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RESEARCH

Genetic variation within the arctic-alpine *Calamagrostis stricta* (Poaceae) species complex in Europe

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Abstract

The *Calamagrostis stricta* (Poaceae) species complex is a circumpolar, boreo-arctic and montane taxon that includes numerous subspecies and varieties. The recent discovery of *Calamagrostis lonana* in the Alps calls for a thorough assessment of relationships within *C. stricta*. The main aim of our study was to elucidate the phylogenetic position, genetic structure and ploidy level of *C. lonana*, as compared to the other members of the *C. stricta* species complex from Central Europe to the Arctic. Fifteen populations of the *C. stricta* species complex were sampled across Central and Northern Europe, and their ploidy level was estimated using flow cytometry. Genetic variation was characterized using double digest RAD sequencing reads (ddRAD-seq) on a total of 115 individuals genotyped at 1157 single-nucleotide polymorphisms. Based on flow cytometric measurements, tetraploidy was observed in Arctic populations from Northern Europe and *C. lonana* in the Alps, in contrast to other populations exhibiting higher ploidy levels. *Calamagrostis lonana* was genetically closely related to the arctic *C. stricta* subsp. *groenlandica*, while *C. stricta* subsp. *stricta* formed a second genetic cluster across Central Europe. A third, very distinct genetic cluster was observed in the northern Svalbard archipelago. Despite lacking evidence of sexual reproduction, substantially more genetic diversity than expected under asexual reproduction was detected within populations in *C. lonana* and other taxa. The distribution and genetic structure of the *C. stricta* species complex has been shaped by major post-glacial environmental changes having affected cold regions and specifically highlights *C. lonana* as a valuable relict taxon for the Alps.

Keywords Arctic-alpine disjunctions · *Calamagrostis lonana* · Glacial relicts · Narrow endemism · Phylogeography · Polyploidy

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Introduction

Glacial cycles of the Pleistocene shaped the current distribution of species across Europe (Taberlet et al. 1998; Hewitt 2000; Brochmann et al. 2003). The Alps in particular have acted as a major barrier constraining the expansion of thermophilous species but may also have served as a refugium for many cold-adapted taxa (Schönswetter et al. 2006; Holderegger and Thiel-Egenter 2009; Laenen et al. 2018). Although the spatial dynamics of plants from the last glacial maximum (LGM) to nowadays have been extensively investigated in the context of climate-induced range shifts and speciation (Ronikier et al. 2012; Kadereit and Abbott 2021; Parisod 2022), the glacial and post-glacial history of plants currently presenting an arctic-alpine distribution remains largely elusive. Several studies based on fossil records as well as molecular markers indicated that several cold-adapted species with an arctic-alpine distribution survived the LGM across vast tundra occurring between the ice sheets protruding from the North and the Alps, and then shifted their distribution range towards higher latitudes and/or elevation during warmer phases (e.g. *Dryas octopetala*; Skrede et al. 2006; Birks 2008; Kadereit 2024). How such range shifts have shaped genetic diversity across the Arctic versus the Alps remains poorly understood. To what extent warm phases supporting allopatric divergence within arctic-alpine species have been long enough to enable the evolution of reproductive isolation or to promote the origin of polyploid species (Stebbins 1984) shall accordingly be further investigated.

The *Calamagrostis stricta* (Poaceae) species complex is a circumpolar, boreo-arctic and montane taxon that is abundant in Arctic and subarctic regions but much rarer in Western and Central Europe where it is considered a glacial relic (Böhling et al. 1998; Conert et al. 1998; Guyonneau et al. 2007). Although the lack of phylogenetic and taxonomic understanding largely hinders investigations (Schiebold et al. 2009; Saarela et al. 2017; Soreng et al. 2017; Peterson et al. 2022), this species complex includes six to ten subspecies and varieties indicating a high degree of variation (Böhling et al. 1998; Guyonneau et al. 2007; Flora of Svalbard 2023; Panarctic Flora 2023). In Europe and European Arctic, two taxa of this complex have been reported: *C. stricta* subsp. *stricta* (Timm) Koeler [= *C. neglecta* (Ehrh.) Gaertn.] and *C. stricta* subsp. *groenlandica* (Schrank) Á.Löve (Flora of Svalbard 2023; Panarctic Flora 2023).

The recent discovery of the narrow endemic *Calamagrostis lonana* Eggenb. & Leibundg. in the central part of the Pennine Alps, at 2588 m a. s. l. (in Pas de Lona near Grimentz in Switzerland; Eggenberg et al. 2023) and its description as a taxon showing morphological and ecological similarities with *C. stricta*, and especially with its arctic subspecies *C. stricta* subsp. *groenlandica* (Böhling et al. 1998; Conert et al. 1998; Flora of Svalbard 2023; Panarctic Flora 2023; Aiken et al. 2023), calls for an assessment of taxonomic relationships within *C. stricta*. In total, five ploidy levels, namely 4x (the most common one), 6x, 8x, 10x and 12x have been reported the *C. stricta* species complex (Aiken et al. 2023 and original references therein). Several northern European tetraploid taxa were shown to be sexual, whereas taxa with a higher chromosome number appeared mainly consistent with clonal growth and/or asexual seed production (agamospermy) (Kershaw 1962; Greene 1984; Crackles 1997; Sato 2014; Flora of Svalbard 2023). Accordingly, the present study is the first to investigate genetic variation within the *C. stricta* complex at a continental scale, with special focus on the newly discovered *C. lonana,* in order to understand its origin and offer guidelines for its conservation. We address the following questions: (1) What is the distribution of ploidy levels and genetic diversity within the *C. stricta* species complex across Europe and the Arctic? (2) What are the evolutionary and biogeographic relationships of the newly described *C. lonana* with other European members of the *C. stricta* complex (*C. stricta* subsp. *stricta* and *C. stricta* subsp. *groenlandica*)?

Methods

Plant material and data collection

We sampled 16 populations of the *C. stricta* species complex from seven countries (Table 1, Online Resource 1 and 2). Whole plants were individually sampled in each population, wrapped in coffee filters, and dried in ziplock bags filled with silica gel.

In the Swiss valley of Pas de Lona, 16 whole plants of *C. lonana* were collected for genetic analysis across an area of approximately 600 × 150 m in numerous subgroups distributed along the alluvial plain (Online Resource 1). In order to limit relatedness between individuals, samples were collected in distant grassy tufts, from 20 to 140 m apart, across the 600-m-long plain and were georeferenced. Additionally, two voucher specimens were collected and deposited in the herbarium of the Conservatory and Botanic Garden of the City of Geneva (CJBG), Switzerland. Similar habitats were prospected across the neighbouring Vallon de Réchy (Le Louché, Combacondoi, Ar du Tsan) to eventually find other populations. According to unreliable sources, *C. stricta* could have been previously growing in Switzerland around the Lac des Taillères (Jura Mountains, Canton of Neuchâtel), but remediation works destroyed the tiny peninsula possibly hosting *C. stricta* in 1926 (Feuilles d'Avis de

Table 1 Characteristics of all sampled populations of the *Calamagrostis stricta* species complex used for genetic analysis, showing the number of detected genotypes, observed heterozygosity (H_o), expected heterozygosity within population (H_s), genetic differentiation (G_{IS}) and the ploidy level estimated through flow cytometry (Ploidy)

Population name	Country/Region	Location	Elevation a. s. l	Sample size for analysis	Number of genotypes	H_o	H_s	G_{IS}	Ploidy	Taxonomic assignment
Lona (CH)	Switzerland	46° 09′ 16″ N 07° 32′ 29″ E	2580 m	14 (+1 replicate)	10	0.247	0.167	−0.475	4x	*C. lonana*
Finndalen (NO)	Norway	61° 56′ 11.9″ N 08° 51′ 26″ E	768 m	10	9	0.239	0.165	−0.449	4x	*C. stricta* subsp. *groenlandica*
Möðrudalur (IS)	Iceland	65° 22′ 20″ N 15° 52′ 31″ W	445 m	1	1	–	–	–	4x	*C. stricta* subsp. *groenlandica*
Stiflisdalsvatn (IS)	Iceland	64° 14′ 49″ N 21° 19′ 30″ W	178 m	1 (+1 replicate)	1	–	–	–	4x	*C. stricta* subsp. *groenlandica*
Hornsund (SJ)	Svalbard	77° 00′ 08″ N 15° 22′ 06″ E	11 m	6	2	0.221	0.151	−0.459	4x	*C. stricta* subsp. *groenlandica*
Longyearbyen_1 (SJ)	Svalbard	78° 13′ 20″ N 15° 39′ 27″ E	8 m	10 (+1 replicate)	2	0.232	0.156	−0.486	4x	*C. stricta* subsp. *groenlandica*
Longyearbyen_2 (SJ)	Svalbard	78° 14′ 57″ N 15° 30′ 46″ E	3 m	3 (+1 replicate)	1	0.202	0.138	−0.468	4x	*C. stricta* subsp. *groenlandica*
Censure (FR)	France	46° 53′ 54″ N 06° 16′ 17″ E	830 m	8 (+1 replicate)	8	0.236	0.161	−0.463	10x*	*C. stricta* subsp. *stricta*
Corne (FR)	France	46° 51′ 27″ N 06° 11′ 20″ E	840 m	10	9	0.244	0.167	−0.461	10x*	*C. stricta* subsp. *stricta*
Grand Mont (FR)	France	46° 53′ 20″ N 06° 15′ 51″ E	820 m	8 (+1 replicate)	6	0.230	0.157	−0.465	10x*	*C. stricta* subsp. *stricta*
Federsee (DE)	Germany	48° 04′ 47″ N 09° 36′ 43″ E	580 m	12 (+1 replicate)	7	0.230	0.158	−0.455	10x*	*C. stricta* subsp. *stricta*
Görbelmoos (DE)	Germany	48° 6′ 35″ N 11° 14′ 35″ E	820 m	2	2	0.249	0.172	−0.450	10x*	*C. stricta* subsp. *stricta*
Kaliště (CZ)	Czech Republic	49° 14′ 28″ N 15° 18′ 06″ E	691 m	10 (+1 replicate)	8	0.237	0.162	−0.467	10x*	*C. stricta* subsp. *stricta*
Lovětín (CZ)	Czech Republic	49° 12′ 19″ N 15° 03′ 19″ E	500 m	9 (+1 replicate)	8	0.237	0.162	−0.462	10x*	*C. stricta* subsp. *stricta*
Lidzbark (PL)	Poland	53° 17′ 56″ N 19° 52′ 16″ E	148 m	7 (+1 replicate)	3	0.216	0.148	−0.458	10x*	*C. stricta* subsp. *stricta*

*10x: higher polyploids, most likely decaploid

Neuchâtel n°230, 1928) and no occurrence of *C. stricta* was therefore known in Switzerland and in the Alps (Aeschimann et al. 2004; Info Flora 2023) before the discovery of a population of *C. lonana* (Eggenberg et al. 2023).

Sampling of *C. stricta* subsp. *stricta* in France and Germany (Quinger 1987; André and Ferrez 2003; Ferrez and Dehondt 2004; Guyonneau et al. 2007) proceeded in a similar way, although ten samples and one voucher were collected in the Drugeon Basin (Franche-Comté) in the Jura mountains and in Görbelmoos (Bavaria), and 24 samples were collected in Federsee (Baden-Württemberg). In all other European, subarctic and Arctic regions, plants were collected and sent by local specialists (between two and 15 plants per population).

Samples from the taxonomically closely related *C. arundinacea* and *C. epigejos* were collected in the Botanical Garden of the University of Fribourg and used as outgroups for genetic analyses (Online Resource 3).

In order to assess pollen and seed production in *C. lonana*, a total of 40 panicles was collected, with 10 panicles collected each time, repeated four times (in September 2021, October 2021, July 2022 and September 2022). These were cut and collected to perform binocular observations.

To investigate the abiotic conditions suitable for the growth of *C. lonana* at root level over a full year period, two TMS-4 dataloggers (Wild et al. 2019, TOMST® s.r.o., Czech Republic) were placed in the Lona marsh from August 2021 to August 2022. They were used to measure the soil temperature in a depth of 6 cm below ground surface every 15 min.

Ploidy level

For each sampled population, a few randomly-selected silica gel dried leaves were sent to Plant Cytometry Services (Didam, The Netherlands, www.plantcytometry.nl) to estimate genome size based on propidium iodide (PI) staining following Bourge et al. (2018) and accordingly estimate the ploidy level of samples. A total of six samples from the Swiss population of Lona and two to three individuals from each of remaining populations were analysed. For each sample, the 2C-value was estimated in picograms of DNA (pg) following one to three runs using external standards (i.e. *Ophiopogon planiscapus*, 2C = 11.90 pg; *Clivia miniata*, 2C = 35.77 pg; *Monstera deliciosa*, 2C = 8.90 pg; and *Allium schoenoprasum*, 2C = 15.03 pg).

The ploidy level of samples was inferred by comparing measured DNA content with values published for *Calamagrostis* taxa in Šmarda et al. (2019) and Zonneveld (2019). Literature reports a majority of tetraploid ($2n = 4x = 28$) *Calamagrostis* species in Europe and the Arctic with 2C-values ranging between 6.35 and 8.80 pg. A 2C-value of 6.94 pg has been estimated for *C. stricta* from Norway, and a value of 14.57 pg for the decaploid ($2n = 10x = 70$) *C. villosa* (Šmarda et al. 2019; Zonneveld 2019).

DNA extraction and ddRAD sequencing

DNA was extracted using the DNeasy® Plant Mini kit (QIAGEN, Venlo, The Netherlands), following the manufacturer's instructions. DNA concentration and quality were evaluated with the NanoDrop™ One/OneC Microvolume UV–Vis Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, U.S.A) and electrophoresis on a 1% agarose gel, followed by a more accurate DNA quantification using Qubit™ dsDNA Assay Kits (Thermo Fisher Scientific Inc., Waltham, U.S.A).

ddRAD-seq libraries were prepared following a protocol adapted from Peterson et al. (2012) as described in Grünig et al. (2021) for 132 high-quality DNA samples among which 12 samples were selected as technical replicates, yielding 144 samples corresponding to 15 populations (and two outgroup populations) scattered across Europe and Svalbard (Table 1, Online Resource 4). In brief, 150 ng of DNA per sample was digested with EcoR1 and Mse1 restriction enzymes (New England Biolabs, Ipswich, U.S.A) at 37 °C for 50 min. Digested DNA fragments were individually tagged by ligation to a unique combination of EcoRI adapters including one of 48 five base pairs barcode and a library specific MseI index that further included four degenerate bases to identify PCR duplicates and a biotin tag. Library pools were size-selected targeting fragments around 550 bp using a ratio of 0.6 to 1 AMPure XP beads (Agilent, Santa Clara, U.S.A.), and sequences with the biotin tag (i.e. properly ligated to the MseI adapter) were retrieved with Dynabeads M-270 Streptavidin (Invitrogen, Waltham, U.S.A.). Selected fragments were amplified with 12–15 PCR cycles before being purified using 1 × AMPure beads. Concentration and size distribution of final libraries were measured using the Qubit® 2.0 Fluorimeter and Bioanalyzer 2200 TapeStation System (Agilent Technologies, Santa Clara, U.S.A.). The three libraries were sequenced as 2 × 250 bp paired-end on the NovaSeq 6000 (500 cycles; Illumina inc., San Diego, U.S.A.) at the NGS platform of the University of Bern, Switzerland.

SNP calling

Raw reads were demultiplexed and checked for intact restriction associated DNA cut sites and barcodes using process radtags (Catchen et al. 2011; 2013). PCR clones were identified and removed with clone filter (Catchen et al. 2011; 2013). Trimmomatic (Bolger et al. 2014) was used to trim Illumina adapters and discard reads shorter than 100 bp and/or with a Phred quality score below 15 within a four base sliding windows.

A de novo reference catalogue of RAD tags was created following the dDocent pipeline (Puritz et al. 2014) using reads from the 48 samples from library 3 (Online Resource 4) that were found at least once in eight samples (Parameters: dDocent Cutoff1 = 1, dDocent Cutoff2 = 8, first clustering rate 80%, second clustering rate 80%). Then, reads from all 144 samples were mapped against the resulting catalogue using the mem algorithm from the Burrows-Wheeler Alignment tool (Li and Durbin 2009). SNPs were called with the Genome Analysis Toolkit v. 4.1.0.0 (McKenna et al. 2010) by local re-assembly of haplotypes, merging of single-sample GVCFs and joint genotyping, using successively the HaplotypeCaller, GenomicsDBImport and GenotypeGVCFs tools. The resulting vcf file was filtered to only keep biallelic SNPs fulfilling the GATK best practices quality filtering (“QD < 2.0”, “QUAL < 30.0”, “SOR > 3.0”, “FS > 60.0”, “MQ < 40.0”, “MQRankSum < -12.5”, “ReadPosRankSum < − 8.0”). Genotypes with a depth below 5 and above 50 were set as no-calls and only positions with a maximal proportion of 20% missing data were kept. The dataset was further pruned using PLINK2 (Purcell et al. 2007) based on linkage disequilibrium (using the parameters –indep-pairwise 1000 100 0.2) and only positions with a minor allele count (MAC) of at least 3 were kept. Accordingly, 19 samples with numerous missing data were discarded, reducing the dataset from 144 to 125 samples (including ten technical replicates). Loci with more than 5% missing data (i.e. in seven or more samples) were removed from the dataset, and loci prone to sequencing errors were further eliminated based on the 10 technical replicates, reducing the number of SNPs to 1157 hi-quality loci. The genotyping error rate was estimated following Bonin et al. (2004). Subsequent analyses were hence performed using 1157 biallelic loci among 115 individual samples.

Genetic structure analysis

Observed heterozygosity (Ho), within population heterozygosity (Hs), number of observed alleles, effective number of alleles (Eff_num) and degree of deviation in heterozygosity from the Hardy–Weinberg equilibrium (Gis) were estimated using Genodive (Meirmans 2020). Standard deviation was estimated by jackknifing over loci, and the upper and lower bounds of the 95% confidence interval were calculated by bootstrapping over loci. Both jackknifing and bootstrapping only take place when there are at least six loci in the dataset (Meirmans 2020).

Clonal diversity within population was estimated based on pairwise SNP differences between individuals to evaluate the number of mutations among genotypes under the infinite allele model, using Genodive (Meirmans 2020). The threshold value was selected objectively, adhering closely to the methodology proposed in Douhovnikoff and Dodd (2003). Nucleotide differences among biological replicates were also used to select the threshold distinguishing mutations from genotyping errors and identify samples to be considered as ramets of the same genet (Rogstad et al. 2002; Meirmans and Van Tienderen 2004, Online Resource 5). In this study, as regards to the genotyping error rate (Online Resource 5), the upper threshold to consider clones was set at 170. The Nei-corrected diversity index was used to check whether the sample shows a clonal population structure (Nei 1973; Meirmans and Van Tienderen 2004) and estimate clonal diversity indices as well as the number of different effective genotypes in each population.

The principal component analysis (PCA) was computed using Genodive (Meirmans 2020) and plotted using the ggplot2 package in R (Wickham 2016). Pairwise Fst between pairs of populations was tested with 999 permutations in Genodive and plotted as a heatmap following hierarchical clustering based on principal components in R (Garnier et al. 2021). Population genetic structure was further quantified using a hierarchical analysis of molecular variance (AMOVA; Excoffier and Slatkin 1995), using squared Euclidean distances and assuming an infinite allele model. Accordingly, differentiation among populations (Fst) was partitioned among different grouping schemes to highlight groups of populations resulting in lowest variation among populations within groups (Fsc) and the highest variation among groups (Fct). The two Icelandic populations (Stiflisdalsvatn and Möðrudalur), as well as one in Germany (Görbelmoos), had not enough individuals to calculate Fst-Pairwise values with other populations and were therefore excluded from corresponding analyses.

Isolation by distance

Isolation by distance was estimated by associating genetic and geographic distances between pairs of populations using a Mantel Test as implemented in the ade4 package in R (Mantel 1967; Dray and Dufour 2007). As proposed in Rousset (1997), genetic distances were linearized as [Fst/(1-Fst)], whereas geographic distances were log-transformed and significance was determined by 9999 Monte Carlo permutations (Metropolis and Ulam 1949). Data were plotted with ggplot2 in R (Wickham 2016).

Results

Genome size variation

Among the 16 sampled populations of the *C. stricta* species complex (Table 1, Fig. 1), only the one from Pas de Lona was identified as *C. lonana* (Online Resource 1), whereas seven populations from subarctic and arctic areas

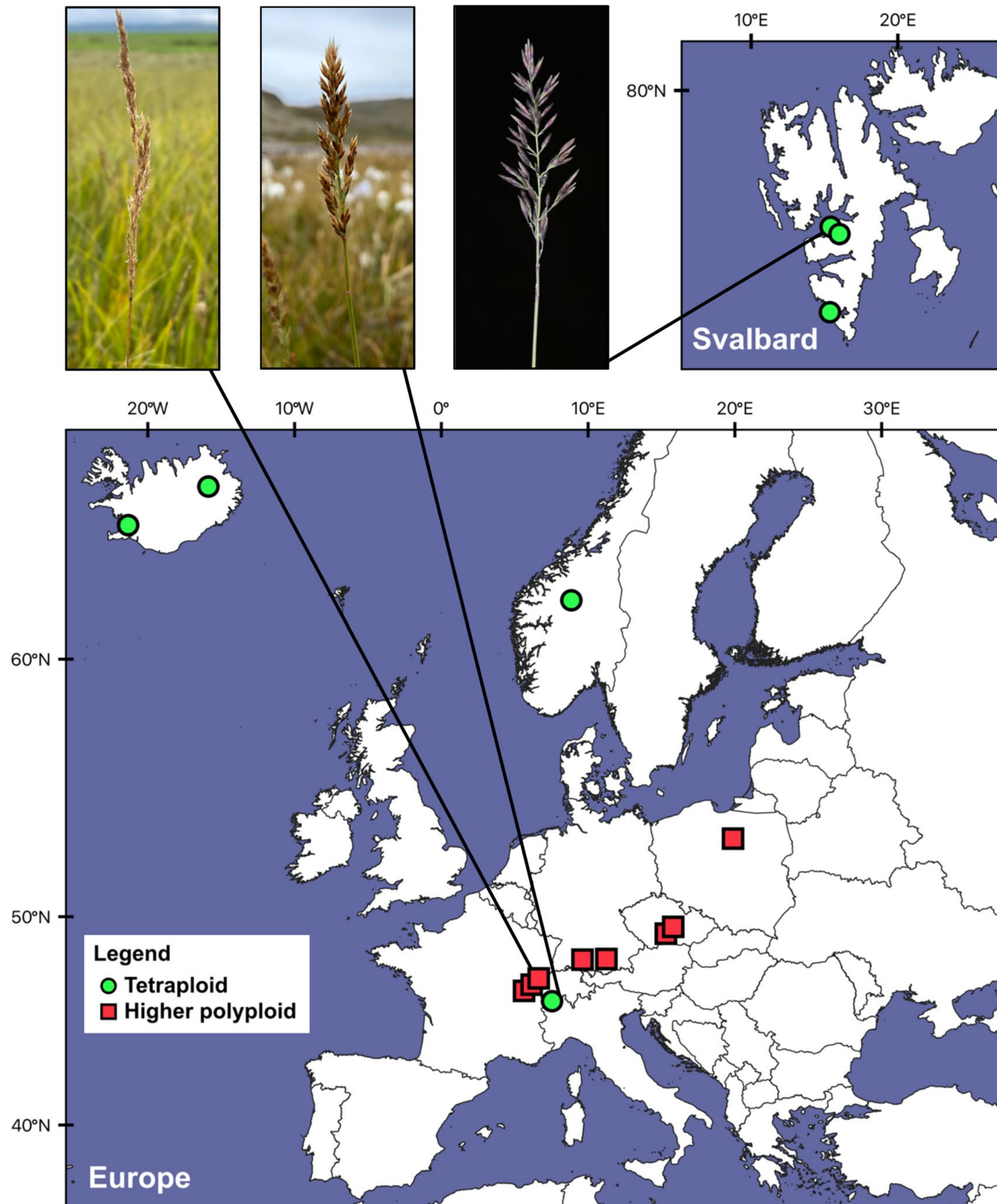

Fig. 1 Map of collection sites and the population ploidy levels (QGIS Development Team 2021)

were belonging to *C. stricta* subsp. *groenlandica* and eight populations from Central Europe were identified as *C. stricta* subsp. *stricta* (Online Resource 6). Estimates of genome size using flow cytometry unambiguously separated the samples of the *C. stricta* complex into two groups (Fig. 1, Table 1). Samples from Switzerland, Norway, Iceland and Svalbard showed 2C-values ranging between 7.2 and 8.9 pg of DNA, matching published estimates for tetraploid *Calamagrostis* taxa (2n = 4x = 28). In more detail, the within-species 2C-values variation in *C. lonana* ranged from 7.8 to 8.3 pg, whereas 2C-values of arctic and subarctic samples of *C. stricta* subsp. *groenlandica* varied between 7.2 and 8.9 pg. Contrastingly, samples of *C. stricta* subsp. *stricta* from France, Germany, the Czech Republic and Poland showed higher 2C-values, ranging from 13.6 to 16.6 pg and rather matched decaploid taxa of *Calamagrostis* such as *C. villosa* (2n = ca. 70; Online Resource 7).

Populations genetic structure

Average indices of total genetic diversity for the *C. stricta* species complex are found in Online Resource 8. Total heterozygosity (Ht), the expected frequency of heterozygotes over all populations assuming Hardy–Weinberg equilibrium, was estimated to be 0.165. Value for the heterozygosity within populations (Hs) was 0.160. The observed heterozygosity (Ho) had a value of 0.234. Inbreeding coefficient (Gis) was exceptionally low with a value of -0.469. Hs, Ho and Gis were also calculated separately for each population (Table 1). These population specific values were all very close to the averages obtained for the whole *Calamagrostis* complex. Ho varied between 0.202 and 0.249, Hs varied between 0.138 and 0.172 and Gis varied between −0.521 and −0.449.

Clonal diversity revealed multiple multilocus genotypes in most populations that presented both clonal and non-clonal samples (Table 1). In *C. lonana*, up to ten effective genotypes (i.e. different genets) were identified among the 14 samples analysed and this newly discovered taxon hence revealed high diversity. In contrast, both populations from Longyearbyen had much lower clonal diversity with less than 20% of samples being different genotypes (Online Resource 9).

Axes one and two of the PCA among genotypes of the 115 samples explained 4.02% and 3.22% of the total genetic variance among the five *Calamagrostis* taxa under scrutiny (Fig. 2). As expected, samples from two outgroup species (*C. epigejos* and *C. arundinacea*) appeared well differentiated from samples of the *C. stricta* complex that was highlighted as belonging to three main genetic clusters. All samples from the higher polyploid *C. stricta* subsp. *stricta* clustered together and appeared differentiated from others. Samples from the Lona population (i.e. *C. lonana*) clustered with samples from the four northern *C. stricta* subsp. *groenlandica* populations from Finndalen (NO), Hornsund (SJ), Stiflisdalsvatn (IS) and Möðrudalur (IS). Samples collected around Longyearbyen in Svalbard (i.e. populations Longyearbyen_1 and Longyearbyen_2) formed a third, distinct genetic cluster indicating that two differentiated lineages assigned to the taxon *C. stricta* subsp. *groenlandica* are co-occurring in the remote archipelago of Svalbard.

As could be expected for polyploid taxa, populations presented limited genetic differentiation. The heatmap based on pairwise Fst between populations highlighted the same three genetic groups as the PCA among individual samples, with the two populations of Longyearbyen being slightly but significantly differentiated from one another (Fst = 0.011; p-value = 0.019), and considerably more differentiated from all other populations (Fst from 0.052 to 0.068). The second group included samples from Lona (CH), Finndalen (NO) and Hornsund (SJ) that were only slightly differentiated with significant Fst ranging from 0.020 to 0.021). The third group included all populations assigned to *C. stricta* subsp. *stricta* with a higher ploidy level and that were significantly differentiated with Fst between 0.017 and 0.034. (Fig. 3; Online Resource 10 and 11).

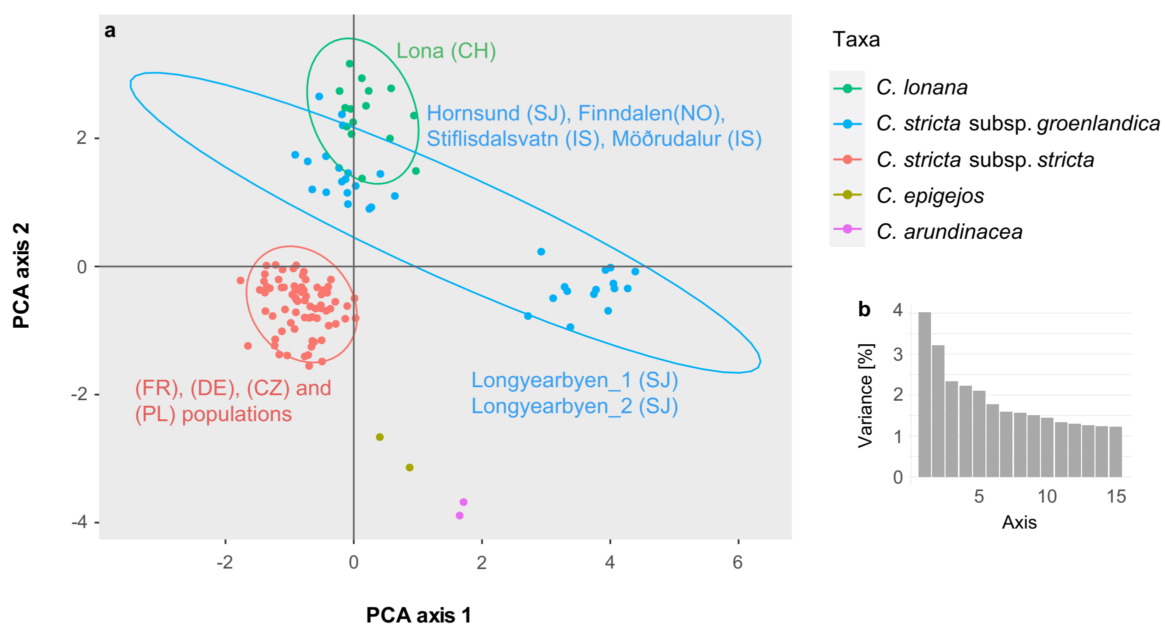

Fig. 2 **a** Principal component analysis (PCA) plotted with axis 1 and 2, taxonomic separation in colours. **b** Percentage of PCA explained variance plotted for axis 1–15. The list of populations is available in the Table 1

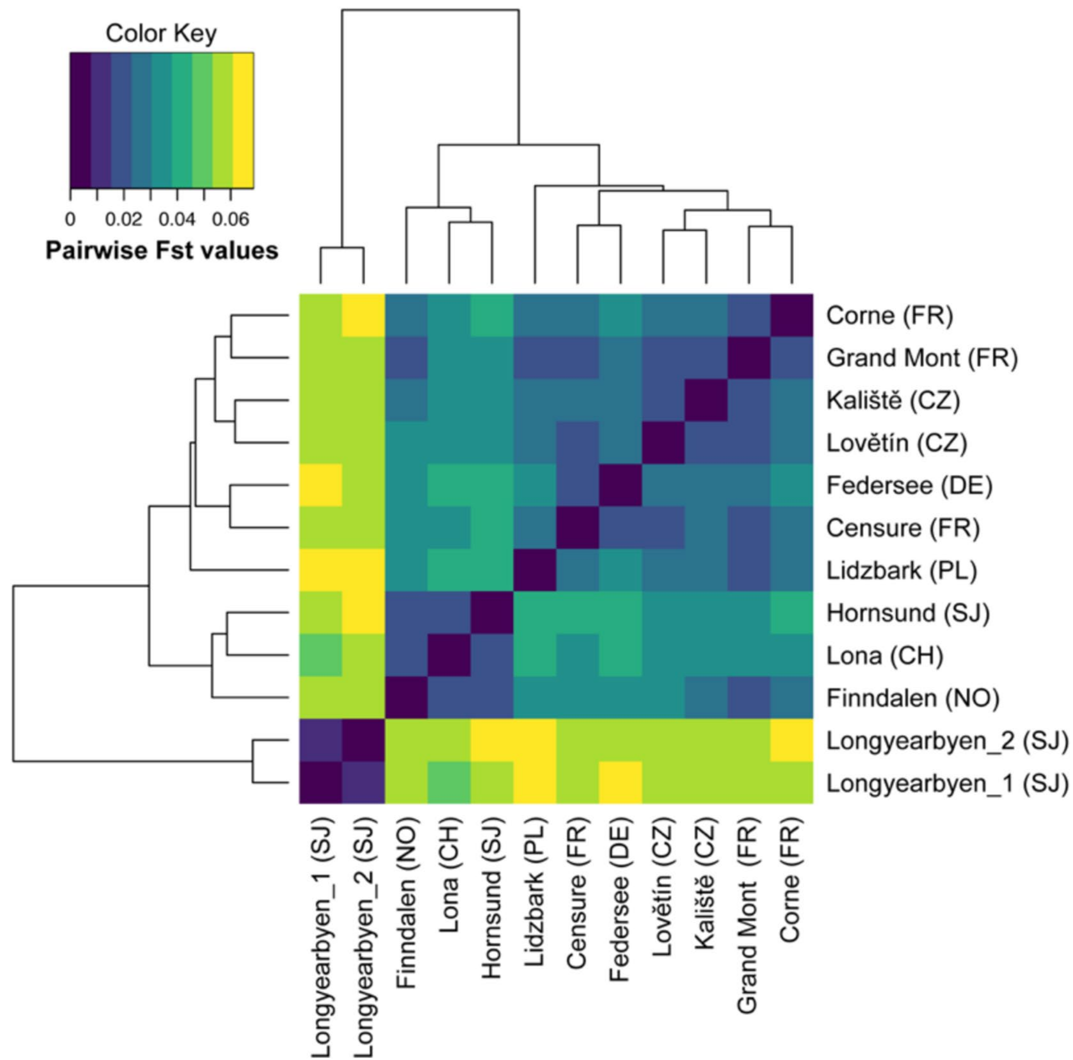

Fig. 3 Heatmap generated with Fst values between pairs of *Calamagrostis* populations. The list of populations is available in Table 1

Analysis of molecular variance (AMOVA) among all populations supported a significant genetic structure (Fst = 0.036, $p < 0.001$; Table 2). Following the grouping of populations in multiple combinations (Online Resource 12), partitioning of the genetic variance between a group made of only the two populations Longyearbyen_1 (SJ) and Longyearbyen_2 (SJ) *versus* a group made of all other populations of the *C. stricta* complex showed lowest variation among populations within groups (Fsc = 0.028, *p*-value = 0.001) and highest variation among groups (Fct = 0.032, *p*-value < 0.001; Table 3; Online Resource 13).

Genetic evidence thus convergently pointed to samples of the *C. stricta* species complex being partitioned into a Longyearbyen cluster (both populations near Longyearbyen in Svalbard), and the European cluster, which can be divided further into an arctic-alpine cluster (*C. lonana* and *C. stricta*

Table 2 AMOVA across all populations with significance tested using 999 permutations

Source of variation	Nested in	%var	F-stat	*F*-value	Std.Dev.[a]	c.i.2.5%[b]	c.i.97.5%[b]	*p*-value
Within individual	Total	1.413	F_{it}	−0.413	0.015	−0.442	−0.383	–
Among individual	Population	−0.448	F_{is}	−0.465	0.014	−0.492	−0.438	1.000
Among population	Total	0.036	F_{st}	0.036	0.003	0.031	0.042	0.001

[a]Standard deviations of F-statistics obtained through jackknifing over loci

[b]95% confidence intervals of F-statistics obtained through bootstrapping over loci

Table 3 Hierarchical AMOVA with population grouped as Longyearbyen populations versus all other populations, with significance tested using 999 permutations

Source of variation	Nested in	%var	F-stat	*F*-value	Std.Dev. [a]	c.i.2.5%[b]	c.i.97.5% [b]	*p*-value
Within individual	–	1.378	F_it	−0.378	0.016	−0.408	−0.345	–
Among individual	Population	−0.437	F_is	−0.465	0.014	−0.492	−0.438	1.000
Among population	Groups	0.027	F_sc	0.028	0.003	0.023	0.035	0.001
Among groups	–	0.032	F_ct	0.032	0.004	0.025	0.040	0.001

[a]Standard deviations of F-statistics obtained through jackknifing over loci

[b]95% confidence intervals of F-statistics obtained through bootstrapping over loci

subsp. *groenlandica* from Norway, Iceland and Hornsund in Svalbard) and a continental, higher polyploid cluster *cluster* (all higher polyploids of *C. stricta* subsp. *stricta* from France, Germany, the Czech Republic and Poland).

Isolation by distance

Isolation by distance tested among populations of the three taxa *C. lonana*, *C. stricta* subsp. *stricta* and *C. stricta* subsp. *groenlandica* together was significant ($r = 0.642$; p-value < 0.001). Although the ln of distances between pairs of populations and linearized genetic distances [Fst/(1-Fst)] were strongly associated (Online Resource 14), clear exceptions were noticeable. In particular, Lona (CH) was genetically close to the northern populations of Hornsund (SJ) and Finndalen (NO) despite long geographical distances, whereas Hornsund (SJ) was geographically close but genetically differentiated from Longyearbyen_1 (SJ) and Longyearbyen_2 (SJ).

Environment in the Alps

Dataloggers provided results showing that the soil (6 cm below ground) in the Lona marsh is frozen from November to the end of May and beginning of June (Online Resource 15). Thus, Lona's environmental conditions seem to be very similar to those in the Arctic.

Discussion

This first assessment of the distribution of genome-wide variation across the range of the *C. stricta* species complex provides insights on its evolutionary history, including the putative origin of the recently described *C. lonana* (Eggenberg et al. 2023).

Genome size estimates revealed a clear differentiation into two ploidy groups within the species complex, with arctic and subarctic populations being tetraploid (i.e. *C. stricta* subsp. *groenlandica*), whereas populations from lower latitudes across Central Europe were of higher ploidy (likely decaploids; i.e. *C. stricta* subsp. *stricta*). As an exception to this general pattern, the *C. lonana* population is tetraploid and closely related to arctic populations (i.e. *C. stricta* subsp. *groenlandica*) despite its allopatric distribution range at low latitude but high elevation in the Alps. Such disjunct distribution is coherent with an ancestral tetraploid stock having persisted to current times in extremely cold and wet habitats still existing in the Arctic and the Alps (Brochmann et al. 2004). Although the origin of interspersed populations of higher ploidy levels is beyond the scope of this study, this taxon has likely expended across Central Europe after the LGM as was demonstrated for several polyploid complexes (e.g. Huynh et al. 2020). Accordingly, with a distribution of populations of lower ploidy level in the vicinity of glacial refugia and derived polyploids of higher ploidy level presenting a widespread distribution, *C. stricta* matches with predictions of climate-driven range shifts typical of artic-alpine polyploid species complexes (Brochmann et al. 2004).

Populations of ancestral tetraploids currently found at high latitude in Finndalen (Norway), Stiflisdalsvatn and Möðrudalur (Iceland) form a clear genetic cluster that reached the Svalbard archipelago (e.g. Hornsund). Following Alsos et al. (2003) and assuming Svalbard as inhospitable for survival during the LGM, this indicates that this tetraploid taxon has achieved long-distance dispersal. Furthermore, consistently supporting the Longyearbyen populations as a differentiated genetic cluster, the genetic structure here highlighted is consistent with this archipelago having been repeatedly colonized from different sources as was shown for several other species (Alsos et al. 2003). Although additional sampling would here be required to identify the circumpolar sources of *C. stricta* in Svalbard, its genetic structure is consistent with considerable climate-driven range shift since the LGM.

On top of the shared ploidy level, the genetic structure highlighted by both model-based and multivariate analyses supported *C. lonana* as closely related to the arctic populations, and especially to populations from Hornsund in Svalbard (i.e. *C. stricta* subsp. *groenlandica*). Those disjunct periglacial environments characterized by non-frozen soil for < 4 months are known to have similarities in flora despite large geographical distances (Billings 1973). Two different scenarios could accordingly explain the origin of *C. lonana* in the Alps. On the one hand, *C. lonana* may be considered a glacial relict having spread in or around the Alps before

the last ice age and having survived in situ, as supported for other cold-adapted plant species such as *Saxifraga oppositifolia* (Abbott and Brochmann 2003; Stehlik 2003). The ecological niche of *C. lonana* being restricted to marshes, isolated populations appear more likely to have survived the LGM in refugia north of the Alps as supported for numerous species (Kadereit 2024), with a possibly widespread population having later contracted and fragmented into a disjunct arctic-alpine distribution in the face of post-glacial warming (Bétrisey et al. 2020). Supporting this hypothesis, *C. lonana* is not the only rare arctic-alpine relict species growing in the region of Pas de Lona (e.g. *Potentilla nivea, Carex bicolor*, *Carex microglochin*; Eggenberg et al. 2023). Such scenario would support the specific status of this newly discovered taxon in the Alps. On the other hand, *C. lonana* would be considered as the result of a more recent colonization event out of large populations in the Arctic. Despite complexities inherent to the characterization of such highly stochastic processes (Nathan 2006), the occurrence of some rare arctic-alpine plants in the Alps has been discussed as the result of long-distance dispersal with source located in Scandinavia and Siberia (e.g. Schönswetter et al. 2006). Bird migration may be among the most likely vectors (Viana et al. 2016). That *C. lonana* is genetically related to populations from Svalbard more than southern populations may support this hypothesis and would also call its taxonomic status as a species rather than a subspecies into question.

Limiting conclusions to be reached, seed production has never been observed in these taxa, neither in Lona nor in Svalbard (Eggenberg et al. 2023; Flora of Svalbard 2023), casting doubts on the interpretation of generation time to the most common ancestor. Mimicking the situation in Svalbard, where plants rarely reach full anthesis and where seed production is absent (Flora of Svalbard 2023), not a single plant was presenting either well-developed pollen or seed in the small and isolated population of *C. lonana*. Nevertheless, in contrast to the assumption of strictly clonal populations, clonality tests here revealed multiple different genotypes coherent with sexual reproduction among taxa of the *C. stricta* complex here considered. Such indication that these polyploid populations may have gone through only few sexual generations in their recent history likely explains observed deviation from Hardy–Weinberg equilibrium (Toeckel et al. 2006) and, more importantly, seems to indicate early isolation of *C. stricta* subsp. *groenlandica* and *C. lonana* as would be expected for a glacial relict more than a recent event of long-distance dispersal.

As a newly described taxon based on its morphological differentiation from other taxa of *Calamagrostis* (Eggenberg et al. 2023), *C. lonana* is currently known from only the site of Lona to date. Raising concerns regarding its conservation status in the Alps, it unfortunately also limits quantitative insights on its taxonomic status within the *C. stricta* species complex. Being morphologically distinct (Eggenberg et al. 2023) and genetically weakly differentiated (this study), the described *C. lonana* highlights a valuable unit for the biodiversity of the Alps that is in patent allopatry with closely related taxa. Provided a mixed-ploid species complex, taxa nested within *C. stricta* deserve a specific status to promote further revision based on a comprehensive sampling of the species complex (Doyle and Sherman-Broyles 2017). However, provided the lack of reported sexual reproduction, conclusions on the extent to which it represents a biological species will remain debatable (Coyne and Orr 2004). Finding new populations across similar high-altitude communities in the Alps (e.g. *Caricion bicolori-atrofuscae*, *Cratoneurion*, *Caricion fuscae*, *Caricion davallianae* and *Caricion loasiocarpae*; Eggenberg et al. 2023) shall accordingly be a priority. That said, with its locally high density over relatively large surface occupied by multiple genotypes, *C. lonana* does not appear naturally threatened in the short term.

Information on Electronic Supplementary Material

Online Resource 1.: Lona marsh satellite imagery in map.geo.admin. Red dots represent sampling location of *C. lonana*.

Online Resource 2.: Sample labels.

Online Resource 3.: Outgroup sample labels.

Online Resource 4.: Sample labels and their position on the plate during libraries preparation.

Online Resource 5.: Genotyping errors between original samples and their replicate among the 1157 remaining while discarding missing data.

Online Resource 6.: Elevation above sea level of the sampled populations.

Online Resource 7.: Flow cytometry data.

Online Resource 8.: Indices of total genetic diversity for the *C. stricta* species complex calculated with 121 individuals and 1157 loci (Nei 1987).

Online Resource 9.: Clonal diversity indices values per population.

Online Resource 10.: Fst values between pairs of populations and their p-values.

Online Resource 11: F'st (standardized) Heatmap – A standardized measure of population differentiation, estimated using an AMOVA (Meirmans 2006).

Online Resource 12.: Different grouping schemes tested to highlight groups of populations resulting in lowest variation among populations within groups (Fsc) and the highest variation among groups (Fct).

Online Resource 13.: AMOVA for both different subgroups of the "*Longyearbyen cluster*" and the "*European cluster*" revealed with the heatmap and PCA.

Online Resource 14.: Mantel test of the relationship between pairs of population genetic distance Fst/(1-Fst) and their geographic distance LN km.

Online Resource 15.: Soil temperature (-6 cm below ground) in the Lona marsh (August 2021 to August 2022).

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Data availability Raw sequencing reads are available under ENA accession PRJEB75425 (https://www.ebi.ac.uk/ena/browser/view/PRJEB75425)

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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