Origin and Evolutionary Dynamic of *Elymus ciliaris*, *E. pendulinus* and *E. longearistatus*

(Triticeae: Poaceae)

by

Qianni Hu

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Approved:	Dr. Genlou Sun Supervisor Department of Biology
Approved:	Dr. Sean Myles External Examiner Department of Plant & Animal Sciences, Agricultural Campus Dalhousie University
Approved:	Dr. Ronald Russell Supervisory Committee Member Department of Biology
Approved:	Dr. Pierre Jutras Supervisory Committee Member Department of Geology
Approved:	Dr. Hai Wang Graduate Studies Representative
Date:	August 20, 2013

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Abstract

Evidence accumulated over the last decade has shown that allopolyploid genomes may undergo complex reticulate evolution. *Elymus*, a genus with rampant interspecific hybridization, is an ideal model for examining the impact of gene introgression and polyploidization on species diversification. Although five basic genomes (St, H, Y, P and W) have so far been identified in species of the genus *Elymus*, the origin of the Y genome in species with a StY genome is still unknown and under debate. Previous studies suggested that the St and Y genomes may share a common progenitor genome. To test this hypothesis and explore genome evolutionary dynamic, we analyzed three tetraploid StY *Elymus* species, *E. ciliaris*, *E. pendulinus* and *E. longearistatus*, using molecular markers. The results rejected the suggestion of the same origin of the St and Y genomes. Our data revealed multiple origins and complex reticulate evolutionary dynamic of each species, also indicated that geographic isolation strongly influenced the evolution of the Y genome in these *Elymus* species.

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Table of Contents

Content of Tables

Content of Figures

Origin and evolutionary dynamic of *Elymus ciliaris*, *E. pendulinus* and *E. longearistatus* (Triticeae: Poaceae)

1. Introduction

Phylogenies are important for addressing various biology questions such as relationships among genes, the origin and spread of viral infection, and the demographic changes and migration patterns of species. In addition, the most popular application is to describe relationships among groups of organisms in systematics and taxonomy, which are discovered through molecular sequencing data and morphological data matrices. An interesting challenge facing plant systematists during the last decade is untangling reticulate phylogenetic relationships at all levels and uncovering previous undetected evolutionary processes.

1.1. Hybridization, Polyploidization and Introgression

Hybridization and polyploidization have played an important role in the history of plant evolution, and have contributed greatly to speciation (Cui *et al*., 2006), and are widespread in plants (Masterson, 1994). Polyploids are the result of a fusion between two or more genomes into the same nucleus and can be classified into allopolyploids or autopolyploids, based on the origin of the duplicated genomes (Stebbins, 1947). A recent evaluation of chromosome numbers has led to the conclusion that 15% of speciation events in angiosperms involve polyploidization (Wood *et al*., 2009). Polyploidization is a very common process, especially in plants. Many economically important crops such as wheat, cotton and potatoes are polyploids. The Triticeae group (e.g. *Elymus*, *Aegilops*,

and *Triticum*) emphasizes the impact of hybridization and polyploidization on species evolution.

Introgression through hybridization and segmental gene duplication events are also reported to play critical roles in driving speciation (Antunes *et al*., 2007; Laura Kavanaugh *et al*., 2006; Ragupathy *et al*., 2008). Ragupathy *et al*. (2008) demonstrated that a segmental duplication event mediated by an LTR retrotransposon occurred prior to the polyploidization resulting in hexaploid wheat speciation. Introgression has been shown in some instances to cause widespread genomic and epigenomic changes in a recipient species similar to those caused by the merger of divergent genomes during allopolyploid speciation (Liu and Wendel, 2000; Shan *et al*., 2005; Liu *et al*., 2004; Wang *et al*., 2005). Mallet (2005) estimated that up to 25% of plant species produce viable offspring from interspecific mating, which leads to simple hybridization and introgression. Numerous studies suggested that introgression has resulted in range expansion and niche shifts (Klier *et al*., 1991; Neuffer *et al*., 1999; Milne and Abbott, 2000; Rieseberg *et al*., 2007). The occurrence of introgression events may also confound reconstruction of individual polyploidization events by creating complex reticulate patterns (Mason-Gamer *et al*., 2010). Previous studies indicated that introgression clearly has the potential for inducing significant evolutionary change in recipient species.

1.2. *Elymus* and its genome constitutions

1.2.1. Poaceae, Triticea, *Elymus*

The Poaceae is a large and nearly ubiquitous family of monocotyledonous flowering plants, which are often used as research materials in the studies of evolution, phylogeny, or taxonomy, since they include a number of plants of major economic importance and they have a complex evolutionary history worldwide.

The tribe Triticeae in the grass family (*Poaceae*) is a fairly big group of grasses with more than 15 genera and 300 species, including not only the world's most economically important grain crops (wheat, barley, and rye) but also some valuable forage grasses. While most crop plants are annuals, the majority of species within Triticeae are perennials, and they are potential sources of genes for crop and forage improvement. The economic importance has resulted in its taxonomy being more thoroughly studied than any other tribe of grasses. The tribe combines a wide variety of biological mechanisms and genetic systems which make it an excellent group for research in evolution, and speciation in plants (Bothmer and Salomon, 1994).

Elymus L. is the largest and most morphologically diverse taxon in the Triticeae tribe with approximately 150 species identified worldwide (Löve, 1984). Although predominately a northern temperate genus, *Elymus* species occur from the Arctic and temperate to subtropical regions. These species inhabit various ecological niches, including semi-desert, grassland, forests, mountain slopes and valleys among bushes. Taxonomy of *Elymus* is extremely complex because of the massive morphological

variation within the species, the polyploid origin of the genus, and the frequent spontaneous hybridizations between species. As an exclusively allopolyploid genus, *Elymus* has origins from a few related genera in the Triticeae through natural hybridization (Dewey, 1984), and thus it has close relationships with such genera. It has been considerably effective for facilitating the introgression of useful genes from wild to cultivated species (Sears, 1983; Sharma and Gill, 1983). Due to its worldwide distribution, great economic value and complex genetic composition, *Elymus* is an ideal candidate for studying the evolution and polyploidy in plants.

1.2.2. Genome constitution of *Elymus* species

Five basic genomes (St, H, Y, P, and W) have been cytogenetically identified in different combinations in the genus *Elymus* with all its members containing at least one set of the pivotal St genome, which is donated by *Psedoroegneria* (Nevski) Á Löve (Dewey, 1984; Löve, 1984). The H, P, and W genomes are derived from *Hordeum* L., *Agropyron* Gaetn., and *Australopyrum* (Tzvelev) Á Löve, respectively. The origin of the Y genome is still unclear and under debate (Dewey, 1971; Torabinejad and Mueller, 1993; Jensen and Salomon, 1995).

1.2.3. Three tetraploid *Elymus* species with StY genome

Elymus ciliaris L. (2n = 4x = 28), is a perennial, self-pollinating allotetraploid species with wide distribution in China, Japan and the Russian Federation (Löve, 1984). This species is well adapted to high-humidity environments (Zhou *et al*., 1999), and is a

valuable gene pool for resistance to wheat scab, which may be useful in wheat improvement (Wan *et al*., 1997).

Elymus pendulinus (2n = 4x = 28) is a short-lived perennial, self-pollinating allotetraploid species. It inhabits central Asia and is characterized by drooping to strongly nodding spikes. Löve (1984) classified this species into four subspecies (i.e., *pendulinus*, *brachypodioides*, *multiculmis*, and *pubercaulis*), based on both ecological-geographical and morphological criteria.

Elymus longearistatus (Boiss.) Tzvelev (2n = 4x = 28) is a short-lived perennial, self-pollinating allotetraploid species. It inhabits stony slopes and rocks in the middle and upper mountain belts of eastern Alia, and the western Pamir of the USSR, Turkmainia, and Iran (Tzvelev, 1976).

1.2.4. Debate of the Y genome origion

Polyploid in *Elymus* includes tetraploidy (StH, StY, StP) and hexaploidy (StHP, StYP, StHY, StStY, StStH, etc.) (Dubcovsky *et al.*, 1997). The most common *Elymus* genome combination in Asia is StY, which is present in more than 75% of the known Asiatic tetraploid. Recently, one of the controversial debates is on the origin of the Y genome. Chromosome pairing analyses show low affinity between the St and Y genomes (Sakamoto, 1964; Dewey, 1971; Lu and Bothmer, 1990), and Dewey suggested that the Y genome had an independent origin from a Y diploid species that is now extinct or undiscovered. Internal transcribed spacer (ITS) sequence data suggest that Y genome has gradually differentiated from the St genome, and they may share the same progenitor

genome (Lu and Liu, 2005; Liu *et al*., 2006). More recently, a random amplified polymorphic DNA (RAPD) based sequence tagged site (STS) study suggested one accession of *Pseudoroegneria spicata* (Pursh) Á Löve (St genome) as a potential Y genome donor candidate in tetraploid *Elymus longearistatus* (Boiss.) Tzvelev (StStYY) (Okito *et al*., 2009), which is consistent with the hypothesis based on the ITS. However, accumulating evidence from studies looking at single copy of nuclear genes, including the phosphoenolpyruvate carboxylase (*PepC*), *β-amylase*, the granule-bound starch synthase I (*GBSSI*), the second largest subunit of RNA polymerase II (*RPB2*) and the translation elongation factor G (*EF-G*), rejected the same origination hypothesis of the St and Y genome, and supported Dewey's hypothesis (Mason-Gamer *et al*., 2005, 2010; Sun *et al*., 2008, 2010).

1.3. The molecular markers in phylogenetic study

Over the last two decades, the introduction of molecular genetic markers has provided unprecedented insights into the origin of polyploid species. Although chloroplast DNA was thought to evolve slowly, moderate to high levels of genetic variation have been frequently detected in non-coding spacer regions even within species (Ohsako and Ohnishi, 2000; Huang *et al.*, 2001; Chiang *et al*., 2001). The cpDNA trees remain important components of phylogenetic analyses of polyploids, which are suitable for investigating the origin of maternal lineage (Song *et al*., 2002; Guggisberg *et al*., 2006; Hodge *et al*., 2010; Ni *et al*., 2011).

The ITS region of nuclear DNA, as a member of a multigene family, is a classic marker of concerted evolution and has been widely used in some of early phylogenetic analyses (Buckler *et al*., 1997), recent studies suggest that ITS often fails as a marker in phylogenetic and hybrid speciation reconstructions because it may suffer from sequence homogenization due to concerted evolution (Wendel *et al*., 1995; Li and Zhang, 2002; Kovarik *et al*., 2005; Mahelka and Kopecky, 2010). In contrast to repeat sequences, single or low copy nuclear genes are supposed to undergo no extensive homogenization or even none at all (Mahelka and Kopecky, 2010), and provide higher resolution than ITS (Sang, 2002), which makes them ideal candidates for phylogenetic study (Small *et al*., 2004; Sun and Salomon, 2009).

1.4. The purpose of this study

In order to explore genome evolutionary dynamics and the origin of tetraploid StY *Elymus* species, three tetraploid StY *Elymus* species (*E. ciliaris*, *E. pendulinus* and *E. longearistatus*, respectively) were analyzed in this study, together with diploid species from *Pseudoroegneria* (St) and other Triticeae diploid species. Single copy nuclear genes and chloroplast genes were used. The objectives of this study were to: (1) understand the possible maternal and paternal origin of these three tetraploid species; (2) explore the intra-species evolutionary dynamics of these three *Elymus* species; (3) confirm or refuse the hypothesis that St and Y share a common progenitor genome; (4) investigate the possible relationship between species sequence variation and their geographic regions.

2. Materials and Methods

2.1. Plant materials and DNA extraction

Thirteen accessions of *E. ciliaris* from China, Japan and the Russian Federation, 13 accessions of *E. pendulinus* from southern borders of Altai, Eastern Siberia and the Far East of Russia, and eight accessions of *E. longearistatus* from Pakistan and Iran were used in this study (Tables 1, 2, 3). The seeds were kindly provided by the Germplasm Resources Information Network (GRIN) of the United States Department of Agriculture (USDA). DNA was extracted from fresh young leaf tissues from 5-10 plants of each accession using the method of Junghans and Metzlaff (1990). The sequences for some diploid Triticeae species representing the St, H, I, Xu, W, P, E, Ns, Ta, A, S, Xp, F, O, Q, K, R, and D genomes along with *Bromus* were obtained from published data (Sun *et al*., 2008; Helfgott and Mason-Gamer, 2004), and included in the analyses. Plant materials with accession numbers, genomic constitutions, geographical origins, and GenBank identification numbers are presented in Table 1, 2 and 3.

2.2. DNA amplification

2.2.1. *E. ciliaris*

The single copy nuclear genes including the second largest subunit of RNA polymerase II (*RPB2*), the phosphoglycerate kinase (*PGK1*) and cpDNA gene *RPS16* sequences were amplified by polymerase chain reaction (PCR) using the primers P6F/P6FR (Sun *et al*., 2007), PGKF1/PGKF2 (Huang *et al*., 2002), and RPS16F/RPS16R (Popp and Oxelman,

2007), respectively. The amplification profile for the *RPB2* gene was as follows: an initial denaturation at 95 °C for 4 min; 35-40 cycles of 95 °C for 40 sec, 52 °C for 40 sec, 72 °C for 90 sec, and a final cycle of 72 °C for 10 min. The PCR profile for amplifying *PGK1* gene was based on Huang *et al*. (2002). The PCR protocol for RPS16F/R followed Popp and Oxelman (2007).

Table 1. Taxa from *E. ciliaris*, *Bromus*, *Aegilops*, *Eremopyrum*, *Heteranthelium*, *Psathyrostachys*, *Secale*, *Taeniatherum*, *Agropyron*, *Australopyrum*, *Dasypyrum*, *Thinopyrum*, *Triticum*, *Pseudoroegneria* and *Hordeum* used in this study

Species	Accession No.	Genome*	Origin	*RPB2*	*PGK1*	*RPS16*
Bromus catharticus Vahl	CN 32048			HQ014410	-	+
Bromus inermis	PI 618974		Xinjiang, China	GQ848517	FJ711014	-
Bromus sterilis	PI 229595		Iran	HQ231839	+	+
Aegilops bicornis		S^b	The Middle East	-	AF343485	-
Aegilops longissima Schweinf. & Muschl.		S^l	The Middle East	-	AF343487	-
	PI 542196	S^l		-	-	+
Aegilops searsii Feldman & Kislev		S^S	The Middle East	-	AF343489	-
	PI 599150	S^S		-	-	+
Aegilops sharonensis		S^{sh}	The Middle East	-	AF343486	-
Aegilops sharonensis Eig	PI 542237	S^l		-	-	+
Aegilops speltoides Tausch.		S	The Middle East	-	AF343491	-
	PI 499261	S		-	-	+
Aegilops tauschii Coss.		D	The Middle East	-	AF343479	-
	PI 486265	D		-	-	+
Aegilops umbellulata Zhuk.	PI 276994	U		-	-	+
Aegilops uniaristata Vis.	PI 554418	N		-	-	+
Agropyron cristatum	PI 277352	P	Russian Federation	-	FJ711023	-
	PI 486160	P	Kazakstan	-	JF965622	-
	PI 547347	P	Urumqi, Xinjiang, China	-	JF965620	-
	ZY 09088	P	Dulan, Qinghai, China	-	JF965630	-
Agropyron cristatum (L.) Gaertn.	PI 383534	P	Kars, Turkey	EU187438	-	-
Agropyron fragile (Roth) P. Candargy	PI 598674	P		-	-	+
Agropyron monglicum Keng.	PI 531543	P	Inner Mongolia, China	-	JF965627	-
	PI 598460	P		-	-	+
Australopyrum retrofractum (Vickery) Á. Löve	PI 533014	W	New South Wales, Australia	EU187482	-	+

	PI 547363	W	New South Wales, Australia	EU187470	-	+
	PI 533013	W	New South Wales, Australia	-	FJ711025	-
Crithopsis delileana		K	Greece	-	FJ711026	-
Dasypyrum villosum (L.) P. Candargy	PI 368886	V	Gaziemir, Turkey	EU187471	-	-
	PI 251478	V	Turkey	-	FJ711027	-
Eremopyrum bonaepartis (Spreng.) Nevski	PI 203442	F		-	-	+
Eremopyrum distans	TA 2229	F	Afghanistun	-	FJ711018	-
	PI 193264	F		-	-	+
Eremopyrum orientale (L.) Jaub. & Spach	PI 203440	F		-	-	+
Eremopyrum triticeum	Y 206	F	Xinjiang, China	-	FJ711028	-
Henrardia persica	PI 401349	O	Turkey	-	FJ711029	-
Henrardia persica (Boiss.) C.E. Hubb.	PI 577112	Q		-	-	+
Heteranthelium piliferum (Banks & Sol.) Hochst.	PI 401351	Q	Iran	-	FJ711030	-
	PI 401354	Q		-	-	+
Hordeum bogdanii	PI 531761	H	Xinjiang, China	-	FJ711020	-
H.bogdanii Wilensky	PI 499498	H	Inner Mongolia, China	EF596768	-	-
	H7476	H		-	-	+
H. chilense Roem. and Schult.	PI 531781	H	Chile	-	FJ711017	-
	H1819	H		-	-	+
	H 1816	H	Chile	+	-	-
H.marinum Huds. ssp. *marinum*	H 121	Xa	Greece	+	-	-
H.murinum L. ssp. *glaucum* (Steud.) Tzvel.	H 74	Xu	Egypt	+	-	-
H. vulgare	Betzes	I	The Middle East	-	AF343494	-
H. vulgare ssp. *vulgare*	H 7514A	I	China	+	-	-
Lophopyrum elongatum	PI 531719	E[e]	St. Angulf, France	-	FJ711035	-
Peridictyon sanctum	H 3841	Xp	Greece	-	FJ711037	-
Psathyrostachys fragilis	Y 882	Ns	Iran	-	FJ711016	-
Psathyrostachys juncea (Fischer)	PI 406469	Ns	Former Soviet Union	+	-	+

Nevski						
	PI 222050	Ns	Afghanistun	-	FJ711031	-
Pseudoroegneria ferganensis Drobow	H 10248	St		-	-	+
P. geniculata (Trin.) Á. Löve	PI 632554	St		-	-	+
P. libanotica (Hack.) D. R. Dewey	PI 330688	St	Sirak-Sar, Iran	EF596751	-	+
	PI 228389	St	Iran	HQ231837	-	-
	PI 228390	St	Iran	HQ231838	-	-
	PI 228392	St	Iran	-	FJ711032	-
P. spicata (Pursh) Á. Löve	PI 506274	St	Washington, United States	EF596746	-	+
	PI 610986	St	Utah, United States	EF596747	-	-
	PI 232128	St	Idaho, United States	HQ231840	-	-
	PI 563869	St	Oregon, United States	HQ231856	-	-
	PI 563872	St	Montana, United States	HQ231857	-	-
	PI 619445	St	Nevada, United States	HQ231859	-	-
	PI 232123	St	Washington, USA	-	FJ711015	-
P. stipifolia (Czern. ex Nevski) Á. Löve	PI 325181	St	Stavropol, Russian Federation	EF596748	-	+
	PI 440095	St	Russian Federation	+	-	-
	PI 440095	St	Yankulskaya, Russian	-	FJ711033	-
P. strigosa	PI 531752	St	Estonia	HQ231850	-	-
	W6 14049	St	Russian Federation	HQ231836	-	-
	PI 499637	St	Xinjiang, China	-	FJ711034	-
P. strigosa subsp. *aegilopoides*	W6 13089	St	Xinjiang, China	HQ231835	-	-
	PI 420842	St	Former Soviet Union	HQ231846	-	+
	PI 440000	St	Stavro, Russian Federation	HQ231847	-	-
P. tauri	PI 401324	St	Iran	HQ231844	-	-
	PI 401326	St	Iran	HQ231845	-	-
Secale cereale L.	Imperial	R	The Middle East	-	AF343493	-
	PI 573710	R		-	-	+
Taeniatherum caput-medusae	PI 220591	Ta	Afghanistan	-	FJ711021	-
	PI 208075	Ta		-	-	+
Ta. caput-medusae ssp. *asperum*	PI 561091	Ta		-	-	+

melderis						
Ta. caput-medusae subsp. asperum	PI561091	Ta	Siirt, Turkey	+	-	-
Ta. caput-medusae subsp. *caput-medusae*	PI 208075	Ta	Kars, Turkey	+	-	-
	PI 220591	Ta	Afghanistan	+	-	-
	PI 222048	Ta	Afghanistan	+	-	-
	PI 222048	Ta		-	-	+
Thinopyrum bessarabicum (Savul. & Rayss) Á. Löve	PI 531712	E^b	Estonia	EU187474	-	-
Thinopyrum elongatum (Host) D.R. Dewey	PI 142012	E^e	Odessa, Russian Federation	EU187439	-	-
Triticum monococcum	TA 2025	A^M	The Middle East	-	FJ711022	-
	PI 191146	A^M		-	-	+
Triticum urartu	TA 763	A	Lebanon	-	AF343474	-
Elymus ciliaris (Trin.) Tzvelev	BKA-0931	StY	Siberian Botanical Garden, Novosibirsk	St, Y	St, Y	+
	BKA-0939	StY	Siberian Botanical Garden, Novosibirsk	St, Y	St, Y	+
	PI 377532	StY	Japan	Y, ?	Y, ?	+
	PI 531574	StY	China	St, Y	St, Y	+
	PI 531575	StY	China	St, Y	St, Y	+
	PI 531576	StY	Estonia	St, Y	St, Y	+
	PI 531577	StY	Japan	St, Y	St, Y	+
	PI 564917	StY	Vladivostock, Soviet Far East	St, Y	St, Y	+
	VBG-0844	StY	Siberian Botanical Garden, Novosibirsk	St, Y	St, Y	+
	W6 14463	StY	Unknown	St, Y	St, Y	+
	PI 547303	StY	Near Hasan, far east Primorsky region	St, Y	St, Y	+
	W6 10267	StY	Siberian Botanical Garden,	St, Y	St, Y	+

			Novosibirsk			
Elymus ciliaris (Trin.) Tzvelev	PI 632544	StY	China	St, Y	St, Y	+
	Pr87-88-337	StY		GQ867851	-	-
				GQ867851		

*Note: The genome designations are according to Wang *et al.* (1994). +, the sequence data has been recovered; -, the sequence data does not be recovered.

2.2.2. *E. pendulinus*

The low copy nuclear genes *RPB2* and phosphoenolpyruvate carboxylase (*PepC*), and cpDNA genes *RPS16, and* non-coding chloroplast DNA region *TrnD/T* were amplified using the primers P6F/P6FR (Sun *et al*., 2007), PEPC-F and PEPC-R (Helfgott and Mason-Gamer 2004), RPS16F/RPS16R (Popp and Oxelman 2007), and TrnD and TrnT (Sun, 2002), respectively. The amplification profile for the *RPB2* and *RPS16* genes were the same as 2.2.1. *E. ciliaris*. The PCR profile for amplifying *PepC* gene was based on Helfgott and Mason-Gamer (2004). The PCR protocol for *TrnD/T* followed Sun (2002).

Table 2. Taxa from *E. pendulinus*, *Bromus*, *Aegilops*, *Eremopyrum*, *Heteranthelium*, *Psathyrostachys*, *Secale*, *Taeniatherum*, *Agropyron*, *Australopyrum*, *Dasypyrum*, *Thinopyrum*, *Triticum*, *Pseudoroegneria*, and *Hordeum* used in this study

Species	Accession No.	Genome*	Origin	*RPB2*	*PepC*	*RPS16*	*TrnD/T*
Bromus catharticus Vahl	CN 32048			HQ014410	-	-	-
Bromus sterilis	PI 229595		Iran	HQ231839	-	-	+
B. tectorum	Kellogg s.n.			-	AY553239	-	-
Aegilops comosa Sibth. and Smith	G602	M		-	AY553236	-	-
Aegilops comosa Sibth. and Smith	PI 551032	M		-	-	+	+
Aegilops longissima Schweinf. & Muschl.	PI 542196	S^I		-	-	+	+
Aegilops searsii Feldman & Kislev	PI 599150	S^S		-	-	+	-
Aegilops sharonensis Eig	PI 542237	S^I		-	-	+	+
Aegilops speltoides Tausch.	PI 499261	S		-	-	+	+
Aegilops tauschii Coss.	PI 486265	D		-	-	+	-
Aegilops umbellulata Zhuk.	PI 276994	U		-	-	+	-
Aegilops uniaristata Vis.	PI 276996	N	Istanbul, Turkey	+	-	-	-
Aegilops uniaristata Vis.	PI 554418	N	Former Soviet Union	+	-	+	-
Agropyron cristatum (L.) Gaertn.	PI 383534	P	Kars, Turkey	EU187438	-	-	-
Agropyron fragile (Roth) P. Candargy	PI 598674	P		-	-	+	+
Agropyron fragile (Roth) P. Candargy	PI 598694	P	Kazakhstan	+	-	-	-
Agropyron mongolicum Keng.	PI 598460	P	China	+	-	+	+
Aust. retrofractum (Vickery) Á. Löve	PI 531553	W	Austr. Capital, Australia	-	-	-	+
Australopyrum retrofractum (Vickery) Á. Löve	PI 533013	W	New South Wales, Australia	-	-	-	+
Australopyrum retrofractum (Vickery) Á. Löve	PI 533014	W	New South Wales, Australia	EU187482	-	+	+
Australopyrum retrofractum (Vickery) Á. Löve	PI 547363	W	New South Wales, Australia	EU187470	-	+	+
Eremopyrum bonaepartis (Spreng.) Nevski	PI 203442	F	Ankara	+	-	+	+
Eremopyrum bonaepartis (Spreng.) Nevski	PI 219966	F	Girishk	+	-	-	-
Eremopyrum distans	PI 193264	F		-	-	+	-
Eremopyrum orientale (L.) Jaub. & Spach	PI 203440	F		-	AY553254	-	+
Eremopyrum orientale (L.) Jaub. & Spach	PI 203440	F		-	-	+	-
H. bogdanii	PI 531760	H		-	EU282293	-	-
H. bogdanii Wilensky	H4014	H	Pakistan	+	-	-	-
H. bogdanii Wilensky	H7476	H		-	-	+	-
H. bogdanii Wilensky	PI 499498	H	Inner Mongolia, China	EF596768	-	-	-
H. bogdanii Wilensky	PI 499645	H	Xinjiang, China	EU18747	-	-	-
H. bogdanii Wilensky	PI 531762	H	Tajikistan	-	-	-	+
H. brachyantherum Nevski ssp. *californicum* (Covas and Stebbins) Bothm. *et al*	H 3317	H	U. S. A.	+	-	-	-
H. bulbosum L.	H 3878	I	Italy	+	-	-	-
H. bulbosum L.	PI 440417	I		-	EU282294,EU282295, EU282296	-	-

H. chilense Roem. and Schult.	H 1816	H	Chile	+	EU282297	-	-
H. chilense Roem. and Schult.	H1819	H		-	-	+	-
H. comosum Presl.	H 1181	H	Argentina	+	-	-	-
H. cordobense Bothmer, Jacobsen and Nicora	H 6460	H	Argentina	+	-	-	-
H. flexuosum Steud.	H 2127	H	Uruguay	+	-	-	-
H. marinum	PI 304347	Xa		-	EU282298	-	-
H. marinum Huds.	PI 304346	Xa		-	AY553258	-	-
H. marinum Huds. ssp. *gussoneanum* (Parl.)Thell.	H 581	Xa	Greece	+	-	-	-
H. marinum Huds. ssp. *marinum*	H 121	Xa	Greece	+	-	-	-
H. murinum	CIho 15683	Xu		-	AY553259	-	-
H. murinum L.	PI 247054	Xu		-	EU282299, EU282300	-	-
H. patagonicum (Haumann) Covas ssp. *magellanicum* (Parodi and Nicora) Bothm. *et al.*	H 1342	H	Argentina	+	-	-	-
H. patagonicum (Haumann) Covas ssp. *mustersii* (Nicora) Bothm. *et al.*	H 1358	H	Argentina	+	-	-	-
H. patagonicum (Haumann) Covas ssp. *patagonicum*	H 6052	H	Argentina	+	-	-	-
H. patagonicum (Haumann) Covas ssp. *santacrucense* (Parodi and Nicora) Bothm. *et al.*	H 1353	H	Argentina	+	-	-	-
H. patagonicum (Haumann) Covas ssp. *setifolium* (Parodi and Nicora) Bothm. *et al.*	H 1352	H	Argentina	+	-	-	-
H. pubiflorum Hook. f.	H 1236	H	Argentina	+	-	-	-
H. pusillum Nutt.	CIho 15684	H		-	EU282301	-	-
H. roshevitzii Bowden	H 10070	H		-	-	-	+
H. roshevitzii Bowden	H 7754	H		-	-	-	+
H. roshevitzii Bowden	H9152	H	China	+	-	-	-
H. stenostachys Godr.	H 1780	H	Argentina	+	-	-	-
H. stenostachys Godr.	H 6439	H	Argentina	+	-	-	+
H. stenostachys Godr.	PI 531791	H		-	EU282302	-	-
H. vulgare	RJMG 107	I		-	AY553260	-	-
H. vulgare ssp. *spontaneous* (K.Koch) Thell	H 3140A	I	Cyprus	+	-	-	-
H. vulgare ssp. *vulgare*	H 7514A	I	China	+	-	-	-
Henrardia persica (Boiss.) C.E. Hubb.	PI 577112	Q		-	-	+	-
Heteranthelium piliferum (Banks & Sol.) Hochst.	PI 401351	Q	Iran	+	-	-	-
Heteranthelium piliferum (Banks & Sol.) Hochst.	PI 401354	Q	Iran	+	AY553255	+	-
Pseudoroegneria ferganensis Drobow	H 10248	St		-	-	+	-
P. geniculata (Trin.) Á. Löve	PI 632554	St		-	-	+	-
P. libanotica (Hack.) D. R. Dewey	PI 228389	St	Iran	HQ231837	-	-	+
P. libanotica (Hack.) D. R. Dewey	PI 228390	St	Iran	HQ231838	-	-	+
P. libanotica (Hack.) D. R. Dewey	PI 228391	St		-	EU282304	-	-
P. libanotica (Hack.) D. R. Dewey	PI 282392	St		-	EU282305	-	-
P. libanotica (Hack.) D. R. Dewey	PI 330687	St	Kandavan Pass, Iran	EF596753	-	-	-
P. libanotica (Hack.) D. R. Dewey	PI 330688	St	Sirak-Sar, Iran	EF596751	-	+	+
P. libanotica (Hack.) D. R. Dewey	PI 401274	St	Saqqez, Iran	EF596752	-	-	-

P. spicata (Pursh) Á. Löve	D 2844	St		-	AY553264	-	-
P. spicata (Pursh) Á. Löve	PI 232128	St	Idaho, United States	HQ231840	-	-	+
P. spicata (Pursh) Á. Löve	PI 232134	St	Whoming, United States	HQ231841	-	-	-
P. spicata (Pursh) Á. Löve	PI 232140	St	U. S. A.	-	-	-	+
P. spicata (Pursh) Á. Löve	PI 236669	St	British Columbia, Canada	HQ231842	-	-	-
P. spicata (Pursh) Á. Löve	PI 286198	St	Washington, United States	HQ231843	-	-	-
P. spicata (Pursh) Á. Löve	PI 506274	St	Washington, United States	EF596746	-	+	-
P. spicata (Pursh) Á. Löve	PI 516184	St	Oregon, United States	HQ231848	-	-	-
P. spicata (Pursh) Á. Löve	PI 537379	St	Oregon, United States	HQ231851	-	-	-
P. spicata (Pursh) Á. Löve	PI 537389	St	Washington, United States	HQ231852	-	-	-
P. spicata (Pursh) Á. Löve	PI 539873	St	Idaho, United States	HQ231853	-	-	-
P. spicata (Pursh) Á. Löve	PI 547154	St	Idaho, United States	HQ231854	-	-	-
P. spicata (Pursh) Á. Löve	PI 547162	St	Oregon, United States	HQ231855	-	-	-
P. spicata (Pursh) Á. Löve	PI 563869	St	Oregon, United States	HQ231856	-	-	+
P. spicata (Pursh) Á. Löve	PI 563872	St	Montana, United States	HQ231857	-	-	+
P. spicata (Pursh) Á. Löve	PI 598818	St	Oregon, United States	-	-	-	+
P. spicata (Pursh) Á. Löve	PI 598822	St	Colorado, United States	HQ231858	-	-	+
P. spicata (Pursh) Á. Löve	PI 610986	St	Utah, United States	EF596747	AY553263	-	-
P. spicata (Pursh) Á. Löve	PI 619445	St	Nevada, United States	HQ231859	-	-	+
P. stipifolia	PI 531751	St		-	EU282307, EU282308	-	-
P. stipifolia (Czern. ex Nevski) Á. Löve	PI 313960	St		-	EU282306	-	-
P. stipifolia (Czern. ex Nevski) Á. Löve	PI 325181	St	Stavropol, Russian Federation	EF596748	-	+	+
P. stipifolia (Czern. ex Nevski) Á. Löve	PI 440095	St	Russian Federation	+	-	-	-
P. strigosa	PI 531752	St	Estonia	HQ231850	-	-	+
P. strigosa	W6 14049	St	Russian Federation	HQ231836	-	-	+
P. strigosa (M.Bieb.) Á. Löve	PI 499637	St		-	EU282309, EU282310	-	-
P. strigosa subsp. *aegilopoides*	PI 420842	St	Former Soviet Union	HQ231846	-	+	+
P. strigosa subsp. *aegilopoides*	PI 440000	St	Stavro, Russian Federation	HQ231847	-	-	+
P. strigosa subsp. *aegilopoides*	W6 13089	St	Xinjiang, China	HQ231835	-	-	+
P. strigosa subsp. aegilopoides (Drobow) Á. Löve	PI 531755	St		-	EU282311	-	-
P. tauri	PI 380644	St		-	EU282314, EU282315	-	-
P. tauri	PI 401319	St		-	EU282313	-	-
P. tauri	PI 401324	St	Iran	HQ231844	-	-	+
P. tauri	PI 401326	St	Iran	HQ231845	-	-	+
P. tauri	PI 401330	St	Toward Ahar, Iran	-	-	+	-
P. tauri (Boiss. & Balansa) Á. Löve	PI 380652	St		-	EU282312	-	-
Psathyrostachys juncea (Fischer) Nevski	PI 406469	Ns	Former Soviet Union	+	-	+	+
Psathyrostachys juncea (Fischer) Nevski	PI 430871	Ns	Former Soviet Union	+	-	-	-
Secale cereale L.	Kellogg s.n.	R		-	AY553266	-	-
Secale cereale L.	PI 573710	R		-	-	+	+
Ta. caput-medusae	RJMG 189	Ta		-	AY553268	-	-
Ta. caput-medusae subsp. asperum	PI561091	Ta	Siirt, Turkey	+	-	+	-
Ta. caput-medusae subsp. *caput-medusae*	PI 208075	Ta	Kars, Turkey	+	-	+	-

Ta. caput-medusae subsp. *caput-medusae*	PI 220591	Ta	Afghanistan	+	-	-	-
Ta. caput-medusae subsp. *caput-medusae*	PI 222048	Ta	Afghanistan	+	-	+	-
Thinopyrum bessarabicum (Savul. & Rayss) Á. Löve	PI 531712	E^b	Estonia	EU187474	-	-	-
Thinopyrum elongatum (Host) D.R. Dewey	PI 142012	E^e	Odessa, Russian Federation	EU187439	-	+	+
Thinopyrum elongatum (Host) D.R. Dewey	RJMG 113	E^e		-	AY553269	-	-
Triticum monococcum	PI 191146	A^M		-	-	+	-
Triticum monococcum L.		A^M		-	AJ007705	-	-
E. abolinii (Drobow) Tzvelev	PI531555	StY		-	GQ844927, GQ844928	-	-
E. abolinii (Drobow) Tzvelev	PI 531554	StY	Xinjiang, China	EU187443, EU18744	-	-	-
E. antiques (Nevski) Tzvelev	PI632564	StY		-	GQ844931, GQ844932	-	-
E. caucasicus (Koch) Tzvelev	PI 531573	StY	Estonia	EU187454, EU187453	GQ844940, GQ844941	-	-
E. ciliaris (Trin.) Tzvelev	PI531575	StY		-	GQ844942, GQ844943	-	-
E. ciliaris (Trin.) Tzvelev	PI 564917	StY	Vladivostock, Soviet Far East	EF596749 , EU187483	-	-	-
E. longearistatus (Boiss.) Tzvelev	PI401277	StY		-	GQ844950, GQ844951	-	-
E. longearistatus (Boiss.) Tzvelev	PI 401280	StY	North of Tehran, Iran	EU187447, EU187448	-	-	-
E. nevskii Tzvelev	PI314620	StY		-	GQ844952, GQ844953	-	-
E. semicostatus (Nees ex Steud.) Melderis	PI271522	StY		-	GQ844956, GQ844957	-	-
E. semicostatus (Nees ex Steud.) Melderis	PI 207452	StY	Afghanistan	EU187445, EU187446	-	-	-
E. pendulinus (Nevski) Tzvelev	PI499452	StY		-	GQ844954, GQ844955	-	-
	BKA 0921	StY		Y, ?	St1, St2, Y	+	+
	VOK 0728	StY		St, Y	Y	+	+
	VBG 0727	StY		St, Y, ?	?, ?	+	+
	VOK 0724	StY		St, Y	St, Y	+	+
	VBG 0722	StY		St, Y, ?	St, Y	+	+
	MES 0721	StY		St, Y, ?	St, Y	+	+
	USS 0720	StY		St	St, Y	+	+
	VLA 0719	StY		St, Y	St, Y, ?	+	+
	VLA 0718	StY		Y	St, Y	+	+
	RUS 0716	StY		St, Y	St, Y	+	+
	ZAR 0715	StY		St, Y	St, Y	+	+
	ZAR 0714	StY		St, Y, ?	St, Y, ?	+	+
	AND 0713	StY		St, ?, ?	St, Y, ?	+	+

*Note: The genome designations are according to Wang *et al.* (1994). +, the sequence data has been recovered; -, the sequence data does not be recovered.

2.2.3. *E. longearistatus*

Two single copy nuclear genes: the translation elongation factor G (*EF-G*) closely linked to the *VRS1* locus and the thioreoxin-like gene (*HTL*); along with two chloroplast DNA sequences: *RPS16* and *TrnD/T* were amplified. The *EF-G*, *HTL*, *RPS16* and *TrnD/T* sequences were amplified using the primers of cMWG699T3-2 and cMWG699T7-2 (Komatsuda *et al*., 1999), trxF/R (Kakeda *et al*., 2008), RPS16F/R (Popp and Oxelman, 2007), and TrnD/T (Sun, 2002), respectively. The amplification profile for *EF-G* was based on Yan and Sun (2011), and the protocols for the other three genes followed Sun (2002), Popp and Oxelman (2007) and Kakeda *et al*. (2008).

Table 3. Taxa from *E. longearistatus*, *Bromus*, *Aegilops*, *Eremopyrum*, *Heteranthelium*, *Psathyrostachys*, *Secale*, *Taeniatherum*, *Agropyron*, *Australopyrum*, *Thinopyrum*, *Triticum*, *Pseudoroegneria*, and *Hordeum* used in this study

Species	Accession No.	Genome*	Origin	*EF-G*	*HTL*	*RPS16*	*TrnD/T*
Bromus catharticus Vahl	CN 32048			-	-	+	-
Bromus sterilis	PI 229595		Iran	-	-	-	+
B. sterilis	55777			AY836187	-	-	-
Aegilops comosa Sibth. and Smith	PI 551032	M		-	-	+	+
Aegilops longissima Schweinf. & Muschl.	PI 542196	S^I		-	-	+	+
Aegilops searsii Feldman & Kislev	PI 599150	S^S		-	-	+	-
Aegilops sharonensis Eig	PI 542237	S^I		-	-	+	+
Aegilops speltoides Tausch.	PI 499261	S		-	-	+	+
Aegilops tauschii Coss.	PI 486265	D		-	-	+	-
Aegilops umbellulata Zhuk.	PI 542378	U	Turkey	-	+	-	-
Aegilops umbellulata Zhuk.	PI 276994	U		-	-	+	-
Aegilops uniaristata Vis.	PI 276996	N	Istanbul, Turkey	-	+	-	-
Aegilops uniaristata Vis.	PI 554418	N	Former Soviet Union	-	+	+	-
Agropyron cristatum (L.) Gaertn.	PI 383534	P	Kars, Turkey	GU982325	-	-	-
Agropyron fragile (Roth) P. Candargy	PI 598674	P		-	-	+	+
Agropyron mongolicum Keng.	PI 598460	P	China	-	-	+	+
Australopyrum retrofractum (Vickery) Á. Löve	PI 531553	W	Austr. Capital, Australia	-	-	-	+
Aust. retrofractum (Vickery) Á. Löve	PI 533013	W	New South Wales, Australia	-	-	-	+
Aust. retrofractum (Vickery) Á. Löve	PI 533014	W	New South Wales, Australia	GU982345	-	+	+
Aust. retrofractum (Vickery) Á. Löve	PI 547363	W	New South Wales, Australia	GU982347	-	+	+
Eremopyrum bonaepartis (Spreng.) Nevski	PI 203442	F	Ankara	-	-	+	+
Eremopyrum distans	PI 193264	F		-	-	+	-
Eremopyrum orientale (L.) Jaub. & Spach	PI 203440	F		-	-	-	+
Eremopyrum orientale (L.) Jaub. & Spach	PI 203440	F		-	-	+	-
Hordeum bogdanii Wilensky	H7476	H		-	-	+	-
H. bogdanii Wilensky	PI 499498	H	Inner Mongolia, China	GU982334	-	-	-
H. bogdanii Wilensky	PI 499645	H	Xinjiang, China	GU982335	-	-	-
H. bogdanii Wilensky	PI 531762	H	Tajikistan	-	-	-	+
H. chilense Roem. and Schult.	H1819	H		-	-	+	-
H. roshevitzii Bowden	H 10070	H		-	-	-	+
H. roshevitzii Bowden	H 7754	H		-	-	-	+
H. stenostachys Godr.	H 6439	H	Argentina	-	-	-	+
Henrardia persica (Boiss.) C.E. Hubb.	PI 577112	Q		-	-	+	-
Heteranthelium piliferum (Banks & Sol.) Hochst.	PI 401351	Q	Iran	-	+	-	-
Heteranthelium piliferum (Banks & Sol.) Hochst.	PI 401354	Q	Iran	-	+	+	-
Pseudoroegneria ferganensis Drobow	H 10248	St	Gissar mtns, Tadzhikistan	GU982369	-	+	-
P. geniculata (Trin.) Á. Löve	PI 632554	St		-	-	+	-

P. libanotica (Hack.) D. R. Dewey	PI 228389	St	Iran	-	-	-	+
P. libanotica (Hack.) D. R. Dewey	PI 228390	St	Iran	HQ231862	+	-	+
P. libanotica (Hack.) D. R. Dewey	PI 330688	St	Sirak-Sar, Iran	HQ231866	+	+	+
P. spicata (Pursh) Á. Löve	PI 232128	St	Idaho, United States	HQ231863	+	-	+
P. spicata (Pursh) Á. Löve	PI 232140	St	U. S. A.	-	-	-	+
P. spicata (Pursh) Á. Löve	PI 506274	St	Washington, United States	GU982338	-	+	-
P. spicata (Pursh) Á. Löve	PI 563869	St	Oregon, United States	-	-	-	+
P. spicata (Pursh) Á. Löve	PI 563872	St	Montana, United States	-	-	-	+
P. spicata (Pursh) Á. Löve	PI 598818	St	Oregon, United States	-	-	-	+
P. spicata (Pursh) Á. Löve	PI 598822	St	Colorado, United States	-	-	-	+
P. spicata (Pursh) Á. Löve	PI 619445	St	Nevada, United States	-	-	-	+
P. stipifolia (Czern. ex Nevski) Á. Löve	PI 325181	St	Stavropol, Russian Federation	GU982324	+	+	+
P. strigosa	PI 531752	St	Estonia	-	-	-	+
P. strigosa	W6 14049	St	Russian Federation	HQ231861	+	-	+
P. strigosa subsp. *aegilopoides*	PI 420842	St	Former Soviet Union	GU982329	+	+	+
P. strigosa subsp. *aegilopoides*	PI 440000	St	Stavro, Russian Federation	HQ231867	+	-	+
P. strigosa subsp. *aegilopoides*	W6 13089	St	Xinjiang, China	-	-	-	+
P. tauri	PI 401324	St	Iran	-	+	-	+
P. tauri	PI 401326	St	Iran	-	+	-	+
P. tauri	PI 401330	St	Toward Ahar, Iran	GU982328	-	+	-
Psathyrostachys juncea (Fischer) Nevski	PI 406469	Ns	Former Soviet Union	-	-	+	+
Psathyrostachys juncea (Fischer) Nevski	H10108	Ns	Russia	-	AB509256	-	-
Secale cereale L.	PI 573710	R		-	-	+	+
Ta. caput-medusae subsp. asperum	PI561091	Ta	Siirt, Turkey	-	-	+	-
Ta. caput-medusae subsp. *caput-medusae*	PI 208075	Ta	Kars, Turkey	-	-	+	-
Ta. caput-medusae subsp. *caput-medusae*	PI 222048	Ta	Afghanistan	-	-	+	-
Thinopyrum bessarabicum (Savul. & Rayss) Á. Löve	PI 531712	E^b	Estonia	GU982344	-	-	-
Triticum monococcum	PI 191146	A^M		-	-	+	-
E. abolinii (Drobow) Tzvelev	PI 531554	StY	Xinjiang, China	GU982339, GU982340	-	-	-
E. caucasicus (Koch) Tzvelev	PI 531573	StY	Estonia	GU982342, GU982343	-	-	-
E. semicostatus (Nees ex Steud.) Melderis	PI 207452	StY	Afghanistan	GU982318, GU982319	-	-	-
E. gmelinii (Ledeb.) Tzvelev	PI 610898	StY	Xinjiang, China	GU982352, GU982353	-	-	-
E. strictus (Keng) Á. Löve	PI 499476	StY	Lanzhou, China	GU982330, GU982331	-	-	-
E. antiquus (Nevski) Tzvelev	PI 619528	StY	Sichuan, China	GU982355, GU982356	-	-	-
E. longearistatus (Boiss.) Tzvelev	PI 401276	StY	Iran	St, Y	St, Y	+	+
	PI 401277	StY	Iran	St, Y	St, Y	+	+

E. longearistatus (Boiss.) Tzvelev	PI 401278	StY	Iran	St, Y	St, Y	+	+
	PI 401279	StY	Iran	St, Y	St, Y	+	+
	PI 401280	StY	Iran	St, Y	St, Y	+	+
	PI 401282	StY	Iran	St, Y	St, Y	+	+
	PI 401283	StY	Iran	St, Y	St, Y	+	+
	PI 564942	StY	Pakistan	St, Y	St, Y	+	+

*Note: The genome designations are according to Wang *et al.* (1994). +, the sequence data has been recovered; -, the sequence data does not be recovered.

2.3. Cloning and sequencing

The PCR products for the nuclear genes were cloned into the pGEM-easy T vector (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. The resulting plasmids were used to transform *Escherichia coli* DH5α, and at least 20 colonies for each accession were randomly selected for screening. Each colony was transferred to 10 μL of LB broth with 0.1 mg/ml ampicillin. The solutions were incubated at 37°C for 30 min before using 2 μL for PCR to check the presence of an insert using the same primers that were used for the original PCR amplification. For the solutions that were confirmed to contain the insert, the remaining 8 μL of solution was transferred to 5 mL LB broth and incubated at 37°C overnight. Plasmid DNA was isolated using Promega Wizard ® *Plus* Minipreps DNA Purification System (Promega Corporation, Madison, WI, USA) according to manufacturer's instructions.

The PCR products amplified by cpDNA primers RPS16F/R and TrnD/T were purified and then directly sequenced. Both the PCR products and plasmid DNAs were commercially sequenced by either MACROGEN (Seoul, Korea) or Taihe Biotechnology (Beijing, China). Both forward and reverse strands of PCR products or plasmid DNAs were sequenced independently to enhance the sequence quality. Since *Taq* errors that cause substitutions are mainly random and it is unlikely that any two sequences would share identical *Taq* errors to create a false synapomorphy, each PCR product amplified by cpDNA primer was independently amplified twice and sequenced to avoid any error which would be caused by *Taq* DNA polymerase during PCR amplification.

2.4. Data analysis

Automated sequence outputs were visually inspected with chromatographs. Multiple sequence alignments were made using ClustalX using default parameters and adjusted manually to minimize gaps (Thompson *et al*., 1997). Phylogenetic analysis using the maximum-parsimony (MP) method was performed with the computer program PAUP* ver. 4 beta 10 (Swofford, 2003). All characters were specified as unweighted and unordered, and gap-only columns were excluded in the analyses. The most parsimonious trees were constructed by performing a heuristic search using the Tree Bisection-Reconnection (TBR) with the following parameters: MulTrees on and ten replications of random addition sequences with the stepwise addition option. Multiple parsimonious trees were combined to form a strict consensus tree. Overall character congruence was estimated by the consistency index (CI), and the retention index (RI). To test the robustness of clades, bootstrap values with 1,000 replications (Felsenstein, 1985) were calculated by performing a heuristic search using the TBR option with Multree on.

In addition to MP analysis, maximum-likelihood (ML) and Bayesian analyses were also performed in the study of *E. ciliaris* and *E. longearistatus*. Because of huge amount of sequence data (greater than 100 sequence), the Bayesian analysis was not suitable for the analysis of *E. pendulinus.* For ML analysis, eight nested models of sequence evolution were tested for each data set using PhyML 3.0 (Guindon and Gascuel, 2003). For each data set, the general time-reversible (GTR) (Lanave *et al*., 1984) substitution model led to the largest ML score compared to the other 7 substitution models: JC69 (Jukes and Cantor, 1969), K80 (Kimura, 1980), F81 (Felsenstein, 1981), F84 (Felsenstein, 1993), HKY85

(Hasegawa *et al*., 1985), TN93 (Tamura and Nei, 1993) and custom (data not shown). For ML, the support of clades was assessed with the approximate likelihood ratio test method (aLRT), which is an alternative to the bootstrap method for evaluating tree reliability (Anisimova and Gascuel, 2006).

As the result, the GTR model was used in the Bayesian analysis using MrBayes 3.1 (Ronquist and Huelsenbeck, 2005). MrBayes 3.1 was run with the program's standard setting of two analyses in parallel, each with four chains, and estimates of convergence of results by calculating standard deviation of split frequencies between analyses. In order to make the standard deviation of split frequencies fall below 0.01, so that the occurrence of convergence could be certain. Samples were taken every 1000 generations under the GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites. For all analyses, the first 25% of samples from each run were discarded as burn-in to ensure the stationary of the chains. Bayesian posterior probability (PP) values were obtained from a majority rule consensus tree generated from the remaining sampled trees.

3. Results

3.1. *E. ciliaris*

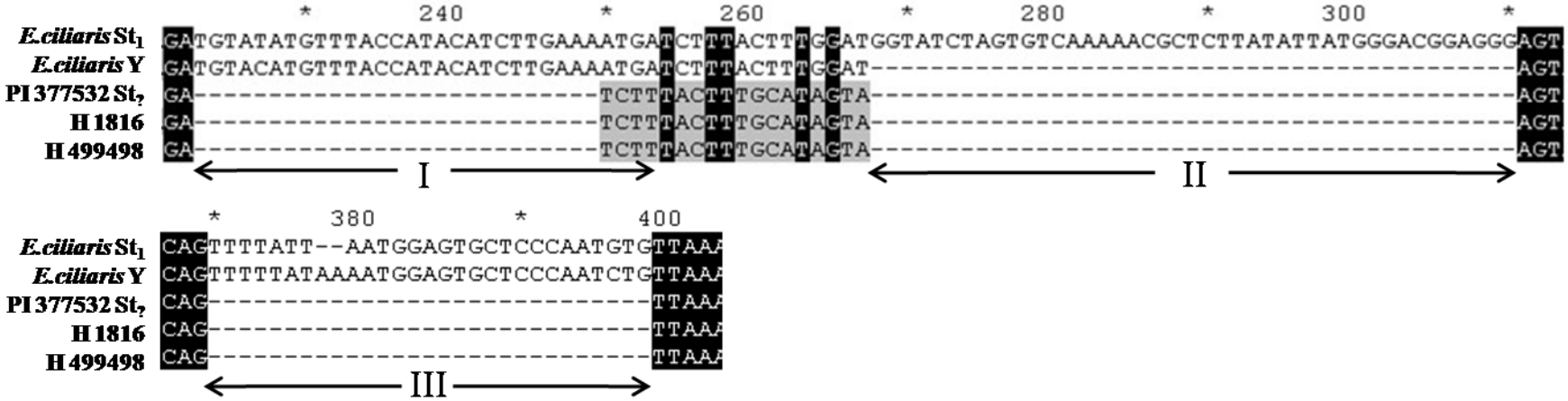
3.1.1. Sequence variation

The amplified patterns from 13 accessions of tetraploid *E. ciliaris* species showed a single band for each gene, *RPB2*, *PGK1*, and *RPS16*, with size of approximately 1000bp, 1400bp and 900bp, respectively, which corresponded well with previous findings (Sun,

2002; Fan *et al*., 2012; Hodge *et al*., 2010). Sequence comparison of cloned PCR fragments identified two distinct copies each for *RPB2* and *PGK1* genes from all *E. ciliaris* accessions analyzed. The amplified regions of *RPB2* are ~1,000 bp long in the all St genome except a sequence from accession PI 377532, where it is approximately 900 bp in length (named $St_?$).

Extensive sequence variation was detected among *E. ciliaris* for each gene, with the variability of the *RPB2* sequences being the highest. *RPB2* sequence alignment showed three large transposable-like element insertion/ deletions. This corresponded well with previous findings (Sun *et al*., 2007, 2008). The first indel occurred at position 233, with H and $St_?$ genomes having a deletion compared to the St and Y genome in this region (Fig. 1). All sequences from *E. ciliaris* St genome have a 43 bp insertion at position 268 compared to the sequences from H, Y and $St_?$ genomes. A third insertion of about 29-31bp occurred in the sequences from all *E. ciliaris* St and Y genomes except for the $St_?$ and H genome.

Figure 1. Partial alignment of the amplified sequences of *RPB2* from *E. ciliaris* and *Hordeum* species. Note that except St_1 genome sequence, sequences from $St_?$, and H have the deletions at three indels, the Y genome sequence has a deletion at the second indel as well. All the St_1 and Y sequences are the same in these two alignment regions.


240
260
280
300
E.ciliaris St1
E.ciliaris Y
PI 377532 St2
H 1816
H 499498
I
II
380
400
III

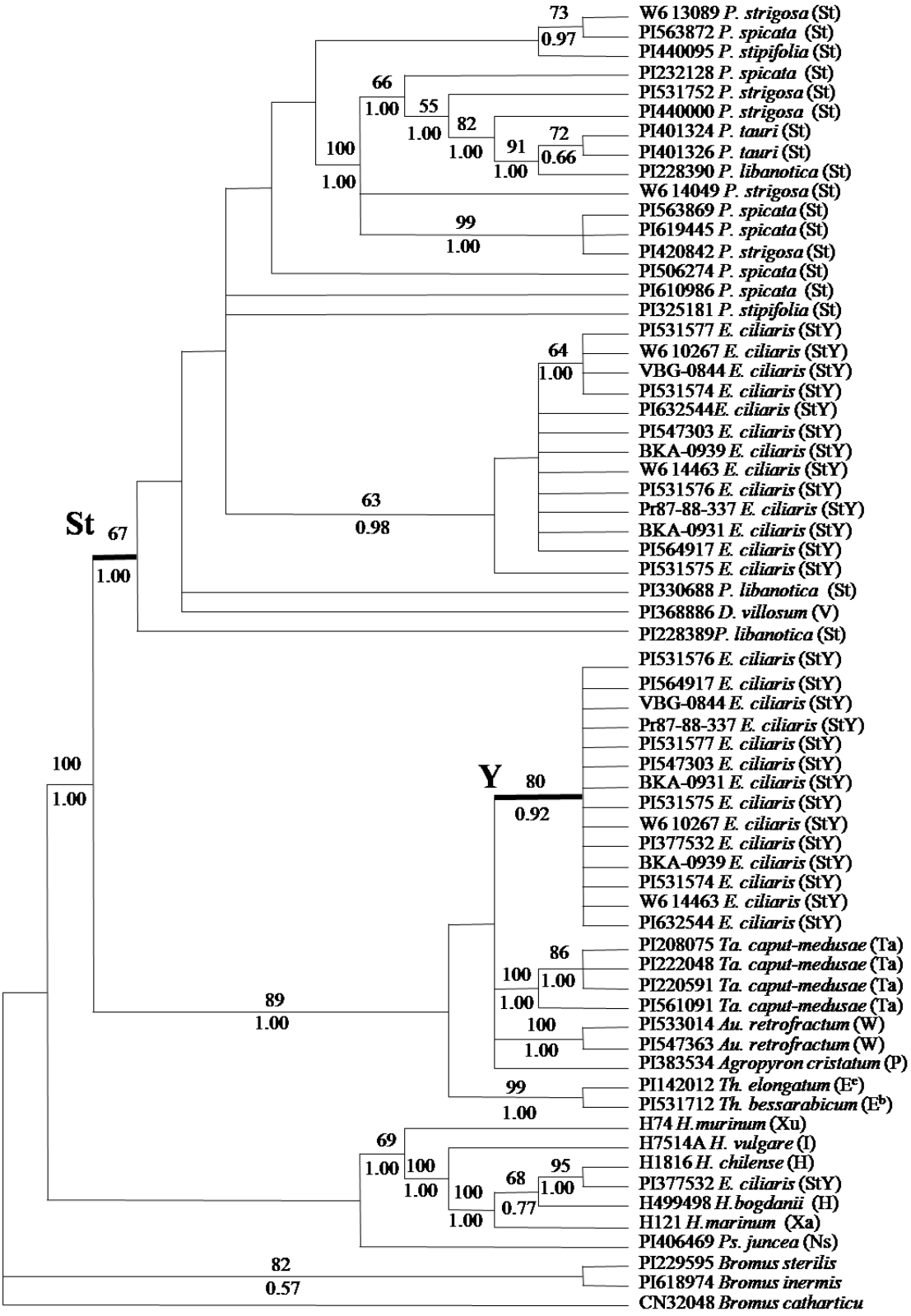
3.1.2. Phylogenetic analyses of *RPB2* sequences

MP analysis using *Bromus sterilis*, *B. inermis and B. catharticus* as the outgroup was conducted (304 parsimony-informative characters, 1189 equally most parsimonious trees, CI = 0.639; RI = 0.826). The separated Bayesian analyses using the GTR model resulted in identical trees with mean log-likelihood values -7235.30 and -7255.86 (data not shown). The tree topologies were almost identical in both ML and Bayesian trees and were similar to those generated by MP. Strict consensus trees with bootstrap (1000 replicates) values and Bayesian PP are shown in Figure 2.

The phylogenetic tree showed three different clades. The sequences from *Hordeum* species (H, Xa, I and Xu genomes) were grouped into a clade (BS = 69%, PP = 1.00). As expected, two distinct copies of sequences obtained from each tetraploid *E. ciliaris* accessions of StStYY genomes that were amplified and sequenced were well separated into two different clades, one in the St genome (*Pseudoroegneria*) clade and the other in the Y genome clade (BS = 80%, PP = 0.92). However, one sequence from the accession PI 377532 of *E. ciliaris* ($St_?$) was grouped with the *Hordeum* species. All *E. ciliaris* St sequences were grouped together into a subclade (BS = 63%, PP = 0.98), which was nested within the *Pseudoroegneria* species St genome clade. The St sequences from accession PI 531577, W6 10267, VBG-0844 and PI 531574 are closer to each other than the St sequences from other *E. ciliaris* accessions and formed a weakly supported group (BS=64%, PP=1.00). The sequence from accession PI 531575 is distinct from the St

sequences from other accessions of *E. ciliaris*. The Y-genome sequences formed a polytomous clades.

Figure 2. Strict consensus trees derived from *RPB2* sequence data was conducted using heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and Bayesian posterior probability (PP) values, respectively. *Bromus sterilis, Bromus inermis, and Bromus catharthartic*u were used an outgroups. Consistency index (CI) = 0.639, retention index (RI) = 0.826, rescale consistency index (RCI) = 0.528.


W6 13089 P. strigosa (St)
PI563872 P. spicata (St)
PI440095 P. stipifolia (St)
PI232128 P. spicata (St)
PI531752 P. strigosa (St)
PI440000 P. strigosa (St)
PI401324 P. tauri (St)
PI401326 P. tauri (St)
PI228390 P. libanotica (St)
W6 14049 P. strigosa (St)
PI563869 P. spicata (St)
PI619445 P. spicata (St)
PI420842 P. strigosa (St)
PI506274 P. spicata (St)
PI610986 P. spicata (St)
PI325181 P. stipifolia (St)
PI531577 E. ciliaris (StY)
W6 10267 E. ciliaris (StY)
VBG-0844 E. ciliaris (StY)
PI531574 E. ciliaris (StY)
PI632544E. ciliaris (StY)
PI547303 E. ciliaris (StY)
BKA-0939 E. ciliaris (StY)
W6 14463 E. ciliaris (StY)
PI531576 E. ciliaris (StY)
Pr87-88-337 E. ciliaris (StY)
BKA-0931 E. ciliaris (StY)
PI564917 E. ciliaris (StY)
PI531575 E. ciliaris (StY)
PI330688 P. libanotica (St)
PI368886 D. villosum (V)
PI228389P. libanotica (St)
PI531576 E. ciliaris (StY)
PI564917 E. ciliaris (StY)
VBG-0844 E. ciliaris (StY)
Pr87-88-337 E. ciliaris (StY)
PI531577 E. ciliaris (StY)
PI547303 E. ciliaris (StY)
BKA-0931 E. ciliaris (StY)
PI531575 E. ciliaris (StY)
W6 10267 E. ciliaris (StY)
PI377532 E. ciliaris (StY)
BKA-0939 E. ciliaris (StY)
PI531574 E. ciliaris (StY)
W6 14463 E. ciliaris (StY)
PI632544 E. ciliaris (StY)
PI208075 Ta. caput-medusae (Ta)
PI222048 Ta. caput-medusae (Ta)
PI220591 Ta. caput-medusae (Ta)
PI561091 Ta. caput-medusae (Ta)
PI533014 Au. retrofractum (W)
PI547363 Au. retrofractum (W)
PI383534 Agropyron cristatum (P)
PI142012 Th. elongatum (Ee)
PI531712 Th. bessarabicum (Eb)
H74 H.murinum (Xu)
H7514A H. vulgare (I)
H1816 H. chilense (H)
PI377532 E. ciliaris (StY)
H499498 H.bogdanii (H)
H121 H.marinum (Xa)
PI406469 Ps. juncea (Ns)
PI229595 Bromus sterilis
PI618974 Bromus inermis
CN32048 Bromus catharticu
St
Y

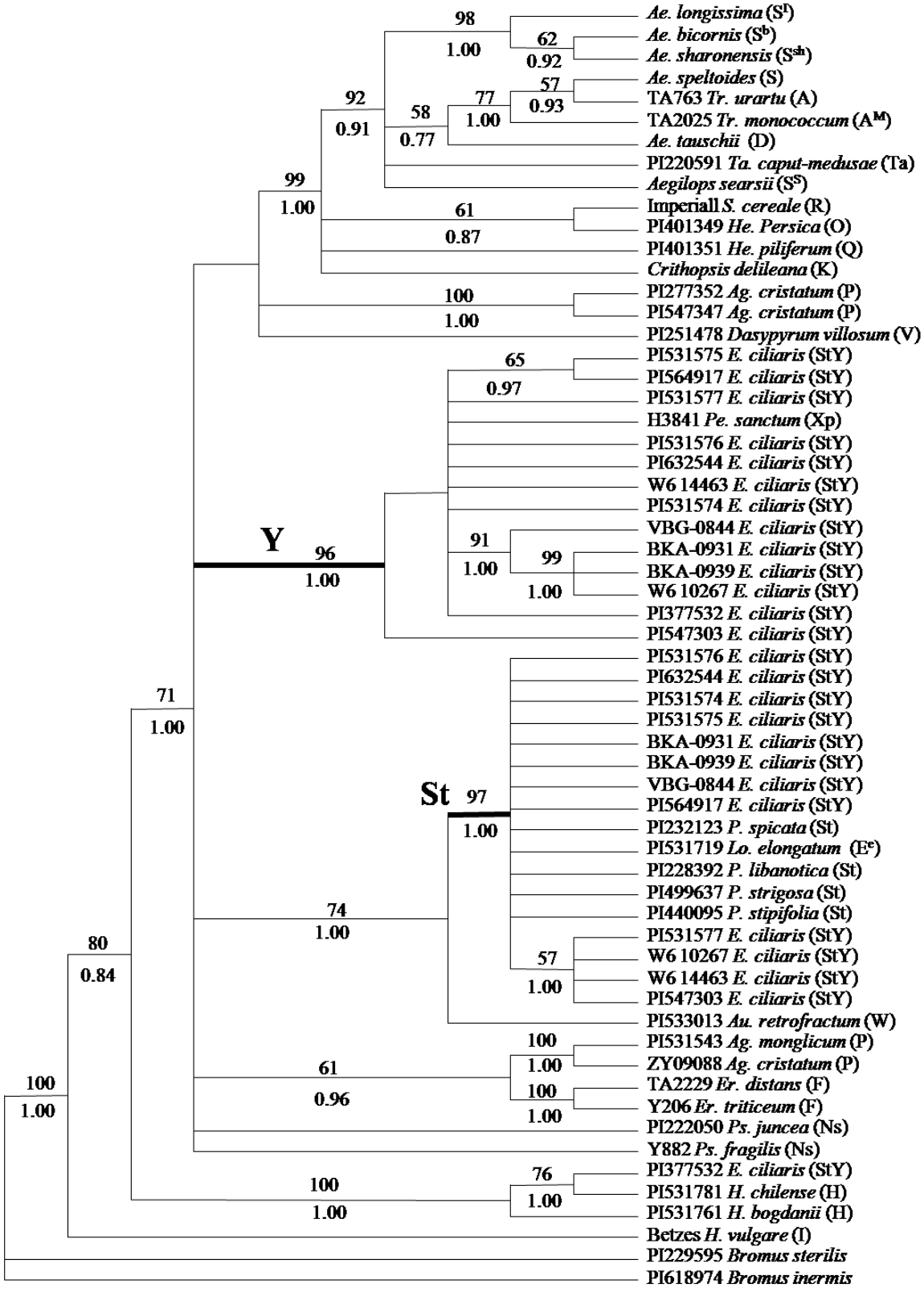
3.1.3. Phylogenetic analyses of *PGK1* sequences

Thirteen accessions of *E. ciliaris* were analyzed using the *PGK1* gene. Two distinct copies of sequences were detected for each accession of *E. ciliaris* studied. Phylogenetic analysis of the 60 sequences was performed using *B. sterilis* and *B. inermis* as outgroups. The data matrix contained 1403 characters, of which 957 were constant, 222 were parsimony uninformative, and 224 were parsimony informative. Heuristic searches resulted in 827 most parsimonious trees with a CI (excluding uninformative characters) = 0.642 and RI = 0.806. The Bayesian analyses using the GTR model results in identical trees with mean log-likelihood values -7140.45 and -7163.61 (data not shown). The tree topologies generated by ML, MP and Bayesian analyses were similar to each other. The strict consensus tree with BS and PP values is shown in Figure 3.

Two copies of sequences from each accession of tetraploid *E. ciliaris* were separated into two different clades, one into the Y genome clade and the other into the St genome clade, except for one copy of sequence from accessions PI 377532 ($St_?$ copy). The St-genomic sequences from tetraploid *E. ciliaris* were grouped together with St genome sequences from *Pseudoroegneria* species with a 97% bootstrap support (PP = 1.00; Fig. 3). Within this clade, the St sequences from *E. ciliaris* accession PI 531577, W6 10267, W6 14463, and PI 547303 formed a subclade (BS = 57%, PP = 1.00). Similarly to the phyologenetic result of *RPB2* data, phylogenetic analysis based on *PGK1* data also grouped one copy sequence from the accession PI 377532 together with H genome species, *Hordeum chilense* and *H. bogdanii* in high support (BS = 100%, PP = 1.00). Our *PGK1* sequence data also generated an obvious Y genome specific clade (BS = 96%, PP =

1.00; Fig. 3), which contained the Y genome copies sequences from all the *E. ciliaris* accessions analyzed, and is distinct from the St clade. Within the Y genome clade, four *E. ciliaris* accessions (VBG-0844, BKA-0931, BKA0939, and W6 10267) from Siberian, Russia formed a subclade with a strong bootstrap support of 91% (PP = 1.00 in ML).

Figure 3. Strict consensus tree derived from *PGK1* sequence data was conducted using heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and Bayesian posterior probability (PP) values, respectively. *B. sterilis* and *B. inermis* were used as outgroups. Consistency index (CI) = 0.642, retention index (RI) = 0.806.

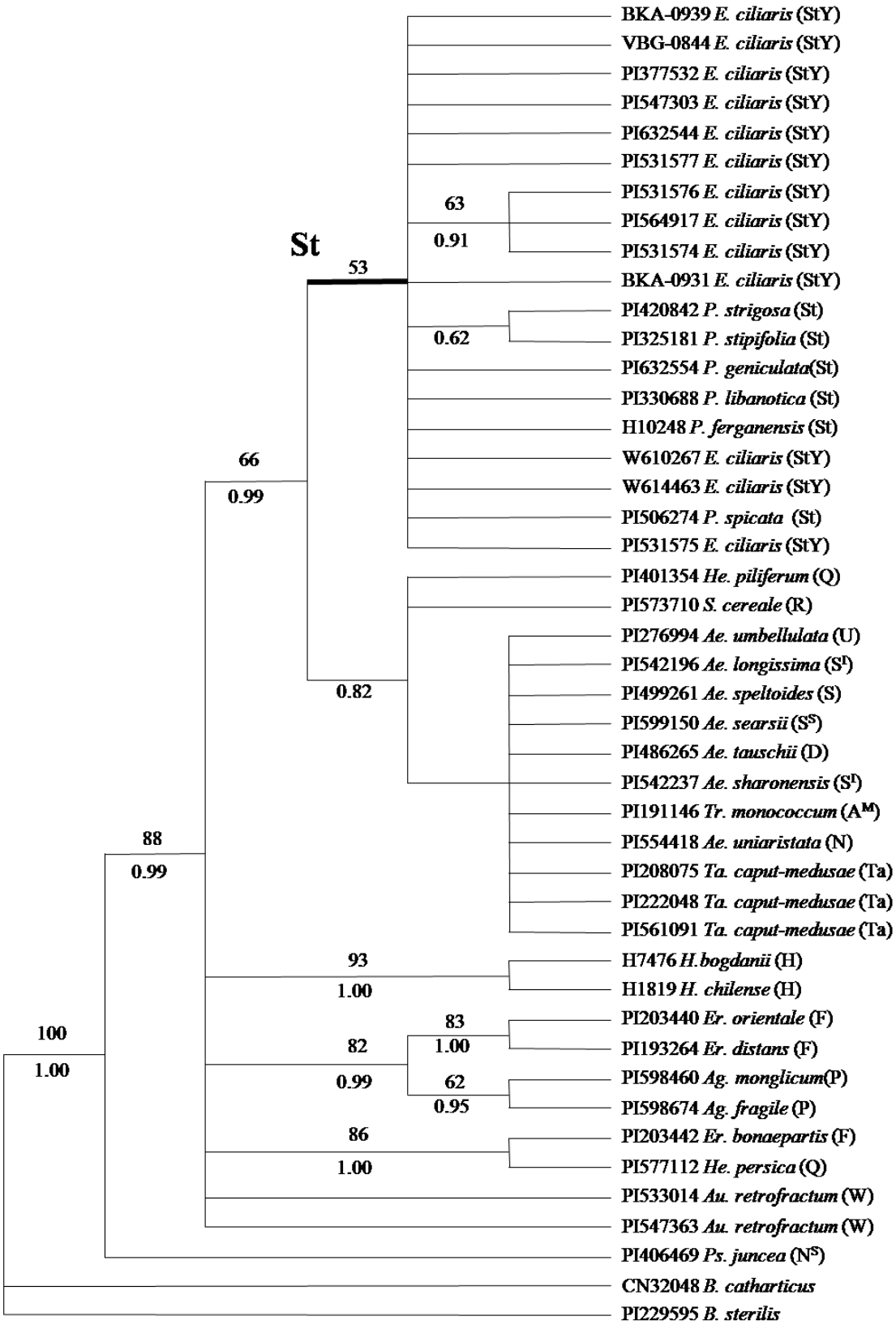

Ae. longissima (S^l)
Ae. bicornis (S^b)
Ae. sharonensis (S^sh)
Ae. speltoides (S)
TA763 Tr. urartu (A)
TA2025 Tr. monococcum (A^M)
Ae. tauschii (D)
PI220591 Ta. caput-medusae (Ta)
Aegilops searsii (S^S)
Imperiall S. cereale (R)
PI401349 He. Persica (O)
PI401351 He. piliferum (Q)
Crithopsis delileana (K)
PI277352 Ag. cristatum (P)
PI547347 Ag. cristatum (P)
PI251478 Dasypyrum villosum (V)
PI531575 E. ciliaris (StY)
PI564917 E. ciliaris (StY)
PI531577 E. ciliaris (StY)
H3841 Pe. sanctum (Xp)
PI531576 E. ciliaris (StY)
PI632544 E. ciliaris (StY)
W6 14463 E. ciliaris (StY)
PI531574 E. ciliaris (StY)
VBG-0844 E. ciliaris (StY)
BKA-0931 E. ciliaris (StY)
BKA-0939 E. ciliaris (StY)
W6 10267 E. ciliaris (StY)
PI377532 E. ciliaris (StY)
PI547303 E. ciliaris (StY)
PI531576 E. ciliaris (StY)
PI632544 E. ciliaris (StY)
PI531574 E. ciliaris (StY)
PI531575 E. ciliaris (StY)
BKA-0931 E. ciliaris (StY)
BKA-0939 E. ciliaris (StY)
VBG-0844 E. ciliaris (StY)
PI564917 E. ciliaris (StY)
PI232123 P. spicata (St)
PI531719 Lo. elongatum (E^e)
PI228392 P. libanotica (St)
PI499637 P. strigosa (St)
PI440095 P. stipifolia (St)
PI531577 E. ciliaris (StY)
W6 10267 E. ciliaris (StY)
W6 14463 E. ciliaris (StY)
PI547303 E. ciliaris (StY)
PI533013 Au. retrofractum (W)
PI531543 Ag. monglicum (P)
ZY09088 Ag. cristatum (P)
TA2229 Er. distans (F)
Y206 Er. triticeum (F)
PI222050 Ps. juncea (Ns)
Y882 Ps. fragilis (Ns)
PI377532 E. ciliaris (StY)
PI531781 H. chilense (H)
PI531761 H. bogdanii (H)
Betzes H. vulgare (I)
PI229595 Bromus sterilis
PI618974 Bromus inermis
Y
St
98
1.00
62
0.92
57
0.93
92
0.91
77
1.00
58
0.77
99
1.00
61
0.87
100
1.00
65
0.97
91
1.00
99
1.00
96
1.00
71
1.00
97
1.00
74
1.00
80
0.84
57
1.00
100
1.00
61
0.96
100
1.00
100
1.00
76
1.00
100
1.00

3.1.4. Phylogenetic analyses of *RPS16* sequences

Forty-eight *RPS16* sequences were analyzed. The data matrix contained 774 characters, of which 710 characters were constant, 35 variable characters were parsimony uninformative, and 29 were parsimony informative. MP analysis was conducted by using *B. sterilis* and *B. catharticus* as outgroups. MP analysis produced 79 equally parsimonious trees (CI excluding uninformative characters = 0.886; RI = 0.915). The Bayesian analyses using the GTR model resulted in identical trees with mean log-likelihood values -1690.64 and -1706.12 (data not shown). The tree topologies generated by ML, MP and Bayesian analyses were similar to each other. Strict consensus tree with BS and PP values is shown in Figure 4.

Phylogenetic analyses based on *RPS16* sequence data grouped all sequences from *E. ciliaris* into the St genome clade (Fig. 4). The sequence from the *E. ciliaris* accession PI 377532 was also included in the St clade. Within this clade, the sequences from three accessions of *E. ciliaris* (PI 531576, PI 564917 and PI 531574) formed a subclade in 65% BS and PP = 0.92.

Figure 4. Strict Consensus tree derived from *RPS16* sequence data was conducted using heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and Bayesian posterior probability (PP) values, respectively. *B. sterilis* and *B. catharticus* were used as outgroups. Consistency index (CI) = 0.909, retention index (RI) = 0.939.


St
BKA-0939 E. ciliaris (StY)
VBG-0844 E. ciliaris (StY)
PI377532 E. ciliaris (StY)
PI547303 E. ciliaris (StY)
PI632544 E. ciliaris (StY)
PI531577 E. ciliaris (StY)
PI531576 E. ciliaris (StY)
PI564917 E. ciliaris (StY)
PI531574 E. ciliaris (StY)
BKA-0931 E. ciliaris (StY)
PI420842 P. strigosa (St)
PI325181 P. stipifolia (St)
PI632554 P. geniculata(St)
PI330688 P. libanotica (St)
H10248 P. ferganensis (St)
W610267 E. ciliaris (StY)
W614463 E. ciliaris (StY)
PI506274 P. spicata (St)
PI531575 E. ciliaris (StY)
PI401354 He. piliferum (Q)
PI573710 S. cereale (R)
PI276994 Ae. umbellulata (U)
PI542196 Ae. longissima (S^l)
PI499261 Ae. speltoides (S)
PI599150 Ae. searsii (S^s)
PI486265 Ae. tauschii (D)
PI542237 Ae. sharonensis (S^l)
PI191146 Tr. monococcum (A^M)
PI554418 Ae. uniaristata (N)
PI208075 Ta. caput-medusae (Ta)
PI222048 Ta. caput-medusae (Ta)
PI561091 Ta. caput-medusae (Ta)
H7476 H.bogdanii (H)
H1819 H. chilense (H)
PI203440 Er. orientale (F)
PI193264 Er. distans (F)
PI598460 Ag. monglicum(P)
PI598674 Ag. fragile (P)
PI203442 Er. bonaepartis (F)
PI577112 He. persica (Q)
PI533014 Au. retrofractum (W)
PI547363 Au. retrofractum (W)
PI406469 Ps. juncea (N^S)
CN32048 B. catharticus
PI229595 B. sterilis
53
63
0.91
0.62
66
0.99
0.82
88
0.99
93
1.00
83
1.00
82
0.99
62
0.95
86
1.00
100
1.00

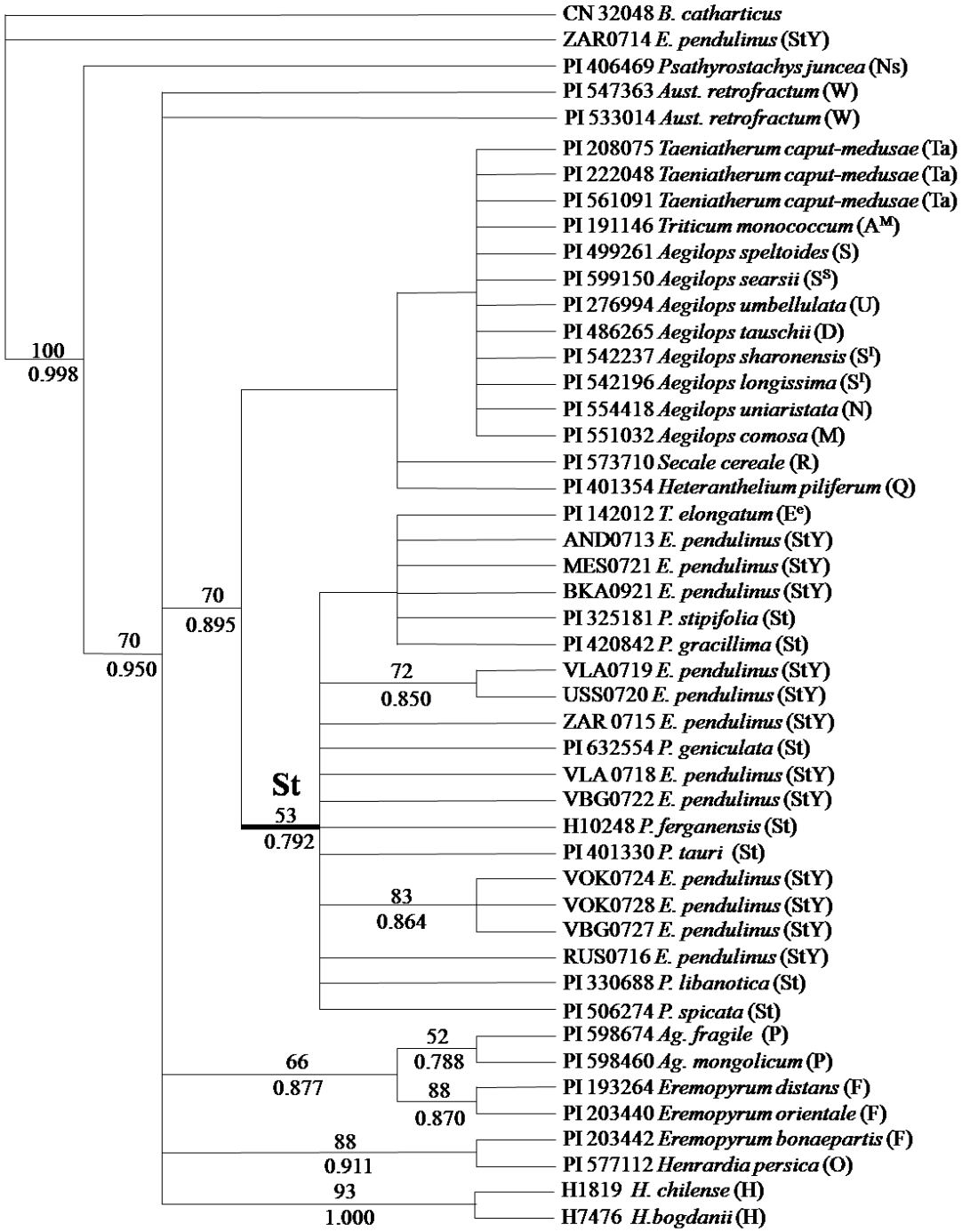
3.2. *E. pendulinus*

3.2.1. *RPS16* analysis

Thirteen accessions of *E. pendulinus* were analyzed together with an additional 34 *RPS16* sequences. The data matrix contained 733 characters, of which 659 characters were constant, 43 variable characters were parsimony-uninformative, and 31 were parsimony informative. MP analysis was conducted by using *Bromus catharticus* as outgroup. MP analysis produced 88 equally parsimonious trees (CI excluding uninformative characters = 0.886; RI = 0.913). The ML heuristic search under GTR model resulted in identical trees. The tree topologies generated by MP and ML analyses were similar to each other. A strict consensus tree with BS and ML aLRT values is shown in Figure 5.

Phylogenetic analyses based on *RPS16* sequence data grouped all sequences from *E. pendulinus* into the *Pseudoroegneria* St genome clade except for the sequence from the accession ZAR0714 of *E. pendulinus* (Fig. 5). Within this clade, the sequences from three accessions of *E. pendulinus* (VOK0724, VOK0728 and VBG0727) formed a subclade in 83% BS and aLRT = 0.864, and the accession VLA0719 and USS0720 were put into a subclade (BS=72, aLRT = 0.850). The accessions AND0713, MES0721 and BKA0921 were grouped with two *Pseudoroegneria* species (*P. stipifolia* PI 325181 and *P. gracillima* PI 420842) and *T. elongatum* (PI 142012) with weakly support. Unexpectedly, the sequence from the *E. pendulinus* accession ZAR0714 was not included in the St clade.

Figure 5. Strict Consensus tree derived from *RPS16* sequence data was conducted using heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and ML aLTR values, respectively. *B. catharticus* was used as outgroup. Consistency index (CI) = 0.886, retention index (RI) = 0.913.

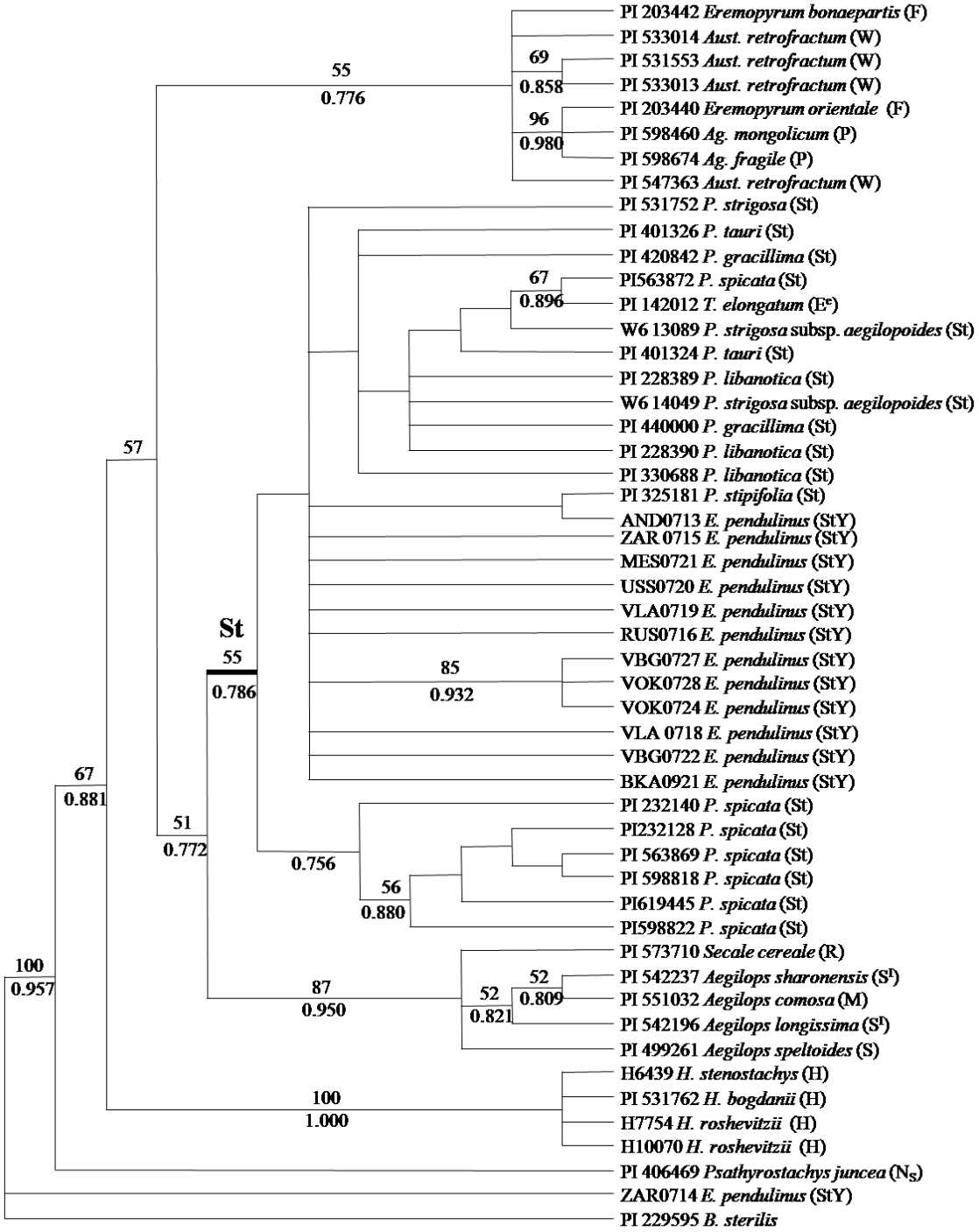

CN 32048 B. catharticus
ZAR0714 E. pendulinus (StY)
PI 406469 Psathyrostachys juncea (Ns)
PI 547363 Aust. retrofractum (W)
PI 533014 Aust. retrofractum (W)
PI 208075 Taeniatherum caput-medusae (Ta)
PI 222048 Taeniatherum caput-medusae (Ta)
PI 561091 Taeniatherum caput-medusae (Ta)
PI 191146 Triticum monococcum (A^M)
PI 499261 Aegilops speltoides (S)
PI 599150 Aegilops searsii (S^S)
PI 276994 Aegilops umbellulata (U)
PI 486265 Aegilops tauschii (D)
PI 542237 Aegilops sharonensis (S^l)
PI 542196 Aegilops longissima (S^l)
PI 554418 Aegilops uniaristata (N)
PI 551032 Aegilops comosa (M)
PI 573710 Secale cereale (R)
PI 401354 Heteranthelium piliferum (Q)
PI 142012 T. elongatum (E^e)
AND0713 E. pendulinus (StY)
MES0721 E. pendulinus (StY)
BKA0921 E. pendulinus (StY)
PI 325181 P. stipifolia (St)
PI 420842 P. gracillima (St)
VLA0719 E. pendulinus (StY)
USS0720 E. pendulinus (StY)
ZAR 0715 E. pendulinus (StY)
PI 632554 P. geniculata (St)
VLA 0718 E. pendulinus (StY)
VBG0722 E. pendulinus (StY)
H10248 P. ferganensis (St)
PI 401330 P. tauri (St)
VOK0724 E. pendulinus (StY)
VOK0728 E. pendulinus (StY)
VBG0727 E. pendulinus (StY)
RUS0716 E. pendulinus (StY)
PI 330688 P. libanotica (St)
PI 506274 P. spicata (St)
PI 598674 Ag. fragile (P)
PI 598460 Ag. mongolicum (P)
PI 193264 Eremopyrum distans (F)
PI 203440 Eremopyrum orientale (F)
PI 203442 Eremopyrum bonaepartis (F)
PI 577112 Henrardia persica (O)
H1819 H. chilense (H)
H7476 H.bogdanii (H)
100
0.998
70
0.950
70
0.895
St
53
0.792
72
0.850
83
0.864
52
0.788
66
0.877
88
0.870
88
0.911
93
1.000

3.2.2. *TrnD/T analysis*

Thirteen *TrnD/T* sequences from *E. pendulinus* were used in this analysis. The *TrnD/T* data matrix of 51 sequences contained 900 characters, of which 73 were parsimony informative. MP analysis produced 295 equally parsimonious trees (CI excluding uninformative characters = 0.797; RI = 0.764). The tree topologies generated by ML were similar to those generated by MP. Strict consensus trees with bootstrap (1000 replicates) values and ML is shown in Figure 6.

Phylogenetic analyses based on *TrnD/T* sequence data also grouped all sequences from *E. pendulinus* into the St genome clade except accession ZAR0714 (BS = 55, aLRT = 0.786) (Fig. 6). Interestingly, the cpDNA *TrnD*/*T* data tree also positioned the accession ZAR0714 of *E. pendulinus* outside of Triticeae species analyzed here. Within this clade, three accessions of *E. pendulinus* (VOK0724, VOK0728 and VBG0727) formed an independent subclade in 85% BS and aLRT = 0.932. The accession AND0713 of *E. pendulinus* grouped with one accession of *P. stipifolia* (PI 325181).

Figure 6. Strict consensus tree derived from *TrnD/T* sequence data was conducted using heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and ML aLTR values, respectively. *Bromus sterilis* was used as an outgroup. Consistency index (CI) = 0.797, retention index (RI) = 0.764.


PI 203442 Eremopyrum bonaepartis (F)
PI 533014 Aust. retrofractum (W)
PI 531553 Aust. retrofractum (W)
PI 533013 Aust. retrofractum (W)
PI 203440 Eremopyrum orientale (F)
PI 598460 Ag. mongolicum (P)
PI 598674 Ag. fragile (P)
PI 547363 Aust. retrofractum (W)
PI 531752 P. strigosa (St)
PI 401326 P. tauri (St)
PI 420842 P. gracillima (St)
PI563872 P. spicata (St)
PI 142012 T. elongatum (Ee)
W6 13089 P. strigosa subsp. aegilopoides (St)
PI 401324 P. tauri (St)
PI 228389 P. libanotica (St)
W6 14049 P. strigosa subsp. aegilopoides (St)
PI 440000 P. gracillima (St)
PI 228390 P. libanotica (St)
PI 330688 P. libanotica (St)
PI 325181 P. stipifolia (St)
AND0713 E. pendulinus (StY)
ZAR 0715 E. pendulinus (StY)
MES0721 E. pendulinus (StY)
USS0720 E. pendulinus (StY)
VLA0719 E. pendulinus (StY)
RUS0716 E. pendulinus (StY)
VBG0727 E. pendulinus (StY)
VOK0728 E. pendulinus (StY)
VOK0724 E. pendulinus (StY)
VLA 0718 E. pendulinus (StY)
VBG0722 E. pendulinus (StY)
BKA0921 E. pendulinus (StY)
PI 232140 P. spicata (St)
PI232128 P. spicata (St)
PI 563869 P. spicata (St)
PI 598818 P. spicata (St)
PI619445 P. spicata (St)
PI598822 P. spicata (St)
PI 573710 Secale cereale (R)
PI 542237 Aegilops sharonensis (Sl)
PI 551032 Aegilops comosa (M)
PI 542196 Aegilops longissima (Sl)
PI 499261 Aegilops speltoides (S)
H6439 H. stenostachys (H)
PI 531762 H. bogdanii (H)
H7754 H. roshevitzii (H)
H10070 H. roshevitzii (H)
PI 406469 Psathyrostachys juncea (NS)
ZAR0714 E. pendulinus (StY)
PI 229595 B. sterilis
St
55
0.776
69
0.858
96
0.980
67
0.896
57
55
0.786
85
0.932
67
0.881
51
0.772
0.756
56
0.880
100
0.957
87
0.950
52
0.821
52
0.809
100
1.000

3.2.3. *RPB2* analysis

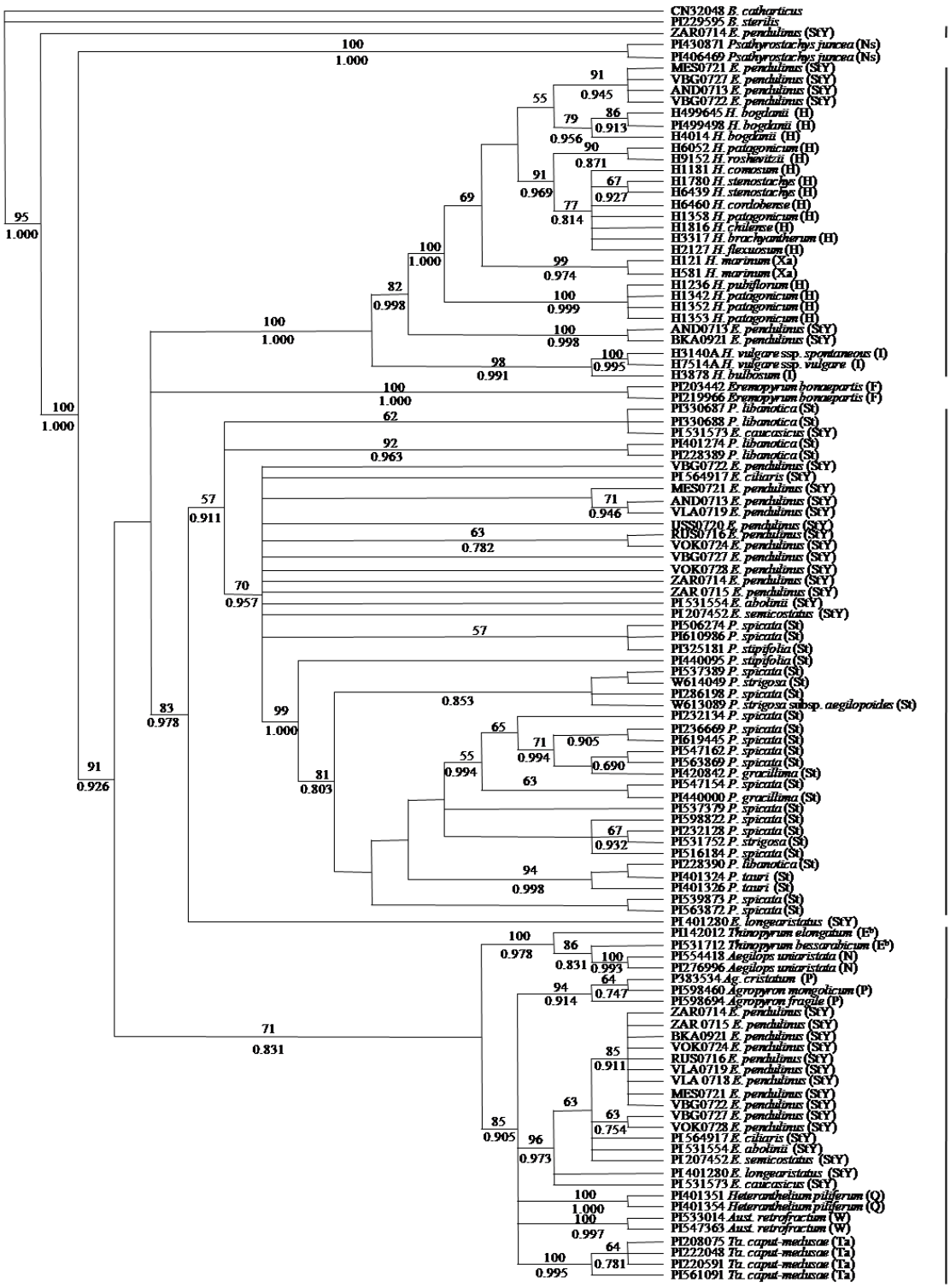
As expected for allotetraploids, two different copies were recovered from six accessions (BKA0921, VOK0728, VOK0724, VLA0719, RUS0716, and ZAR0715); however, three distinct sequences were recovered from five accessions of *E. pendulinus* (VBG0727, VBG0722, MES0721, ZAR0714, and AND0713), and one copy was recovered from two accessions (USS0720 and VLA0718). MP analysis using 100 *RPB2* sequences together with two outgroups, *Bromus sterilis* and *B. catharticus*, was conducted (332 parsimony-informative characters, 1320 equally most parsimonious trees, CI = 0.573; RI = 0.859). The separated ML analyses using the GTR model resulted in identical trees. The tree topologies were almost identical in ML trees and similar to those generated by MP. Strict consensus trees with ML aLTR and MP bootstrap (1000 replicates) value is shown (Fig. 7).

The phylogenetic tree showed three different clades, representing St, Y, and H genome groups, respectively. As expected, two distinct copies of sequences obtained from nine accessions of *E. pendulinus* (ZAR0714, ZAR0715, RUS0716, VLA0719, MES0721, VBG0722, VOK0724, VBG0727, VOK0728) that were amplified and sequenced were well separated into two different clades, one in the *Pseudoroegneria*-like (St genome) clade (BS=83%, aLTR = 98%), another in the Y genome clade (BS = 96%, aLTR = 97%). Only St genome sequence from accession USS0720 and only the Y genome sequence from accession VLA0718 were obtained. Within the Y genome clade, the *E.*

pendulinus sequences were separated into two subclades in 85%BS and 63%BS, respectively.

Unexpectedly, one of each of the sequences from five *E. pendulinus* accessions (AND0713, MES0721, VBG0722, VBG0727, and BKA0921) was grouped with *Hordeum* species (H, Xa, and I genomes) (BS = 100%, aLTR = 100%). These *Hordeum*-like sequences from *E. pendulinus* were put into two subclades. The first (91% BS) is comprised of accession AND 0713, MES0721, VBG0722, and VBG0727 along with *H. bogdanii*; the second subclade contained BKA0921 and AND0713 *Hordeum*-like copy.

Figure 7. Strict Consensus tree derived from *RPB2* sequence data was conducted using heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and ML aLTR values, respectively. *B. catharticus* was used as outgroup. Consistency index (CI) = 0.573, retention index (RI) = 0.859.


CN32048 B. catharticus
PI229595 B. sterilis
ZAR0714 E. pendulinus (StY)
PI430871 Psathyrostachys juncea (Ns)
PI406469 Psathyrostachys juncea (Ns)
MES0721 E. pendulinus (StY)
VBG0727 E. pendulinus (StY)
AND0713 E. pendulinus (StY)
VBG0722 E. pendulinus (StY)
H499645 H. bogdanii (H)
PI499498 H. bogdanii (H)
H4014 H. bogdanii (H)
H6052 H. patagonicum (H)
H9152 H. roshevitzii (H)
H1181 H. comosum (H)
H1780 H. stenostachys (H)
H6439 H. stenostachys (H)
H6460 H. cordobense (H)
H1358 H. patagonicum (H)
H1816 H. chilense (H)
H3317 H. brachyantherum (H)
H2127 H. flexuosum (H)
H121 H. marinum (Xa)
H581 H. marinum (Xa)
H1236 H. pubiflorum (H)
H1342 H. patagonicum (H)
H1352 H. patagonicum (H)
H1353 H. patagonicum (H)
AND0713 E. pendulinus (StY)
BKA0921 E. pendulinus (StY)
H3140A H. vulgare ssp. spontaneum (I)
H7514A H. vulgare ssp. vulgare (I)
H3878 H. bulbosum (I)
PI203442 Eremopyrum bonaepartis (F)
PI219966 Eremopyrum bonaepartis (F)
PI330687 P. libanotica (St)
PI330688 P. libanotica (St)
PI531573 E. caucasicus (StY)
PI401274 P. libanotica (St)
PI228389 P. libanotica (St)
VBG0722 E. pendulinus (StY)
PI564917 E. ciliaris (StY)
MES0721 E. pendulinus (StY)
AND0713 E. pendulinus (StY)
VLA0719 E. pendulinus (StY)
USS0720 E. pendulinus (StY)
RUS0716 E. pendulinus (StY)
VOK0724 E. pendulinus (StY)
VBG0727 E. pendulinus (StY)
VOK0728 E. pendulinus (StY)
ZAR0714 E. pendulinus (StY)
ZAR 0715 E. pendulinus (StY)
PI531554 E. alaskanus (StY)
PI207452 E. semicostatus (StY)
PI506274 P. spicata (St)
PI610986 P. spicata (St)
PI325181 P. stipifolia (St)
PI440095 P. stipifolia (St)
PI537389 P. spicata (St)
W614049 P. strigosa (St)
PI286198 P. spicata (St)
W613089 P. strigosa subsp. aegilopoides (St)
PI232134 P. spicata (St)
PI236669 P. spicata (St)
PI619445 P. spicata (St)
PI547162 P. spicata (St)
PI563869 P. spicata (St)
PI420842 P. gracillima (St)
PI547154 P. spicata (St)
PI440000 P. gracillima (St)
PI537379 P. spicata (St)
PI598822 P. spicata (St)
PI232128 P. spicata (St)
PI531752 P. strigosa (St)
PI516184 P. spicata (St)
PI228390 P. libanotica (St)
PI401324 P. tauri (St)
PI401326 P. tauri (St)
PI539873 P. spicata (St)
PI563872 P. spicata (St)
PI 401280 E. longearistatus (StY)
PI142012 Thinopyrum elongatum (E^b)
PI531712 Thinopyrum bessarabicum (E^b)
PI554418 Aegilops uniaristata (N)
PI276996 Aegilops uniaristata (N)
P383534 Ag. cristatum (P)
PI598460 Agropyron mongolicum (P)
PI598694 Agropyron fragile (P)
ZAR0714 E. pendulinus (StY)
ZAR 0715 E. pendulinus (StY)
BKA0921 E. pendulinus (StY)
VOK0724 E. pendulinus (StY)
RUS0716 E. pendulinus (StY)
VLA0719 E. pendulinus (StY)
VLA 0718 E. pendulinus (StY)
MES0721 E. pendulinus (StY)
VBG0722 E. pendulinus (StY)
VBG0727 E. pendulinus (StY)
VOK0728 E. pendulinus (StY)
PI564917 E. ciliaris (StY)
PI531554 E. alaskanus (StY)
PI207452 E. semicostatus (StY)
PI 401280 E. longearistatus (StY)
PI531573 E. caucasicus (StY)
PI401351 Heteranthelium piliferum (Q)
PI401354 Heteranthelium piliferum (Q)
PI533014 Aust. retrofractum (W)
PI547363 Aust. retrofractum (W)
PI208075 Ta. caput-medusae (Ta)
PI222048 Ta. caput-medusae (Ta)
PI220591 Ta. caput-medusae (Ta)
PI561091 Ta. caput-medusae (Ta)
?
Hordeum+Elymus
Pseudoroegneria+Elymus
Y+Triticeae

3.2.4. *PepC analysis*

Fourteen accessions of *E. pendulinus* were analyzed using the *PepC* gene sequence. Expectedly, two distinct *PepC* copies were recovered from nine accessions of *E. pendulinus* (PI499452, ZAR0715, RUS0716, VLA0718, VLA0719, USS0720, MES0721, VBG0722, VOK0724, and VBG0727). Unexpectedly, three different copies of *PepC* sequences were detected from four accessions of *E. pendulinus* (AND0713, ZAR0714, VLA0719, and BKA0921), and only one copy was identified from accession VOK0728. Phylogenetic analysis of the 80 sequences was performed using *B. tectorum* as outgroup. The data matrix contained 1054 characters, of which 267 were constant, 395 were parsimony uninformative, and 392 were parsimony informative. Heuristic searches resulted in 1575 most parsimonious trees with a CI (excluding uninformative characters) = 0.705 and RI = 0.838. The tree topologies generated by ML using GTR model and MP analyses were similar to each other. Strict consensus tree with BS and aLRT values is shown in Figure 4.

The phylogentic analyses generated three large clades (Fig. 8), representing St genome sequences, Y genome sequences together with Triticeae genome sequences (M, Ta, A^M, R, and E^e), and a *Hordeum*-like sequence. Thirteen *E. pendulinus* sequences were grouped with *Pseudoroegneria* (St) diploid species. The second clade was composed of the identified Y copies from tetraploid *Elymus* species, *E. pendulinus* sequences and the sequences from M, Ta, A^M, R, and E^e genomes in Triticeae. In this clade, nine accessions from *E. pendulinus* together with seven Y copies from additional tetraploid *Elymus*

species constructed a subclade, and the other three *E. pendulinus* sequences were grouped with R and E^e genomes sequences. Four sequences from *E. pendulinus* fell into the H clade together with diploid *Hordeum* species, named *Hordeum*-like copies. Interestingly, a copy from *E. pendulinus* ZAR0714 was also put outside of Triticeae species analyzed here which is consistent with the *RPB2* nuclear gene and two cpDNA data. Two different *Hordeum*-like copies with obvious sequence variation from the accession VBG0727, and two distinct St copies from the accession BKA0921 were also recovered.

Within the St (*Pseudoroegneria* + *Elymus*) clade, all *E. pendulinus* accessions, except AND0713, BKA0921b, and VLA0719, formed a well supported subclade (93% BS, 94% aLRT). The sequences from AND0713 and BKA0921b were grouped into a separated subclade (99% BS, 99% aLRT). The St copy from VLA0719 was distinct from the other St copies. In the second clade, AND0713, BKA0921b, and VLA0719 were grouped with Triticeae diploid species with R, Ta, A^M, E^e, and M genomes. The rest of the Y genome sequences of *E. pendulinus* were grouped with other tetraploid *Elymus* with strong support (100% BS and aLRT), within this clade, and all accessions from Russia were placed into one subclade (BS= 62%, aLRT = 87%).

Figure 8. Strict Consensus tree derived from *PepC* sequence data was conducted using heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and ML aLTR values, respectively. *B. tectorum* was used as outgroup. Consistency index (CI) = 0.705, retention index (RI) = 0.838.

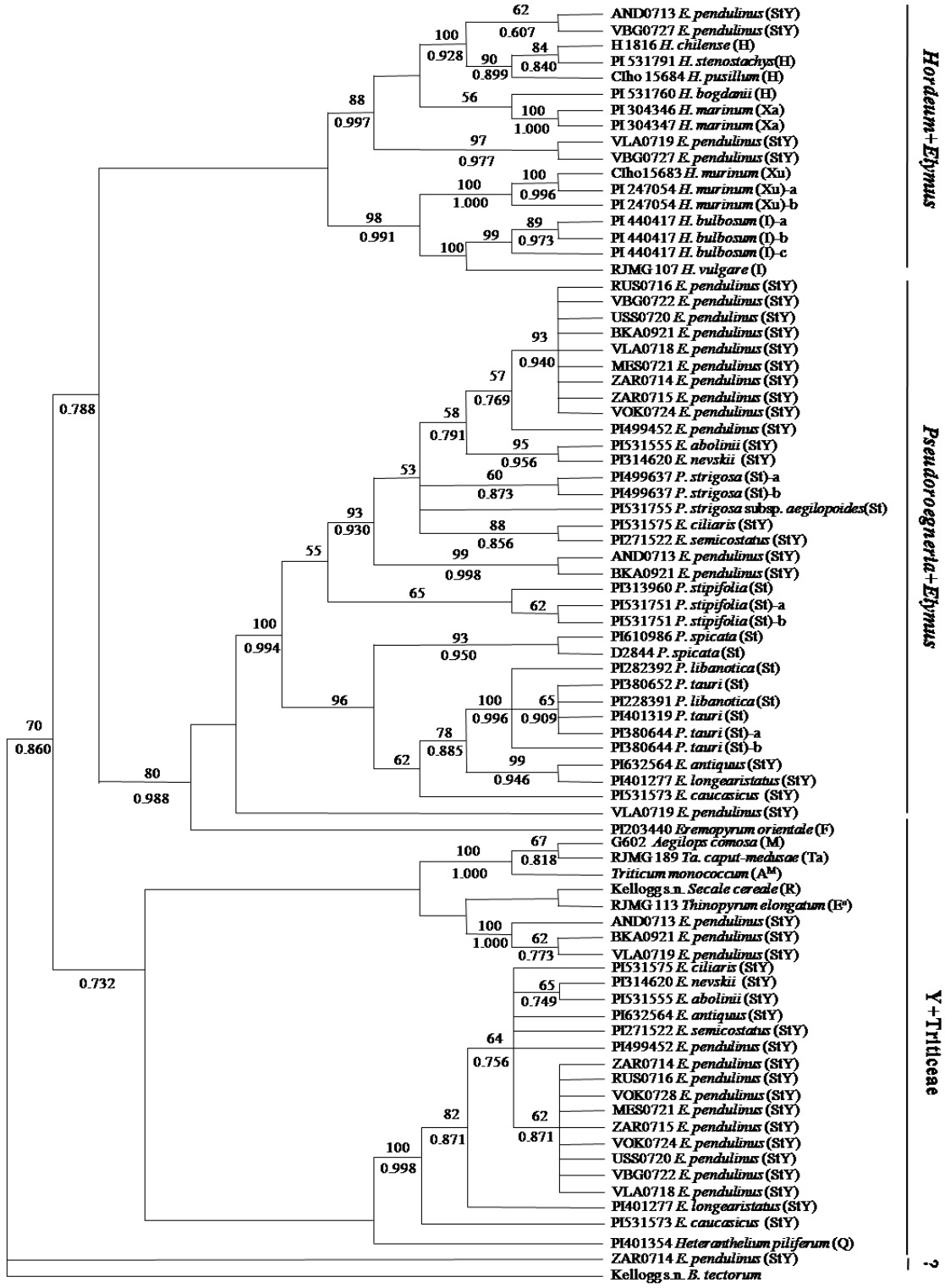
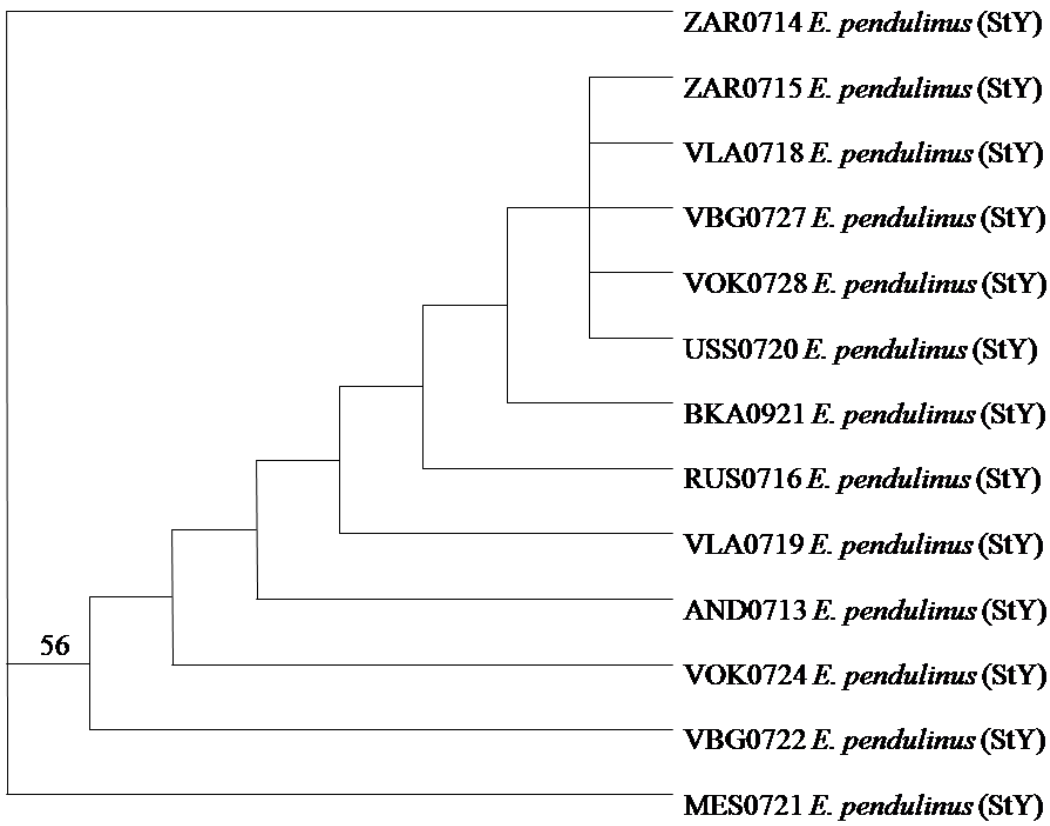

AND0713 E. pendulinus (StY)
VBG0727 E. pendulinus (StY)
H 1816 H. chilense (H)
PI 531791 H. stenostachys (H)
CIho 15684 H. pusillum (H)
PI 531760 H. bogdanii (H)
PI 304346 H. marinum (Xa)
PI 304347 H. marinum (Xa)
VLA0719 E. pendulinus (StY)
VBG0727 E. pendulinus (StY)
CIho15683 H. murinum (Xu)
PI 247054 H. murinum (Xu)-a
PI 247054 H. murinum (Xu)-b
PI 440417 H. bulbosum (I)-a
PI 440417 H. bulbosum (I)-b
PI 440417 H. bulbosum (I)-c
RJMG 107 H. vulgare (I)
RUS0716 E. pendulinus (StY)
VBG0722 E. pendulinus (StY)
USS0720 E. pendulinus (StY)
BKA0921 E. pendulinus (StY)
VLA0718 E. pendulinus (StY)
MES0721 E. pendulinus (StY)
ZAR0714 E. pendulinus (StY)
ZAR0715 E. pendulinus (StY)
VOK0724 E. pendulinus (StY)
PI499452 E. pendulinus (StY)
PI531555 E. abolinii (StY)
PI314620 E. nevskii (StY)
PI499637 P. strigosa (St)-a
PI499637 P. strigosa (St)-b
PI531755 P. strigosa subsp. aegilopoides (St)
PI531575 E. ciliaris (StY)
PI271522 E. semicostatus (StY)
AND0713 E. pendulinus (StY)
BKA0921 E. pendulinus (StY)
PI313960 P. stipifolia (St)
PI531751 P. stipifolia (St)-a
PI531751 P. stipifolia (St)-b
PI610986 P. spicata (St)
D2844 P. spicata (St)
PI282392 P. libanotica (St)
PI380652 P. tauri (St)
PI228391 P. libanotica (St)
PI401319 P. tauri (St)
PI380644 P. tauri (St)-a
PI380644 P. tauri (St)-b
PI632564 E. antiquus (StY)
PI401277 E. longearistatus (StY)
PI531573 E. caucasicus (StY)
VLA0719 E. pendulinus (StY)
PI203440 Eremopyrum orientale (F)
G602 Aegilops comosa (M)
RJMG 189 Ta. caput-medusae (Ta)
Triticum monococcum (AM)
Kellogg s.n. Secale cereale (R)
RJMG 113 Thinopyrum elongatum (Ee)
AND0713 E. pendulinus (StY)
BKA0921 E. pendulinus (StY)
VLA0719 E. pendulinus (StY)
PI531575 E. ciliaris (StY)
PI314620 E. nevskii (StY)
PI531555 E. abolinii (StY)
PI632564 E. antiquus (StY)
PI271522 E. semicostatus (StY)
PI499452 E. pendulinus (StY)
ZAR0714 E. pendulinus (StY)
RUS0716 E. pendulinus (StY)
VOK0728 E. pendulinus (StY)
MES0721 E. pendulinus (StY)
ZAR0715 E. pendulinus (StY)
VOK0724 E. pendulinus (StY)
USS0720 E. pendulinus (StY)
VBG0722 E. pendulinus (StY)
VLA0718 E. pendulinus (StY)
PI401277 E. longearistatus (StY)
PI531573 E. caucasicus (StY)
PI401354 Heteranthelium piliferum (Q)
ZAR0714 E. pendulinus (StY)
Kellogg s.n. B. tectorum
Hordeum+Elymus
Pseudoroegneria+Elymus
Y+Triticeae
?

Figure 9. Strict consensus tree derived from morphological data matrix (glume trichomes, lemma margin cilia, lemma trichomes, rachilla trichomes, leaf-sheath villosity, blade upper surface villosity, stem node) was conducted using heuristic search with TBR branch swapping. Numbers above are bootstrap values. Consistency index (CI) = 0.675, retention index (RI) = 0.618.


ZAR0714 E. pendulinus (StY)
ZAR0715 E. pendulinus (StY)
VLA0718 E. pendulinus (StY)
VBG0727 E. pendulinus (StY)
VOK0728 E. pendulinus (StY)
USS0720 E. pendulinus (StY)
BKA0921 E. pendulinus (StY)
RUS0716 E. pendulinus (StY)
VLA0719 E. pendulinus (StY)
AND0713 E. pendulinus (StY)
56
VOK0724 E. pendulinus (StY)
VBG0722 E. pendulinus (StY)
MES0721 E. pendulinus (StY)

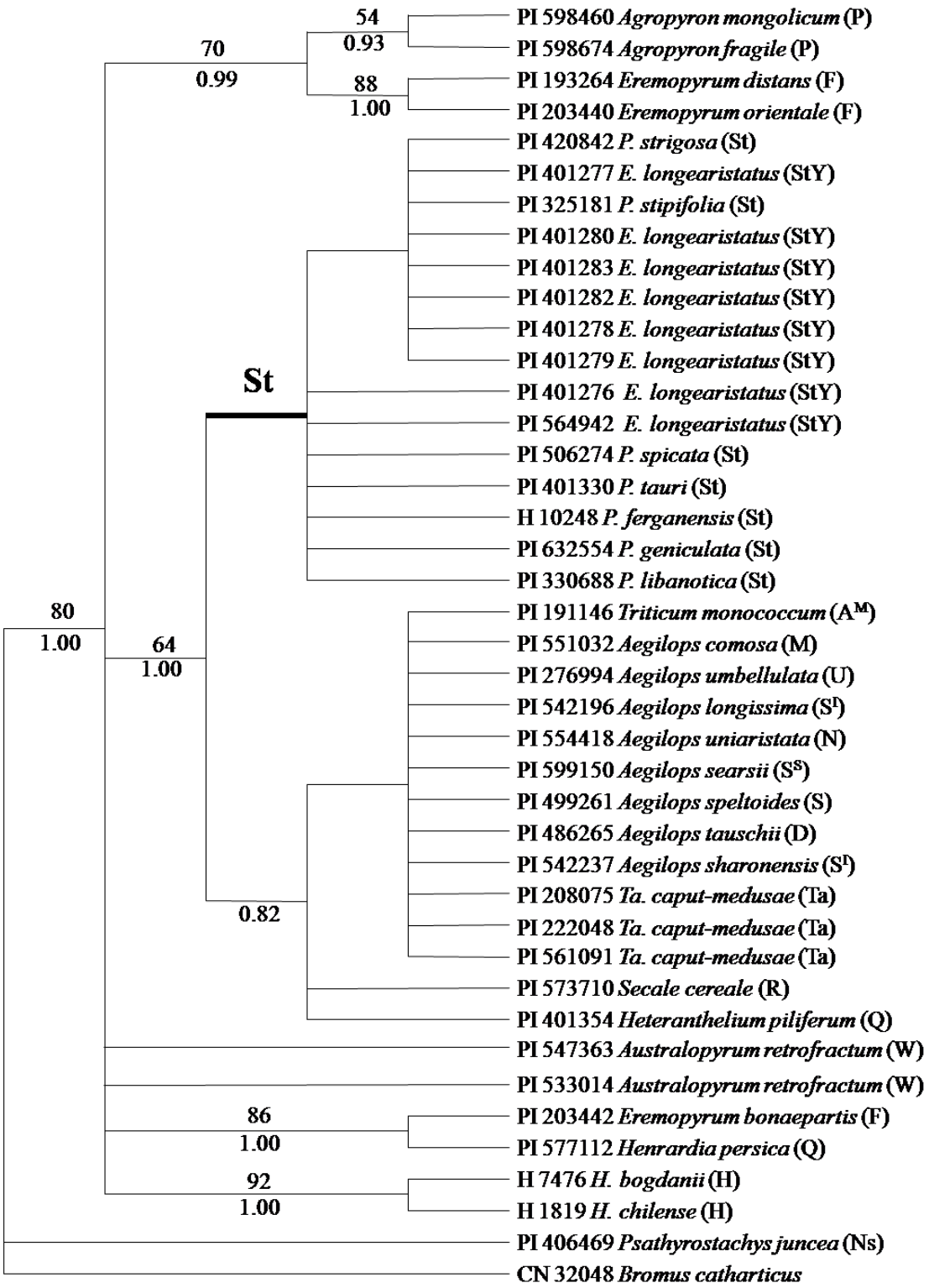
3.3. *E. longearistatus*

3.3.1. Phylogenetic analyses of *RPS16* sequences

The *RPS16* data matrix of 41 sequences contained 736 characters, of which 680 were constant, 35 were parsimony uninformative, and 21 were parsimony informative. MP analysis produced 66 equally parsimonious trees (CI = 0.894, RI = 0.923, RCI = 0.825). The separated Bayesian analyses using GTR model resulted in identical trees with mean log-likelihood values -1552.69 and -1563.64 (data not shown).

Phylogenetic analyses based on *RPS16* sequence data grouped all sequences from *E. longearistatus* into the St clade with the St genome from *Pseudoroegneria* (Fig. 10). Within this clade, six accessions of *E. longearistatus* originated from Iran (PI 401277, PI 401278, PI 401279, PI 401280, PI 401282, PI 401283) formed a subclade with the accession PI 420842 of *P. strigosa* and PI 325181 of *P. stipifolia*. The accessions PI 401276 (from Iran) and PI 564942 (from Pakistan) were placed out of this subclade with other *Pseudoroegneria* diploid species.

Figure 10. Strict consensus trees derived from *RPS16* sequence data was conducted using heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and Bayesian posterior probability (PP) values, respectively. *Bromus catharticu* was used as an outgroup. Consistency index (CI) = 0.894, retention index (RI) = 0.923, rescale consistency index (RCI) = 0.825.

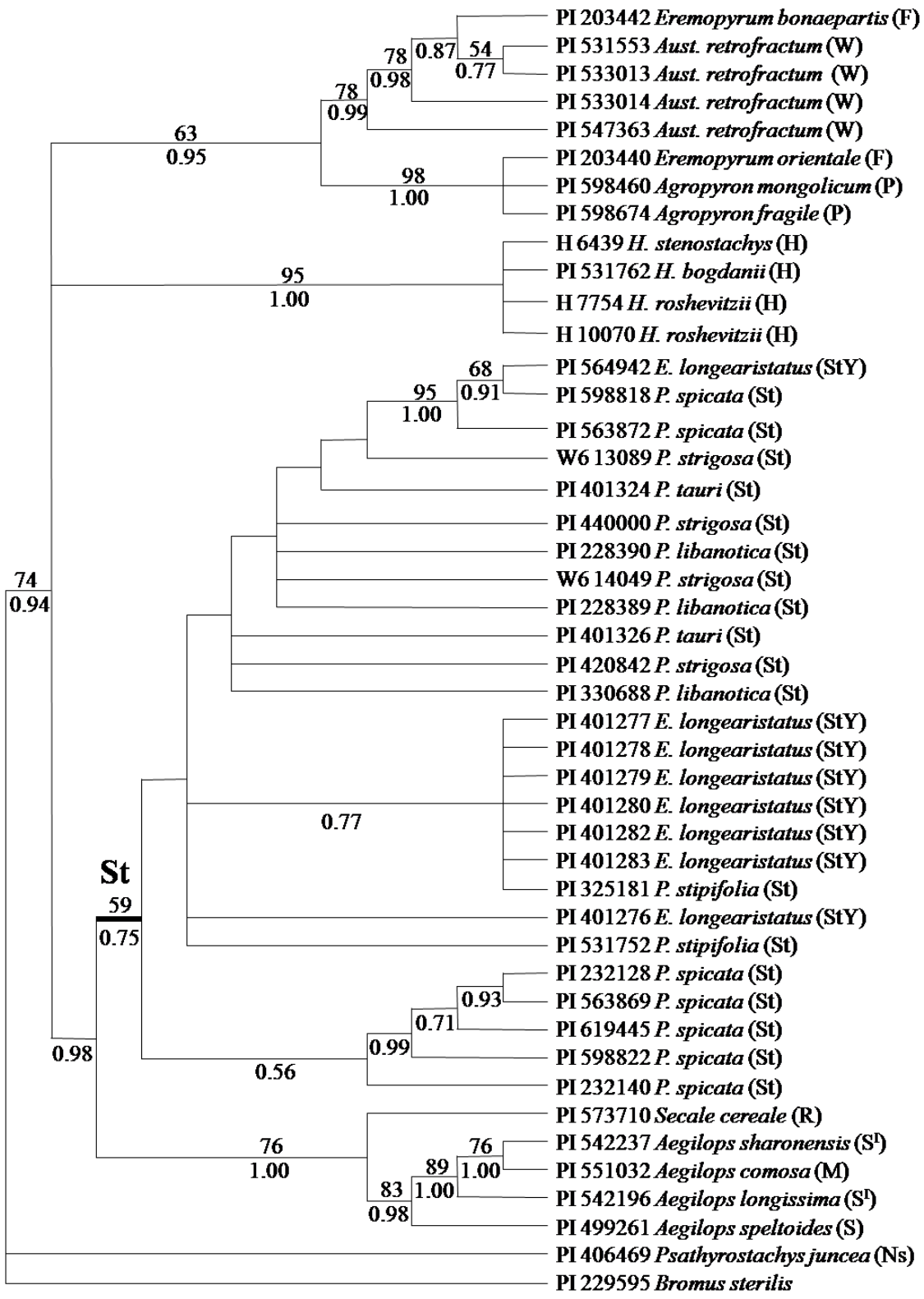

54
0.93
70
0.99
88
1.00
PI 598460 Agropyron mongolicum (P)
PI 598674 Agropyron fragile (P)
PI 193264 Eremopyrum distans (F)
PI 203440 Eremopyrum orientale (F)
PI 420842 P. strigosa (St)
PI 401277 E. longearistatus (StY)
PI 325181 P. stipifolia (St)
PI 401280 E. longearistatus (StY)
PI 401283 E. longearistatus (StY)
PI 401282 E. longearistatus (StY)
PI 401278 E. longearistatus (StY)
PI 401279 E. longearistatus (StY)
St
PI 401276 E. longearistatus (StY)
PI 564942 E. longearistatus (StY)
PI 506274 P. spicata (St)
PI 401330 P. tauri (St)
H 10248 P. ferganensis (St)
PI 632554 P. geniculata (St)
PI 330688 P. libanotica (St)
80
1.00
64
1.00
PI 191146 Triticum monococcum (A^M)
PI 551032 Aegilops comosa (M)
PI 276994 Aegilops umbellulata (U)
PI 542196 Aegilops longissima (S^l)
PI 554418 Aegilops uniaristata (N)
PI 599150 Aegilops searsii (S^s)
PI 499261 Aegilops speltoides (S)
PI 486265 Aegilops tauschii (D)
PI 542237 Aegilops sharonensis (S^l)
PI 208075 Ta. caput-medusae (Ta)
0.82
PI 222048 Ta. caput-medusae (Ta)
PI 561091 Ta. caput-medusae (Ta)
PI 573710 Secale cereale (R)
PI 401354 Heteranthelium piliferum (Q)
PI 547363 Australopyrum retrofractum (W)
PI 533014 Australopyrum retrofractum (W)
86
1.00
PI 203442 Eremopyrum bonaepartis (F)
PI 577112 Henrardia persica (Q)
92
1.00
H 7476 H. bogdanii (H)
H 1819 H. chilense (H)
PI 406469 Psathyrostachys juncea (Ns)
CN 32048 Bromus catharticus

3.3.2. Phylogenetic analyses of *TrnD/T* sequences

Eight accessions of *E. longearistatus* were analyzed together with an additional 37 *TrnD/T* sequences. The data matrix contained 1027 characters, of which 758 characters were constant, 99 were parsimony informative, and 170 variable characters were parsimony-uninformative. MP analysis was conducted by using *Bromus sterilis* as the outgroup. MP analysis produced 423 most parsimonious trees (CI = 0.773, RI = 0.725, RCI =0.560). The separated Bayesian analyses using GTR model resulted in identical trees with mean log-likelihood values -3962.49 and -3983.07 (data not shown).

As shown in Figure 11, MP and Bayesian analyses clearly grouped the sequences from *E. longearistatus* with diploid *Pseudoroegneria* species into one clade (BS = 59%, PP = 0.75). Within this clade, the sequences from six accessions of *E. longearistatus* (PI 401277, PI 401278, PI 401279, PI 401280, PI 401282, PI 401283) formed a subclade with a *P. stipifolia* accession PI 325181 in 0.77 Bayesian PP value, the accession PI 564942 was grouped with one *P. spicata* accession PI 598818 (BS = 68%, PP = 0.91), and PI 401276 was placed outside these subclades with other diploid *Pseudoroegneria* species.

Figure 11. Strict consensus trees derived from *TrnD/T* sequence data was conducted using heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and Bayesian posterior probability (PP) values, respectively. *Bromus sterilis* was used as an outgroup. Consistency index (CI) = 0.773, retention index (RI) = 0.725, rescale consistency index (RCI) = 0.560.


PI 203442 Eremopyrum bonaepartis (F)
PI 531553 Aust. retrofractum (W)
PI 533013 Aust. retrofractum (W)
PI 533014 Aust. retrofractum (W)
PI 547363 Aust. retrofractum (W)
PI 203440 Eremopyrum orientale (F)
PI 598460 Agropyron mongolicum (P)
PI 598674 Agropyron fragile (P)
H 6439 H. stenostachys (H)
PI 531762 H. bogdanii (H)
H 7754 H. roshevitzii (H)
H 10070 H. roshevitzii (H)
PI 564942 E. longearistatus (StY)
PI 598818 P. spicata (St)
PI 563872 P. spicata (St)
W6 13089 P. strigosa (St)
PI 401324 P. tauri (St)
PI 440000 P. strigosa (St)
PI 228390 P. libanotica (St)
W6 14049 P. strigosa (St)
PI 228389 P. libanotica (St)
PI 401326 P. tauri (St)
PI 420842 P. strigosa (St)
PI 330688 P. libanotica (St)
PI 401277 E. longearistatus (StY)
PI 401278 E. longearistatus (StY)
PI 401279 E. longearistatus (StY)
PI 401280 E. longearistatus (StY)
PI 401282 E. longearistatus (StY)
PI 401283 E. longearistatus (StY)
PI 325181 P. stipifolia (St)
PI 401276 E. longearistatus (StY)
PI 531752 P. stipifolia (St)
PI 232128 P. spicata (St)
PI 563869 P. spicata (St)
PI 619445 P. spicata (St)
PI 598822 P. spicata (St)
PI 232140 P. spicata (St)
PI 573710 Secale cereale (R)
PI 542237 Aegilops sharonensis (S[I])
PI 551032 Aegilops comosa (M)
PI 542196 Aegilops longissima (S[I])
PI 499261 Aegilops speltoides (S)
PI 406469 Psathyrostachys juncea (Ns)
PI 229595 Bromus sterilis
St
78
0.87
54
0.77
78
0.98
63
0.95
78
0.99
98
1.00
95
1.00
68
0.91
95
1.00
74
0.94
0.77
59
0.75
0.98
0.56
0.93
0.71
0.99
76
1.00
76
1.00
89
1.00
83
0.98

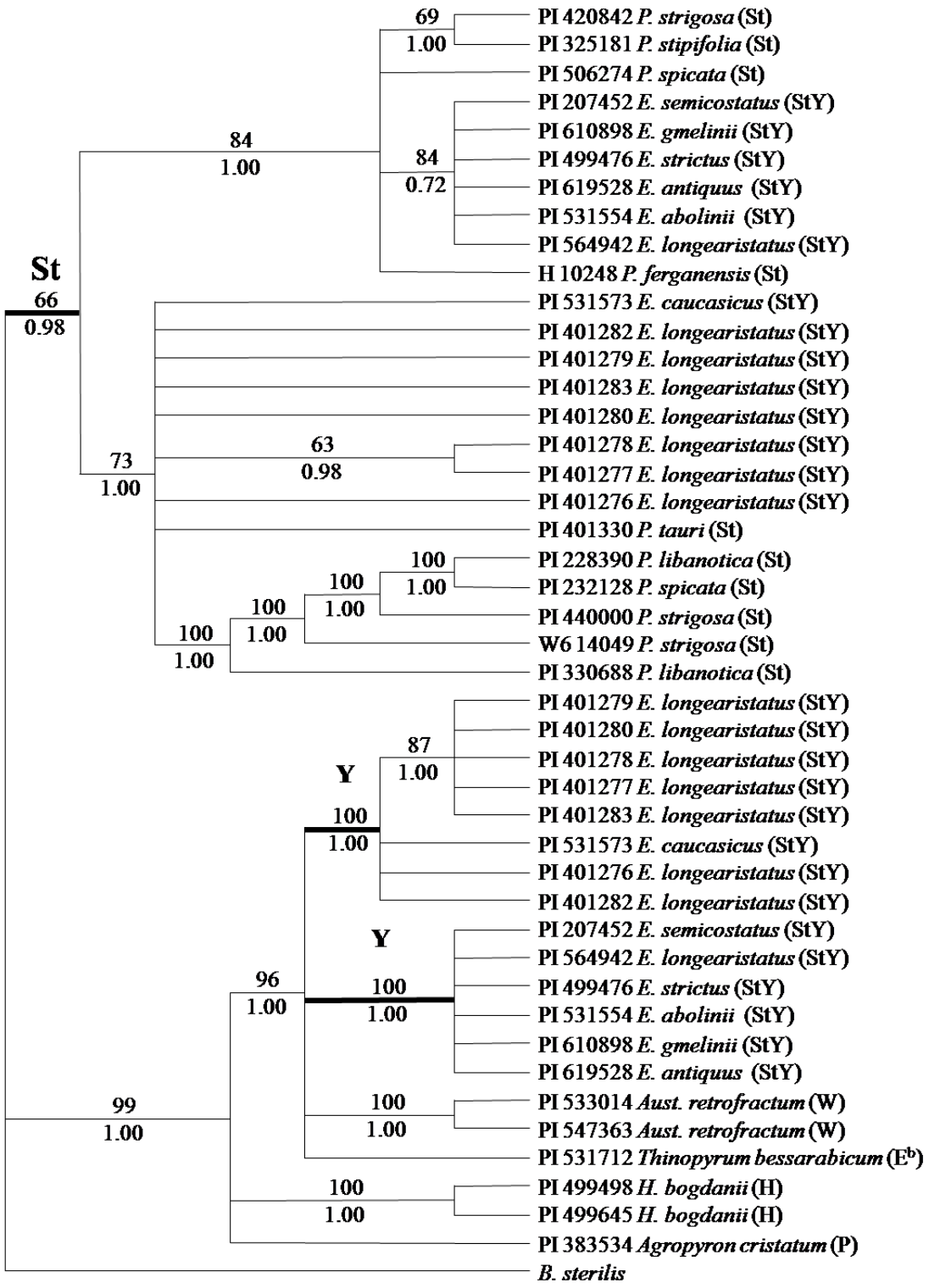
3.3.3. Phylogenetic analyses of *EF-G* sequences

Total of 45 sequences from 11 diploid Triticeae species/subspecies, six tetraploid StY *Elymus*, and eight accessions of *E. longearistatus* were analyzed. MP analysis using *Bromus sterilis* as the outgroup was conducted (258 parsimony-informative characters, 509 equally most parsimonious trees, CI = 0.884; RI = 0.964). The separated Bayesian analyses using the GTR model resulted in identical trees with mean log-likelihood values -4012.01 and -4026.92 (data not shown). The tree topologies were almost identical in both ML and Bayesian trees and were similar to those generated by MP. A strict consensus tree with bootstrap (1000 replicates) values and Bayesian PP are shown in Figure 12.

Phylogenetic analyses clearly separated the two copies of sequences from each StY tetraploid (*E. abolinii*, *E. antiquus*, *E. genelinii*, *E. semicostatus*, *E. strictus*, *E. caucasicus*, and *E. longearistatus*) accession into two different clades, one in the St genome clade, the other in the Y genome clade. All diploid *Pseudoroegneria* species (St genome) together with St copy from tetraploid StY *Elymus* species were grouped into a clade (BS = 66%, PP = 0.98). Within the St (*Pseudoroegneria* + *Elymus*) clade, the St genome from eight *E. longearistatus* accessions were clearly separated into two groups, the first one containing one *E. longearistatus* accession (PI 564942, origin from Pakistan) and five StY tetraploid *Elymus* species (BS = 84%, PP = 1.00), the second one containing the rest of seven *E. longearistatus* accessions (PI 401276, PI 401277, PI 401278, PI 401279, PI 401280, PI 401282, PI 401282, all originating from Iran) and one accession of *E. caucasicus* (StY) (BS = 73%, PP = 1.00).

The Y copy sequences from tetraploid StY *Elymus* species formed another clade with the W, P, H, and E^b genome species in 99% BS (PP = 1.00). The Y genome sequences from *Elymus* were also separated into two clades both with a strong bootstrap support of 100% and PP = 1.00, the accession PI 564942 originating from Pakistan was grouped with five StY tetraploid *Elymus* species (*E. abolinii*, *E. antiques*, *E. gmelinii*, *E. semicostatus*, *E. strictus*), the other seven accessions of *E. longearistatus*, which all originate from Iran, were placed with *E. caucasicus*. Within the Iranian Y clade, five *E. longearistatus* accessions (PI 401277, PI 401278, PI 401279, PI 401280, PI 401283) formed a well supported subclade (BS = 87%, PP = 1.00).

Figure 12. Strict consensus trees derived from *EF-G* sequence data was conducted using heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and Bayesian posterior probability (PP) values, respectively. *Bromus sterilis* was used as an outgroup. Consistency index (CI) = 0.884, retention index (RI) = 0.964, rescale consistency index (RCI) = 0.853.


St
Y
Y
PI 420842 P. strigosa (St)
PI 325181 P. stipifolia (St)
PI 506274 P. spicata (St)
PI 207452 E. semicostatus (StY)
PI 610898 E. gmelinii (StY)
PI 499476 E. strictus (StY)
PI 619528 E. antiquus (StY)
PI 531554 E. abolinii (StY)
PI 564942 E. longearistatus (StY)
H 10248 P. ferganensis (St)
PI 531573 E. caucasicus (StY)
PI 401282 E. longearistatus (StY)
PI 401279 E. longearistatus (StY)
PI 401283 E. longearistatus (StY)
PI 401280 E. longearistatus (StY)
PI 401278 E. longearistatus (StY)
PI 401277 E. longearistatus (StY)
PI 401276 E. longearistatus (StY)
PI 401330 P. tauri (St)
PI 228390 P. libanotica (St)
PI 232128 P. spicata (St)
PI 440000 P. strigosa (St)
W6 14049 P. strigosa (St)
PI 330688 P. libanotica (St)
PI 401279 E. longearistatus (StY)
PI 401280 E. longearistatus (StY)
PI 401278 E. longearistatus (StY)
PI 401277 E. longearistatus (StY)
PI 401283 E. longearistatus (StY)
PI 531573 E. caucasicus (StY)
PI 401276 E. longearistatus (StY)
PI 401282 E. longearistatus (StY)
PI 207452 E. semicostatus (StY)
PI 564942 E. longearistatus (StY)
PI 499476 E. strictus (StY)
PI 531554 E. abolinii (StY)
PI 610898 E. gmelinii (StY)
PI 619528 E. antiquus (StY)
PI 533014 Aust. retrofractum (W)
PI 547363 Aust. retrofractum (W)
PI 531712 Thinopyrum bessarabicum (Eb)
PI 499498 H. bogdanii (H)
PI 499645 H. bogdanii (H)
PI 383534 Agropyron cristatum (P)
B. sterilis
69
1.00
84
1.00
84
0.72
66
0.98
73
1.00
63
0.98
100
1.00
100
1.00
100
1.00
100
1.00
87
1.00
100
1.00
96
1.00
100
1.00
99
1.00
100
1.00
100
1.00

3.3.4. Phylogenetic analyses of *HTL* sequences

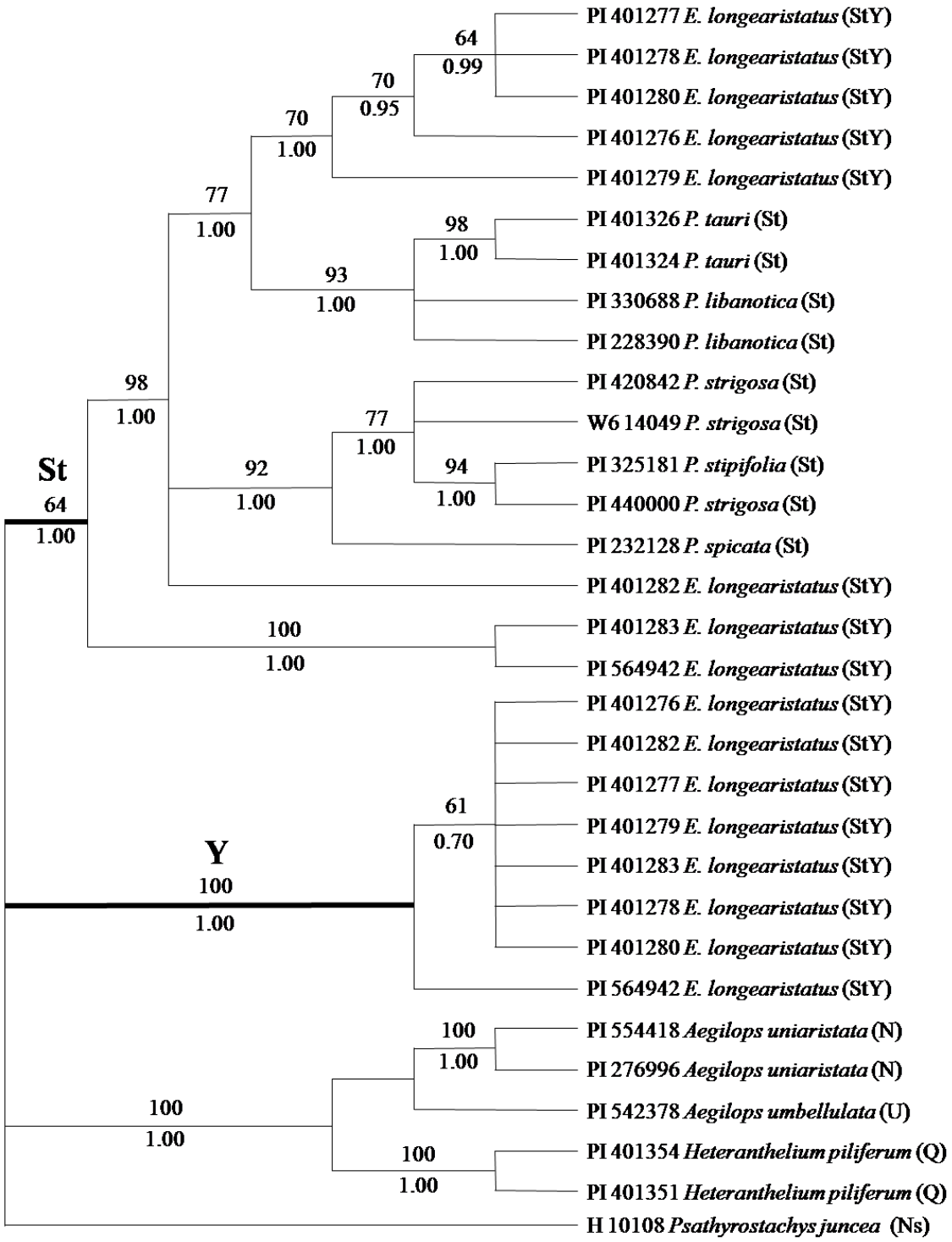
Previous studies revealed that Ns genome of *Psathyrostachy* is basal in the Triticeae tribe (Petersen and Seberg 1997, Mason-Gamer and Kellogg 2000). *Psathyrostachy juncea* (Fish.) Nevski was used as the outgroup in the phylogenetic analysis on *Hordeum* species based on the thioreoxin-like gene (*HTL*) (Wang *et al*., 2011).

Phylogenetic analysis of the 31 sequences was performed to determine the phylogenetic relationship of the St and Y genomes. MP analysis was conducted using *Psathyrostachys juncea* as the outgroup. The data matrix contained 1002 characters, of which 718 were constant, 91 were parsimony uninformative, and 193 were parsimony informative. Heuristic searches results in 403 most parsimonious trees with a CI of 0.811, RI of 0.921 and RCI of 0.747. The Bayesian analyses using the GTR model results in identical trees with mean log-likelihood values -3806.60 and -3824.07 (data not shown). The tree topologies generated by ML, MP and Bayesian analyses were similar to each other. A strict consensus tree with BS and Bayesian PP is shown in Figure 13.

A clear separation between St and Y genome was observed (Fig. 13). The sequences from the St genome of *E. longearistatus* was grouped with St genome from *Pseudoroegneria* (BS = 64%, PP = 1.00). Within the *Pseudoroegneria+Elymus* clade, the St sequences of *E. longearistatus* PI 564942 and PI 401283 were sister to the sequences from the other six *E. longearistatus* accessions and diploid *Pseudoroegneria* species with a bootstrap value of 100% and PP value of 1.00. The St sequences from *E. longearistatus* accessions PI 401276, PI 401277, PI 401278, PI 401279, and PI 401280 formed another

subclade (BS = 70%, PP = 1.00). Within the Y genome clade, seven accessions of Iranian *E. longearistatus* formed a subclade (BS = 61%, PP = 0.70), and the accession from Pakistan was sister to this subclade (Fig. 13).

Figure 13. Strict consensus trees derived from *HTL* sequence data was conducted using heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and Bayesian posterior probability (PP) values, respectively. *Psathyrostachys juncea* was used as an outgroup. Consistency index (CI) = 0.811, retention index (RI) = 0.921, rescale consistency index (RCI) = 0.747.


PI 401277 E. longearistatus (StY)
PI 401278 E. longearistatus (StY)
PI 401280 E. longearistatus (StY)
PI 401276 E. longearistatus (StY)
PI 401279 E. longearistatus (StY)
PI 401326 P. tauri (St)
PI 401324 P. tauri (St)
PI 330688 P. libanotica (St)
PI 228390 P. libanotica (St)
PI 420842 P. strigosa (St)
W6 14049 P. strigosa (St)
PI 325181 P. stipifolia (St)
PI 440000 P. strigosa (St)
PI 232128 P. spicata (St)
PI 401282 E. longearistatus (StY)
PI 401283 E. longearistatus (StY)
PI 564942 E. longearistatus (StY)
PI 401276 E. longearistatus (StY)
PI 401282 E. longearistatus (StY)
PI 401277 E. longearistatus (StY)
PI 401279 E. longearistatus (StY)
PI 401283 E. longearistatus (StY)
PI 401278 E. longearistatus (StY)
PI 401280 E. longearistatus (StY)
PI 564942 E. longearistatus (StY)
PI 554418 Aegilops uniaristata (N)
PI 276996 Aegilops uniaristata (N)
PI 542378 Aegilops umbellulata (U)
PI 401354 Heteranthelium piliferum (Q)
PI 401351 Heteranthelium piliferum (Q)
H 10108 Psathyrostachys juncea (Ns)
St
Y
64
0.99
70
0.95
70
1.00
77
1.00
98
1.00
93
1.00
98
1.00
77
1.00
92
1.00
94
1.00
64
1.00
100
1.00
61
0.70
100
1.00
100
1.00
100
1.00
100
1.00

4. Discussion

4.1. Phylogenetic analysis revealed reticulate evolution of *E. ciliaris*

4.1.1. On the origin of *E. ciliaris*

It has been proposed that *Pseudoroegneria* (St genome) and an unknown diploid (Y genome) species could be the constituents of the tetraploid *E. ciliaris* genome (Zhou *et al*., 1999; Redinbaugh *et al*., 2000; Sun and Salomon, 2009; Mason-Gamer *et al*., 2010). Both *RPB2* and *PGK1* phylogenetic trees showed an obvious Y genome specific clade which is distinct from the St clade. Our results further confirmed that *E. ciliaris* is a StStYY tetraploid, with the St genome coming from the maternal parent, but has also shown that the St genome in *E. ciliaris* species has a complex evolutionary history (it will be discussed later).

The *RPB2* sequence data indicated that the Y genome in *E. ciliaris* species were not highly differentiated from each other, all sequences from the Y genomes formed an unresolved polytomy (Fig. 2). Although the strict consensus tree from *PGK1* data showed that all sequences from the *E. ciliaris* Y-genome also formed a clade and within this clade accessions PI 531575 and PI 564917 were grouped together, and four additional accessions (VBG-0844, BKA-0931, BKA-0939 and W6 10267) formed a well supported subclade (BS = 91%; PP = 1.00). The Y copy sequence of PI 547303 is sister to the Y copy sequences from the remaining accessions, indicating that the *PGK1* sequences in the Y genome were highly differentiated from each other. Both *RPB2* and *PGK1* data showed that the St copy sequences from *E. ciliaris* formed a clade except the sequence from *E.*

ciliaris PI 377532 which was grouped into the H genome (Figs. 2, 3). Within the St clade, *RPB2* data revealed that the St copy sequences from PI 531577, W6 10267, VBG-0844 and PI 531574 formed a subclade, and PI 531575 was sister to other *E. ciliaris* species (Fig. 2). The *PGK1* tree showed that PI 531577, W6 10267, W6 14463 and PI 547303 were grouped together, indicating that the St genome sequences from *E. ciliaris* were also differentiated from each other. Although two single copy nuclear genes, *RPB2* and *PGK1*, showed some degree of difference in evolutionarily pattern between *E. ciliaris* species, the data suggested multiple origins of *E. ciliaris*. Both *RPB2* and *PGK1* data supported the finding that *E. ciliairs* has originated from the *Pseudorogneria* (St) and unknown donor (Y) diploids, however, the particular diploid progenitors could not be determined.

4.1.2. Reticulate origin of StY *E. ciliaris*

The St genome is present in all *Elymus* species and is the most important constituent of the genus. Cytological and molecular data have confirmed that *Pseudoroegneria* species is the donor of the St genome to *Elymus* (Dewey, 1984; Jensen, 1990; Torabinejad and Mueller, 1993; Jensen and Salomon, 1995; Jones *et al*., 2000; Redinbaugh *et al*., 2000; Mason-Gamer *et al*., 2002; McMillan and Sun, 2004; Xu and Ban, 2004; Liu *et al*., 2006; Yan *et al*., 2011). Our result based on chloroplast and nuclear DNA data also suggested that the St genome is one constituent of *Elymus ciliaris* species (Figs. 2, 3, and 4).

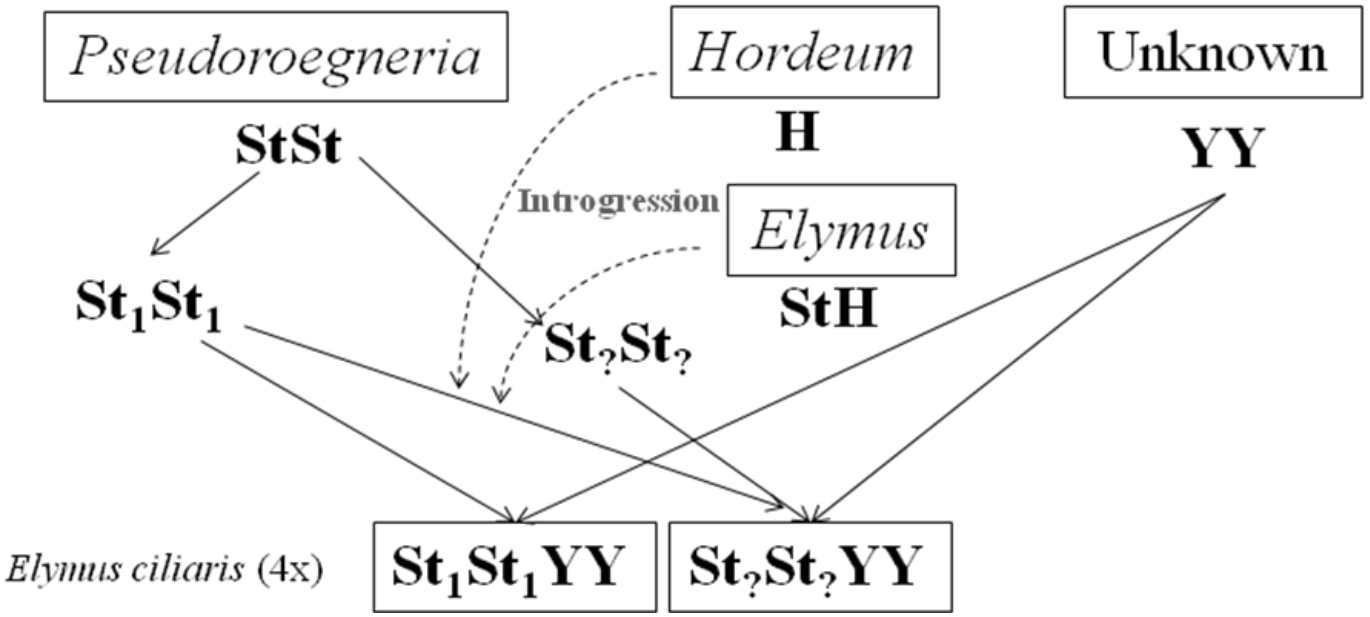
Interestingly, our phylogenetic data from two single copy nuclear genes, *RPB2* and *PGK1*, suggested that the St genome is not present in the accession PI 377532 of *E. ciliaris*, but may contain a YH genome. However, cpDNA *RPS16* have clearly indicated

that its maternal origin is the same as other *E. ciliaris* accessions, and is a St genomic diploid species (Fig. 4). Since there is no YH genome species reported in the genus *Elymus*, or in the tribe Triticeae so far, this accession cannot be a YH genome species. There are many StHY allopolyploid species in the genus *Elymus* and this accession might contain StHY genomes. A copy of the St sequence may have been missed in the sampling or lost since single copy genes may more easily suffer from locus loss due to stochastic events after polyploidization (Mahelka and Kopecky, 2010). One possible explanation is that this accession (PI 377532) contains StY genomes, in which the St genome highly differentiated from the St genome in other *E. ciliaris* accessions and the *Pseudoroegneria* species (St donors) studied here. Considerable variation existed among the St genome sequences, and the relationships among them are far more complicated (Mason-Gamer *et al*., 2010). Nuclear gene data (*RPB2* and *EF-G*) clearly showed a high level of nucleotide polymorphism in *Pseudoroegneria* species, and significant differentiation between *P. libanotica* and *P. tauri* groups with other St genome *Pseudoroegneria* diploid species (Yan and Sun, 2011). In a study of allohexaploid *Elymus repens* (StStH) by Mahelka and Kopecky (2010), the *GBSSI* data indicated that besides the "original" *Pseudoroegneria* clade (St) and *Hordeum* (H) clade species, another "unknown" *Pseudoroegneria*-like clade species may be the third genome lineage within hexaploid *E. repens*. There is accumulating evidence for multiple contributions of *Pseudoroegneria* taxa to various tetraploid *Elymus* species (Sun *et al*., 2007). A study of tetraploid *Elymus caninus* (StH) using *RPB2* gene data indicates that the St genome in *Elymus caninus* originated from two distinct sub-genomes, St_1 and St_2 (Yan and Sun, 2012). Moreover, grouping of St

genome species *P. stipofolia* and *P. strigosa* within the H- genome has been reported (Mason-Gamer and Kellogg, 2000). Liao *et al*. (2011) also found that *RPB2* sequences from *Roegneria alashanica* (StSt--) and *Pseudoroegneria geniculata* (StStStSt) form a subclade with the H clade. Thus, it is more likely that there are two versions of the St genome present in *E. ciliaris* species; one is grouped with *Pseudoroegneria* diploid species, the other is grouped with *Hordeum* species (named $St_?$) (Figs. 2 and 3).

The possibility that the St genome in accession PI 377532 of *E. ciliaris* has acquired both *RBP2* and *PGK1* sequences from H genome either from *Hordeum* H genome species or from *Elymus* StH genome species through introgression after polyploidization also cannot be ruled out since natural hybridization among different Triticeae is common in the tribe (Dewey, 1984). Thus, we propose a scenario of tetraploid *Elymus ciliaris* reticulate origin inferred from *RPB2*, *PGK1*, and *RPS16* sequences as shown in Figure 14.

Figure 14. Hypothetical scenario of tetraploid *E. ciliaris* origin as inferred from *RPB2*, *PGK1*, and*RPS16* sequences.


Pseudoroegneria
StSt
Hordeum
H
Unknown
YY
Introgression
Elymus
StH
St1St1
St?St?
Elymus ciliaris (4x)
St1St1YY
St?St?YY

4.1.3. Relationship among *E. ciliaris* Y genome from different geographic regions

With respect to the geographic origin of the *E. ciliaris* accessions considered here, the accessions investigated were generally collected from Eastern Russian (Siberian Botanical Garden, Novosibirsk; and Vladivostock), China, eastern Asia (Japan) (Table 1). A previous genetic diversity study by Yan *et al*. (2011) on StY *Elymus* species using two nuclear genes *RPB2* and *EF-G* demonstrates different evolutionarily diversities of the Y genome among different species. The *Elymus* Y genome clade was divided into two subclades by *EF-G* sequences, the first contained *E. semicostatus* (Afghanistan), *E. stricus* (China), *E. abolinii* (China), *E. antiques* (China), *E. pendulinus* (China), and *E. gmelinii* (China) (BS = 100%, PP = 1.00), the second group was comprised of species from western Asia, *E. longearistatus* (Iran) and *E. caucasicus* (Armenia) with 100% bootstrap support (PP = 1.00). The *RPB2* data group all different *Elymus* species into one single clade, and did not show a link between Y genome diversities and their geographic origin. A study on *Kengyilia* (StYP) from a wide distribution by Fan *et al*. (2012) pointed out that the Y genome copies are separated into several subclades. Seven central Asia accessions group into three independent subclades with BS= 100%, 99%, and 100% respectively. Two accessions from Qinghai-Tibetan plateau constructed a subclade with BS = 99% and one accession from central Asia grouped together with two Qinghai-Tibetan plateau accessions while the other accession from central Asia did not group into any of the subclades. In the present study, the *PGK1* Y genome sequences from the Siberian accessions (VBG-0844, BKA-0931, BKA-0939, and W6 10267),

formed a well supported subclade (BS=91, PP=1.00, Fig. 3). This might suggest that geographic isolation might have strongly influenced the evolution of Siberian population of *E. ciliaris*.

4.2. Introgression and complex evolution of *E. pendulinus*

4.2.1. Maternal donor of *E. pendulinus*

As expected, the cpDNA data indicated that most of *E. pendulinus* accessions analyzed here have the St genome donor as maternal parent (Figs. 5 and 6), which is consistent with previous results. However, both *RPS16* and *TrnD/T* sequence trees revealed that the *E. pendulinus* accession ZAR0714 is present outside of the Triticeae clade, indicating two possible maternal donors to *E. pendulinus*.

Interestingly, both nuclear gene data also found a copy of sequence from ZAR0714 present outside the Triticeae in the phylogenetic trees (Figs. 7 and 8). Based on the morphological characteristics we collected from *E. pendulinus*, phenotypic relationships among them are constructed by MP analysis. Coincidently, a phylogenetic tree based on morphology also places ZAR0714 together with MES 0721 outside of the main clade (Fig. 9). It is not clear if an unknown donor from outside of the Triticeae, if any, represented an entire genome from a third donor, or whether both cpDNA and nuclear gene are acquired through introgression by natural hybridization. The former hypothesis can be ruled out since our cytological observation found that this accession is tetraploid (data not shown). We suggest an alternative interpretation that introgression events occurred within the donor of ZAR0714 for the chloroplast and nuclear genes in ancient

time, as has been reported in several instances (Martinsen *et al*., 2001; Heuertz *et al*., 2006). A recent study by Koch and Matschinger (2007) suggested that chloroplast introgression has become less common in recent times. *E. pendulinus* species have been shown to hybridize with other species (Lu *et al*., 1991; Salomon and Lu, 1994; Zhou *et al*., 1999), demonstrating at least some potential to acquire genetic material through introgression. Moreover, both nuclear gene data of ZAR0714 contained the St copy. The conclusion that *Pseudoroegneria* is the maternal donor of *E. pendulinus* is favored here. We proposed a hypothetical scenario that a successful hybridization event might lead to introgression, which have occurred repeatedly or continually on *E. pendulinus* ZAR0714, leading to a morphologic variation.

4.2.2. Introgression shaped genome diversity within *E. pendulinus*

Results from nuclear gene sequences are more complicated than those from the chloroplast genome. Our results indicated that *E. pendulinus* has experienced a very complex evolutionary history which has involved multiple hybridizations and polyploidization. The analyses of unlinked nuclear *RPB2* and *PepC* gene sequences are interpreted in light of the morphological study, demonstrating intra-species variation in natural *E. pendulinus* populations originated from Russia. The nuclear gene trees revealed more potential donors than were expected, including *Pseudoroegneria* (St), unknown donor (Y), *Hordeum* (H), and an unknown donor from within/outside the tribe Triticeae.

The presence of the St and Y genome within *E. pendulinus* is consistent with previous cytogenetic (Dewey, 1984; Jensen, 1990) and molecular data (Mason-Gamer *et al*., 2002; McMillan and Sun, 2004). However, these two expected copies were not recovered from all accessions. In *RPB2* gene data, one of the expected copies (St, Y) was not recovered from BKA0921 VLA0718, and USS0720. In addition, the *PepC* sequence data showed that St copy sequences were not recovered from accession VOK0728 and VBG0727. While failure to obtain a particular gene copy from a polyploid species may not indicate the lack or loss of that gene copy, in this situation primers specific to the "missing" gene copy may clarify the case (Ge *et al*., 1999; Ferguson and Sang, 2001; Doyle *et al*., 2002; Mahelka and Kopechy, 2010). Furthermore, when we compared the two phylogenetic trees from nuclear genes, the St and Y copies in all accessions of *E. pendulinus* have been recovered, from either *RPB2* or *PepC*, or from both genes.

The apparent involvement of *Hordeum* (genome H) as a third participant in the evolution of *E. pendulinus* comes as a surprise. Five out of thirteen *E. pendulinu*s accessions contained the *Hordeum*-like sequence in *RPB2* data, and three out of fourteen in *PepC* data. There is little cytogenetic evidence for the presence of an H genome in *E. pendulinus*. All of the *RPB2* sequences in the *E. pendulinus* + *Hordeum* clade have three deletions, which is corresponded well with previous findings (Sun *et al*., 2007, 2008), and appear to be non-functional. *Hordeum*-like *PepC* sequences from *E. pendulinus* have numerous insertions and deletions, some of which involved gain or loss of *Strowaway*-like transposable elements, which is consistent with a previous study in *E. repense* (StStH) (Mason-Gamer, 2008). The appearance of *Hordeum*-like copy was only

found for two accessions (AND0713 and VBG0727) out of thirteen accessions with both *RPB2* and *PepC Hordeum*-like copies, another three accessions (MES0721, VBG0722, and VBG0727) with a *Hordeum*-like *RPB2* sequences, and accession VLA0719 with a *Hordeum*-like *PepC* sequence. The hypothesis of a entire H genome present in *E. pendulinus* can be ruled out, not only because of the lack of the presence of an H copy in all individuals, but also because our cytological observation found that all accessions studied here are tetraploids without a third genome (data not shown). A direct contribution from *Hordeum* to *E. pendulinus* remains a possibility. Although StH and StY species are intersterile, there are many StHY allopolyploid species in the genus *Elymus*. The gene exchange may have occurred between the H and Y genome in StHY species. An alternative explanation might be that *E. pendulinus* acquired the sequences from the H genome through introgression. This phenomenon has been revealed for certain loci in *E. repens* (Mason-Gamer, 2008) and in *E. ciliaris* (Hu *et al*., 2013, In press). Another unexpected result is the apparent genetic contribution from species outside the Triticeae to *E. pendulinus.*

The molecular phylogenetic analyses of *E. pendulinus* agree in part with the earlier cytogenetic studies, but uncover an additional genome-level complexity. The four gene trees together with cytological observation have shed light on the origin of tetraploid *E. pendulinus,* confirming the involvement of two distinct genome donors, and likely introgression of additional copies, one of which is a *Hordeum*-like copy, the other is outside the tribe.

4.2.3. Multiple origins of *E. pendulinus*

Our molecular results have demonstrated intra-species variation in *E. pendulinus* in Russian distribution area, which corresponded with morphological results. From the *RPS16* cpDNA gene tree, three subclades composed by *E. pendulinus* accessions were examined in the St clade. The sequences from *E. pendulinus* AND0713, MES0712, and BKA0921 fell into the clade with sequences from diploid *P. stipifolia* and *P. gracillima*, which indicated that they could be potential donors. The other two subclades in the *RPS16* St clade are a monophyletic group with *E. pendulinus* accessions only. On the *TrnD/T* tree, accession AND0713 is grouped with *P. stipifolia* as well, and the same accessions (VBG0727, VOK0728, and VOK0724) formed a subclade in St clade (BS = 85%, aLRT = 93%) as *RPS16* (BS= 83%, aLRT = 86%). Based on our phylogenetic analyses, both the *RPS1*6 and *TrnD/T* cpDNA trees point to more than one potential maternal donor for *E. pendulinus*, suggesting that the Russian *E. pendulinus* used in this study may have originated from multiple sources of *Pseudoroegneria.*

Within the *RPB2* tree, four accessions of *E. pendulinus* (MES0721, VBG0727, AND0713, and VBG0722) are placed in a distinct monophyletic group (BS= 91%, aLRT = 95%) that is sister to the *H. bogdanii*; the other two *Hordeum*-like sequences (AND0713 and BKA0921) are grouped together into a distinguished subclade (BS= 100%, aLRT = 100%). This demonstrates that the *RPB2* sequences in *E. pendulinus* might be introgressed from at least two different *Hordeum* diploids. In the *RPB2* St clade, five accessions were placed into two subclades with weak support; as well as *RPB2* Y clade, all *E. pendulinus* accessions are separated into two distinguished subclades.

In the *PepC* tree, the *Hordeum*-like copies are also separated into two suclades (AND0713 with VBG0727; VLA0719 with VBG0727). In the St clade, nine accessions of *E. pendulinus* from Russia formed a subclade (BS = 93%, aLRT = 94%). The other three accessions (AND0713, BKA0921, and VLA0719) do not have a close relationship with any of the diploid taxa sampled, thus which St genome species was their progenitor remain unidentified. A similar situation occurs in the Y clade as well, where the same three accessions (AND0713, BKA0921, and VLA0719) in *E. pendulinus* are grouped with the Triticeae genome (R, E^e, A^M, Ta, and M), which are separated from the other Y copy of *E. pendulinus*. This phenomenon has been reported for *EF-G* gene (elongation factor) in tetraploid *Elymus* (Sun and Komatsuda, 2010), where Y genome sequences were grouped with W and E sequences as well as sequences from many annual species (M, N, Ta, R, A, Q etc.). All of the gene trees suggested multiple contributions from *Pseudoroegneria*, an unknown Y genome donor, and *Hordeum* species, and reveal sequence divergence after polyploid formation.

4.3. Origin and geographical differentiation of the Y genome in *E. longearistatus*

4.3.1. Maternal donor of *E. longearistatus*

The presence of *Pseudoroegneria*-derived chloroplast sequences is consistent with previous molecular studies that *Pseudoroegneria* (St) is the maternal parent of polyploid containing the St nuclear genome in combination with other genomes (Dewey, 1984; Redinbaugh *et al*., 2000; Hodge *et al*., 2010), and this phenomenon was documented in

numerous cases (Redinbaugh *et al*., 2000; Mason-Gamer, 2001; McMillan and Sun, 2004; Liu *et al*., 2006; Yan and Sun, 2012). Our nuclear sequence data further confirmed the contribution from *Pseudoroegneria* to all *E. longearistatus* accessions studied.

A close relationship among six Iranian accessions of *E. longearistatus* (PI 401277, PI 401278, PI 401279, PI 401280, PI 401282, PI 401283) was revealed by both *RPS16* and *TrnD/T* data, they formed a subclade within the *Pseudoroegneria+E. longearistatus* clade (Figs. 10 and 11). In *RPS16* dataset, these six accessions were grouped with the accession PI 420842 of *P. strigosa* and PI 325181 of *P. stipifolia*; In the *TrnD/T* phylogenetic analysis, they were grouped with a *P. stipifolia* accession PI 325181. The data generated from chloroplasts suggested that the accession of *P. stipifolia* (St) possess sequences that are most closely related to those from these six *E. longearistatus*, making *P. stipifolia* the most likely donor of these six Iranian accessions, although *P. strigosa* could not be excluded. Another Iranian *E. longearistatus* accession PI 401276 and the accession PI 564942 from Pakistan were placed outside of the subclade in both chloroplast sequence trees, indicating multiple maternal donors to *E. longearistatus*.

4.3.2. Intraspecies relationship among *E. longearistatus*

Recent molecular data indicated that polyploid speciation is often more complex than initially thought (Soltis and Soltis, 1993). Allopolyploids are fundamentally hybrids. The same allopolyploid can follow very different evolutionary trajectories (Mahelka and Kopecky, 2010; Yan and Sun, 2012), because each origin can bring together different

combinations of alleles at each homologous locus, leading to different transgressive effects (Doyle *et al*., 1999).

In the present study, the *E. longearistatus* accessions from Iran and Pakistan were sampled and analyzed, both the two cpDNA and nuclear gene data provide a general link between the sequence placement of *E. longaristatus* in the phylogenetic trees and their geographic origins. All the sequences from the Pakistan accession, no matter the cpDNA or nuclear sequences from St or Y genome, were well separated from the sequences from Iranian accessions. Especially in the Y genome sequences, a close relationship was found among Iranian accessions, since they formed a well supported subclade (BS = 100%, PP =1.00, Fig. 12) in the *EF-G* tree, as well as the *HTL* tree. The Y genome sequences from Iranian accessions displayed a single, compact and distinct group from the sequences from the Pakistan accession (BS = 61%, PP =0.70, Fig. 13). This grouping suggested a common origin of Y genome in the accessions of *E. longearistatus* from Iran. Previous cytogenetic studies suggested that the *E. longearistatus* genome showed extraordinary variation from many Eastern and central Asiatic StY *Elymus* (Jensen and Wang, 1991; Lu and von Bothmer, 1993), however, our nuclear data revealed that the accession PI 564942 from Pakistan had a close relationship with Eastern Asiatic *Elymus* species (*E. abolinii*, *E. antiquus*, *E. gmelinii*, *E. semicostatus*, *E. strictus*, Fig. 4). This novelty was based in both St (BS = 84%, PP =0.72) and Y (BS = 100%, PP =1.00) genome sequence data. Our data suggested that the geographic isolation strongly influenced the evolution of the Y genome in *E. longearistatus*. Previous molecular studies also supported the correlation between Y genome differentiation and geographical distribution. In our study of *Elymus*

ciliaris, the *PGK1* Y genome sequences from all the Siberian accessions formed a well supported subclade (BS = 91%, PP = 1.00) apart from the remaining accessions from China, Japan, and Estonia (Hu *et al*. in press). A study on *Kengyilia* (StYP) from a wide distribution by Fan *et al*. (2012) pointed out that the Y genome in Qinghai-Tibetan plateau accessions were separated from the Y genome from central Asia accessions, and showed geographical differentiation.

The *EF-G* sequence data indicated that the St genomes in the StY-genomic *E. longearistatus* from Iran were not highly differentiated from each other as observed in the Y genomes. Indeed, all sequences from the St genome formed an unresolved polytomy (Fig. 12). Although the phylogenetic tree from *HTL* data placed all the sequences from the *E. longearistatus* St-genomes into a clade (BS = 64%, PP = 1.00), within the clade accessions PI 564942 and PI 401283 were grouped together, and five accessions (PI 401276, PI401277, PI401278, PI401279, PI 401280) formed a well supported subclade with *P. tauri* and *P. libanotica* (BS = 77%, PP = 1.00). The St sequence of PI 401282 was sister to the St sequences from the remaining diploid *Pseudoroegneria* accessions (PI 420842, W6 14049, PI 325181, PI 440000, PI 232128) (Fig. 13), indicating that the *HTL* sequences in the St genome were highly differentiated from each other and displayed low correlation between accessions separated by geographical distance.

4.3.3. The relationship between St and Y genome

The results convincingly showed that the homologous genomes in the StY tetraploid *E. longearistatus* were derived from phylogenetically distinct donors. All of the *E. longearistatus* accessions studied here yielded two distinct copies for each nuclear gene. The results confirmed cytogenetic results (Dewey, 1974; Jensen and Wang, 1991; Jensen and Salomon, 1995; Lu *et al*., 1990) that the StY tetraploid species *Elymus* are allotetrapolids. However, ITS sequences suggested that the St and Y genome sets were both derived from *Pseudoroegneria* (Liu *et al*., 2006). Concerted evolution might explain the lack of a distinct Y genome ITS sequence (Mason-Gamer *et al*., 2010). Okito *et al*. (2009) suggested that one accession of *P. spicata* (PI 232134) might be the donor of the Y genome and a prime candidate for the origin of the Y genome to two *E. longearistatus* accessions (PI 401278, PI 401282) which were also included in this study. In a previous study, both the *RPB2* and *EF-G* phylogenetic tree placed this accession of *P. spicata* (PI 232134) in the St genome together with other *Pseudoroegneria* species (Yan *et al*., 2011), and indicated that there was not a close link between St genome in *P. spicata* and the Y genome in *E. longearistatus* and in other StY *Elymus* species.

5. Summary

In the study of *E. ciliaris*, both *RPB2* and *PGK1* data supported that *E. ciliairs* has multiple origins, and originated from the *Pseudorogneria* (St) and unknown donor (Y) diploids. The St genome in *E. ciliaris* species has a complex evolutionary history. Both nuclear data suggested the absence of St genome in the accession PI 377532 of *E. ciliaris*.

However, cpDNA *RPS16* clearly indicated that its maternal origin is the same as other *E. ciliaris* accessions, and is St genomic diploid species. Results suggest that there are two lineages of St genome present in *E. ciliaris* species; one is grouped with *Pseudoroegneria* diploid species, the other is grouped with *Hordeum* (H) species (named $St_?$). The Japanese accession PI 377532 might have introgression either from *Hordeum* H genome species or from *Elymus* StH genome species with replacement of at least some nuclear St-loci by H-loci. The *PGK1* data suggest that geographic isolation might have strongly influenced the evolution of Siberian population of *E. ciliaris.*

In the analysis of *E. pendulinus*, our results revealed an extreme reticulate pattern, at least four distinct gene lineages coexisting within this species which might be acquired through a possible combination of allotetraploidization and introgression from both within and outside the Triticeae. Chloroplast DNA data identified two potential maternal genome donors (*Pseudoroegneria* and unknown species outside Triticeae) to *E. pendulinus*, Nuclear gene data indicated that both *Pseudoroegneria* and unknown Y diploid have contributed to the nuclear genome of *E. pendulinus*, in agreement with cytogenetic data. However, unexpected contributions from *Hordeum*, and unknown aliens within/outside Triticeae to *E. pendulinus* without genome duplication were observed. *E. pendulinus* provides a remarkable instance of the previously unsuspected chimerical nature of some plant genomes and the resulting phylogenetic complexity produced by multiple historical reticulation events.

In the study of *E. longearistatus*, the two single copy nuclear gene sequence data (*EF-G* and *HTL*) provided strong support for the independent origin of the Y genome in

StY tertraploid *E. longearistatus*. Phylogenetic analyses well separated the Y genome from the St genome in both phylogenetic trees (with BS = 96% PP = 1.00 in *EF-G* tree, BS = 100% PP = 1.00 in *HTL* tree) (Figs. 12 and 13). These results are consistent with previous studies (Mason-Gamer *et al*., 2005; Sun *et al*., 2008; Sun and Komatsuda, 2010;Yan *et al*., 2011). These nuclear data rejected the hypothesis that the Y genome was derived from the *Pseudoroegneria* species, instead supporting different origins of the St and Y genomes (Mason-Gamer *et al*., 2005; Sun *et al*., 2008; Mason-Gamer *et al*., 2010; Sun and Komatsuda, 2010; Yan *et al*., 2011).

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Publications

1. **Q. Hu**, C. Yan, G. Sun. 2013 Phylogenetic analysis revealed reticulate evolution of allotetraploid *Elymus ciliaris*. *Molecular Phylogenetics and Evolution* (In press)
2. C. Yan[1], **Q. Hu**[1], G. Sun[*]. 2013 Nuclear and chloroplast DNA phylogeny reveals introgression and complex evolution of *Elymus pendulinus*. *Molecular Phylogenetics and Evolution* (Submitted) ([1] contributed equally)
3. **Q. Hu**, G. Sun[*]. 2013 Origin and geographical differentiation of the Y genome in *Elymus longearistatus* (Triticeae: Poaceae). *Proceeding of the Royal Society B* (Submitted)

[*] Corresponding author