Phylogenetic Analysis of the Genus *Pseudoroegneria* and the Triticeae Tribe Using the *rbcl* Gene

By

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Abstract

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Pseudoroegneria are diploid perennial grasses of the Triticeae tribe. The haplome "St" of *Pseudoroegneria* is shared with several important polyploid species such as *Elymus, Kengyilia* and, *Elytrigia*. Due to frequent hybridizations and complex genetic mechanisms, the relationships within *Pseudoroegneria*, and within the Triticeae, have been heavily disputed. Using the chloroplast *rbcL* gene I estimated the nucleotide diversity of 8 *Pseudoroegneria* species, and additionally examined the phylogenetic relationships within *Pseudoroegneria* and of *Pseudoroegneria* within the larger context of Triticeae. The estimates of nucleotide diversity indicated that *P. tauri* and *P. spicata* species had the highest diversity, while *P. gracillima* had the lowest. The phylogenetic analysis of *Pseudoroegneria* placed all *P. spicata* sequences into a clade separate from the other *Pseudoroegneria*, while the relationship of the other *Pseudoroegneria* species could not be determined. Due to the groupings of *Pseudoroegneria* with the polyploid *Elymus*, our results strongly support *Pseudoroegneria* as the maternal genome donor to *Elymus*. There was also weak support for the idea that *P. spicata* may be the maternal genome donor to the StH *Elymus* species.

April 17th, 2013

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1. Introduction

1.1-Triticeae

The Triticeae are an economically important grass tribe in the Poaceae family. They contain many important cereal grasses such as barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and rye *(Secale cereale*), as well as many forage grasses such as *Elymus* and *Pseudoroegneria* (Hodge, Wang & Sun, 2010; Petersen & Seberg, 1997). The large variety of biological and genetic mechanisms, including frequent hybridizations, introgression and rapid speciation that occur in the Triticeae make them a very useful group for studies in evolution, genetics and plant speciation (Petersen & Seberg, 1997; Sun, 2007).

There has been, and continues to be, much disagreement in regards to the taxonomic classification of genera within the Triticeae. In the past, genera were classified solely on morphological characteristics, such as Krause (1898) who analyzed morphological traits and placed all the Triticeae in one genus (as cited in Yen et al, 2005). Other classifications relied on disputable key characteristics such as the number of spikelets per rachis nodes in *Elymus,* which were considered key by some taxonomists while others used different key characteristics (McMillan & Sun, 2004). Analysis of these traits resulted in the reclassification of a large number of species into other genera, leaving the genera *Elymus* with only a handful of species. Similar classifications were identified in the studies of Hitchcock (1951), Keng (1959), and Baum(1983) (as cited in McMillan & Sun, 2004). In 1984 two important publications by Löve (1984) and Dewey (1984) helped create a classification of Triticeae based on genomic data, using

chromosome pairing (cytological) to define the boundaries of genera. Due to the indiscrete nature of the genome, even between the publications by Löve (1984) and Dewey (1984) there were large variations in the number of genera reported. Further, molecular studies are highly congruent with the cytological data used by Löve and Dewey in their classifications, which are now used to provide species level taxonomic separation (Barkworth & Jacobs, 2011). The key morphological characteristics, such as spikelets per rachis nodes, that had been previously used to define species and genera are not correlated with cytological or molecular data. However, there are some morphological characteristics that when examined together do, corroborate with cytological and molecular data and can be used in classification (Barkworth & Jacobs, 2011; Mason-Gamer, 2001). Molecular trees have, however, produced several inconsistencies between different gene studies. These inconsistencies, are often attributed to poorly supported clades or reticulation events, and as a result there is still no unanimous classification scheme for the Triticeae (Kellogg, Appels, & Mason-Gamer, 1996; Yen et al., 2005).

1.2-Pseudoroegneria

The genus *Pseudoroegneria (Nevski) Á. Löve* contains approximately 15 different species, with the type species being *P. strigosa (M. Bieb) Á. Löve* (Dewey, 1984; Löve, 1984; Watson & Dallwitz, 1992 onwards; Yan & Sun, 2011). A perennial Triticeae, *Pseudoroegneria,* has culms (stems) between 30-90 cm tall, flat or convoluted leaves, long anthers and they often display dense cespitose (many stems). The spikes of *Pseudoroegneria* are single and wide apart with 5-8 mm glumes (leaf like structure) which can be of equal length as in *P. strigosa*, or unequal as *in P. spicata, tauri* and *libanotica*. The awns (hairs) of *Pseudoroegneria* are between 8-30 mm, or may be absent

as in *P. tauri* and *libanotica*. (Watson & Dallwitz, 1992 onwards; Yan & Sun, 2011; Yen, Yang & Baum, 2005). *Pseudoroegneria* are predominately cool-season grasses found on rocky hillsides in the Middle East, Transcaucasia, central Asia, northern China and western North America at latitudes above 35°N. These grasses are palatable to animals and are drought and salt tolerant. This makes them highly desirable for plant breeders (Dewey, 1984; Löve, 1984; Yan & Sun, 2011; Yu et al., 2008).

As with many of the grasses in the Triticeae, interspecific hybridization is common, often resulting in increased ploidy or the formation of a new species when two or more of the approximately 30 distinct haplomes in the perennial Triticeae hybridize (Redinbaugh, Jones & Zhang, 2000). The cytological studies by Löve (1984) and Dewey (1984) designated the genome of *Pseudoroegneria,* as the St genome. Although the genus *Pseudoroegneria* has a relatively small number of species, its St genome is one of the most important genomes in the Triticeae as it is the donor genome to several important polyploid genera such as *Roegneria, Kengyilia, Douglasdeweya, Elymus, Elytrigia,* and *Pascopyrum* (Dewey, 1984; Löve, 1984; Luo et al., 2012; Yan & Sun, 2011; Yen et al., 2005; Zeng et al., 2010).

1.3- Relationship of Elymus and Pseudoroegneria

The genus *Elymus* is the largest, most widely distributed, and also the most morphologically diverse genus in the Triticeae with approximately 150 species, all of which are allopolyploids, containing at least one St haplome (Dewey, 1984; Okito, Mott, Wu & Wang, 2009; Sun, Ni, & Daley, 2008; Yan, Sun & Sun, 2011). Cytological analyses have shown that there are five basic genomes within *Elymus* (St, H, P, W, and

Y). The H genome is believed to have originated from the genus *Hordeum*, which include the species *H. vulgare* (barley). The P genome is believed to be from *Agropyron* (crested wheat grass). The W genome is believed to be from *Australopyrum*, and the origin of the Y genome is unknown (Dewey, 1984; Yan et al. 2011).

There are approximately 30 *Elymus* species with an StY genome, found through central and east Asia (Liu et al., 2006; Okito et al, 2009). In recent years there have been several studies attempting to determine the origin of the Y genome, of which there are two predominate hypotheses. One hypothesis is that the Y genome is a highly diverged St genome of *Pseudoroegneria*. This hypothesis is based on *internal transcribed spacer sequences* (*ITS*) of nuclear rDNA, which found that StY genomes grouped only with St and not with any of the other basic genomes (W, P, or H), which is argued to indicate a common origin between St and Y (Liu et al., 2006). Okito et al. (2009) found one accession of *P. spicata* (PI 232134) to be very closely related to *E. longearistatus* which made *P. spicata* the candidate for the Y genome donor. The other hypothesis, as proposed by Dewey (1984), is that the Y genome is a distinct diploid genome of a rare and undiscovered or extinct Asian species. Studies of the *RNA polymerase II* (*RPB2)* gene (Sun et al., 2008; Yan et al., 2011) and the *translation elongation factor-G (EF-G)* gene (Sun et al., 2008; Sun & Komatsuda, 2010) clearly separated the St and Y genomes into distinct groups. The *P. spicata* (PI 232134) strain, found by Okito et al. (2009) to be the possible donor to *E. longearistatus*, was included in the St groups during the study by Yan et al. (2011), indicating that it is unlikely the St and Y genomes share a common origin.

There have been many studies on the chloroplast DNA of *Elymus*, with a consistent finding that the St genome of *Pseudoroegneria* is the maternal donor of the St haplome in North American, Middle Eastern and Asian *Elymus* species (Hodge et al., 2010; Liu et al., 2006; Mason-Gamer, Orme & Anderson, 2002; McMillan & Sun, 2004; Sun & Salomon, 2009).

1.4- Relationship within Pseudoroegneria

Interspecies hybridizations of the diploid *Pseudoroegneria* often results in sterile offspring. A cytological study by Wang et al. (1995), found that the St genomes of different *Pseudoroegneria* species have become highly diverged. This indicates that there may be more than one form of the St genome within the *Pseudoroegneria* genus, and new genomic designation may be necessary (Yu et al., 2008). Further studies, including those on the *granule-bound starch synthase I (GBSSI)* gene (Mason-Gamer, 2001; Mason-Gamer et al., 2010), the *RPB2* genes (Sun et al., 2008; Yan & Sun, 2011), *ITS* of nuclear rDNA (Yu et al., 2008), the *EF-G* genes (Sun & Komatsuda, 2010; Yan & Sun, 2011) *phosphoenolpyruvate carboxylase (Pep-C)* (Mason-Gamer et al., 2010) and *ß-amylase* (Mason-Gamer et al., 2010), have found two distinct clades within the St genome of *Pseudoroegneria*. In the *GBSSI, RPB2, EF-G,* and *ß-amylase* gene phylogenies, *P. libanotica* and *P. tauri* have very similar sequences and are sufficiently different from the other diploid *Pseudoroegneria* so as to form a separate clade (Mason-Gamer et al., 2010; Sun et al., 2008; Sun & Komatsuda, 2010; Yan & Sun, 2011). This being said, *ITS* sequences have indicated that *P. libanotica, P. tauri, P. spicata* and *P. strigosa ssp. aegilopoides* are more closely related than they are to others in the St genome (Yu et al.,

2008), while *Pep-C* placed *P. libanotica, P. tauri,* and *P. spicata* into one clade (Mason-Gamer et al., 2010).

There have been only a few phylogenetic studies based on cpDNA in Pseudoroegneria. These studies include chloroplast intergenic regions *TrnT-F* (Mason-Gamer et al., 2002), and *TrnD/T* (Yan & Sun, 2011), several chloroplast restriction sites (Mason-Gamer et al., 2002), and the cpDNA gene *RNA polymerase α-subunit (RpoA)* (Mason-Gamer et al., 2002). In Mason-Gamer et al. (2002) study, the combined data of the *RpoA, TrnT-F,* and restriction sites grouped *P. libanotica,* and *P. strigosa* into a clade separate from *P. spicata*. This study, however, included only a small number of *Pseudoroegneria* species and did not include *P. tauri* (Mason-Gamer et al., 2002*).* Similarly, when the *TrnD/T* data were examined, almost all of the *P. spicata* grouped separately from the other *Pseudoroegneria* species, with one exception PI 563872, while the relationships of the other groups could not be determined (Yan & Sun, 2011).

Relative to other Triticeae diploid or polyploid species, such as *Hordeum* and *Elymus,* there have been only a few studies on the inter- and intraspecific nucleotide divergence of *Pseudoroegneria.* Studies on the *RPB2* gene (Sun et al., 2007) and the *cytochrome oxidase subunit II (CoxII)* gene (Zeng et al., 2010) found there to be a low level of interspecific nucleotide diversity, however these studies only sampled 2 to 3 *Pseudoroegneria* species. A study by Yan and Sun (2011) using the *TrnD/T,* the *EF-G* gene and the *RPB2* gene, while including eight *Pseudoroegneria* species and over 40 strains, found the rate of interspecific nucleotide diversity was much higher than previously reported. The rate of intraspecific nucleotide variation in the study by Yan and Sun (2011) was the highest within *P. spicata* and *P. gracillima*, which is consistent with

studies on the genetic diversity of P. *spicata* (Fu & Thompson, 2006; Larson et al., 2000; Larson, Jones, & Jensen, 2004). The rate of divergence in the chloroplast (cpDNA) *TrnD/T* region, and that of the mitochondrial (mtDNA) *CoxII* gene was significantly lower than that of the nuclear (nDNA) genes studied, within similar sample sizes (Sun et al., 2007; Yan, & Sun, 2011; Zeng et al., 2010). These large differences may be partially explained through the different evolutionary histories that occur between nuclear, chloroplast and mitochondrial genomes.

The discrepancies that are observed, between different gene trees, when examining the phylogenetic relationship of *Pseudoroegneria* as well as other members of the Triticeae are thought to occur for several reasons. One of the major reasons for the discrepancies is due to low support of clades between studies. This could occur as different genes have a higher or lower substitution rate which may or may not provide a accurate picture for each species/genra if they are recently diverged or very distantly related (Kellogg et al., 1996). Specimen selection is also important as reticulation events such introgressions are known to cause incongruences in tree construction, and as such, phylogenies should be interpreted from all known gene trees (Kellogg et al., 1996; Mason-Gamer et al., 2002). For these reasons, further studies adding more genes and including a greater number of specimens may help researchers clarify the discrepancies that occur between gene trees, leading to a more accurate description of the evolutionary history of the Triticeae.

1.5-cpDNA and the *rbcL* Gene

The use of cpDNA is common in many phylogenetic and genetic diversity studies. cpDNA has several advantages over nDNA, cpDNA is clonally (non-Mendelian) inherited, which is useful as cpDNA trees may display different evolutionary histories than nDNA trees, due to introgression and its mode of inheritance. These differences can provide greater insight into the history of the Triticeae than could be inferred if only nDNA was used (Mason-Gamer et al., 2002). The cpDNA of the Triticeae, as with most grasses, is maternally inherited (McMillan & Sun, 2004). As well, the rate of nucleotide subsitution is typically very conservative between species and genera, which is useful when studying phylogenies at higher taxonomic levels (Doebley et al., 1990; Gielly & Taberlet, 1994; Sun, 2002).

This study used the ~1300bp, *large subunit of the ribulose 1,5-bisphosphate carboxylase (rbcL)* gene which encodes for RUBISCO, an enzyme involved in photosynthesis, which is located in the chloroplast (Gielly & Taberlet, 1994). This gene was chosen because it is present as a single copy in all land plants, and its highly conserved nature allows for simpler amplification among a wide variety of species and genera (Doebley et al., 1990). Several studies have indicated that the *rbcL* gene may be too conserved to be used at the intergenic or interspecies level (Doebley et al., 1990; Gielly & Taberlet, 1994), while more recent studies have indicated that the *rbcL* gene can be used at lower taxonomic levels depending on the methodology and the types of questions being asked (Sun, 2007).

1.6- Objectives & Predictions

The objectives of this study are to: (1) investigate the phylogenetic relationship of the genus *Pseudoroegneria*. Based on previous genic studies it is predicted that *P. spicata* sequences will be very similar and group into a separate clade from the rest of the *Pseudoroegneria,* while P. *libanotica*, and *P. strigosa* will group together; (2) investigate the phylogenetic relationship of the Triticeae tribe with specific focus on the relationship of *Pseudoroegneria* within the tribe. As *Elymus* is known to contain an St haplome, it is predicted that *Pseudoroegneria* will form a closely associated group with *Elymus* indicating that *Pseudoroegneria* is the maternal genome donor, as cpDNA is maternally inherited in the Triticeae; (3) examine the degree of interspecific and intraspecific nucleotide divergence of the *rbcL* gene within the genus *Pseudoroegneria*. It is predicted that *P. spicata* will have a much larger nucleotide diversity than other species in *Pseudoroegneria,* and that due to the conservative nature of the *rbcL* gene there will be much fewer nucleotide polymorphisms than found in previous studies using other coding, and non-coding regions within the chloroplast genome (Yan & Sun, 2011).

This study is important because a greater understanding of the genomic variation within *Pseudoroegneria* can help in future sampling, as highly conserved species may require fewer samples and more divergent species may require greater sampling to obtain adequate representation; which may also have implications for plant breeders and resources management (Fu & Thompson, 2006; Larson et al., 2000). This study may provide valuable information for *Pseudoroegneria* species selections during future work with polyploid speciation, in species containing or possibly containing the St genome. As there is differential success in hybridization based on which species is the female parent

(Redinbaugh, Jones, & Zhang, 2000). Furthermore, this study may also provide greater insight into the phylogenetic history of *Pseudoroegneria* and of the Triticeae.

2. Materials and Methods

2.1-Plant Material & DNA Extraction

Thirty eight accessions (strains) from 8 species of *Pseudoroegneria*, and 3 accessions from 1 species of *Australopyrum* (Table 1) were grown in a sand-peat mixture at Saint Mary's University in Halifax, Nova Scotia. Young leaves were harvested and DNA was extracted using the GeneJet™ Plant Genomic DNA Purification Mini Kit (#K0791, #K0792).

2.2- Sequence Amplification

The chloroplast *ribulose 1,5-bisphosphate carboxylase (rbcL*) gene was amplified with the $rbcL_1$ primer (5'-TGTCACCAAAAACAGAGACT-3') and $rbcL_2$ primer (5'-TTCCATACTTCACAAGCAGC-3') for each of the 41 previously extracted accessions (McMillan & Sun, 2004). The amplifications for each accession contained a 40µL mixture which was separated into two 20µL mixtures during polymerase chain reaction (PCR) amplification, then pooled together after the PCR was complete. This separation was necessary, as it reduces the chance of PCR drift and PCR selection which may occur due to random Taq errors during the amplifications process (Yan & Sun, 2011; Zeng et al., 2010). Each 20µL mixture contained 4µL of dNTPs, 2µL of 10x KAPA Taq Buffer A (1.5mM $MgCl_2$ at 1x), 2µL of $rbcL_1$ primer, 2µL of $rbcL_2$ primer, 0.12µL of 5U/µL KAPA Taq, and 2µL of cellular DNA. PCR amplification was performed using the BIO RAD T100™ Thermal Cycler with a thermal cycling profile of: Initial denaturation at 95°C for 4 minutes, 40 cycles of denaturation at 95°C for 30-45 seconds, annealing at 51°C for 30-

45seconds, and extension at 72°C for 1.5- 2 minutes. After 40 cycles, a final extension at 72°C for 7-10 minutes was also completed.

2.3-Sequencing

Seven microliters of the 40μL of amplified mixture were mixed with 3-5μL of 10x loading dye and analysed by agarose gel electrophoresis to determine if the amplification was successful, before being sequenced. The samples were sent to Beijing, China where they were commercially purified and sequenced at the Taihe Biotechnology Company.

Table 1: *Pseudoroegneria* and *Australopyrum* species, with plant accession number, and country of origin for the *rbcL* sequences used in this study

Species	Accession	Country of Origin
P. gracillima	PI 440000	Russia
	PI 420842	Russia
P. libanotica	PI 228389	Iran
	PI 228390	Iran
	PI 229583	Iran
	PI 330687	Iran
	PI 330688	Iran
	PI 330689	Iran
	PI 330690	Iran
	PI 343188	Iran
	PI 401274	Iran
P. kosaninii	PI 237636	Turkey
P. spicata	PI 232128	Idaho, USA
	PI 232134	Wyoming, USA
	PI 232134	Wyoming, USA
	PI 232135	Wyoming, USA
	PI 236669	British Columbia
	PI 286198	Washington, USA
	PI 516184	Oregon, USA
	PI 537379	Washington, USA
	PI 537389	Washington, USA
	PI 539873	Idaho, USA
	PI 547162	Oregon, USA
	PI 563872	Montana, USA
	PI 598818	Oregon, USA
	PI 598822	Colorada, USA
	PI 619445	Nevada, USA
	PI 632480	Montana, USA
P. stipifolia	PI 440095	Russia
	PI 636641	Ukraine
	w6 21759	Ukraine
P. strigosa	PI 531752	Estonia
	W6 14049	Russia
P. strigosa subsp. aegilopoides	PI 595164	Xinjiang, China
P. tauri	PI 380645	Iran
	PI 401320	Iran
	PI 401326	Iran
	PI 401328	Iran
A. retrofractum	PI 533014	Australia
	PI 533013	Australia
	PI 531553	Australia

2.4-Data Analysis

2.4.1-Nucleotide Diversity

To examine the nucleotide diversity in *Pseudoroegneria*, the *Pseudoroegneria rbcL* sequences were input into the MEGA v 5.10 computer program (Tamura et al., 2011), where a multiple sequence alignment (MSA) of the sequence was performed using the ClustalW program, under the default settings (Higgins et al., 1994). The aligned sequences were then placed into the DnaSP 4.0 software (Rozas et al., 2005) which was used to calculate the average pairwise diversity between the aligned sequences, Tajima π (Tajima, 1989), the diversity based on the number of polymorphic segregating sites, Watterson's θ (Watterson, as cited in Yan & Sun, 2011), Tajima's D (1989) and Fu & Li's D (1993) tests of neutral evolution.

2.4.2- *Pseudoroegneria* Phylogenetic Analysis

Using the MEGA interface, gaps in the aligned sequences were manually removed and the sequences were entered into Gblocks (Talavera, & Castresana, 2007). This removed poorly aligned and divergent regions within the alignment, creating a more suitable alignment for the phylogenetic analysis. The phylogenetic analysis was conducted using the Neighbour-Joining Method (Saitou & Nei, 1987) in MEGA with the maximum composite likelihood (Tamura et al., 2004) substitution model, with uniform rates and 1000 bootstrap (BS) replicates (Felsenstein, 1985). A Maximum Parsimony analysis was also conducted with the Subtree-Pruning-Regrafting (Nei & Kumar, 2000) search method, a search level of 1, and 1000 BS replicates. *Psathyrostachys juncea* was used as the outgroup in both models.

As the MEGA program has not been used as extensively in the literature within this field, MP and NJ trees were also constructed using PAUP 4.0 (Swofford, 2002) to allow for greater comparability between studies. To do this the aligned sequences were input into the PAUP 4.0 program and a heuristic search of the sequences was conducted using Tree Bisection-Reconnection option with MulTree's on and 10 replications of random addition sequences with the stepwise addition option. The characters were unordered and unweighted, with gaps uncorrected and *Psathyrostachys juncea* designated as the outgroup. The NJ method was conducted using the Tajima-Nei distance. 1000 BS replications were calculated on both the MP and NJ tree through a heuristic search using the Tree Bisection-Reconnection option with MulTrees on.

2.4.3-Triticiea Phylogenetic Analysis

The *rbcL* sequence of *P. libanotica* (PI 33068) was used to perform a Genbank, BLASTn search (http://www.ncbi.nlm.nih.gov/blast/) to obtain *rbcL* accessions from other genera within the Triticeae tribe. This was restricted to sequences of at least 1000bp in length, in order to avoid small partial sequences which may be phylogenetically uninformative (Table 2). Neighbour-Joining Trees of the *Hordeum* and *Leymus* genera were created in order to choose representative sequences for each species because many species had multiple sequences available. Within the species with multiple accessions, the strains that appeared sufficiently divergent from each other were kept, while those that were highly similar were discarded. The *Pseudoroegneria* species in Table 1 were sampled in a similar manner and those sufficiently divergent were included in Table 2 for the Triticeae phylogeny. The phylogenetic analysis of the Triticeae was conducted in the

same manner as that of the *Pseudoroegneria* phylogenies, however, *Bromus hordeaceus* was used as the outgroup in all trees.

Table 2: Triticeae species, Genbank accession numbers of *rbcL* sequences, genomic designations and species accession numbers, used in this study.

Species	*rbcL* Accession	Genome	Accession
Aegilops comosa	AY836161.1	M	H6673
Aegilops speltoides	AY836183.1	B	H10681
Aegilops tauschii	AY836175.1	D	H6668
Agropyron cristatum	AY836175.1	P	H6668
Agropyron Hodkinson	EF125160.1	P	Hodkinson62 TCD
Amblyopyrum muticum	AY836157.1	T	H5572
Australopyrum calcis subsp. calcis	AY691636.1	W	AK296501
Australopyrum pectinatum	AY836158.1	W	H6771
Australopyrum velutinum	AY836160.1	W	H6724
Crithopsis delileana	AY836177.1	K	H5558
Dasyspyrum villosum	AY836163.1	V	H5561
Elymus canadensis	HM770840.1	StH	H3994
Elymus caucasicus	HM770839.1	StY	H3207
Elymus glaucescens	Z49837.1	StH	H6102
Elymus patagonicus	Z49838.1	StH	H6020
Elymus semicostatus	HM770841.1	StY	H4104
Elymus solandri	AY691642.1	StYW	AK281159
Elymus trachycaulus	Z49839.1	StH	H4228
Eremopyrum bonaepartis	EF125162.1	F	
Eremopyrum distans	AY836164.1	F	H5552
Eremopyrum orientale	FJ346562.1	F	
Eremopyrum triticeum	AY836165.1	F	H5553
Festucopsis serpentine	AY836166.1	L	H6511
Henrardia persica	AY836167.1	O	H5556
Heteranthelium piliferum	AY836168.1	Q	H5557
Hordelymus europaeus	EU376159.1	XoXr	H6778
Hordeum bogdanii	AY137455.1	H	H4014
Hordeum brachyantherum subsp. californicum	AY137451.1	H	H1942
Hordeum brevisubulatum subsp. violaceum	AY137435.1	H	H315
Hordeum bulbosum	AY137454.1	I	H3878
Hordeum capense	AY601671.1	HXa	H334
Hordeum erectifolium	AY836170.1	H	H1150
Hordeum chilense	AY137449.1	H	H1819
Hordeum comosum	AY137441.1	H	H1181
Hordeum cordobense	AY137458.1	H	H6429
Hordeum erectifolium	AY137440.1	H	H1150
Hordeum euclaston	AY137442.1	H	H1263
Hordeum flexuosum	AY137439.1	H	H1133
Hordeum intercedens	AY137450.1	H	H1940

Hordeum jubatum	Z49841.1	H	H1918
Hordeum lechleri	Z49842.1	H	H2164
Hordeum marinum subsp. gussoneanum	AY137425.1	Xa	H820
Hordeum marinum subsp. gussoneanum	AY137428.1	Xa	H826
Hordeum marinum subsp. gussoneanum	AY137432.1	Xa	H161
Hordeum marinum subsp. marinum	AY137436.1	Xa	H546
Hordeum marinum subsp. marinum	AY137421.1	Xa	H607
Hordeum murinum subsp. Glaucum	AY137437.1	Xu	H801
Hordeum muticum	AY137438.1	H	H958
Hordeum patagonicum subsp. magellanicum	AY137457.1	H	H6209
Hordeum patagonicum subsp. mustersii	AY137446.1	H	H1358
Hordeum patagonicum subsp. Patagonicum	AY137444.1	H	H1319
Hordeum patagonicum subsp. santacrucense	AY137447.1	H	H1493
Hordeum patagonicum subsp. setifolium	AY137445.1	H	H1357
Hordeum pubiflorum	HQ619241.1	H	H1236
Hordeum pusillum	AY137443.1	H	GP-2003
Hordeum secalinum	AY601672.1	HXa	H231
Hordeum roshevitzii	AY137459.1	H	H7202
Hordeum stenostachys	AY137448.1	H	H1783
Hordeum vulgare subsp. vulgare	AY137456.1	I	H5867
Hordeum vulgare subsp. spontaneum	AY137453.1	I	H3139
Leymus akmolinensis	GU140021.1	NsXm	PI440306
Leymus angustus	EU636660.1	NsXm	Z2215
Leymus arenarius	GU140017.1	NsXm	PI272126
Leymus cinereus	GU140019.1	NsXm	PI469229
Leymus chinensis	EU636661.1	NsXm	Z2027
Leymus erianthus	GU140015.1	NsXm	W6 13826
Leymus innovates	GU140014.1	NsXm	PI236818
Leymus karelinii	EU636664.1	NsXm	R354
Leymus mollis	EU636666.1	NsXm	Z1398
Leymus paboanus	EU636662.1	NsXm	Z2040
Leymus pseudoracemosus	GU140020.1	NsXm	PI531810
Leymus racemosus	EU636663.1	NsXm	R361
Leymus sabulosus	EU636665.1	NsXm	R366
Leymus salinus	GU140018.1	NsXm	PI636574
Leymus secalinus	EU636667.1	NsXm	Z2618
Leymus triticoides	GU140016.1	NsXm	PI537357
Peridictyon sanctum	AY836176.1	G	H5575
Psathyrostachys fragilis subsp. villosus	AY137461.1	Ns	H4372
Psathyrostachys fragilis subsp. secaliformis	AY607054.1	Ns	H4348
Psathyrostachys fragilis subsp. fragils	AY137460.1	Ns	H917
Psathyrostachys lanuginosa	AY607053.1	Ns	H8803

Psathyrostachys caduca	AY607060.1	Ns	H6702
Psathyrostachys stoloniformis	AY836180.1	Ns	H9182
Psathyrostachys rupestris subsp. daghestanica	AY607058.1	Ns	H6703
Psathyrostachys huashanica	AY607057.1	Ns	H3087
Psathyrostachys juncea	AY607050.1	Ns	H7544
Pseudoroegneria gracillima		St	PI440000
Pseudoroegneria kosaninii		St	PI237636
Pseudoroegneria libanotica		St	PI330688
Pseudoroegneria libanotica		St	PI229583
Pseudoroegneria spicata		St	PI598818
Pseudoroegneria spicata		St	PI537379
Pseudoroegneria spicata		St	PI537389
Pseudoroegneria spicata		St	PI286198
Pseudoroegneria spicata		St	PI236669
Pseudoroegneria spicata		St	PI232134
Pseudoroegneria stipifolia		St	PI636641
Pseudoroegneria strigosa		St	PI531752
Pseudoroegneria strigosa		St	W614049
Pseudoroegneria strigosa subsp. aegilopoides		St	PI595164
Pseudoroegneria tauri		St	PI380645
Pseudoroegneria tauri		St	PI401326
Secale strictum	AY836181.1	R	H4342
Stenostachys enysii	HM770838.1	HW	H3153
Stenostachys gracilis	HM770843.1	HW	H10624
Stenostachys laevis	AY691640.1	HW	AK281985
Thinopyrum bessarabicum	AY836185.1	E^{e}	H6729
Thinopyrum distichum	AM235075.1	$E^{b}E^{e}$	Forest F. et al. 658 (NBG)
Thinopyrum elongatum	AY836174.1	E^{e}	H6692
Thinopyrum farctus	AM849350.1	E^{b}	P.A. Christin 06-2005
Taeniatherum caput medusae	AY836184.1	Ta	H10254
Triticum aestivum	AY328025.1	$A^{u}BD$	
Triticum monococcum	AY836162.1	A^{M}	H4547
Bromus hordeaceus	HM849826.1		BM 2008/17

Genomic designation according to Wang et al., (1995)

3. Results

3.1- Nucleotide Diversity of Pseudoroegneria

The *rbcL* sequences for *Pseudoroegneria* were 1322-1338bp in length. All the *P. spicata* species shared a C to T substitution at the nucleotide position 202, and a T-A substitution at the 1000 nucleotide position (Figures 1 and 2) which separated *P. spicata* from the other *Pseudoroegneria* species. *P. kosanni* displayed two deletions at nucleotide positions 84 and 91, as well as a TT to AA substitution at position 86 and 87, which were unique to this species. The *Australopyrum* genus had unique single nucleotide substitutions at 10 sites (248, 261, 267, 643, 673, 728, 790, 799, 953, and 1000) which clearly separated them from the *Pseudoroegneria* species.

The genetic diversity from Table 3 indicates that *P. tauri* has the highest nucleotide diversity of the *Pseudoroegneria* analyzed at the rbcL gene (π=0.0046, θ=0.00536), while *P. gracillima* had the lowest Tajima π value (π=0.00227), and *P. strigosa* had the lowest Watterson's θ (θ=0.00252). The pairwise diversity of the entire genus (π=0.00259) was lower than that of all the individual species with the exception of *P. gracillima* (π=0.00227). However, in the segregating site diversity (θ) the entire genus had the highest diversity (θ=0.00419) with the exception of *P. tauri* (θ=0.00536). The two tests of neutrality were conducted on those species with four or more samples. The negative values recorded in Table 3 for the analyzed samples in the Fu and Li's D and Tajimia's D indicate that there are many low-frequency variations within the sample (Fu & Li, 1993; Tajima, 1989). This being said, only two of the tests were found to be significant. The Fu and Li's D in *Pseudoroegneria* and Tajima's D in *P. tauri* indicate

that the *rbcL* gene in *these* populations have undergone an intense selectional pressure, such as a bottlenecking event, however, neither test was significant in both the Fu and Li D and Tajima D tests.

Table 3: Estimates of the nucleotide diversity, and tests of neutral evolution for the *rbcL* gene in Pseudoroegneria.

Species	N	n	s	π	θ	Fu and Li's D	Tajima D
Pseudoroegneria	38	1308	18	0.00259	0.00419	-2.53458	-1.29148
P. libanotica	9	1315	10	0.00272	0.00336	-0.91781	-0.89272
P. spicata	16	1318	14	0.00369	0.00389	-0.15019	-0.19859
P. tauri	4	1322	11	0.00466	0.00536	-0.84307	-1.31864
P. gracillima	2	1321	3	0.00227	0.00227		
P. stipifolia	3	1318	7	0.00378	0.00378		
P. strigosa	2	1320	5	0.00277	0.00252		

The N is the number of sequences used, n is the number of sites, s is the number of segregating/polymorphic sites, Tajima's π is the average pairwise diversity, Watterson θ is the diversity based on s, Fu and Li's D and Tajima's D are tests of neutral evolution, which are indicated in red if significant ($p<0.05$).

Figure 1: Partial alignment of *rbcL* gene for *Pseudoroegneria* and *Australopyrum* with polymorphic sites at nucleotide positions 202, 248, 261, and 267 highlighted.

Species/Abbrv
4. Pseudoroegneria libanotica-330688
5. Pseudoroegneria libanotica-330687
6. Pseudoroegneria libanotica-401274
7. Pseudoroegneria libanotica-228389
8. Pseudoroegneria libanotica-330690
9. Pseudoroegneria libanotica-228390
10. Pseudoroegneria libanotica-229583
11. Pseudoroegneria libanotica-343188
12. Pseudoroegneria libanotica-330689
13. Pseudoroegneria spicata-232128
14. Pseudoroegneria spicata-232134
15. Pseudoroegneria spicata-236669
16. Pseudoroegneria spicata-563972
17. Pseudoroegneria spicata-632480
18. Pseudoroegneria spicata-598318
19. Pseudoroegneria spicata-547162
20. Pseudoroegneria spicata-619445
21. Pseudoroegneria spicata-516184
22. Pseudoroegneria spicata-232134(x)
23. Pseudoroegneria spicata-232135
24. Pseudoroegneria spicata-286198
25. Pseudoroegneria spicata-537379
26. Pseudoroegneria spicata-598822
27. Pseudoroegneria spicata-537389
28. Pseudoroegneria spicata-539973
29. Pseudoroegneria stipifolia-440095
30. Pseudoroegneria stipifolia-x621759
31. Pseudoroegneria stipifolia-636641
32. Pseudoroegneria strigosa-531752
33. Pseudoroegneria strigosa-W614049
34. Pseudoroegneria strigosa subsp. aegil
35. Pseudoroegneria tauri-401328
36. Pseudoroegneria tauri-380645
37. Pseudoroegneria tauri-401320
38. Pseudoroegneria tauri-401326
39. Australopyrum retrofractum531013
40. Australopyrum retrofractum531553
41. Australopyrum retrofractum531014

Figure 2: Partial alignment of *rbcL* gene for *Pseudoroegneria* and *Australopyrum* with significant polymorphic sites at nucleotide positions 953 and 1000 highlighted.

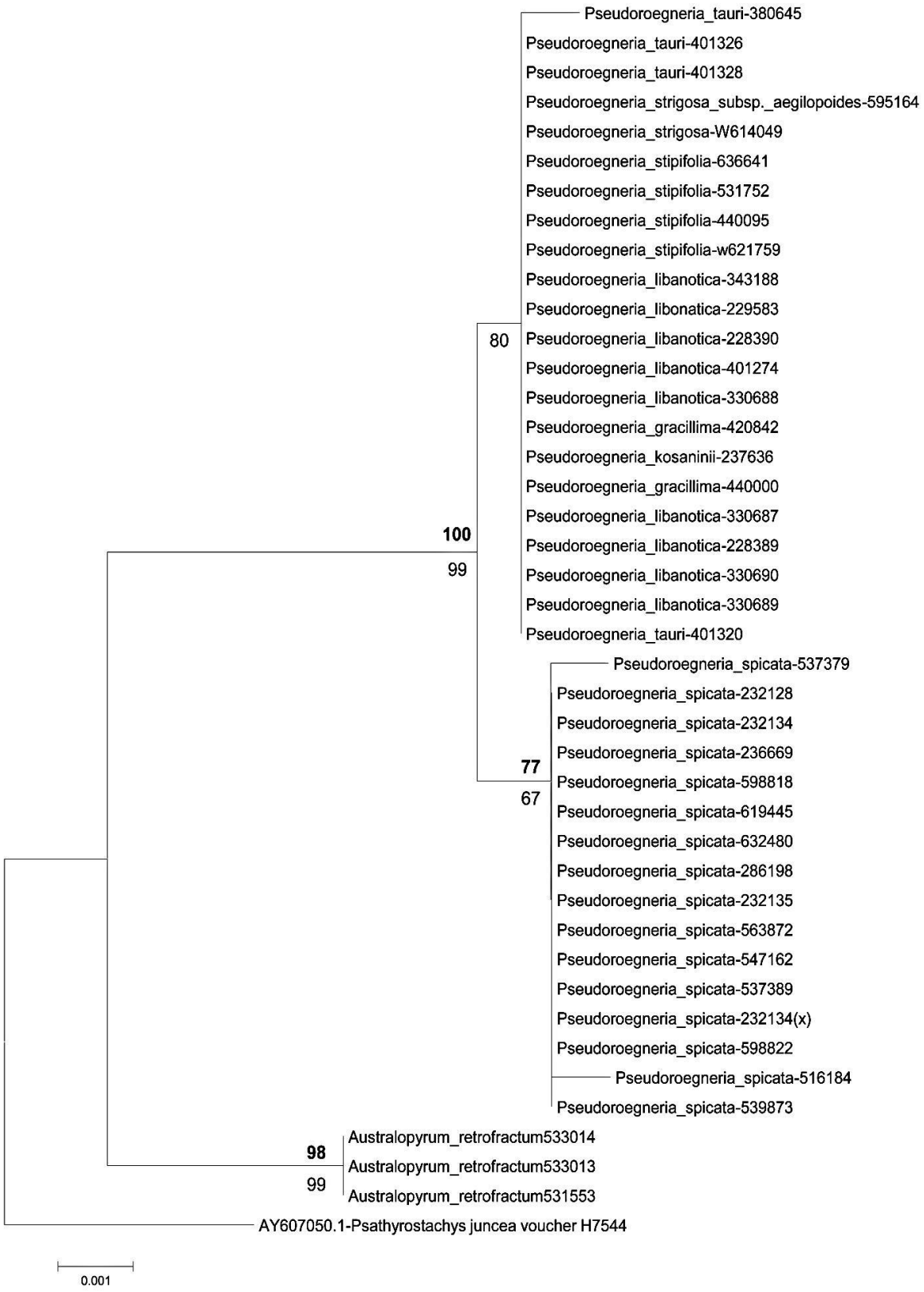
Species/Abbrv
4. Pseudoroegneria libanotica-330698
5. Pseudoroegneria libanotica-330687
6. Pseudoroegneria libanotica-401274
7. Pseudoroegneria libanotica-228339
8. Pseudoroegneria libanotica-330650
9. Pseudoroegneria libanotica-228390
10. Pseudoroegneria libonatica-229583
11. Pseudoroegneria libanotica-343188
12. Pseudoroegneria libanotica-330689
13. Pseudoroegneria spicata-232128
14. Pseudoroegneria spicata-232134
15. Pseudoroegneria spicata-236669
16. Pseudoroegneria spicata-563872
17. Pseudoroegneria spicata-632480
18. Pseudoroegneria spicata-598018
19. Pseudoroegneria spicata-547162
20. Pseudoroegneria spicata-619445
21. Pseudoroegneria spicata-516184
22. Pseudoroegneria spicata-2321134(x)
23. Pseudoroegneria spicata-232135
24. Pseudoroegneria spicata-286198
25. Pseudoroegneria spicata-537379
26. Pseudoroegneria spicata-598822
27. Pseudoroegneria spicata-537389
28. Pseudoroegneria spicata-539873
29. Pseudoroegneria stipifolia-440095
30. Pseudoroegneria stipifolia-v621759
31. Pseudoroegneria stipifolia-636641
32. Pseudoroegneria strigosa-537752
33. Pseudoroegneria strigosa-W614049
34. Pseudoroegneria strigosa subsp. aegil
35. Pseudoroegneria tauri-401328
36. Pseudoroegneria tauri-380645
37. Pseudoroegneria tauri-401320
38. Pseudoroegneria tauri-401326
39. Australopyrum retrofractum53013
40. Australopyrum retrofractum51553
41. Australopyrum retrofractum53014

3.2 Phylogenetic Analysis of *Pseudoroegneria*

The MP consensus tree contained 1179 characters of which 13 were informative for parsimony analysis. The consensus tree was inferred from 22 parsimonious trees with a consistency index (CI) of 0.955, and a retention index (RI) of 0.983. The topologies of the MP and NJ trees were identical, but they varied in their BS values. Due to their similar topologies, only the NJ tree was included in this analysis while the BS values of the MP tree were indicated above the branches. The NJ BS values were recorded below the branches, to highlight the variation in robustness between the different methods (Figure 3).

The phylogenetic analysis placed all the *P. spicata* accessions into a well-supported monophyletic clade. The remaining species formed a clade with no discernibility between the different species, while the three *Australopyrum* accessions grouped separately from the entire *Pseudoroegneria* genus (Figure 3).

Figure 3: Phylogenetic tree derived from the *rbcL* sequence using the NJ method for the 38 *Pseudoroegneria* accessions and 3 *Australopyrum* accession. Bootstrap (BS) values based on 1000 replicates are recorded below the branches. Numbers in bold above the branches are BS values from the topologically similar MP tree. *Psathyrostachys juncea* was used as the outgroup.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Pseudoroegneria_tauri-401326
Pseudoroegneria_tauri-401328
Pseudoroegneria_strigosa_subsp._aegilopoides-595164
Pseudoroegneria_strigosa-W614049
Pseudoroegneria_stipifolia-636641
Pseudoroegneria_stipifolia-531752
Pseudoroegneria_stipifolia-440095
Pseudoroegneria_stipifolia-w621759
Pseudoroegneria_libanotica-343188
Pseudoroegneria_libonatica-229583
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Pseudoroegneria_libanotica-228390
Pseudoroegneria_libanotica-401274
Pseudoroegneria_libanotica-330688
Pseudoroegneria_gracillima-420842
Pseudoroegneria_kosaninii-237636
Pseudoroegneria_gracillima-440000
Pseudoroegneria_libanotica-330687
100
Pseudoroegneria_libanotica-228389
99
Pseudoroegneria_libanotica-330690
Pseudoroegneria_libanotica-330689
Pseudoroegneria_tauri-401320
Pseudoroegneria_spicata-537379
Pseudoroegneria_spicata-232128
Pseudoroegneria_spicata-232134
Pseudoroegneria_spicata-236669
77
Pseudoroegneria_spicata-598818
67
Pseudoroegneria_spicata-619445
Pseudoroegneria_spicata-632480
Pseudoroegneria_spicata-286198
Pseudoroegneria_spicata-232135
Pseudoroegneria_spicata-563872
Pseudoroegneria_spicata-547162
Pseudoroegneria_spicata-537389
Pseudoroegneria_spicata-232134(x)
Pseudoroegneria_spicata-598822
Pseudoroegneria_spicata-516184
Pseudoroegneria_spicata-539873
Australopyrum_retrofractum533014
98
Australopyrum_retrofractum533013
99
Australopyrum_retrofractum531553
AY607050.1-Psathyrostachys juncea voucher H7544
0.001

3.3 Phylogenetic Analysis of the Triticeae

The MP consensus tree contained 1179 characters of which 36 were informative for parsimony analysis. The consensus tree was inferred from 114 parsimonious trees with a consistency index (CI) of 0.746, and a retention index (RI) of 0.955. The topologies for the NJ consensus trees (Figure 4) and the MP trees (Figure 5) were similar, yet contained several notable differences and as such both were included in this section. It was noted that the MP and NJ trees constructed using the MEGA or PAUP interface contained slight difference in BS values, as such the BS values for PAUP are indicated in bold above the branches, while the MEGA BS values are reported below the branches (Figures 4 and 5). The small number of topological differences created due to the use of the different programs will also be mentioned.

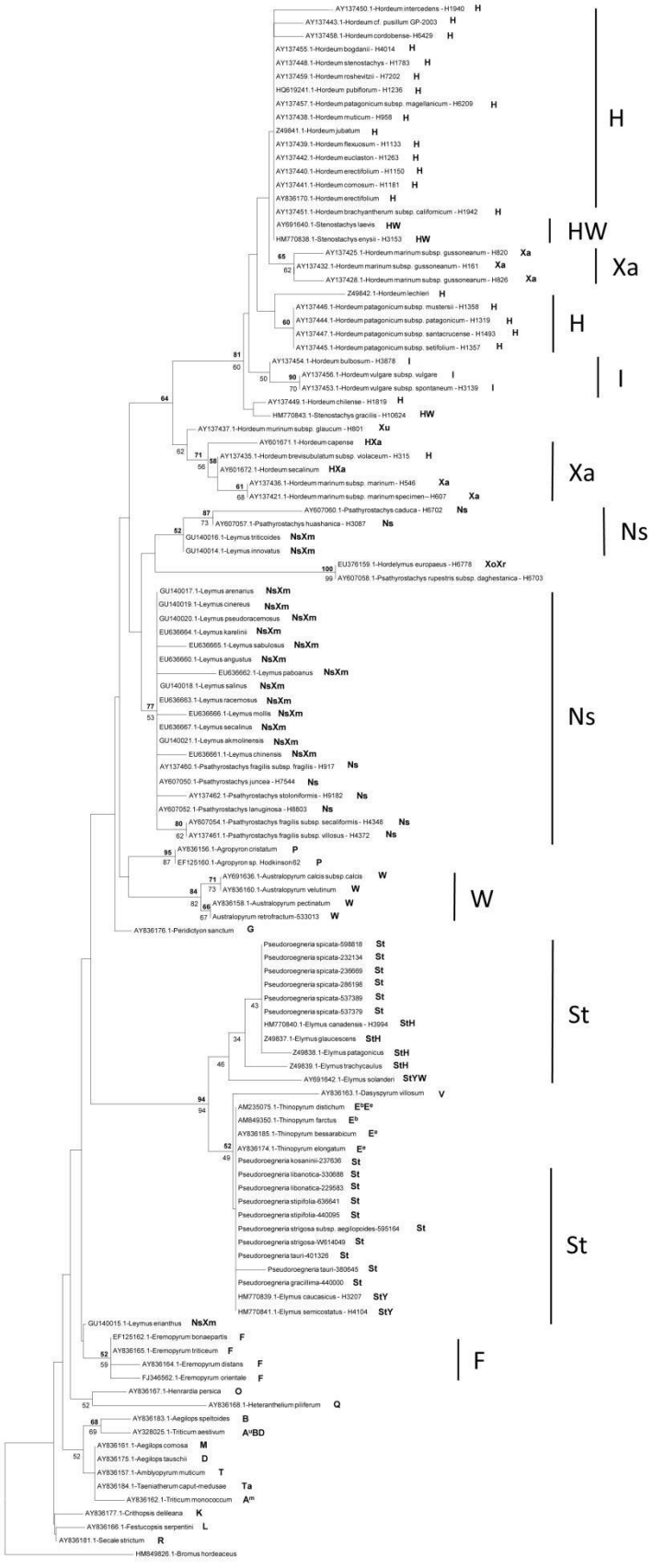
In both the NJ and MP trees (Figures 4 and 5) *Pseudoroegneria* and *Elymus* form a well-supported clade, which also includes species from the *Thinopyrum* and *Daspyrum* genera. In the NJ tree (Figure 4) there is weak support for the separation of a separate sub-clade with *P. spicata*, the StH *Elymus* species and *E. solanderi* from the rest of the clade. The PAUP NJ tree did however also group *Daspyrum* and *Thinopyrum elongatum* into this clade, while the MP tree did not show any separation at all (Figure 5).

All the species of *Hordeum* formed a distinct clade, which contained only one other genera, *Stenostachys* (Figures 4 and 5). In the NJ tree (Figure 4) the majority of the H genome species grouped together with the HW species with the exception of *Stenostachys gracilis*, which grouped with *Hordeum chilense* (H) in its own clade. The *H. marnium subsp. gussoneanum* (Xa) and all of the I genomes formed a larger group with

the H and HW genomes. The remainder of the *Hordeum* formed a separate clade which included the one Xu genome (*H. murinum*), the HXa genomes, the *H. marinum subsp. marinum* (Xa) and one H genome species (*H. brevisubulatum*). In the MP tree's (Figure 5) there were only two major clades, one contained the H,I, Xa (*subsp. gussoneanum),* and HW species, while the second contained Xu, Xa (*subsp. marinum),* one H (*H. brevisubulatum*) and HXa species.

In both the NJ and MP tree's (Figures 4 and 5) all the *Psathyrostachys* and *Leymus* species formed a single clade, with the exception that *Psathyrostachys rupestris* and *Hordelymus* formed a separate well supported clade. As well, *Psathyrostachys caduca, Psathyrostachys huashanica, Leymus triticoides, Leymus innovatus* and *Leymus erianthus* did not group together in the larger *Psathyrostachys –Leymus clade*. There was strong support in all analysis for the grouping of the two P genome species, grouping of the W genome species, and some weak support for the grouping of the F genomes. There was moderate support for the grouping of *Aegilops speltoides* and *Triticum aestivium*.

Figure 4: Phylogenetic tree derived from the *rbcL* sequence using the NJ method for the 114 Triticeae accessions. BS values from the MEGA program based on 1000 replicates are recorded below the branches. Numbers in bold above the branches, are BS values from the topologically similar NJ tree produced in the PAUP program. Genomic designations are indicated to the right of accession numbers. *Bromus hordeaceus* was used as the outgroup.
.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AY137443.1-Hordeum cf. pusillum GP-2003 H
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AY137455.1-Hordeum bogdanii - H4014 H
AY137448.1-Hordeum stenostachys - H1783 H
AY137459.1-Hordeum roshevitzii - H7202 H
HQ619241.1-Hordeum pubiflorum - H1236 H
AY137457.1-Hordeum patagonicum subsp. magellanicum - H5209 H
AY137438.1-Hordeum muticum - H958 H
Z49841.1-Hordeum jubatum H
AY137439.1-Hordeum flexuosum - H1133 H
AY137442.1-Hordeum euclaston - H1263 H
AY137440.1-Hordeum erectifolium - H1150 H
AY137441.1-Hordeum comosum - H1181 H
AY836170.1-Hordeum erectifolium H
AY137451.1-Hordeum brachyantherum subsp. californicum - H1942 H
AY691640.1-Stenostachys laevis HW
HM770838.1-Stenostachys enysii - H3153 HW
AY137425.1-Hordeum marinum subsp. gussoneanum - H820 Xa
AY137432.1-Hordeum marinum subsp. gussoneanum - H161 Xa
AY137428.1-Hordeum marinum subsp. gussoneanum - H826 Xa
Z49842.1-Hordeum lechleri H
AY137446.1-Hordeum patagonicum subsp. mustersii - H1358 H
AY137444.1-Hordeum patagonicum subsp. patagonicum - H1319 H
AY137447.1-Hordeum patagonicum subsp. santacrucense - H1493 H
AY137445.1-Hordeum patagonicum subsp. setifolium - H1357 H
AY137454.1-Hordeum bulbosum - H3878 I
AY137456.1-Hordeum vulgare subsp. vulgare I
AY137453.1-Hordeum vulgare subsp. spontaneum - H3139 I
AY137449.1-Hordeum chilense - H1819 H
HM770843.1-Stenostachys gracilis - H10624 HW
AY137437.1-Hordeum murinum subsp. glaucum - H801 Xu
AY601671.1-Hordeum capense HXa
AY137435.1-Hordeum brevisubulatum subsp. violaceum - H315 H
AY601672.1-Hordeum secalinum HXa
AY137436.1-Hordeum marinum subsp. marinum - H546 Xa
AY137421.1-Hordeum marinum subsp. marinum specimen - H607 Xa
AY607060.1-Psathyrostachys caduca - H6702 Ns
AY607057.1-Psathyrostachys huashanica - H3087 Ns
GU140016.1-Leymus triticoides NsXm
GU140014.1-Leymus innovatus NsXm
EU376159.1-Hordelymus europaeus - H6778 XoXr
AY607058.1-Psathyrostachys rupestris subsp. daghestanica - H6703
GU140017.1-Leymus arenarius NsXm
GU140019.1-Leymus cinereus NsXm
GU140020.1-Leymus pseudoracemosus NsXm
EU636664.1-Leymus karelinii NsXm
EU636665.1-Leymus sabulosus NsXm
EU636660.1-Leymus angustus NsXm
EU636662.1-Leymus paboanus NsXm
GU140018.1-Leymus salinus NsXm
EU636663.1-Leymus racemosus NsXm
EU636666.1-Leymus mollis NsXm
EU636667.1-Leymus secalinus NsXm
GU140021.1-Leymus akmolinensis NsXm
EU636661.1-Leymus chinensis NsXm
AY137460.1-Psathyrostachys fragilis subsp. fragilis - H917 Ns
AY607050.1-Psathyrostachys juncea - H7544 Ns
AY137462.1-Psathyrostachys stoloniformis - H9182 Ns
AY607052.1-Psathyrostachys lanuginosa - H8803 Ns
AY607054.1-Psathyrostachys fragilis subsp. secaliformis - H4348 Ns
AY137461.1-Psathyrostachys fragilis subsp. villosus - H4372 Ns
AY836156.1-Agropyron cristatum P
EF125160.1-Agropyron sp. Hodkinson62 P
AY691636.1-Australopyrum calcis subsp.calcis W
AY836160.1-Australopyrum velutinum W
AY836158.1-Australopyrum pectinatum W
Australopyrum retrofractum-533013 W
AY836176.1-Peridictyon sanctum G
Pseudoroegneria spicata-598618 St
Pseudoroegneria spicata-232134 St
Pseudoroegneria spicata-236669 St
Pseudoroegneria spicata-286198 St
Pseudoroegneria spicata-537389 St
Pseudoroegneria spicata-537379 St
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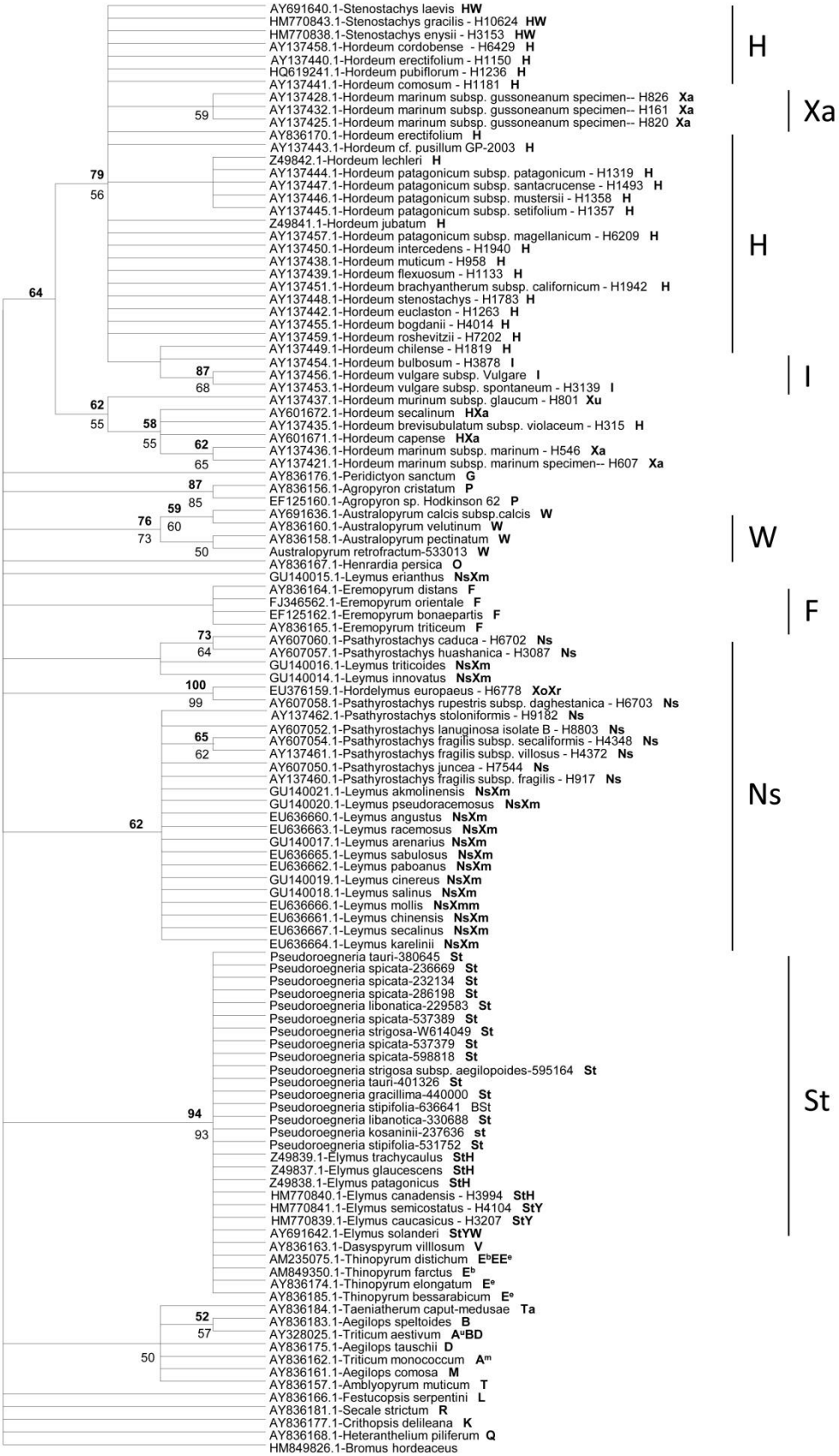
Z49837.1-Elymus glaucescens StH
Z49838.1-Elymus patagonicus StH
Z49839.1-Elymus trachycaulus StH
AY691642.1-Elymus solanderi StYW
AY836163.1-Dasyspyrum villosum V
AM235075.1-Thinopyrum distichum E^bE^e
AM849350.1-Thinopyrum farctus E^b
AY836185.1-Thinopyrum bessarabicum E^e
AY836174.1-Thinopyrum elongatum E^e
Pseudoroegneria kosaninii-237636 St
Pseudoroegneria libanotica-330688 St
Pseudoroegneria libanotica-229583 St
Pseudoroegneria stipifolia-636641 St
Pseudoroegneria stipifolia-440095 St
Pseudoroegneria strigosa subsp. aegilopoides-598164 St
Pseudoroegneria strigosa-W514049 St
Pseudoroegneria tauri-401326 St
Pseudoroegneria tauri-380645 St
Pseudoroegneria gracilima-440000 St
HM770839.1-Elymus caucasicus - H3207 StY
HM770841.1-Elymus semicostatus - H4104 StY
GU140015.1-Leymus erianthus NsXm
EF125162.1-Eremopyrum bonaepartis F
AY836165.1-Eremopyrum triticeum F
AY836164.1-Eremopyrum distans F
FJ346562.1-Eremopyrum orientale F
AY836167.1-Henrardia persica O
AY836168.1-Heteranthelium piliferum Q
AY836183.1-Aegilops speltoides B
AY328025.1-Triticum aestivum A^uBD
AY836161.1-Aegilops comosa M
AY836175.1-Aegilops tauschii D
AY836157.1-Amblyopyrum muticum T
AY836184.1-Taeniatherum caput-medusae Ta
AY836162.1-Triticum monococcum A^m
AY836177.1-Crithopsis delileana K
AY836166.1-Festucopsis serpentini L
AY836181.1-Secale strictum R
HM849826.1-Bromus hordeaceus
H
HW
Xa
H
I
Xa
Ns
Ns
W
St
St
F

Figure 5: One of the 114 most parsimonious trees derived from the *rbcL* sequence using the MP method for the 114 Triticeae accessions (CI=0.746, RI= 0.955). BS values from the MEGA program based on 1000 replicates are recorded below the branches. Numbers in bold above the branches, are BS values from the topologically similar MP tree produced in the PAUP program. Genomic designations are indicated to the right of accession numbers. *Bromus hordeaceus* was used as the outgroup.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HM770838.1-Stenostachys enysii - H3153 HW
AY137458.1-Hordeum cordobense - H6429 H
AY137440.1-Hordeum erectifolium - H1150 H
HQ619241.1-Hordeum pubiflorum - H1236 H
AY137441.1-Hordeum comosum - H1181 H
AY137428.1-Hordeum marinum subsp. gussoneanum specimen-- H826 Xa
AY137432.1-Hordeum marinum subsp. gussoneanum specimen-- H161 Xa
AY137425.1-Hordeum marinum subsp. gussoneanum specimen-- H820 Xa
AY836170.1-Hordeum erectifolium H
AY137443.1-Hordeum cf. pusillum GP-2003 H
Z49842.1-Hordeum lechleri H
AY137444.1-Hordeum patagonicum subsp. patagonicum - H1319 H
AY137447.1-Hordeum patagonicum subsp. santacrucense - H1493 H
AY137446.1-Hordeum patagonicum subsp. mustersii - H1358 H
AY137445.1-Hordeum patagonicum subsp. setifolium - H1357 H
Z49841.1-Hordeum jubatum H
AY137457.1-Hordeum patagonicum subsp. magellanicum - H6209 H
AY137450.1-Hordeum intercedens - H1940 H
AY137438.1-Hordeum muticum - H958 H
AY137439.1-Hordeum flexuosum - H1133 H
AY137451.1-Hordeum brachyantherum subsp. californicum - H1942 H
AY137448.1-Hordeum stenostachys - H1783 H
AY137442.1-Hordeum euclaston - H1263 H
AY137455.1-Hordeum bogdanii - H4014 H
AY137459.1-Hordeum roshevitzii - H7202 H
AY137449.1-Hordeum chilense - H1819 H
AY137454.1-Hordeum bulbosum - H3878 I
AY137456.1-Hordeum vulgare subsp. Vulgare I
AY137453.1-Hordeum vulgare subsp. spontaneum - H3139 I
AY137437.1-Hordeum murinum subsp. glaucum - H801 Xu
AY601672.1-Hordeum secalinum HXa
AY137435.1-Hordeum brevisubulatum subsp. violaceum - H315 H
AY601671.1-Hordeum capense HXa
AY137436.1-Hordeum marinum subsp. marinum - H546 Xa
AY137421.1-Hordeum marinum subsp. marinum specimen-- H607 Xa
AY836176.1-Peridictyon sanctum G
AY836156.1-Agropyron cristatum P
EF125160.1-Agropyron sp. Hodkinson 62 P
AY691636.1-Australopyrum calcis subsp.calcis W
AY836160.1-Australopyrum velutinum W
AY836158.1-Australopyrum pectinatum W
Australopyrum retrofractum-533013 W
AY836167.1-Henrardia persica O
GU140015.1-Leymus erianthus NsXm
AY836164.1-Eremopyrum distans F
FJ346562.1-Eremopyrum orientale F
EF125162.1-Eremopyrum bonaepartis F
AY836165.1-Eremopyrum triticeum F
AY607060.1-Psathyrostachys caduca - H6702 Ns
AY607057.1-Psathyrostachys huashanica - H3087 Ns
GU140016.1-Leymus triticoides NsXm
GU140014.1-Leymus innovatus NsXm
EU376159.1-Hordelymus europaeus - H6778 XoXr
AY607058.1-Psathyrostachys rupestris subsp. daghestanica - H6703 Ns
AY137462.1-Psathyrostachys stoloniformis - H9182 Ns
AY607052.1-Psathyrostachys lanuginosa isolate B - H8803 Ns
AY607054.1-Psathyrostachys fragilis subsp. secaliformis - H4348 Ns
AY137461.1-Psathyrostachys fragilis subsp. villosus - H4372 Ns
AY607050.1-Psathyrostachys juncea - H7544 Ns
AY137460.1-Psathyrostachys fragilis subsp. fragilis - H917 Ns
GU140021.1-Leymus akmolinensis NsXm
GU140020.1-Leymus pseudoracemosus NsXm
EU636660.1-Leymus angustus NsXm
EU636663.1-Leymus racemosus NsXm
GU140017.1-Leymus arenarius NsXm
EU636665.1-Leymus sabulosus NsXm
EU636662.1-Leymus paboanus NsXm
GU140019.1-Leymus cinereus NsXm
GU140018.1-Leymus salinus NsXm
EU636666.1-Leymus mollis NsXmm
EU636661.1-Leymus chinensis NsXm
EU636667.1-Leymus secalinus NsXm
EU636664.1-Leymus karelinii NsXm
Pseudoroegneria tauri-380645 St
Pseudoroegneria spicata-236669 St
Pseudoroegneria spicata-232134 St
Pseudoroegneria spicata-286198 St
Pseudoroegneria libonatica-229583 St
Pseudoroegneria spicata-537389 St
Pseudoroegneria strigosa-W614049 St
Pseudoroegneria spicata-537379 St
Pseudoroegneria spicata-598818 St
Pseudoroegneria strigosa subsp. aegilopoides-595164 St
Pseudoroegneria tauri-401326 St
Pseudoroegneria gracillima-440000 St
Pseudoroegneria stipifolia-636641 BSt
Pseudoroegneria libanotica-330688 St
Pseudoroegneria kosaninii-237636 st
Pseudoroegneria stipifolia-531752 St
Z49839.1-Elymus trachycaulus StH
Z49837.1-Elymus glaucescens StH
Z49838.1-Elymus patagonicus StH
HM770840.1-Elymus canadensis - H3994 StH
HM770841.1-Elymus semicostatus - H4104 StY
HM770839.1-Elymus caucasicus - H3207 StY
AY691642.1-Elymus solanderi StYW
AY836163.1-Dasyspyrum villosum V
AM235075.1-Thinopyrum distichum EbEEe
AM849350.1-Thinopyrum farctus Eb
AY836174.1-Thinopyrum elongatum Ee
AY836185.1-Thinopyrum bessarabicum Ee
AY836184.1-Taeniatherum caput-medusae Ta
AY836183.1-Aegilops speltoides B
AY328025.1-Triticum aestivum AuBD
AY836175.1-Aegilops tauschii D
AY836162.1-Triticum monococcum Am
AY836161.1-Aegilops comosa M
AY836157.1-Amblyopyrum muticum T
AY836166.1-Festucopsis serpentini L
AY836181.1-Secale strictum R
AY836177.1-Crithopsis delileana K
AY836168.1-Heteranthelium piliferum Q
HM849826.1-Bromus hordeaceus
H
Xa
H
I
W
F
Ns
St

4. Discussion

4.1- Nucleotide Diversity

Low rates of nucleotide diversity in our study (Table 3) conformed well to our predictions, as these were closely related taxa and the *rbcL* gene is known to be highly conserved (Doebley et al., 1990; Gielly & Taberlet, 1994; Olmstead & Palmer, 1994; Sun, 2007). The inter- and intra-species genetic diversity from our study displayed many differences to a similar study by Yan & Sun (2011), who examined the nucleotide diversity using the *TrnD/T* chloroplast intergenic region, as well as two nuclear genes. Yan & Sun found that the species with the most nucleotide diversity was *P. spicata* (*TrnD/T*, $\pi = 0.03743$, $\theta=0.04811$) followed by *P. gracillima* (*TrnD/T*, $\pi = 0.02466$, $\theta= 0.02466$), while the least nucleotide diversity was in *P. tauri* (TrnD/T, $\pi = 0.00183$, $\theta= 0.00183$). In contrast, our results indicated that *P. tauri* ($\pi=0.0046$, $\theta=0.00536$) had the highest nucleotide diversity while *P. gracillima* had the lowest pairwise diversity ($\pi=0.00227$) and the second lowest segregating site diversity ($\theta=0.00227$) within the *Pseudoroegneria* (Table 3). *P. spicata* did have the second highest nucleotide diversity ($\pi=0.00369$, $\theta=0.00389$) in our study which is congruent with the high nucleotide diversity seen by Yan & Sun (2011), as well as the high genetic diversity seen in previous studies (Fu & Thompson, 2006; Larson et al., 2000, 2004). This discrepancy between our findings and that of Yan & Sun may be due to the small number of *P. tauri*, and *P. gracillima* accessions used in both the Yan & Sun analysis and in our study. Our study used only four accessions of *P. tauri* and two of *P. gracillima*, while Yan & Sun used two accessions for both *P. tauri and P. gracilima.* This small sample size may have caused biases between the species analyzed, leading to the discrepancies of genetic diversity

between our studies. Future studies with more samples could help better estimate the genetic diversity between *Pseudoroegneria* taxa.

4.2- Pseudoroegneria Phylogeny

The low number of nucleotide polymorphisms of the *rbcL* gene within the *Pseudoroegneria* genus did not allow for any differentiation between the majority of the species in this study (Figure 3). There was, however, strong support for the grouping of all the *P. spicata* sequences into their own monophyletic group, as was predicted would occur. This separation of *P. spicata* has also been reported with the *TrnT/F*, *TrnD/T*, and restriction sites of cpDNA studies (Mason-Gamer et al., 2002; Yan & Sun, 2011). This relationship was, however, not observed in the *RpoA* and *TrnL/F* gene trees (Liu et al., 2006; Mason-Gamer et al., 2002), as the *P. spicata* in both studies both grouped with *P. libanotica*. This being said, both studies used only a small number of species and samples in their analysis. When the *RpoA* tree was combined with the *TrnT/F* and restriction site tree, the separation of *P. spicata* again was seen (Mason-Gamer et al., 2002). The Mason-Gamer et al. (2002) study was, however, not able to provide a thorough analysis of the genus as they used only three different species, two of which were represented by only one sample. The Yan and Sun (2011) study on the *TrnD/T*, which used many species and multiple samples, did find that all the *P. spicata* species grouped together with one exception; the *P. spicata* accession PI 563872 formed a weakly supported group with *Pseudoroegneria strigosa subsp. aegilopoides* and not with the other *P. spicata*. Our study also included the same accession of *P. spicata*, however, we did not observe this same relationship as all our *P. spicata* grouped together (Figure 3). The distinct separation of *P. spicata* from the other *Pseudoroegneria* species may be largely due to the

geographic separation of *P. spicata* from the other *Pseudoroegneria* species, as *P. spicata* is the only N. American species while other species are found in parts of Asia, the Middle East and Europe (Fu & Thompson, 2006; Larson et al., 2004; Yan & Sun, 2011; Yu et al., 2008).

4.3-Triticeae Phylogeny

The nDNA gene trees of the diploid Triticeae have produced numerous discrepancies, while studies using cpDNA have been slightly more congruent (Kellogg, Appels, & Mason-Gamer, 1996; Mason-Gamer et al., 2002; Petersen & Seberg, 1997; Petersen et al., 2011; Wang et al., 2011). Overall our *rbcL* gene trees (Figures 4 and 5) were similar to that of previous cpDNA studies, indicating that the *rbcL* gene can be used at lower taxonomic levels than previously thought (Doebley et al., 1990; Escobar et al., 2011; Petersen & Seberg, 1997; Petersen et al., 2011).

The topology of the *Hordeum/Stenostachys* clade in our tree was highly congruent between previous cpDNA studies on *Hordeum* (Jakob & Blattner, 2006; Nishikawa et al., 2002; Petersen & Seberg, 2003). The one exception was that *H. murinum* did not group with *H. marinum marinum* in the *matK, atpB-rbcL, trnL/F* combined tree (Nishikawa et al., 2002) as was observed in our data (Figures 4 and 5) and in previous studies. The four genomes of Hordeum H, I Xa and Xu displayed similar phylogenies to previous studies, as the H and Xa genomes were paraphyletic while the I genome was monophyletic (Jakob & Blattner, 2006; Nishikawa et al., 2002; Petersen & Seberg, 2003). The phylogeny of Xu could not be examined as there was only one sample. Our results support previous studies which have indicated that *Hordeum* is the maternal genome donor of

Stenostachys, and are in agreement with the recent reclassification of *S. enysii* from *Elymus* to *Stenostachys* (Barkworth & Jacobs, 2011; Petersen et al., 2011).

The highly supported clade of *Hordelymus* and *Psathyrostachys* (Figure 4 and 5) is consistent with a previous study by Petersen & Seberg (2008). Using a combined *ndhF* and *rbcL* tree, Petersen & Seberg found that *Hordelymus* formed a sister group to *P. stolonformis* and *P. fragilis*, while *P. rupestris* was not included in the study. In contrast, our study found *P. rupestris* formed a clade with *Hordelymus* while *P. stolonformis* and *P. fragilis* grouped with the other *Psathyrostachys* and *Leymus* as a weakly supported sister group (Figure 4). The association between *Hordelymus* and *Psathyrostachys* is believed to be indicative of a species ancestral to *Psathyrostachys* being the maternal genome donor of *Hordelymus* (Petersen & Seberg, 2008).

The majority of the *Psathyrostachys* and *Leymus* species formed a weakly supported clade (Figures 4 and 5), while the species that did not group within the large clade, formed a separate very weakly supported clade (Figures 4 and 5). These findings are consistent with previous studies and indicates that *Psathyrostachys* may be the maternal genome donor of *Leymus*, and that there may be multiple origins of *Leymus* (Liu et al., 2008; Zhou, Yang, Li, & Li, 2010).

Past studies have found that *Eremopyrum*, *Australopyrum*, and *Agropyron* have tended to group together in cpDNA gene trees, indicating the close relationships between these genera (Escobar et al., 2011; Hodge et al., 2010; Mason-Gamer et al., 2002; Petersen et al., 2006, 2011; Petersen & Seberg, 1997, 2008). Our analyses did not result in this pattern as *Eremopyrum*, *Australopyrum*, and *Agropyron* each formed their own

monophyletic clade (Figure 4 and 5). Additionally, the majority of theses studies found *Henrardia* and *Peridictyon* genera to be sister clades of the *Eremopyrum-Australopyrum-Agropyron* clade. From our *rbcL* gene trees we did not observe this relationship, as *P. sanctum,*and *H. persica* had no support as sister groups to either of the *Eremopyrum, Australopyrum,* or *Agropyron* clades (Figure 4 and 5). These discrepancies may be due to the low evolutionary rate of the rbcL gene, and that many of these studies used combined gene trees to increase their resolution and significance (Doebley et al., 1990; Escobar et al., 2011; Mason-Gamer et al., 2002; Petersen et al., 2011; Petersen & Seberg, 2008).

Our weakly supported *Aegilops-Triticum* clade (Figures 4 and 5) was consistent with previous studies which have found that *Aegilops* and *Triticum* form a paraphyletic clade with *Amblyopyrum*, and *Taeniatherum caput medusae* genera (Escobar et al., 2011; Hodge et al., 2010; Mason-Gamer et al., 2002; Petersen et al., 2006, 2011; Petersen & Seberg, 2008). These studies have also indicated that *Heteranthelium* and *Secale* tend to be included in this clade, which was not observed in our tree (Figures 4 and 5). The weakly supported sub-clade of *A. speltoides* and *T. aestivum* indicate that *A. speltoides* may be the maternal donor to *T. aestivum*. A study by Petersen et al. (2006) using the *ndhF* cpDNA gene found that *A. speltoides* formed a sub-clade with the $A^{u}BD$ *Triticum* species. This is consistent with our tree (Figures 4 and 5) and indicates that *A. speltoides* may be the maternal donor for the $A^{u}BD$ *Triticum* species.

4.4-*Pseudoroegneria* Phylogeny within the Triticeae

The position of *Pseudoroegneria* within the Triticeae *rbcL* gene tree is, in general, consistent with previous studies. As was predicted, the *Pseudoroegneria* species grouped with the *Elymus* species (Figures 4 and 5). This grouping of *Elymus* and *Pseudoroegneria* has been reported in many cpDNA studies, and indicates that *Pseudoroegneria* may be the maternal genome donor to *Elymus* (Hodge et al., 2010; Liu et al., 2006; Mason-Gamer et al., 2002; McMillan & Sun, 2004; Petersen et al., 2011; Redinbaugh et al., 2000; Sun, 2007; Xu & Ban, 2004). We can use cpDNA to predict the maternal genome donor of polyploidy species as only the haplome from the maternal plant species in the polyploid species is observed in cpDNA trees. Thus, close association of polyploidy species with diploid species can be used to infer the maternal genome donor. As *Thinopyrum* and *Dasyspyrum* species were also found in the *Pseudoroegneria* paraphyly, we cannot rule out either as a possible maternal donor to *Elymus* (Figure 4 and 5), however, studies on nuclear genes and *ITS* data have indicated that *Pseudoroegneria* and either *Hordeum, Agropyron, or Australopyrum* (depending on the *Elymus* species) are the paternal genome donors to *Elymus*, while there does not appear to be any additional support for either *Thinopyrum* or *Dasyspyrum* as a genome donor to *Elymus* (Liu et al., 2006; Mason-Gamer, 2001; Mason-Gamer et al., 2010; Sun et al., 2007; Yan et al., 2011).

Studies on other St polyploids, such as *Douglasdeweya* (StP) and *Elytrigia* (StE^b) have indicated that there is a preference for the *Pseudoroegneria* St Genome to be the maternal genome donor (Redinbaugh et al., 2000; Yu et al., 2010). Our study contained only one St polyploid genera, *Elymus*, which contained an H, Y, W, or P genomes, as well as the St genome. All the *Elymus* species grouped with the St and not the H, W or P

containing species (Figures 4 and 5), this does lend some support to the notion of St predominance as maternal donor. This St predominance could indicate that the St chloroplast genome provides some advantage over the chloroplast genomes of the other genera, however, this is only speculative and requires further study (Redinbaugh et al., 2000).

As the *P. spicata* had formed a separate sub-clade in the *Pseudoroegneria* tree (Figure 3), there was also weak support in the Triticeae NJ trees (Figure 4), for a similar separation of *P. spicata*. This sub-clade with *P. spicata* also contained the StH *Elymus* species, and *E. solandri* (StYW), which may indicate that *P. spicata* is the maternal genome donor for the StH *Elymus* species and *E. solandri*, while one or more of the other *Pseudoroegneria* species may be the maternal donor for the StY *Elymus* species. Several previous studies lend support to our findings as studies on the *pep-C, ß- amylase, GBSSI, DMC1, EF-G,* and *RPB-2* nuclear genes have found *P. spicata* to be more closely related to the StH *Elymus* than any other *Pseudoroegneria* species (Mason-Gamer et al., 2010; Sun et al., 2007; Petersen et al., 2011; Yan et al., 2011). In most cpDNA studies the relationship of *P. spicata* with the StH *Elymus* have not been observed, typically due to poor resolution of the trees, as was observed in Figure 5 (Mason-Gamer et al., 2002; McMillan & Sun, 2004; Wang et al., 2011). When the cpDNA genes trees for *rbcL, rpoA, ndhF, matK*, and the mtDNA gene *coxII* were combined with two nuclear gene (*DMC1* and *EF-G*) the *P. spicata* were more closely associated to StH *Elymus* (Petersen et al., 2011). The geographic origin of the StH *Elymus* species also supports our data as the StH *Elymus* species are believed to have originated in N. America and *P. spicata* is the only known N. American *Pseudoroegneria* species (Mason-Gamer et al., 2010). In both nDNA

and cpDNA studies there is indication that there are many species of *Pseudoroegneria* that may have contributed the St genome to StY *Elymus* species, including *P. spicata* (Hodge et al., 2010; Liu et al., 2006; Mason-Gamer et al., 2010; Petersen et al., 2011;Sun & Komatsuda, 2010; Yan, et al, 2011). This is congruent with our NJ tree which has the StY *Elymus* species grouped with different *Pseudoroegneria*, while *E. solandri* (StYW) also shows some weak support for grouping within the *P. spicata* sub-clade (Figure 4).

Summary

This study has indicated that within the *Pseudoroegneria*, *P. tauri* and *P. spicata* may be the most genetically diverse species, while *P. gracillima* may be the least diverse species. We have examined the relationship within the *Pseudoroegneria* and provided strong support that *P. spicata* is more distantly related than are the other species of *Pseudoroegneria*, which is thought to be due to the geographic separation of *P. spicata.* Our *rbcL* gene tree of the Triticeae found similar topologies to that of previous cpDNA studies, indicating *rbcL* genes can be used at lower taxonomic levels. Our results strongly supported the hypothesis that *Pseudoroegneria* is the maternal genome donor of *Elymus*, and moderate support that *Hordeum* is the maternal donor in *Stenostachys*. We also provided weak support that *P. spicata* may be the maternal genome donor to the StH *Elymus* species, which has not been previously observed in cpDNA trees (not combined with nuclear genes).

The Triticeae are known to have a complex phylogenetic history, which has resulted in many incongruent gene trees. Our data supported many previous studies, while also providing support that all *P. spicata* strains are more distantly related to other

Pseudoroegneria species, including *Pseudoroegneria strigosa subsp. aegilopoides*. As well the indication that *P. spicata* is the maternal donor for the StH *Elymus* species could be used to increase the success rate of hybrid crosses. As crosses with *P. spicata* as the maternal donor, to an H genome father may be more successful than if other *Pseudoroegneria* species are used. Although this will require further testing to confirm.

Works Cited

Barkworth, M. E., & Jacobs, S. W. (2011). The Triticeae (Gramineae) in Australasia. *Telopea, 13*, 37-56.

Dewey, D. (1984). The Genomic system of classification as a guide to intergeneric hybridization with perennial Triticeae. In J. P. Gustafsen (Ed.), *Gene Manipulation in Plant Improvement* (pp. 209-279). New York, USA: Plenum Press.

Doebley, J., Durbin, M., Golenberg, E., Clegg, M., & Pow, D. (1990). Evolutionary Analysis of the Large Subunit of Carboxylase (rbcL) Nucleotide Sequence Among the Grasses (Gramineae). *Evolution, 44*, 1097-1108.

Escobar, J., Scornavacca, C., Cenci, A., Guilhaumon, C., Santoni, S., Douzery, E., . . . David, J. (2011). Multigenic phylogeny and analysis of tree incongruences in Triticeae (Poaceae). *BMC Evolutionary Biology, 11*, 181.

Felsenstein, J. (1985). Confidence limits on phylogenetics: an approach using the bootstrap. *Evolution, 39*, 783-791.

Fu, Y., & Li, W. (1993). Statistical tests of neutarilty of mutations. *Genetics, 133*, 693-709.

Fu, Y.-B., & Thompson, D. (2006). Genetic diversity of bluebunch wheatgrass (Pseudoroegneria spicata) in the Thompson River valley of British Columbia. *Canadian Journal of Botany, 84*(7), 1122-1128.

Gielly, L., & Taberlet, P. (1994). The Use of Chloroplast DNA to Resolve Plant Phylogenies: Noncoding versus rbcL Sequences. *Molecular Biology and Evolution, 11*(5), 769-777.

Higgins, D., Thompson, J., Gibson, T., Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res., 22*, 4673-4680.

Hodge, C., Wang, H., & Sun, G. (2010). Phylogenetic analysis of the maternal genome of tetraploid StStYY Elymus (Triticeae: Poaceae) species and the monogenomic Triticeae based on rps16 sequence data. *Plant Science, 178*, 463-468.

Jakob , S., & Blattner, F. (2006). A Chloroplast Genealogy of Hordeum (Poaceae): Long-Term Persisting Haplotypes, Incomplete Lineage Sorting, Regional Extinction, and the Consequences for Phylogenetic Inference. *Molecular Biology and Evolution, 23*, 1602-1612.

Kellogg, E., Appels, R., & Mason-Gamer, R. J. (1996). When Genes Tell Different Stories: The Diploid Genera of Triticeae (Gramineae). *Systematic Botany, 21*, 321-347.

Larson, S. R., Jones, T. A., Hu, Z.-M., McCracken, C. L., & Palazzo, A. (2000). Genetic Diversity of Bluebunch Wheatgrass Cultivars and a Multiple-Origin Polycross. *Crop Science, 40*(4), 1142-1147.

Larson, S. R., Jones, T. A., & Jensen, K. (2004). Population structure in Pseudoroegneria spicata (Poaceae: Triticeae) modeled by Bayesian clustering of AFLP genotypes. *American Journal of Botany, 91*(11), 1789-1801.

Liu, Q., Ge, S., Haibao, T., Zhang, X., Zhu, G., & Lu, B.-R. (2006). Phylogenetic relationships in Elymus (Poaceae: Triticeae) based on the nuclear ribosomal internal transcribed spacer and chloroplast trnL-F sequences. *New Phytologist, 170*, 411-420.

Liu , Z., Chen , Z., Pan, J., Li , X., Su, M., Wang , L., . . . Liu, G. (2008). Phylogenetic relationships in Leymus (Poaceae: Triticeae) revealed by the nuclear ribosomal internal transcribed spacer and chloroplast trnL-F sequences. *Molecular Phylogenetics and Evolution, 46*, 278–289.

Luo, X., Tinker, N., Fan, X., Zhang, H., Sha, L., Kang, H., . . . Zhou, Y. (2012). Phylogeny and maternal donor of Kengyilia species (Poaceae: Triticeae) based on three cpDNA (matK, rbcL and trnH-psbA) sequences. *Biochemical Systematics and Ecology, 44*, 61-69.

Löve, Á. (1984). Conspectus of the Triticeae. *Feddes Repert, 95*, 425-521.

Mason-Gamer, R. J. (2001). Origin of North American Elymus (Poaceae: Triticeae) Allotetraploid Based on Granule-bound Starch Synthase Gene Sequences. *Systematic Botany, 26*(4), 757-768.

Mason-Gamer, R. J., Orme, N., & Anderson, C. M. (2002). Phylogenetic analysis of North American Elymus and the monogenomic Triticeae (Poaceae) using three chloroplast DNA data sets. *Genome, 45*, 991-1002.

Mason-Gamer, R. J., Burns, M., & Naum, M. (2010). Phylogenetic relationships and reticulation among Asian Elymus (Poaceae) allotetraploids: Analyses of three nuclear gene trees. *Molecular Phylogenetics and Evolution, 54*, 10-22.

McMillan, E., & Sun, G. (2004). Genetic relationships of tetraploid Elymus species and their genomic donor species inferred from polymerase chain reaction-restriction length polymorphism analysis of chloroplast gene regions. *Theoretical and Applied Genetics, 108*, 535-542.

Nei, M., & Kumar, S. (2000). *Molecular Evolution and Phylogenetics.* New York: Oxford University Press.

Nishikawa, T., Salomon, B., Komatsuda, T., von Bothmer, R., & Kadowaki, K.-i. (2002). Molecular phylogeny of the genus Hordeum using three chloroplast DNA sequences. *Genome, 45*, 1157-1166.

Okito, P., Mott, I. W., Wu, Y., & Wang, R. R.-C. (2009). A Y genome specific STS marker in Pseudoroegneria and Elymus species (Triticeae:Gramineae). *Genome, 52*, 391-400.

Olmstead, R., & Palmer, J. (1994). Chloroplast DNA Systematics: A Review of Methods and Data Analysis. *American Journal of Botany, 81*, 1205-1224.

Petersen , G., & Seberg, O. (1997). Phylogenetic Analysis of the Triticeae (Poaceae) Based on rpoA Sequence Data. *Molecular Phylogenetics and Evolution, 7*, 217-230.

Petersen , G., & Seberg, O. (2003). Phylogenetic Analyses of the Diploid Species of Hordeum (Poaceae) and a Revised Classification of the Genus. *Systematic Botany, 28*, 293-306.

Petersen, G., Seberg , O., Yde , M., & Berthelsen, K. (2006). Phylogenetic relationships of Triticum and Aegilops and evidence for the origin of the A, B, and D genomes of common wheat. (Triticum aestivum). *Molecular Phylogenetics and Evolution, 39*, 70-82.

Petersen, G., & Seberg, O. (2008). Phylogenetic relationships of allotetraploid Hordelymus europaeus (L.) Harz (Poaceae: Triticeae). *Plant Systematics and Evolution, 273*, 87-95.

Petersen, G., Seberg, O., & Salomon, B. (2011). The origin of the H, St, W, and Y genomes in allotetraploid species of Elymus L. and Stenostachys Turcz. (Poaceae: Triticeae). *Plant Systematics and Evolution, 296*, 197-210.

Redinbaugh, M., Jones, T., & Zhang, Y. (2000). Ubiquity of the St chloroplast genome in St-containing Triticeae polyploids. *Genome, 43*, 846-852.

Rozas, J., Sánchez-DelBarrio, J. C., Messeguer, X., & Rozas, R. (2005). DNA sequence polymorphism, version 4.10.4. . In *DNAsp4 computer software.* Barcelona Spain.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*, 406-425.

Sun, G. (2002). Interspecific polymorphism at non-coding regions of chloroplast, mitochondrial DNA and rRNA IGS region in Elymus species. *Hereditas, 137*, 119-124.

Sun, G. (2007). Genetic diversity of rbcL gene in Elymus trachycaulus complex and their phylogenetic relationships to several Triticeae species. *Genetic Resources and Crop Evolution, 54*, 1737-1746.

Sun, G., Daley, T., & Ni, Y. (2007). Molecular evolution and genome divergence at RPB2 gene of the St and H genome in Elymus species. *Plant Molecular Biology, 64*, 645-655.

Sun, G., Ni, Y., & Daley, T. (2008). Molecular phylogeny of RPB2 gene reveals multiple origin, geographic differentiation of H genome, and the relationship of the Y genome to other genomes in Elymus species. *Molecular Phylogenetics and Evolution, 46*, 898-907.

Sun, G., & Salomon, B. (2009). Molecular evolution and origin of tetraploid Elymus species. *Breeding Science, 59*, 487-491.

Sun, G., & Komatsuda, T. (2010). Origin of the Y genome in Elymus and its relationship to other genomes in Triticeae based on evidence from elongation factor G (EF-G) gene sequences. *Molecular Phylogenetics and Evolution, 56*, 727-733.

Swofford, D. (2002). *Phylogenetic Analysis Using Parsimony, Version 4 beta 10 Win.* Sunderland, MA, USA: Sinauer Press.

Tajima, F. (1989). Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphisms. *Genetics, 123*, 585-595.

Talavera, G., & Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology, 56*, 564-577.

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA), 101*, 11030-11035.

Wang, R.-C., von Bothme, R., Dvorak, J., Fedak, G., Linde-Laursen, I., & Muramatsu, M. (1995). Genome symbols in the Triticeae (Poaceae). In R.-C. Wang, K. Jensen, & C. Jaussi (Eds.), *Proc. Second International Triticeae Symposium* (pp. 29-34). Logan, Utah: Forage and Range Laboratory, U.S.D.A.-A.R.S.

Wang, X.-L., Fan, X., Zhang, C., Sha, L.-N., Kang, H.-Y., Yang, R.-W., . . . Zhou, Y.-H. (2011). Phylogeny of the StH genome species in Triticeae (Poaceae) Evidence from chloroplast trnL-F and mitochondria COXII intron sequences. *Biochemical Systematics and Ecology, 39*, 758-765.

Watson, L., & Dallwitz, M. J. (1992 onwards). The Grass Genera of the World: Descriptions, Illustrations, Identifications, and Information Retrieval; including Synonyms, Morphology, Anatomy, Physiology, Phytochemistry, Cytology, Classification, Pathogens, World and Local Distribution, and References. *Version:27th June 2005*(http://delta-intkey.com).

Xu, D., & Ban, T. (2004). Phylogenetic and evolutionary relationships between Elymus humidus and other Elymus species based on sequencing of non-coding regions of cpDNA and AFLP of nuclear DNA. *Theoretical and Applied Genetics, 108*, 1443-1448.

Yan, C., & Sun, G. (2011). Nucleotide divergence and genetic relationships of Pseudoroegneria species. *Biochemical Systematics and Ecology, 39*, 309-319.

Yan, C., Sun, G., & Sun, D. (2011). Distinct Origin of the Y and St Genome in Elymus Species: Evidence from the Analysis of a Large Sample of St Genome Species Using Two Nuclear Genes. *PLoS ONE, 6*(10), e26853.

Yen, C., Yang, J., & Baum, B. (2005). Douglasdeweya: A new genus, with a new species and a new combination (Triticeae: Poaceae). *Can. J. Bot., 83*, 413-419.

Yu, H., Fan, X., Zhang, C., Ding, C., Wang, X., & Zhou, Y. (2008). Phylogenetic relationships of species in Pseudoroegneria (Poaceae: Triticeae) and related genera inferred from nuclear rDNA ITS (internal transcribed spacer) sequences. *Biologia, 63*(4), 498-505.

Yu, H., Zhang, C., Ding, C., Ma, X., & Zhou, Y. (2010). Maternal donors of polyploids in Pseudoroegneria (Poaceae: Triticeae) and related genera inferred from chloroplast trnL-F sequences. *Turkish Journal of biology, 34*, 335-342.

Zeng, J., Fan, X., Zhang, L., Wang, X., Zhang, H., Kang, H., & Zhou, Y. (2010). Molecular phylogeny and maternal progenitor implication in the genus Kengyilia (Triticeae: Poaceae): Evidence from COXII intron sequences. *Biochemical Systematics and Ecology, 38*, 202-209.

Zhou, X., Yang, X., Li, X., & Li, L. (2010). Genome origins in Leymus (Poaceae: Triticeae): evidence of maternal and paternal progenitors and implications for reticulate evolution. *Plant Systematics & Evolution, 289*, 165-179.