subsp. *carniolicum*, *C. erisithales*, *C. heterophyllum*, *C. monspessulanum*, *C. oleraceum* (L.) Scop., *C. palustre*, *C. pannonicum*, and *C. spinosissimum* (Table S1; DNA ploidy level of the individuals was 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, Dueren, 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 gels, and DNA concentration was measured using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). To prepare a library, we used 200 ng DNA per diploid sample and 300 ng for tetraploid *C. vulgare*. A RADseq library was assembled following the protocol of Etter *et al.* (2011) with minor modifications. To cut the DNA, we used PstI restriction enzyme (New England Biolabs, Ipswich, MA, 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, Brno, Czech Republic).

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 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* (Falc.) Lipsch.: NC_059793.1, *S. inversa* Raab-Straube: NC_066407.1) genomes in Geneious Prime software v. 2023.0.1 (Biomatters, Auckland, 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 2014) and respects ploidy levels (tetraploid *C. vulgare* among diploid species; Wagner *et al.* 2020). In particular, diploid and tetraploid entries were analysed separately and, at the end of the ipyrad analysis, were 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 were subsequently used for further analyses as inputs. The appropriate 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 SNP 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% 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 retained 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% missing data.

representing *Lophiolepis*, *Cirsium*, or *C. vulgare* samples, each locus from the dataset of 51,256 loci was classified as belonging to only one group, to a combination of two groups, or to all three groups (seven distinct categories).

Estimation of willingness to hybridize spontaneously

Herbarium specimens of *Cirsium* hybrids 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 min longitude × 3 min latitude; the total number of quadrants covering the abovementioned studied area was 10,203; Vavrinec 2020). For each species pair (= potential interspecific hybrid within the *Cirsium* or *Lophiolepis* genus or between the genera), 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:

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

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 v. b5.3.0 (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 v. 1.0.1 (Revell 2012), we constructed a heatmap based on the nuclear supermatrix employing the same distance matrix as the NeigborNet tree. Third, we analysed 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 of 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

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 the 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 (Blizňáková 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 Table S2. The analogous analysis of the hybrid willingness in the British Isles was conducted with the corresponding data taken from Preston *et al.* (2002), Stace *et al.* (2015), and 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:

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

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

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

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 Tables S4 and S5.

RESULTS

RADseq data statistics

The 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 analysed individuals (SD 8.43×10^5; range: 0.73–3.74×10^6 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: 0.57–2.99×10^6 raw reads) and 5.25×10^5 raw reads of organellar sequences per individual (SD 3.83×10^5; range: 0.14–1.46×10^6 raw reads). The mean coverages of nuclear and organellar loci were 10.4× (SD 3.1) and 659.1× (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. 5a). On the other hand, NeighborNet based on the organellar SNPs revealed the affiliation of *C. vulgare* samples to *Cirsium*, with the closest relationship to *C. palustre*, *C. monspessulanum*, and *C. alsophilum* (Fig. 5b).

The dist.p matrix heatmap of the nuclear RADseq data suggested that all samples representing either *Lophiolepis* or *Cirsium* genera are more similar to *C. vulgare* than to each other (Fig. 6).

Based on the highest ΔK (Figure S1), the STRUCTURE analysis showed that the nuclear genome of *C. vulgare* is most likely composed of two subgenomes, each of which is attributable to *Cirsium* or *Lophiolepis* genera (K = 2; Fig. 7a). All the analysed *C. vulgare* individuals showed consistent, proportionally similar admixture of both suspected parental genomes. An alternative K = 3, under which *C. vulgare* forms a separate cluster (Fig. 6b), was less likely yet still acceptable (Figure S1).

The numbers of loci that are clade-specific or shared by two or three clades are shown in the sectors of the Venn diagram (Fig. 8). The highest proportion of the nuclear loci was shared by *C. vulgare* and both suspected parental genera (18,892; 36.9%). About one-third fewer loci were shared by *C. vulgare* with *Cirsium* (13,439; 26.2%) or *Lophiolepis* (12,217; 23.8%). The number of loci absent in *C. vulgare* but shared by *Cirsium* and *Lophiolepis* samples was significantly smaller (1,460; 2.8%), as well as the numbers of loci occurring exclusively in samples of *C. vulgare* (2,173; 4.2%), *Lophiolepis* (2,039; 4.0%), or *Cirsium* (1,036; 2.0%).

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

We identified 60 different hybrids involving 15 *Cirsium* species (including *C. vulgare*) and three *Lophiolepis* species, represented by 4,865 herbarium specimens (Table S2). Of these, 27 (0.55%) specimens were hybrids of *C. vulgare* with other species of *Cirsium* (namely, *C. acaulon*, *C. erisithales*, *C. oleraceum*, and *C. palustre*) and eight (0.16%) specimens were hybrids between *C. vulgare* and *Lophiolepis eriophora* (Table S2).

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

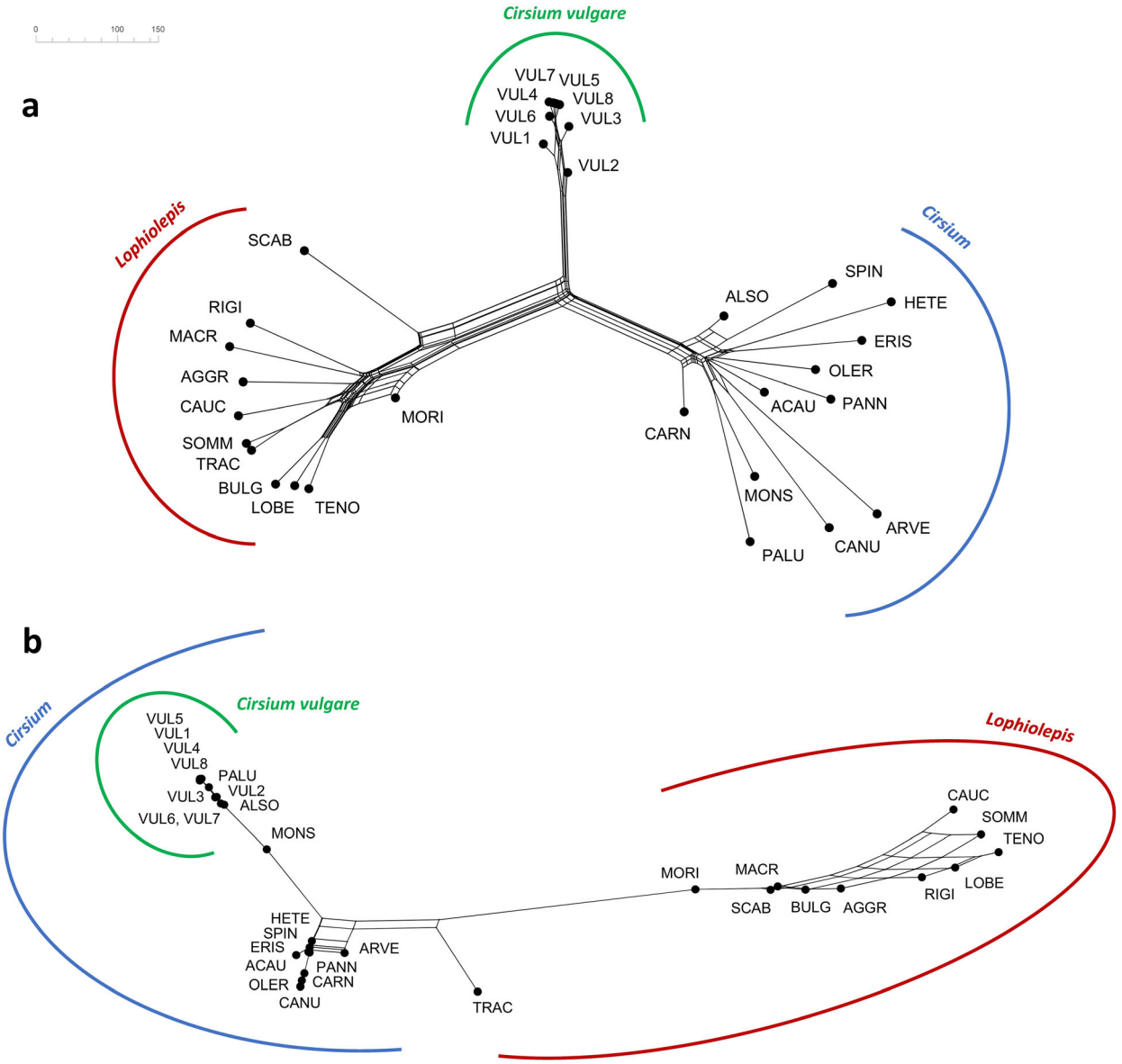

Fig. 5. NeighborNet phylogenetic network visualizing genetic relationship among eight samples of *Cirsium vulgare* (green: VUL1–8) and samples representing species of genera *Cirsium* (blue) and *Lophiolepis* (red) based on nuclear (a) and organellar (b) RADseq data. *Lophiolepis* species: *L. aggregata* (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), and *L. trachylepis* (TRAC). *Cirsium* species: *C. acaulon* (ACAU), *C. alsophilum* (ALSO), *C. arvense* (ARVE), *C. canum* (CANU), *C. carniolicum* subsp. *carniolicum* (CARN), *C. erisithales* (ERIS), *C. heterophyllum* (HETE), *C. monspessulanum* (MONS), *C. oleraceum* (OLER), *C. palustre* (PALU), *C. pannonicum* (PANN), *C. spinosissimum* (SPIN).

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; Table S3).

On a global (world) scale, 103 species of *Lophiolepis* and 321 of *Cirsium* were analysed 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 (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; Table S4).

DISCUSSION

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

Our results based on nuclear RADseq markers showed that *C. vulgare* has a genomic makeup consisting of both *Cirsium* and

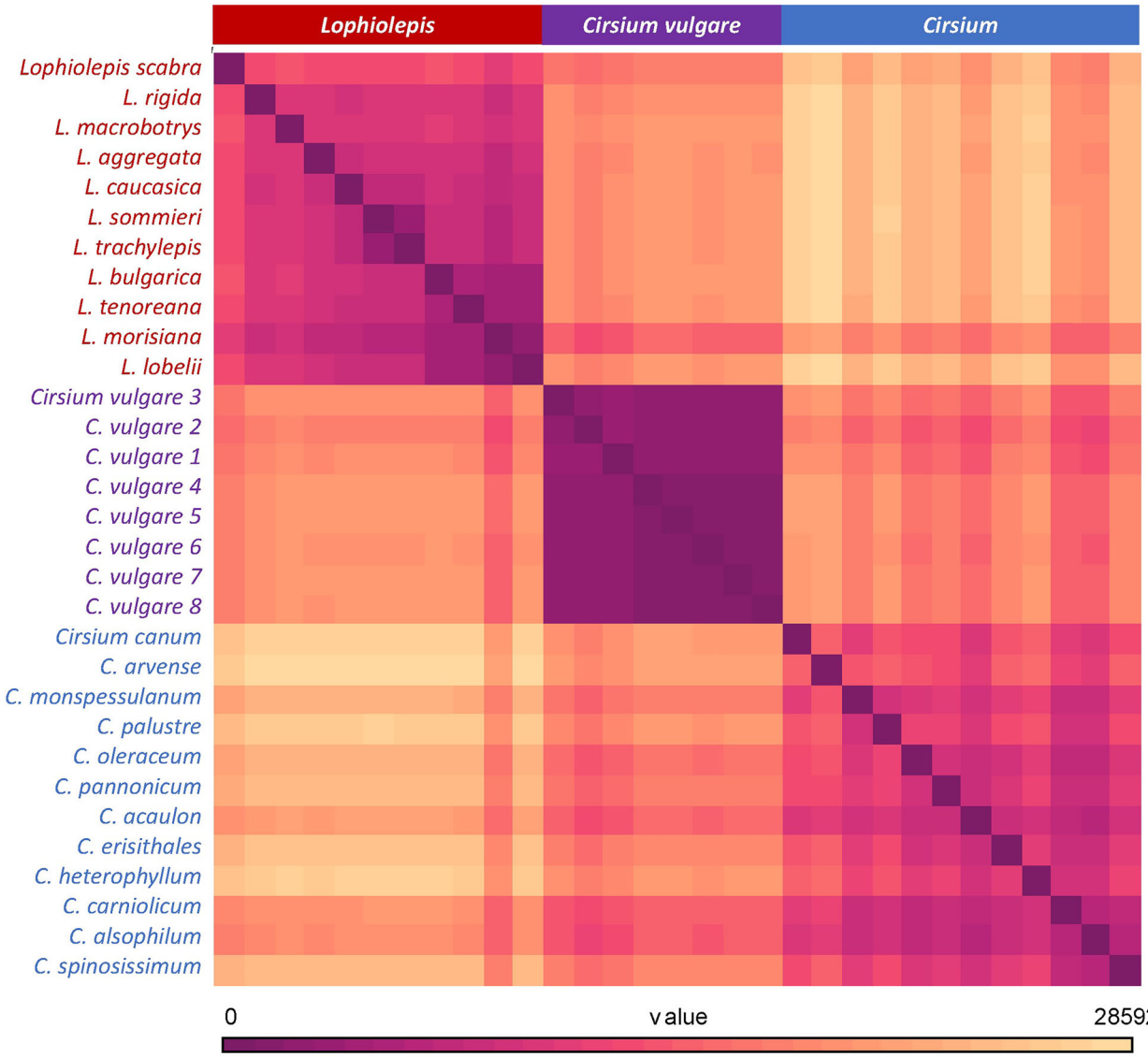

Fig. 6. Heatmap visualizing similarities among the samples representing *Cirsium vulgare* and both suspected parental genera *Lophilepis* and *Cirsium* derived from dist.p matrix based on nuclear RADseq data. Samples of both genera are more similar to *C. vulgare* than to each other. Darker colour in the heatmap indicates greater degree of similarity.

Lophiolepis, and that it is more similar to any of them than these genera are to each other (Figs. 5–8). 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. 8). 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 the divergence of these genera from their most recent common ancestor. When we consider the average number of loci specific for *Cirsium* (1,036 + 13,439) and *Lophiolepis* (2,039 + 12,217) compared to loci specific for *C. vulgare* (2,173), the origin of *C. vulgare* is more recent than the split of *Cirsium* and *Lophiolepis* by a factor of approximately 6.6 (Fig. 8). 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. 7a). The less likely but still acceptable K = 3 in the STRUCTURE analysis makes sense (Fig. 7b), 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. vulgare*'s unique loci in Fig. 8). 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 a sister

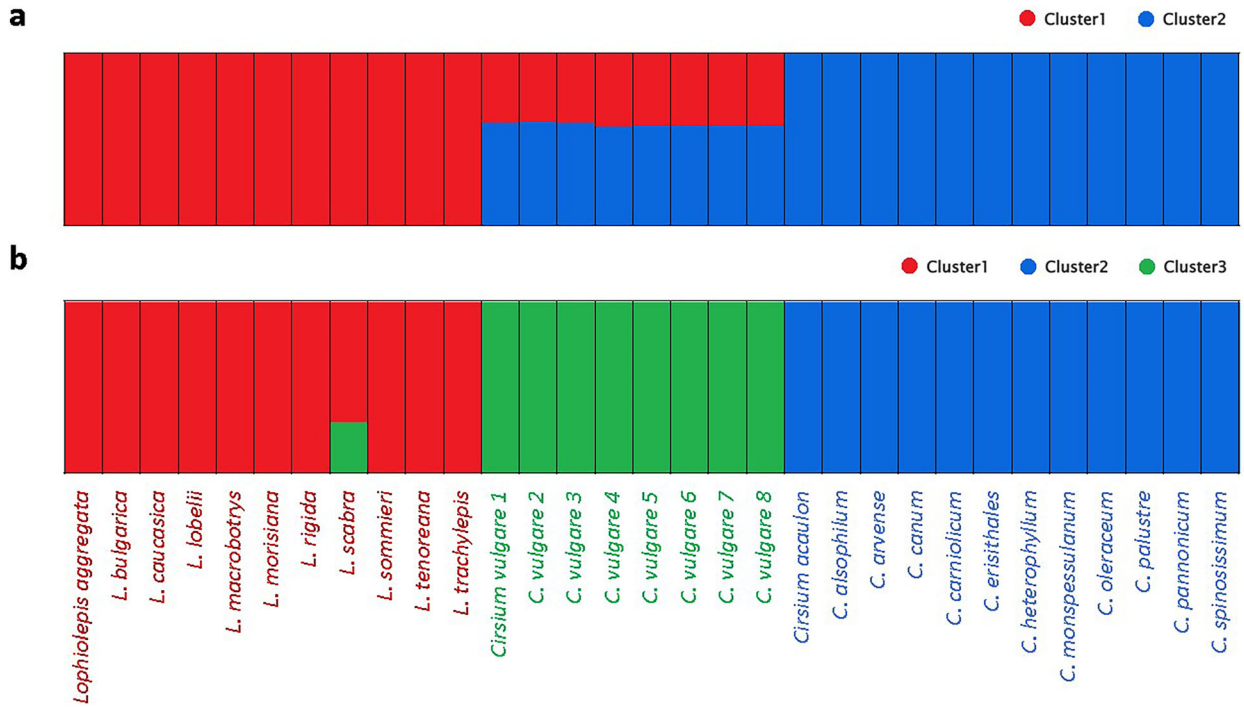

Fig. 7. Results of the STRUCTURE analysis in which nuclear RADseq data are visualized as bar plots showing Bayesian assignment probabilities for two clusters (a) or three clusters (b, the less probable scenario) across the studied samples of *Cirsium vulgare* (associated with the green cluster in b) and representatives of the genera *Lophiolepis* (associated mostly with the red cluster) and *Cirsium* (associated with the blue cluster).

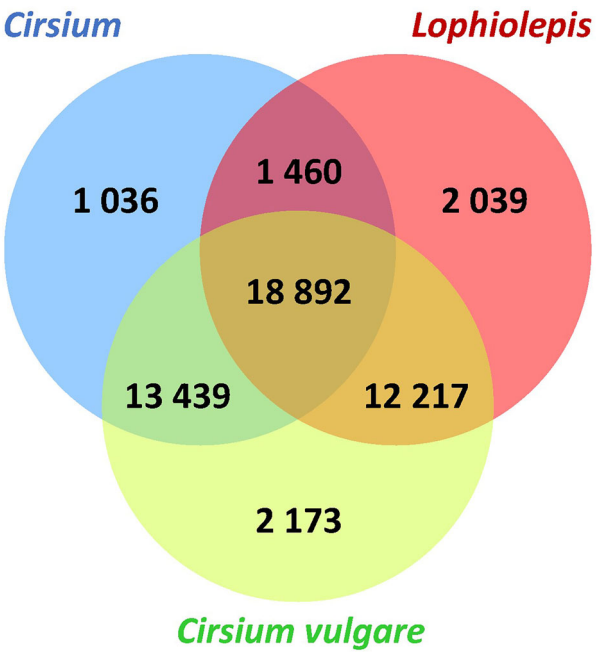

Fig. 8. Venn diagram whose sectors contains the numbers of nuclear loci classified based on specificity and various degrees of sharing within and among eight samples of *Cirsium vulgare* (green circle), 12 *Cirsium* species (excl. *C. vulgare*; blue circle), and 11 *Lophiolepis* species (red circle).

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 a Eurasian clade including taxa belonging to *Cirsium* sect. *Cirsium*; as suggested by several taxa of that clade showing winged stems like those of *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. 5). 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. 4). However, our dataset includes only 12 *Cirsium* species (4.5% of ca 270 Eurasian *Cirsium* species, when African species – recently recognized as *Afrocirsium*, *Nuriaea* – and North American taxa are excluded; cf. POWO 2023). Interestingly, in the species-rich phylogenies of Del Guacchio *et al.* (2022) or Bureš *et al.* (2023), its closest relative was *C. heterotrichum* Pančić, not included in the present study; independently, the same species was identified as the closest relative of *C. vulgare* in the HybSeq-based chloroplast phylogeny of 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.

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 × 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 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 Tables S2 and S3.
[b]Hybridization propensity = percentage of hybridizing species pairs from geographically overlapping species pairs. For primary data, see Tables S4 and S5.
[c]*Cirsium* excl. *C. vulgare*.

Even if the study does not disprove that *C. vulgare* could have arisen multiple times by repeated hybridization (given the extensive distribution of *C. vulgare* and its variability), our results indicate that very likely the hybridization did not involve several species.

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 flowering 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 *C. 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 often with each of these genera than these genera do with each other (Table 1). Although such a behaviour of *C. vulgare* does not directly prove its intergeneric hybridogenous origin, it is at least an 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). This was also true on a regional scale in Central Europe and the British Isles (Table 1; although the British Isles data are less reliable because of the low number of *Lophiolepis* species). 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; 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 morphology-based reassessment calls into question the existence of nearly half of the intergeneric *Cirsium–Lophiolepis* hybrids for which specimens were accessible. During our revision of Central European herbaria, we discovered that among specimens initially identified as hybrids, 9.9% were, in fact, pure species, and 5.3% were other hybrids. Consequently, only 84.8% of the original hybrid identifications were accurate, while for pure species, the reliability of the original determination was 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 (1987), 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 *versus* 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 the British Isles, hybrids are traditionally the focus of taxonomists and regional botanists, and their distribution is 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 *Lophiolepis* were compared to 9,192 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 to parental species (Stebbins 1985; Soltis & 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 (Pandit *et al.* 2011; te Beest *et al.* 2012; Moura *et al.* 2021) or hybridogenous (Ellstrand & Schierenbeck 2000; Hovick & Whitney 2014; Hodgins *et al.* 2018) species. *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* (Figure S2). In addition, a recently published analysis of *C. vulgare*'s current distribution, using models that considered its niche dynamics, revealed a significant potential for this species to further expand into 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. Interglacial fossil records are sometimes reported (cf. Klinkhamer & de Jong 1993), but these are probably based only on very approximately determined material (such as "achenes of *C. vulgare* type") from Watts *et al.* (1963), who detected it in southern Ireland (County 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 analysis, 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 more reliable), *Lophiolepis* was sister to *Picnomon* and both sister in turn to *Lophiolepis*; more relevant, in the concatenated chloroplast phylogeny, *Cirsium* and *Lophiolepis* were 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 noted 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 and 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 of Moreyra *et al.* (2023) might simply be a consequence of the inclusion of *C. vulgare* in 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 *C. 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 & Friesen 2014; Díaz Lifante *et al.* 2023), more often only hypothesized (e.g., Soejima *et al.* 2008; Calvo *et al.* 2013). 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 *C. vulgare* as a separate monotypic genus, distinguished from *Lophiolepis* by the decurrent leaves (Fig. 4c, h *versus* Fig. 4a,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 (detailed taxonomic treatment including hybrids will be given in E. Del Guacchio, P. Bureš, D. Iamonico, F. Zedek, & P. Caputo, in preparation).

The segregation of *C. vulgare* into the separated hybridogenous genus *Ascalea* also makes clear the morphologic delimitation of *Lophiolepis* against *Cirsium*. The previous argument of Ackerfield *et al.* (2020, p. 729) that rigid setae of *C. vulgare* 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 *C. vulgare* are surrounded by neighbouring cells, so the setae grow out of raised areolae or protrusions (Fig. 1a,b), but the sclerenchymatized setae themselves do not differ from *L. eriophora* in base width and shape (Fig. 1c,d *versus* Fig. 1k,l, or Fig. 2e–g *versus* Fig. 2m–p). Moreover, such protrusions are not synapomorphic exclusively for *C. vulgare* 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 the connection between the setae and veins in *C. vulgare* 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 setae often are not (Fig. 1e,m,n). In summary, the rigid setae on the upper leaf surface of *C. vulgare* are very likely homologous to those of *L. eriophora* and other *Lophiolepis* species.

AUTHOR CONTRIBUTIONS

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

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FUNDING INFORMATION

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DATA AVAILABILITY STATEMENT

Raw sequences generated during this study were deposited in the NCBI under the BioProject accession number PRJNA1032292 (accessed 25 October 2023: http://www.ncbi.nlm.nih.gov/bioproject/1032292). BioSample Accession numbers for each sample can be found in Table S1.

SUPPORTING INFORMATION

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

Figure S1. Evanno plots.

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

Table S1. Localities of the studied samples.

Table S2. (A) Willingness to hybridize spontaneously among Central European *Cirsium* and *Lophiolepis* species: Within-*Cirsium* hybrids. (B) Willingness to hybridize spontaneously among Central European *Cirsium* and *Lophiolepis* species: *Cirsium vulgare* × *Cirsium*-hybrids. (C) Willingness to hybridize spontaneously among Central European *Cirsium* and *Lophiolepis* species: *Cirsium* × *Lophiolepis*-hybrids. (D) Willingness to hybridize spontaneously among Central European *Cirsium* and *Lophiolepis* species: *Cirsium vulgare* × *Lophiolepis*-hybrids. (E) Willingness to hybridize spontaneously among Central European *Cirsium* and *Lophiolepis* species: Within-*Lophiolepis* hybrids.

Table S3. (A) Willingness to hybridize spontaneously among British and Irish *Cirsium* and *Lophiolepis* species: Within-*Cirsium* hybrids (data taken from Stace *et al.* 2015). (B) Willingness to hybridize spontaneously among British and Irish *Cirsium* and *Lophiolepis* species: *Cirsium* × *Lophiolepis*-hybrids (data taken from Stace *et al.* 2015). (C) Willingness to hybridize spontaneously among British and Irish *Cirsium* and *Lophiolepis* species: *Cirsium vulgare* × *Cirsium*-hybrids (data taken from Stace *et al.* 2015).

Table S4. Hybrids reported between *Cirsium vulgare* and species of *Lophiolepis* and *Cirsium* genera, within and among these genera.

Table S5. (A) *Cirsium*-species reported as hybridizing with *Cirsium vulgare*. (B) *Lophiolepis*-species reported as hybridizing with *Cirsium vulgare*. (C) Intergeneric hybrids reported between species of genera *Cirsium* and *Lophiolepis*. (D) Survey of congeneric hybrids within *Lophiolepis*.

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