



Genetic structure analyses reveal multiple origins of *Elymus sensu stricto* (Poaceae)

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Abstract *Elymus sensu stricto* includes tetraploid species in the tribe Triticeae with a StH genome combination derived from *Hordeum* (H) and *Pseudoroegneria* (St). The group is related to many important cereals and forage grasses and is considered an important wild genetic resource for crop breeding. The relationships within *Elymus s.s.* have been difficult to resolve due to the large number of widely distributed species, a high degree of morphological diversity, introgression between species, and potential multiple origins. Using genome-wide DArTseqLD data from 57 taxa, including the StH group and associated species, it is possible to gain representative information about the genetic structure of the genus. SplitsTree, STRU CTURE and principal component analysis (PCoA) show that *Elymus s.s.* is divided into two major clades highly correlating with geographical origin separating American species from Eurasians. These findings, together with the contribution of different *Pseudoroegneria* and *Hordeum* species to the two clades, support the conclusion that *Elymus s.s.* has at least two independent origins. The Eurasian and American

clades are further divided into three and two sub-clades, respectively. The results show that *Elymus* species have migrated multiple times from North to South America and between America and Eurasia. The new findings could be used to develop a new classification of *Elymus s.s.* with sections based on phylogenetic data.

Keywords *Hordeum* · Next-generation sequencing · Polyploidy · *Pseudoroegneria* · StH · Triticeae

Introduction

Elymus L. (wheatgrasses) are perennial grasses in the tribe Triticeae (Poaceae) related to some of the most important agricultural cereal crops, such as wheat, barley, and rye. Several *Elymus* species are used as forage crops today, and the genus has an ethnobotanical record as food, medicine, and constructing material (Frawley et al. 2020). The morphologically and physiologically diverse genus makes it interesting as a wild genetic resource in plant breeding. A clear understanding of the intergeneric structure and relationships is required for efficient and precise utilization and conservation. Research on *Elymus* diversity and evolution has evolved together with the development of new techniques and have now entered the field of genomics.

In most contemporary research, generic delimitations of Triticeae are based on genomic combinations

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where species with the same haplome or combination of haplomes are considered to belong to the same genus (Dewey 1984; Löve 1984; Baum et al. 2015). However, *Elymus sensu lato* (*s.l.*) has traditionally been an exception from this convention and includes allopolyploid species that originate from multiple intergeneric allopolyploidization events (Fig. 1). *Elymus s.l.* comprehends five different basic haplomes; St (*Pseudoroegneria* (Nevski) Á.Löve), H (*Hordeum* L.), P (*Agropyron* Gaertn.), W (*Australopyrum* (Tzvelev) Á.Löve), and Y (unknown origin) (Dewey 1984; Lu et al. 1993; Wang et al. 1994; Svitashv et al. 1996; Sun et al. 1997; Liu et al. 2006; Sun et al. 2008; Mason-Gamer et al. 2010a; Petersen et al. 2011). Genome symbols are based on Wang et al. (1994). The St-genome is the only common haplome represented in all *Elymus s.l.* species. The tetraploids have either a StH, StY or StP combination, while the hexaploid and octaploid species include StHH, StStHH, StStYY, StHY, StStY, StPY, StHW, and StWY (Lu 1993; Diaz 1999). The genome assignment to species is derived from cytological research but has been confirmed by genetic methods (Helfgott and Mason-Gamer 2004; Dong et al. 2015). The polyphyletic *Elymus s.l.* is often divided into a more consistent classification splitting the genome combinations into separate genera such as *Roegneria* K. Koch (StY), *Kengyilia* C. Yen & J. L. Yang (StPY), *Campeioestachys* Drobov (StHY), *Anthosachne* Steudel (StYW), *Stenostachys* Turcz. (StHW), and *Elymus sensu stricto* (*s.s.*) (StH) (Yen and Yang 2022). However, several groups are considered as sections rather than having generic status (Salomon and Lu 1992; Barkworth and von Bothmer 2009). The genus *Pseudoroegneria* includes 15–20 species, all

distributed in central and eastern Asia except one, *P. spicata*, which is only found in North America (Dewey 1984; Carlson 1993). Genetic research has shown that *Pseudoroegneria* is the maternal St genome donor of both StStHH and StStYY (Mason-Gamer et al. 2002; McMillan and Sun 2004; Liu et al. 2006; Hodge et al. 2010; Dong et al. 2015). No *Pseudoroegneria* species occurs in South America and hence all *Elymus* species on the continent should originate from North America. Previous research found that gene transfer between North and South American *Elymus* is possible when grown together (Hunziker 1955, 1967; Dewey 1972, 1977; Jensen 1993).

Elymus s.s. (StH) is probably also a polyphyletic group with several involved progenitor species, but our understanding of polyploid evolution in the genus is still limited. Multiple origins have been suggested within single StH species. For example, *Elymus caninus* shows different versions of the *RPB2* gene in both the St and the H genome (Yan and Sun 2012), and polyphyletic *Hordeum*-like sequences have been found in the *Pepc* tree of *E. trachycaulus* (Zuo et al. 2015). Earlier research also suggested multiple origins involving several *Pseudoroegneria* species (Liu et al. 2006, Sun et al. 2008, Fan et al. 2013b, Gao et al. 2015, Lei et al. 2020) and the differentiation of polyploid species with the St genome into three groups based on rDNA ITS sequences (Zhang et al. 2009).

Reticulate evolution through frequent interspecific hybridizations and introgressions is common within *Elymus* genome combinations altering species and population structures and contributing to the difficulties of solving the evolutionary history of the

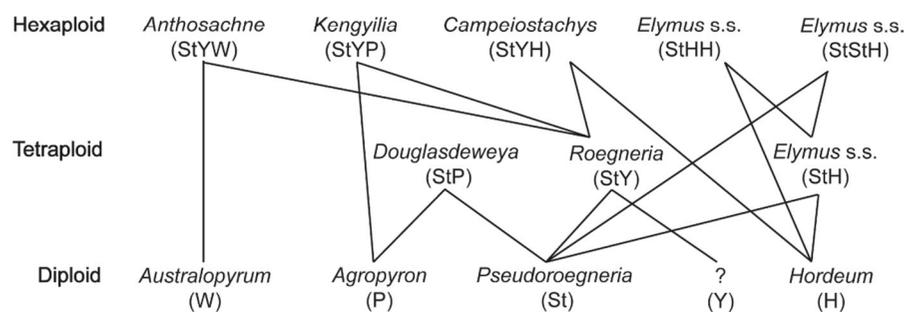


Fig. 1 Generic delimitation and relationships between tetraploid and hexaploid *Elymus s.l.* based on genome combinations proposed by Fan et al. (2013a), Yen et al. (2005), and Yen and

Yang (2009). Lines indicate allopolyploid origins from diploid and tetraploid progenitors. Figure modified from Leo (2022)

genus (Sun 2014; Baum et al. 2015; Wu et al. 2016). Moreover, these factors make it challenging to resolve the genetic relationships even within smaller species groups of *Elymus*, although attempts have been made (Helfgott and Mason-Gamer 2004, McMillan and Sun 2004, Mason-Gamer et al. 2005, 2010b, Liu et al. 2006, Sun et al. 2007, 2008, Mason-Gamer 2007, 2013, Petersen et al. 2011, Wang et al. 2012, Sun 2014, Baum et al. 2015, Gao et al. 2015). However, most research shows poorly resolved gene trees and incomplete sampling, which results in poorly resolved bifurcating trees.

Elymus s.s. (StH) occurs mainly in temperate areas in the northern and southern hemispheres from sea level up to 5 000 m in altitude (Dewey 1984; Löve 1984). The StH genome group contains approximately 50 species and is diverse in habitat preference and morphology (Sun and Salomon 2009). The genus is divided into mainly a boreal and temperate Eurasia-North American disjunction and a South American-North American disjunction (Thorne 1972). The relationships between these two disjunctions are not fully known. The main species diversity occurs in the northern hemisphere, but a high diversity can also be found in southern South America, especially in Chile and Argentina (Seberg and Petersen 1998, Leo et al. unpublished). Two species are circumpolar, *E. alaskanus* and *E. macrourus*, occurring in both North America and Eurasia. Also, *E. sibiricus* and *E. trachycaulus* are often considered circumpolar (Baum et al. 2016), though *E. sibiricus* in North America is probably recently introduced (Bennett 2006), and the main distribution of *E. trachycaulus* is in North America with only *E. trachycaulus* ssp. *novae-angliae* occurring in eastern Russia (Peschkova 1990).

Most research of the genetic relationships in *Elymus* include a small set of loci. This approach is limited by the risk of insufficient information and confusing results, especially in recently diverged lineages where incomplete lineage sorting and/or ongoing hybridization cause conflicting gene-trees (Choi et al. 2019). DArTseqLD™ is a high-throughput genotyping-by-sequencing platform using restriction enzymes to reduce genome complexity and sequencing of the restriction fragments to gain a large number of SNPs (Single Nuclear Polymorphisms) covering the whole genome (Jaccoud et al. 2001; Sansaloni et al. 2011). The technique has become popular for investigating diversity and phylogeny because of its cost efficiency,

the high gain of informative and genome-wide molecular markers, and publicly available bioinformatics pipelines without the need of a reference genome.

The objectives of the present study were to investigate the genetic structure, geographic patterns of diversity, and the phylogeny within *Elymus s.s.* (StH). A clear genetic structure of the genus is important for the future establishment of a more robust circumscription and classification of the genus as well as a framework for further interspecific and population research. Additional objectives were to investigate the potential differentiation in parental origin within the StH combination and the relationships between the two disjunctions (North America-South America and America-Eurasia).

Material and methods

Plant material

This study included 282 individuals from 96 accessions (2–3 individuals per accession) representing 57 taxa from North America, South America, and Eurasia (Table 1). Most accessions are tetraploid *Elymus s.s.* (StH), but several associated species were included: 1) Three hexaploid *Elymus s.s.* (StHH) species and one accession of *Elymus repens* (StStH?) for origin analyses of hexaploids; 2. Six species with the StY genomes (referred to the genus *Roegneria*) for differentiation comparisons; 3. Three *Pseudoroegneria* species (St) and four *Hordeum* (H) species representing parental genomes; 4. Three *Eremium erianthum* (N?) accessions, and one accession each of *Psathyrostachys juncea* (N) and *Brachypodium sylvaticum* from the tribe Brachypodieae used as more distantly related outgroups. The tetraploid StH *Elymus* accessions were selected to maximize the known taxonomical diversity and geographical distribution. The group constitutes several species with wide and narrow distribution ranges, but widespread species are more predominant in the current study. Seed samples were collected from the field or obtained from seed genebank material originally from wild native stands. The genebank material was sown in a greenhouse (20°C, no external light source) at the Swedish University of Agricultural Sciences (SLU). Leaf samples were taken at the seedling and young tillering stage from plants grown in the greenhouse and in the field, respectively, and immediately

Table 1 Analyzed species of *Brachypodium*, *Elymus s.l.*, *Eremium*, *Hordeum*, *Psathyrostachys*, and *Pseudoroegneria*, with accession number (Acc. No), genome, author citation, section affiliation according to Löve (1984) and Yen and Yang (2022), ploidy level, and origin with country and region

Species	Author	Genome	Sections		2n	Acc. no	Origin
			Löve	Yen & Yang			
<i>Brachypodium sylvaticum</i>	P. Beauv				2x	H10811	BUL: Burgas
<i>Hordeum</i>	(Covas & Stebbins)	H			2x	H2408	USA: San Diego co
<i>brachyantherum ssp. californicum</i>	Bothmer, N.Jacobsen & Seberg						
<i>Hordeum comosum</i>	J.Presl	H			2x	H10608	ARG: Bariloche
<i>Hordeum pubiflorum</i>	Hook.f. in Hook	H			2x	H3596	ARG: Jujuy
<i>Hordeum roshevitzii</i>	Bowden	H			2x	H10070	RUS: Altai
<i>Psathyrostachys juncea</i>	(Fisch.) Nevski	Ns			2x, 4x	H10108	RUS: Altai
<i>Eremium erianthum</i>	(Phil.) Seberg & Lindel-Laursen	Ns?			6x	H6428	ARG: Mendoza
					6x	H6509	ARG: Tierra del Fuego
					6x	H6510	CHL: Magallanes
<i>Pseudoroegneria ferganensis</i>	(Drobow) B.Salomon & Bothmer	St			2x	H10244	TJK: Gissar mtn
<i>Pseudoroegneria spicata</i>	(Pursh) Á.Löve	St			2x, 4x	H10481	CAN: Saskatchewan
<i>Pseudoroegneria strigosa</i>	(Drobow) Á.Löve	St			2x	H10147	RUS: Altai
<i>Elymus alaskanus</i>	(Scribn. & Merr.) Á.Löve	StH	G	Et	4x	H10574	NOR: Opdal
					4x	H3669	USA: Alaska
					4x	H10619	CAN: Cape Bathurst
					4x	H10357	ICE: Akureyri
					4x	H10656	RUS: Taymyr
<i>Elymus alopec</i>	B.Salomon	StH			4x	H10360	ICE: Akureyri
<i>Elymus angulatus</i>	J.Presl	StH	D	El	4x	CHL1817	CHL: Magallanes
					4x	CHL1914	CHL: Araucanía
					4x	H6507	CHL: Chiloe
<i>Elymus arizonicus</i>	(Scribn. & J.G.Sm.) Gould	StH	D	G	4x	H3670	USA: New Mexico
<i>Elymus bakeri</i>	(E.E.Nelson) Á.Löve	StH	G	G	4x	H10662	USA: Colorado
<i>Elymus canadensis</i>	L	StH	M	El	4x	H3994	USA: Idaho
<i>Elymus caninus</i>	(L.) L	StH	G	G	4x	H10372	SWE: Västerbotten
					4x	H10426	SWE: Skåne
					4x	H10570	NOR: Uvdal
					4x	H7550	CHN: Xinjiang
					4x	H10355a	SWE: Norrbotten
<i>Elymus caninus</i> var. <i>muticus</i>	(Holmb.) Karlsson	StH	G	G	4x	H3928	SWE: Värmland
<i>Elymus charkeviczii</i>	Prob	StH		G	4x	H10470	RUS: Kamchatka
<i>Elymus confusus</i>	(Roshev.) Tzvelev	StH	El	G	4x	H10460	RUS: Kamchatka
<i>Elymus cordilleranus</i>	Davidse & R.W.Pohl	StH		Et	4x	H6486	PER: Cajamarca
<i>Elymus dentatus</i>	(Hook.f.) Tzvelev	StH	G	G	4x	H4092	PAK: Hozara
<i>Elymus elymoides</i>	(Raf.) Swezey	StH	S	S	4x	H10661	USA: Montana
<i>Elymus fibrosus</i>	(Schrenk) Tzvelev	StH	G	G	4x	H10168	RUS: Altai
					4x	H10339	FIN: Kairala
					4x	H10831	NOR: Finnmark
<i>Elymus glaucescens</i>	Seberg	StH	D	G	4x	CHL1820	CHL: Magallanes
					4x	CHL1839	CHL: Magallanes
					4x	CHL1847	CHL: Magallanes
					4x	CHL1874	CHL: Magallanes

Table 1 (continued)

Species	Author	Genome	Sections		2n	Acc. no	Origin
			Löve	Yen & Yang			
<i>Elymus glaucus</i> ssp. <i>glaucus</i>	Buckley	StH	El	El	4x	H3996	USA: Utah
<i>Elymus jacutensis</i>	(Drobow) Tzvelev	StH	G	G	4x	H3547	RUS: Altai
<i>Elymus komarovii</i>	(Nevski) Tzvelev	StH	G	G	4x	H10394	RUS: Krasnojarsk
					4x	H10612	RUS: Altai
<i>Elymus lanceolatus</i>	(Scribn. & J.G.Sm.) Gould	StH	D	Et	4x	H10663	USA: Nevada
<i>Elymus macrourus</i>	(Turcz. ex Steud.) Tzvelev	StH	G	Et	4x	H10613	CAN: NWT, Mackenzie
					4x	H3549	RUS: Jacutia
<i>Elymus mutabilis</i>	(Drobow) Tzvelev	StH	G	G	4x	H10350a	SWE: Norrbotten
					4x	H10456	RUS: Kamchatka
					4x	H10842	SWE: Lappland
					4x	H3574	KAZ: Alma-Ata
					4x	H7552	CHN: Xinjiang
<i>Elymus parodii</i>	Seberg & G.Petersen	StH	D	El	4x	H6483	ARG: Córdoba
<i>Elymus sajanensis</i>	(Nevski) Tzvelev	StH	G	G	4x	H3933	RUS: Buryatia
<i>Elymus scribneri</i>	(Vasey) M.E.Jones	StH	G	G	4x	H10660	USA: Utah
<i>Elymus sibiricus</i>	L	StH	El	El	4x	H10036	RUS: Altai
					4x	H10238	TJK: Gissar Mtns
					4x	H10403	RUS: Krasnojarsk
					4x	H10438	RUS: Kamchatka
					4x	H7528	CHN: Xinjiang
					4x	H7182	CHN: Sichuan
<i>Elymus sierrae</i>	Gould	StH	G	G	4x	H3626	USA: NWT, Mackenzie
<i>Elymus trachycaulus</i>	(Link) Gould	StH	G	G	4x	H10150	RUS: Altai
					4x	H10485	CAN: BC, Lytton
					4x	H10496	CAN: BC, Bella Colla
					4x	H10497	CAN: BC, Williams lake
					4x	H10664	CAN: Alberta
					4x	H3995	USA: Utah
<i>Elymus transbaicalensis</i>	(Nevski) Tzvelev	StH	G	G	4x	H10054	RUS: Altai
					4x	H10127	RUS: Altai
					4x	H10378	RUS: Krasnojarsk
<i>Elymus vaillantianus</i>	(Wulfen ex Schreb.) K.B.Jensen	StH		Et	4x	H3651	MEX: Toluca
<i>Elymus villosus</i>	Muhl. ex Willd	StH		El	4x	H10505	CAN: BC, Cougar Valley
<i>Elymus violaceus</i>	(Hornem.) J.Feilberg	StH	G	G	4x	H10593	GRL: Julianehåb
					4x	H10514	CAN: Alberta
<i>Elymus virescens</i>	Piper	StH	G	El	4x	H10584	GRL: Julianehåb
<i>Elymus virginicus</i>	L	StH	M	El	4x	H10658	USA: Missouri
<i>Elymus kamezadolorum</i>	(Nevski) Tzvelev	StH	G	G	4x	H3903	RUS: Kamchatka
<i>Elymus patagonicus</i>	Speg	StHH	D	El	6x	H1188	ARG: Rio Negro
					6x	H6342	ARG: Neuquén
<i>Elymus scabriglumis</i>	(Hack.) Á.Löve	StHH	D	G	6x	CHL2002	CHL: Antofagasta
					6x	CHL2004	CHL: Antofagasta
					6x	H6455	ARG: Salta
					6x	H6466	ARG: Jujuy
					6x	H6469	ARG: Jujuy
<i>Elymus transhyrcanus</i>	(Nevski) Tzvelev	StStH	G	G	6x	H10270	TJK: Zeravshan Mtns
					6x	H3756	IRN: Tehran

Table 1 (continued)

Species	Author	Genome	Sections		2n	Acc. no	Origin
			Löve	Yen & Yang			
<i>Elymus repens</i>	(L.) Gould	StStH?		Et	6x	H10205	TJK: Gissar Mtns
<i>Elymus pendulinus</i>	(Nevski) Tzvelev	StY			4x	H8986	CHN: Tibet
<i>Elymus semicostatus</i>	(Nees ex Steud.) Melderis	StY			4x	H4006	PAK: Gilgit
<i>Elymus sikkimensis</i>	(Melderis) Á.Löve	StY?			4x	H10747	BHU: Thimphu
<i>Elymus strictus</i>	(Keng) Á.Löve	StY			4x	H8967	CHN: Sichuan
<i>Elymus tibeticus</i>	(Melderis) G.Singh	StY			4x	H10749	BHU: Timphu
<i>Elymus validus</i>	(Melderis) B.Salomon	StY			4x	H4078	PAK: Gilgit

Sections are: Macrolepis (M), Elymus (El), Elytrigia (Et), Goulardia (G), Sitanion (S), and Dasystachyae (D). Accessions included in Set 2 are indicated in bold font. The chromosome count on the particular accession is indicated with bold font

dried in silica gel (ca 1–3 mm aluminosilicate, Merck KGaA, Darmstadt, Germany). The samples were kept in silica gel until DNA extraction and genotyping. Plants grown in the greenhouse were kept until maturity for taxonomical verification.

Genotyping

The leaf samples were sent to Diversity Arrays Technology (DArT) Pty Ltd., Bruce, Australia, for DNA extraction and genotyping using DArTseqLD™ technology (Jaccoud et al. 2001; Sansaloni et al. 2011). The received data was filtered with the pipeline ‘dartR’ (Gruber et al. 2018) in R v. 4.1.1 (R core team 2021). The filtering setting affected the number of accessions in the analyses resulting in a loss of taxa. Therefore, two sets were created and referred to as Set 1 filtered to include all samples, and Set 2 including only tetraploid StH *Elymus* species. Both sets were filtered on: 1) reproducibility (a measure of the consistency of scoring technical replicates) with a threshold of $\geq 95\%$; 2) locus call rate with a threshold set to $\geq 50\%$; 3) random removal of all but one of the multiple SNPs in the same fragment to avoid the potential influence of linkage (only for the STRUCTURE analyses); 4) individual call rate with the threshold set to zero and $\geq 40\%$ for Set 1 and Set 2, respectively; 5) removal of monomorphic loci; and 6) removal of loci with a minor allele frequency (MAF) $\leq 2\%$. The gl2svdquartets function in ‘dartR’ was used to generate NEXUS files for the genetic structure and relationship analyses, with SNPs converted to haploid states and heterozygous loci coded using standard ambiguity

codes. Based on the results from Set 1 and 2, two additional filtering (Set 3 and 4) were conducted with the same setting as Set 2 representing the two major clades in *Elymus s.s.*. The raw read data is available through the NCBI Short Read Archive (SRA) under BioProject ID PRJNA942607.

PCoA and STRUCTURE analyses

To investigate the genetic structure of the genome combinations a Principal Component Analysis (PCoA) based on Nei’s unbiased genetic distance was carried out using the ade4 package in R (Dray et al. 2007), and visualized by the ggplot2 package (Villanueva and Chen 2019). The two data sets were analyzed using the software STRUCTURE v.2.3.4 (Pritchard et al. 2000) for genomic structure and to infer the number of the most likely groups within the StH genome combination. The method uses a Bayesian iterative algorithm to analyze differences in the distribution of genetic variants amongst populations by placing samples into groups whose members share similar patterns of variation. Each analysis was performed with 100 000 repetitions of the Markov Chain Monte Carlo (MCMC) with a burn-in of 50 000 interactions in 10 independent simulations, and without prior information to define the clusters. The number of clusters (K) was determined using the average likelihood values of the delta K method (Evanno et al. 2005) implemented in the program Structure Harvester (Earl and von Holdt 2012). Bar plots of the ancestry coefficients of each sample were created using ‘ggplot2’ in R (Wickham 2016; Villanueva and Chen 2019).

SplitsTree and coalescence-based (SVDquartets) analyses

To explore the complex relationships of *Elymus s.s.* and detect patterns of reticulation caused by hybridization and incomplete lineage sorting, an unrooted phylogenetic network analysis, including associated species, was performed for Set 1 and Set 2 using SplitsTree 4.0 (Huson and Bryant 2006). Default settings were used, implementing Neighbor-Net analysis with the variance of ordinary least squares, EqualAngle, Uncorrected P, and missing data treated as unknown. Bootstrapping was conducted with 10 000 replicates. Finally, two phylogenetic multispecies coalescent-based analyses with SVDquartets (Chifman and Kubatko 2014) in PAUP* v.4.0a169 (Swofford 2003) were performed to assess the relationships within the identified groups. *Elymus sibiricus* and *E. confuses* were used as an outgroup in the analysis of Eurasian group, and *E. lanceolatus* as an outgroup in the analysis of the American group. All quartets were evaluated, and branch support was assessed through 10 000 nonparametric bootstrap replicates. SVDquartets is computationally efficient when analyzing a large amount of SNP data under the MSC and can be applied without any loss of power to infer the true species tree (Wascher and Kubatko 2021).

Results

DArTseqLD™

The processed sequence dataset provided by DArT Pty Ltd. contained in total 282 genotypes with 20 790 codominant binary SNP markers and 48% missing data, which could be expected from next generation sequences analyses with a taxonomical diverse set of genotypes. For Set 1, the filtering removed 235 unreproducible loci ($\leq 95\%$), 8 624 loci below 50% call rate, and 5017 minor alleles ($\leq 2\%$), resulting in 6914 binary SNPs with 33% of missing data and all genotypes remained. Additionally, 3201 secondary loci were removed prior to the STRUCTURE analyses resulting in 3713 loci left in the analysis. Following initial exploratory analyses with SplitsTree and PCoA, one accession (CHL18-39 *E. glaucescens*) was identified as a hybrid accession and removed

from subsequent analyses. The filtering in Set 2, using a threshold for individual call rate at $\geq 40\%$, resulted in 187 remaining genotypes with in total 6305 binary SNPs and 27% missing data. After the removal of 147 secondaries only, 4888 unlinked loci were left for the STRUCTURE analysis. In Set 3, including species in the Eurasian group, filtering resulted in 104 genotypes and 3591 SNP markers (22% missing), and Set 4, including species in the American group, 83 genotypes and 5589 SNP markers (25% missing data) remained. It was assumed that the amount of missing data could be tolerated without losing accuracy due to the large number of informative genetic markers in the analyses (Philippe et al. 2004).

Phylogenetic analyses

Set 1 and Set 2 were used for network analysis in SplitsTree (Figs. 2 and 3). The results from Set 1 showed a separation between StY and StH species with a further division of the StH species into two major groups, also evident in the analysis of Set 2. The clustering of the StH accessions is highly correlated with geographical origin. One group included all the American species, except *E. kamiczadorum*, not separating between North and South America. The other group included the Eurasian species, as well as the circumpolar species *E. macrourus* and *E. alakanus*. Based on the assumption that the two groups found in the SplitsTree, PCoA and STRUCTURE analyses have independent origins, the SVDquartets analyses in PAUP* (Fig. 4) were performed independently for species in the American and Eurasian clade, respectively. The outgroups were chosen based on the SplitsTree phylogenetic network using Set 2.

In the SplitsTree analysis of Set 1, *Pseudoroegneria spicata* and the American *Hordeum* spp. were grouped together with the “American” *Elymus* StH species, while *P. strigosa* and *H. roshevitzii* grouped together with the Eurasian *Elymus* StH species. In contrast, *P. ferganensis* was intermediate and did not show clear affiliation to any group. The SplitsTree network placed *Eremium erianthum* together with *Psathyrostachys juncea*. *Elymus repens* appeared as an intermediate with a (partial) reticulate relationship between StY and StH genome combinations. The hexaploid *E. scabriglumis* appeared in a network associated with the American *Hordeum*

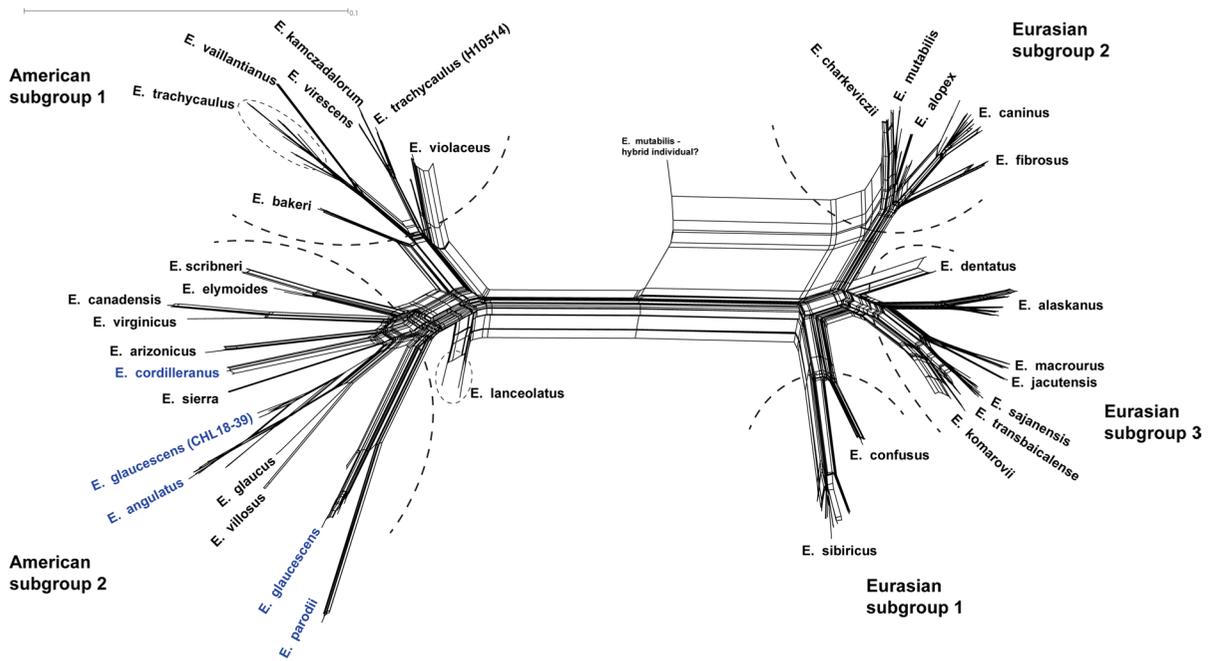


Fig. 2 SplitsTree phylogenetic network (Huson and Bryant 2006) reconstructed using Set 2, including tetraploid genotypes of StH *Elymus* species ($n = 187$) and 6 305 binary SNPs. Blue species names indicate South American origin

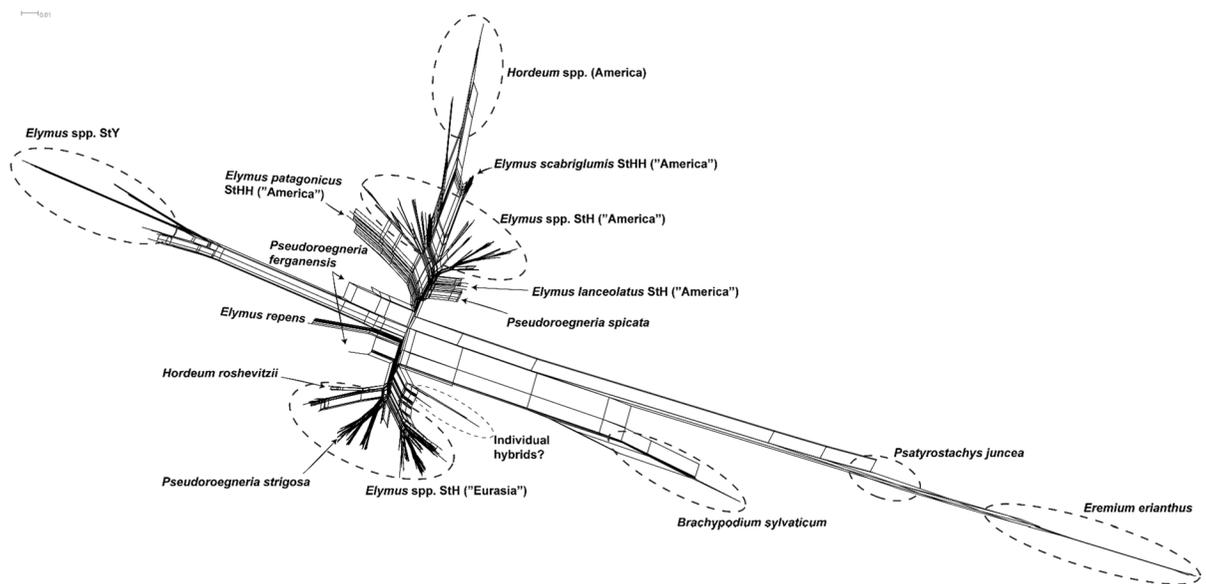


Fig. 3 SplitsTree phylogenetic network (Huson and Bryant 2006) reconstructed using Set 1, including all genotypes, both StH and associated species ($n = 282$) and 6 914 binary SNPs

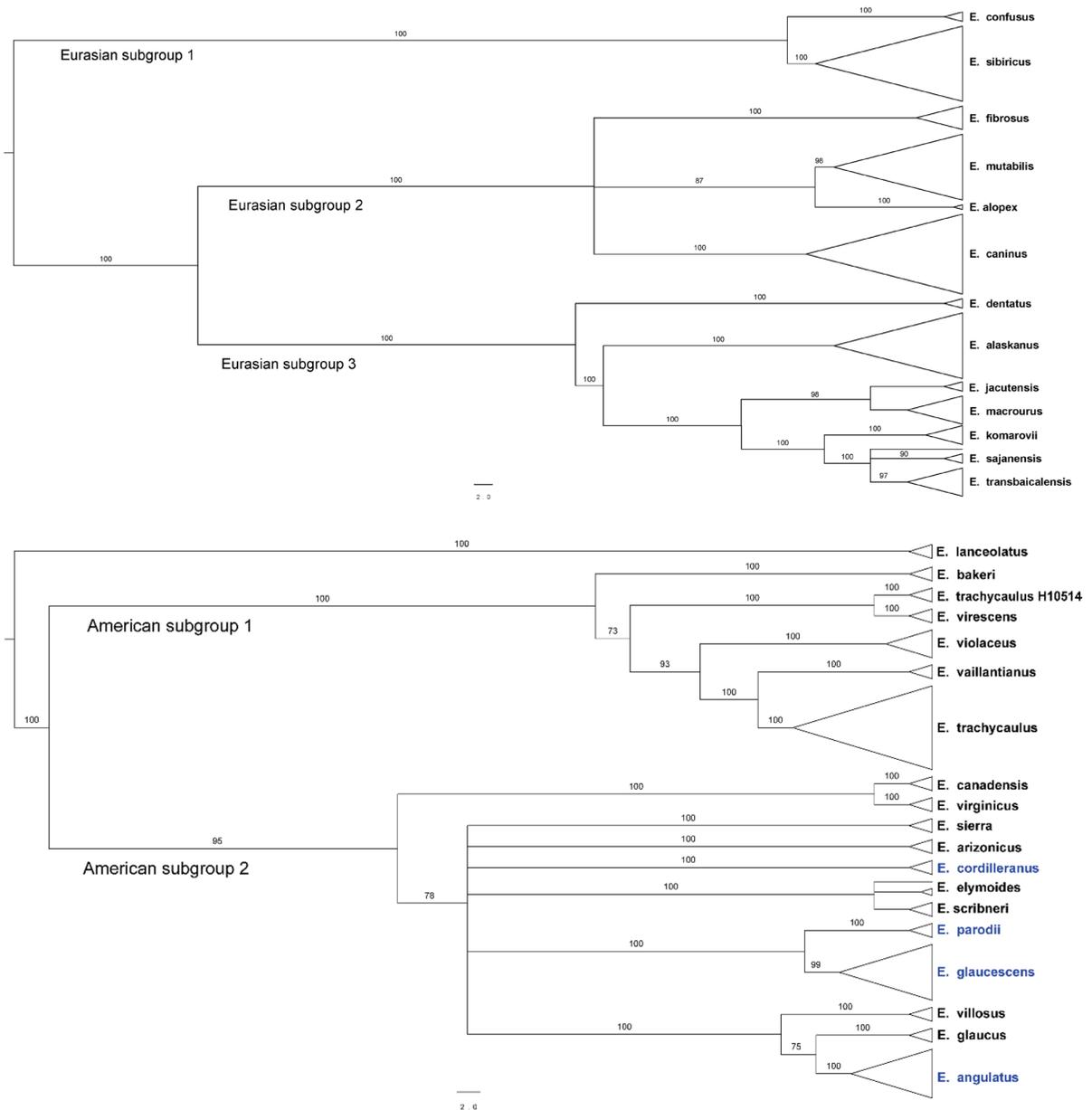


Fig. 4 Phylogenetic trees based on SVDquartets, conducted with PAUP, using DArTseqLD™ SNP data with 10 000 non-parametric bootstrap replicates. The analysis was performed with species identified belonging to the Eurasian (Set 3) and American (Set 4) clade, respectively, with outgroup chosen

based on the SplitsTree phylogenetic network using Set 2. Bootstrap values are indicated above branches and branches with a posterior probability < 0.75 are shown as polytomies. Blue species names indicate South American origin

group and the second American StH subgroup most closely related to *E. trachycaulus*.

The results from the Set 2 SplitsTree analysis and the SVD-quartet analyses showed that the “Eurasian” clade was further divided into three subgroups with

high branch support (100% BS). The correlation between geography and the division into subgroups was not as distinct as for the two main groups. The first subgroup (from central and eastern Asia) included *E. sibiricus* with *E. confusus* as a sister

group. The second subgroup (from primarily western and central Eurasia) included *E. caninus*, *E. fibrosus*, *E. alopex*, and *E. charkeviczii* clustering within most of the *E. mutabilis* accessions. The third subgroup included *E. jacutensis*, *E. komarovii*, *E. sajanensis*, *E. transbaicalensis* (from primarily eastern and central Asia), together with *E. alaskanus* *E. macrourus* (circumpolar species). The phylogenetic results could not separate between *E. sajanensis* and *E. transbaicalensis*. In the SplitsTree analysis, *E. dentatus* appeared as an intermediate between the second and third subgroups, but in the phylogenetic analysis the taxa were placed in the third subgroup. One individual of *E. mutabilis* (H10456) appeared as an intermediate between the “American” and “Eurasian” groups in the SplitsTree analysis of Set 2.

The “American” group was further divided into two main subgroups. The first subgroup included predominantly North American species including *E. bakeri*, *E. kamczadalarum*, *E. trachycaulus*, *E. virescens*, and *E. violaceus*. There was a clear relationship between Asian and American *E. trachycaulus* accessions except *E. trachycaulus* (H10514) forming a monophyletic group (100% BS) together with *E. kamczadalarum* from Kamchatka and *E. virescens* from Northern North America. The second subgroup included both South and North American species intermixed with weak internal branch support (<70% BS) indicating unresolved polytomic relationships. However, accessions of *E. angulatus* formed a well-supported (100% BS) clade together with the two North American species *E. villosus* and *E. glaucus*. In addition, accessions of *E. glaucescens* and *E. parodii* formed a subclade (100% BS).

Analyses of genetic structure

The genetic structures in the PCoA and STRUCTURE analyses (Figs. 5 and 6) were consistent with the results from the SplitsTree and SVDquartets analyses. For Set 1, the first (PCo1 24%) and second (PCo2 12%) axis of variation showed an evident separation of the American StH, Eurasian StH and StY taxa. The PCoA analysis of Set 4 (PCo1 18%, PCo2 10%), including only American StH species, clearly separated the two subgroups as seen in the SplitsTree analyses. In addition, a third subgroup containing *E. glaucescens* and *E. parodii* was separated

from the other species. The PCoA analysis of Set 3 (PCo1 28%, PCo2 20%), including only Eurasian StH species, showed a clear distinction between the three subgroups as seen in the SplitsTree analyses. The STRUCTURE analyses showed the existence of six (K=6) and two groups (K=2) for Set 1 and Set 2, respectively (Fig. 6), based on the ΔK . Samples with an ancestry coefficients >0.75 and <0.75 were assigned to as “pure” and “admixed”, respectively. Set 2 showed low or no admixture levels between the two StH groups. Based on both taxonomical grouping and the geographic distribution of the species, a substructure of the six STRUCTURE groups could be observed: K1=StY, K2=StH 2nd Eurasian subgroup, K3=StH 1st American subgroup, K4=StH 1st and 3rd Eurasian subgroup, K5=Ns and *Brachypodium*, and K6=StH 2nd American subgroup. *Elymus repens* showed a complete admixture. *Elymus bakeri*, *E. violaceus*, *E. virginicus*, *E. canadensis* and *E. lanceolatus* showed a partial introgression between the two American subgroups when analyzing Set 1 but not Set 2. For Set 2, the two groups were consistent with the geographical differentiation between the two major StH groups, and in agreement with the results from the SplitsTree and PCoA analyses. Only one individual of *E. mutabilis* (H10456) was placed as an admixed between the “American” and “Eurasian” groups in the STRUCTURE analysis of Set 2. The assignments of the hexaploid species confirmed the earlier findings from the SplitsTree analyses that *E. scabriglumis* is most closely related to species in the 1st American subgroup, *E. patagonicus* the 2nd American subgroup and *E. transhyrcanus* to the 3rd Eurasian subgroup.

Discussion

Based on a molecular approach using DArTseqLD™, this paper presents a genetic structure and phylogenetic analysis of the complex genus *Elymus* s.s and some associated species. The utilization of a large number of molecular markers, widely distributed over the genome and a large number of taxa from the whole distribution range vouches for an adequate representation of the generic diversity.

The distinct separation of StH and StY genome combinations has previously been demonstrated using molecular markers (Svitashev et al. 1996;

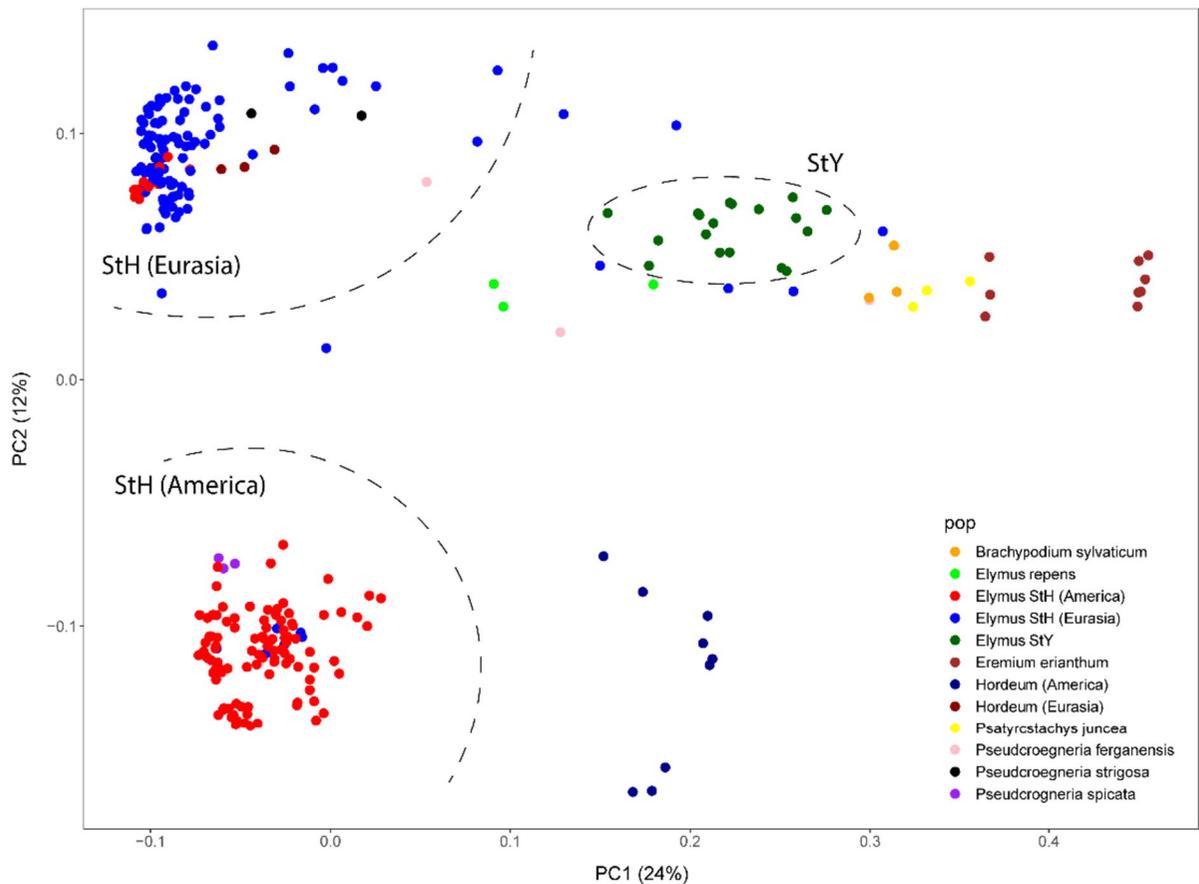


Fig. 5 Principal components analysis (PCoA) based on Nei’s unbiased genetic distance showing the dispersion of samples across the first two principal components of Set 1 (all samples)

Sun et al. 1997) and is also evident in the current study. These results further confirm the affiliation of included species to previously determined genome combinations. The SplitsTree networks together with the PCoA and STRUCTURE analyses in the present study differentiate between two major groups of *Elymus s.s.* which highly correlate with geographical distribution, the “American” group and the “Eurasian” group. The placement of different *Pseudoroegneria* and *Hordeum* species in the different groups suggests the involvement of several progenitor species, making *Elymus s.s.* a polyphyletic taxon with the potential differentiation in a St_1H_1 and a St_2H_2 group. These results are in agreement with earlier research, thereby suggesting that American and Eurasian StH species have evolutionary distinct origins (Jaaska 1992; Linde-Laursen et al. 1994; Dubcovsky et al. 1997; Sun et al. 2008; Sun and Ma

2009; Wang et al. 2012, Fan et al. 2013b). Hence, it contradicts previous results suggesting a common origin (Mason-Gamer et al. 2010b, Wang et al. 2011; Dong et al. 2015). Baum et al. (2015) suggest, based on research of variation in 5S nrDNA, that *Elymus* StH has multiple origins also within continents, but the results from the present study cannot confirm this. Only three species of *Pseudoroegneria* and four from *Hordeum* were included in this study, hence, the results are incomprehensive for the exact progenitor species determination, and other progenitor species cannot be excluded. Different cultivars of the H genome could however indicate introgression by subsequent backcrossing (Zuo et al. 2015), and reciprocal hybridization is possible to have occurred. The origin of the parental species correlates with geography and the origin of the included *Elymus s.s.* species. In addition, the SplitsTree network place

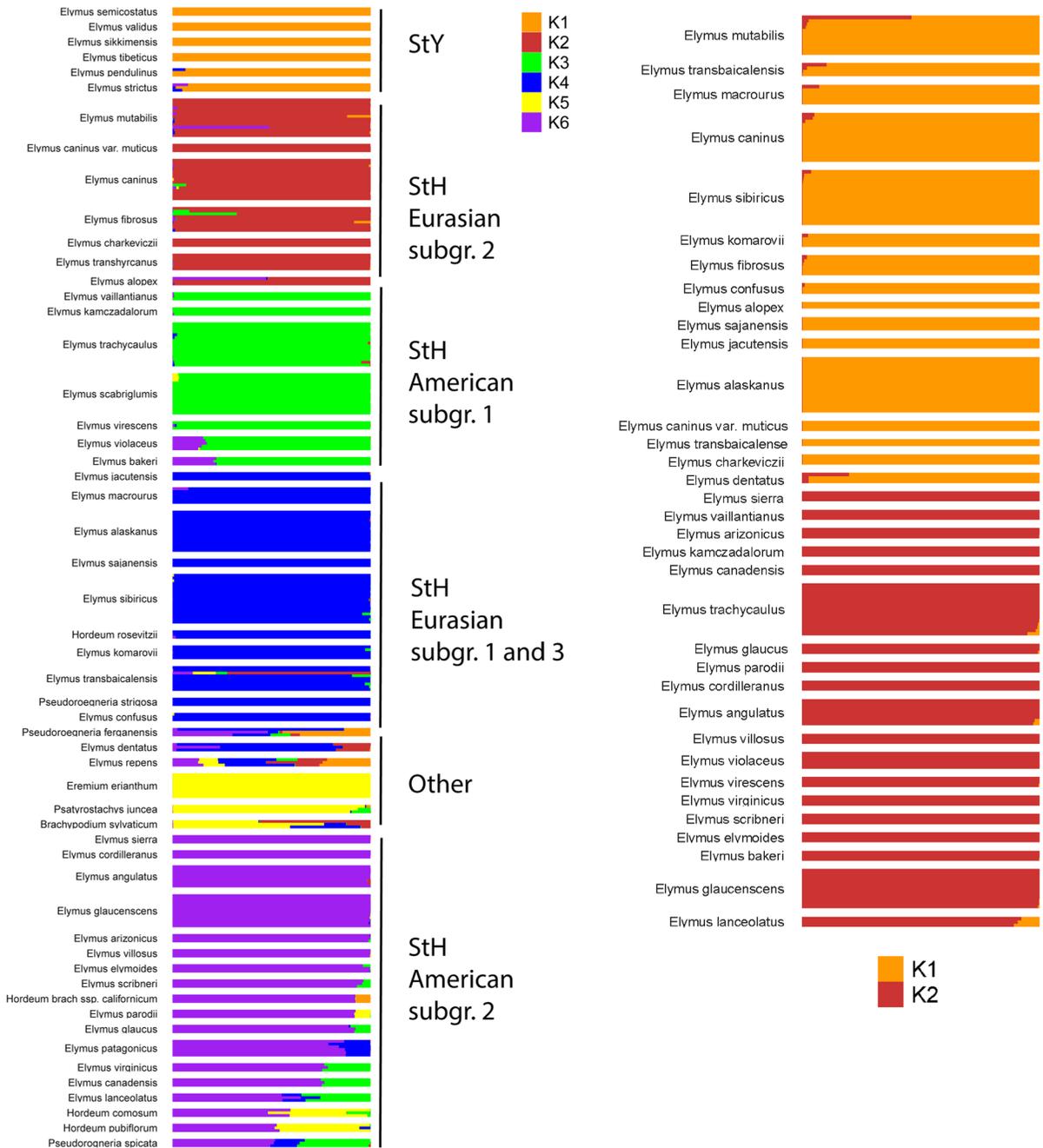


Fig. 6 STRUCTURE bar plots of the estimated membership coefficient (Q) for each individual for K=6 and K=2 based on STRUCTURE results for Set 1 (left) with all species included and Set 2 (right) with only StH species

the South American *Eremium erianthum* together with *Psathyrostachys juncea*, which supports the classification of *Eremium erianthum* as distinct from *Elymus* (Seberg and Linde-Laursen 1996; Svitashv et al. 1996). One individual of *E. mutabilis* in

accession H10456 was interpreted as an intermediate between the “American” and “Eurasian” group in both the STRUCTURE and SplitsTree analysis. This accession derives from Kamchatka, eastern Russia, and the individual is interpreted as a hybrid between

the two major StH groups. In all analyses, the other individuals in the accession group are placed together with the rest of *E. mutabilis* in the Eurasian subgroup. This proves the potential of hybridization between the St₁H₁ and St₂H₂ group but the degree of introgression is not evident.

The Eurasian tetraploid *Elymus* StH group

The first (*sibiricus-confusus*) and the second (*mutabilis-fibrosus-caninus-alopec*) subgroups in Eurasia contain well-separated species. Although distinct in morphology and ecology, the close connection between *E. mutabilis*, *E. fibrosus*, and *E. caninus* is evident in both the biosystematic study by Agafonov and Salomon (2002) and the current study. A high frequency of hybrids has been found in areas where *E. caninus*, *E. fibrosus*, and/or *E. mutabilis* grow together (Diaz 1999; Sun et al. 2001; Wu et al. 2016), which additionally indicates a high degree of introgression and a close relationship, although the species are genetically separate. The present study confirms the separation between *E. mutabilis* and *E. transbaicalensis* (Agafonov 2004; Leo et al. 2022), as well as the inclusion of *E. charkeviczii* in *E. mutabilis* (Agafonov et al. 2005). The species *E. alopec*, endemic to Iceland, was described by Salomon (2005), who proposed that it constitutes a deviating form of *E. caninus*. The results from the SplitsTree analyses suggest an intermediate relationship with *E. mutabilis* and *E. caninus* forming a network. *Elymus alopec* could be of hybrid origin, and several other described taxa are assumed to be *E. caninus* × *E. mutabilis* hybrids (Agafonov and Salomon 2002).

In contrast, the third (*alaskanus-macrourus-transbaicalensis*) subgroup in the Eurasian clade, including two circumpolar species, is diverse and taxonomically more challenging to handle. The close relationship between *E. macrourus*, *E. komarovii*, *E. transbaicalensis*, *E. sajanensis*, and *E. alaskanus*, is in agreement with morphological and isozyme data, as well as biosystematic research showing high crossability between the species (Agafonov et al. 1998; Agafonov 2004). Agafonov and Salomon (2002) conducted a biosystematic study with inter- and intraspecific crosses of Siberian *Elymus* StH taxa to establish a model of recombination gene pools (RGP) and introgression gene pools (IGP). They concluded

that Russian populations of *E. transbaicalensis*, *E. komarovii*, and *E. alaskanus* are connected within the same RGP and in the same IGP as *E. macrourus*. The microevolutionary levels of differentiation between *E. komarovii*, *E. transbaicalensis*, *E. kronokensis*, and *E. sajanensis* have been studied by Agafonov et al. (2019). The species were morphologically variable, with many different biotypes showing complex genetic relationships. The phylogenetic analyses in the present study confirm the relationships and differentiate between *E. alaskanus*, *E. macrourus*, *E. jacutensis*, *E. komarovii*, with *E. transbaicalensis* and *E. sajanensis* in a polytomic clade suggesting incomplete lineage sorting and/or extensive hybridization.

The fact that the two true circumpolar species group in the same subclade is phylogeographically interesting. The transcontinental occurrence of circumpolar species shows that the Bering Sea is a possible dispersal route. No signs of introgression could, however, be found between circumpolar species and co-occurring species in North America. *Elymus alaskanus* is usually morphologically homogenous within regions and populations but variable over its whole distribution area (Wu et al. 2016). The results, however, show all included accessions forming a monophyletic clade with high branch support (100% BS).

The American tetraploid *Elymus* StH group

The first subgroup in the American clade gather many of the species often referred to as the *E. trachycaulus* complex (Barkworth 1994). *Elymus kamczadalarum*, *E. bakeri*, and *E. virescens* have all been treated as subspecies to *E. trachycaulus* (Tzvelev 1973; Löve 1984; Sun et al. 2006a). Sun and Ma (2009) concluded, based on nucleotide variation in chloroplast Asp(GUC)–Thr(GGU) intergenic region, that *E. violaceus* is genetically distinct. Seen in the STRUCTURE analyses, both *E. violaceus* and *E. bakeri*, have a certain influence of genetic structure from the second American subgroup indicating introgression which could explain the deviating variation from *E. trachycaulus*. The *E. trachycaulus* complex remains a taxonomical challenge despite many attempts to unravel the genetic relationships (Gupta et al. 1988; Gaudett et al. 2005; Sun and Li 2005; Sun et al. 2006a, b; Stevens et al. 2007; Sun

2007; Zuo et al. 2015). The present study, however, clearly excludes taxa such as *E. alaskanus*, *E. macrourus*, *E. scribneri*, and *E. sierrae*, which are sometimes considered conspecific (Barkworth 1994). The close relationship between *E. kamczadalarum* from Kamchatka and the North American species indicates a North American origin with a migration of the group over the Bering Sea. Further, the close relationship between Asian and American *E. trachycaulus* indicates a second migration event in the group. However, *E. trachycaulus* has been grown in eastern Eurasia for feeding purposes and the origin of the included population is uncertain.

In the second American subgroup, a polytomic pattern in the SplitsTree and phylogenetic analyses indicates incomplete lineage sorting and potential introgression due to recent and radial speciation events. The unresolved branches make the origin and migration of the South American *Elymus* species uncertain. In all analyses, the two species *E. villosus* and *E. glaucus*, from eastern respectively western North America, group together with *E. angulatus* from southern South America in a well-supported monophyletic subclade. Thus, the four South American species in the American clade, *E. angulatus*, *E. cordilleranus*, *E. glaucescens*, and *E. parodii* constitute a non-monophyletic group indicating either multiple migration events from North to South or a single migration event with a recolonization to North America by *E. villosus* and *E. glaucus*. In southern Chile, *E. angulatus* and *E. glaucescens* sometimes grow sympatric. Individuals from accession CHL18-39 were determined as *E. glaucescens* in the field, with single spikelets per rachis node. In the first exploratory analyses, the individuals in the accession appeared as hybrids between *E. angulatus* and *E. glaucescens* and were subsequently removed from further analyses. Introgression among South American *Elymus* species may obscure the true origin and relationships. Gene introgression through recurrent hybridization could promote a rapid adaptation of these species to different ecological habitats resulting in the formation of many endemic species or genotypes/ecotypes (Sun et al. 2008). The close relationship between *E. virginicus* and *E. canadensis* is confirmed by frequent hybrids occurring in areas where the species grow in sympatry, and introgression is assumed (Pohl 1959; Nelson 1978). As seen in the STRUCTURE analysis, a certain influence from the 1st American subgroup is also evident in *E. virginicus* and

E. canadensis indicating introgression showing the evolutionary dynamics among North American *Elymus* species.

Origin of hexaploid species

The repeated formation of hexaploids is evident from the placement of the three included hexaploid species (*E. transhyrcanus*, *E. scabriglumis*, and *E. patagonicus*) in different phylogenetic groups. *Elymus transhyrcanus* group together with *E. caninus* in the Eurasian clade, while the other two included hexaploid species are found in the American clade. *Elymus scabriglumis* is placed within the first American subgroup in all analyses, apart from the other South American species, showing a close relationship with the Mexican *E. vaillantianus* and the North American *E. trachycaulus*. This suggests a potential origin of *E. scabriglumis* from those North American species and an independent migration from North to South America either before or after the hexaploidization event. Dewey (1977) found that *E. tilcarensis* (syn. *E. scabriglumis* (Seberg and Petersen 1998)) is more closely related to *E. trachycaulus* than *E. lanceolatus*, which further supports the conclusion of origin. The analyses here also show that the tetraploid *E. angulatus* is most closely related to the sympatrically grown hexaploid *E. patagonicus*, and hence *E. angulatus* is the most likely progenitor to *E. patagonicus*. The second genome donor is still unknown. Several potential *Hordeum* progenitor species occur in the same area as *E. angulatus* and *E. patagonicus*, and artificial hybrids between *E. angulatus* and some *Hordeum* species are rather easily obtained (Jacobsen and von Bothmer 1981; Seberg and von Bothmer 1991). Research on morphology, cytology, and reproductive compatibility (biosystematics) between *E. scabriglumis* and *E. patagonicus* show a genetic barrier between the two hexaploid species with sterile artificial hybrids, which further suggests separate origins and the involvement of different *Elymus* and/or *Hordeum* progenitors (Hunziker 1953; Nicora 1978).

Taxonomy

Phylogenetic and diversity data is essential for understanding relationships and evolution, but the results also have taxonomical implications. Petersen et al. (2011) argue that a classification of *Elymus*

should be based on a combination of morphology and cytology as well as molecular data. The phylogenetic results from the present study provide new evidence for a revised classification with the implication of a division into subgenera (St_1H_1 and St_2H_2) and/or sections based on genetic relationships. The results recognize Dewey's (1984) and Löve's (1984) haplome delimiting criteria of Triticeae as an appropriate and pragmatic base for classification and the more consistent genera delimitation based on genome combination proposed by Yen and Yang (2022) as further criteria. However, the results recognize the phylogenetically inaccuracy in the classification system with the pronounced distinction between the two independent American and Eurasian *Elymus s.s.* groups that have originated from different progenitor species. The two major groups could be considered as subgenera, but it is then necessary to find morphological characteristics to distinguish them. The study cannot exclude several different origins also within the St_1H_1 and St_2H_2 groups.

In a diversity study including 24 StH species, Baum et al. (2016) used canonical discriminant analysis based on sequences of the nr5SDNA to identify factors related to genetic relationships and *Elymus s.s.* classification. They found evidence for a classification based on ploidy, section affiliation proposed by Yen and Yang (2022) (*Elymus*, *Elytrigia*, *Goulardia*, *Hystrix*, and *Sitanion*), and geographical regions (Eurasia, circumboreal, North America, and South America). The present study supports the geographical component on a large scale but does not differentiate between North and South America and does not support circumboreal species as a unified group even though the two true circumpolar species belong to the same phylogenetic subgroup. Polyploid species are not associated and group in different clades demonstrated by *E. patagonicus*, *E. scabriglumis*, and *E. transhyrcanus* with different parental origin. The sections earlier proposed by Yen and Yang (2022), Löve (1984), and Tzvelev (1976) are based on cytological and morphological characters (Tab. 1). Neither of the classifications are, however, in accordance with the phylogenetic results. For example, *E. caninus* (from Eurasia) and *E. trachycaulus* (from North America) are placed in the same section, *Goulardia*, by Löve (1984) and Yen and Yang (2022), but the two taxa fall into different clades in current phylogenetic analyses.

These findings show the importance of integrative taxonomy, including taxonomical information from different data types and methodologies.

Conclusion

This study uses molecular DArTseqLD™ data to reveal the genetic structure and relationships within *Elymus s.s.* (StH) and some associated taxa. The analyses demonstrate a major division between predominantly American and Eurasian species with different parental species involved. Species from South America do not form a monophyletic group, which indicates multiple dispersal events from North to South America. The study manages to describe the major features of *Elymus s.s.* diversity but shows the difficulties in resolving evolutionary relationships even when using high throughput techniques with plenty of genetic markers. The complexity of *Elymus s.s.* relationships is shown in the poorly resolved internal branches, indicating a high degree of introgression, incomplete lineage sorting, recent speciation and multiple speciation events. In addition, phenotypic plasticity, cryptic speciation, few reliable morphological diagnostic characters, and a large number of taxa makes it taxonomically difficult to handle in practice. The technical advancements make access of genotypes the limiting factor rather than details of molecular markers. A larger number of species and populations could help resolving the polytomic relationships within the major clades and give a more elaborate understanding of the diversification process and the interspecific interactions among worldwide *Elymus*. In both species and section circumscriptions, molecular data should be used to reexamine morphological characters considered to be of diagnostic importance. A new classification of *Elymus* sections, where phylogenetic relationships are taken into account, is needed. The results from this study have given a deeper understanding of *Elymus* evolution and will form the basis for establishing a more accurate circumscription and classification of the genus and a robust framework for future interspecific and population studies.

Author contributions All authors contributed to the study's conception and design. Material preparation, data collection

and analysis were performed by JL, TB, AM, ASC RvB. The first draft of the manuscript was written by JL and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets analysed during the current study are available in the NCBI Short Read Archive (SRA) repository under BioProject ID PRJNA942607.

Declarations

Conflict of interest This work was supported by Kungliga Fysiografiska Sällskapet (Grant numbers 39809). The authors have no competing interests to declare that are relevant to the content of this article.

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