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Polymorphism of ITS sequences in 35S rRNA genes in *Elymus dahuricus* aggregate species: two cryptic species?

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Nuclear ribosomal internal transcribed spacer (ITS) sequences were sequenced for 23 species and subspecies of *Elymus sensu lato* collected in Russia. The Neighbor-Net analysis of ITS sequences suggested that there are four ribotypes called Core Northern St-rDNA, Core Southern St-rDNA, Northern dahuricus St-rDNA and Southern dahuricus St-rDNA. The Core Southern variant of St-rDNA is closely related to rDNA of diploid *Pseudoroegneria stipifolia* (PI 313960) and *P. spicata* (PI 547161). The Core Northern St-rDNA is closely related to rDNA of *P. cognata* (PI 531720), a diploid species of Kyrgyzstan carrying St^Y variant of the St genome. The Core Northern St-rDNA is widespread among the *Elymus* species of Siberia and the Far East, including Yakutia and Chukotka. The Core Southern St-ribotype is typical of southern *Elymus* and *Pseudoroegneria* of the South Caucasus, Primorye, Pakistan, and South Korea. The Northern dahuricus St-ribotype and Southern dahuricus St-ribotype are derivatives of the Core Northern and Core Southern St-ribotypes, correspondingly. Both of them were found in all four studied species of the *E. dahuricus* aggregate: *E. dahuricus* Turcz. ex Griseb., *E. franchetii* Kitag., *E. excelsus* Turcz. ex Griseb. and Himalayan *E. tangutorum* (Nevski) Hand.-Mazz. In other words, there are at least two population groups (two races) of the *Elymus dahuricus* aggregate species that consistently differ in their ITS-sequences in Siberia, the Far East and Northern China. Each contains all morphological forms, which taxonomists now attribute either to different species of *E. dahuricus* aggr. (*E. dahuricus sensu stricto*, *E. franchetii*, *E. tangutorum*, *E. excelsus*) or subspecies of *Campeiostachys dahurica* (Turcz. ex Griseb.) B.R. Baum, J.L. Yang et C.C. Yen. At the moment it is unknown if there are any morphological differences between plants carrying either Northern or Southern dahuricus rDNA. Probably, they are cryptic species, but it is certain that if differences in morphology between the two races exist, they are not associated with signs that are now considered taxonomically significant and are used to separate *E. dahuricus* s. s., *E. franchetii*, *E. tangutorum*, and *E. excelsus*.
Key words: *Elymus dahuricus* aggr.; interspecific hybridization; rDNA; 35S rRNA; Triticeae.

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Полиморфизм ITS-последовательностей генов 35S рРНК у видов *Elymus dahuricus* aggr.: два криптических вида?

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Секвенированы последовательности внутренних транскрибируемых спейсеров (ITS) 23 видов и подвидов *Elymus sensu lato*. При анализе молекулярно-филогенетической сети Neighbor-Net все последовательности ITS образцов *Elymus* s. l. были разделены на четыре типа: основной северный Elymus-риботип, основной южный Elymus-риботип, северный dahuricus-риботип и южный dahuricus-риботип. рДНК основного южного риботипа родственна рДНК диплоидного вида *Pseudoroegneria stipifolia* (PI 313960) и *P. spicata* (PI 547161). рДНК основного северного риботипа родственна рДНК *P. cognata* (PI 531720), диплоидного вида из Казахстана, несущего St^Y – вариант St-генома. Основной северный риботип широко распространен у видов *Elymus* Сибири и Дальнего Востока, включая Якутию и Чукотку. Основной южный St-риботип характерен для относительно южных популяций *Elymus* и *Pseudoroegneria*, включая Закавказье, Приморье, Пакистан, Южную Корею. Отметим, что северный Elymus dahuricus-риботип и южный Elymus dahuricus-риботип были обнаружены у всех четырех видов группы родства *E. dahuricus* aggr.: *E. dahuricus* Turcz. ex Griseb., *E. franchetii* Kitag., *E. excelsus* Turcz. ex Griseb. и у гималайского вида *E. tangutorum* (Nevski) Hand.-Mazz. Иными словами, молекулярно-филогенетические исследования образцов, относимых к *E. dahuricus* aggr., говорят о том, что в Сибири, на Дальнем Востоке и в Северном Китае существуют по крайней мере две группы популяций (две расы), надежно различающиеся

по ITS-последовательностям, в каждой из которых представлены все морфологические формы, относимые сейчас одними систематиками к четырем разным видам *E. dahuricus* aggr. (*E. dahuricus sensu stricto*, *E. franchetii*, *E. tangutorum*, *E. excelsus*), а другими – к одному виду *Campeiostachys dahurica* (Turcz. ex Griseb.) B.R. Baum, J.L. Yang et C.C. Yen. Имеются ли между этими группами морфологические различия, или это криптические виды (подвиды) – неизвестно, но с уверенностью можно сказать, что если различия в морфологии между этими двумя расами есть, то они не связаны с признаками, которые сейчас считаются таксономически значимыми и используются для разделения *E. dahuricus* s. s., *E. franchetii*, *E. excelsus*, *E. tangutorum*.
Ключевые слова: *Elymus dahuricus* aggr.; межвидовая гибридизация; 35S рРНК; Triticeae.

Introduction

The beginning of the 21 century was marked by very wide using of DNA sequencing in systematics and phylogeny of animals and plants. Remarkable result of this was an exponential rise in the discovery of cryptic species in different groups of animals (Bickford et al., 2007). However, such discoveries are much rarer in plants, especially in angiosperms (Shneyer, Kotseruba, 2015). We suggest that cryptic species may exist in particular, in the genus *Elymus* L. (Triticeae).

Now it is considered the genus *Elymus* is represented in Russia by 53 species (Tzvelev, Probatova, 2010). All these species are allopolyploids with St, Y, H subgenomes and haplomes StY ($2n = 28$), StH ($2n = 28$) and StYH ($2n = 42$), whereas primary diploids ($2n = 14$) are absent in the genus (Agafonov et al., 2001; Agafonov, 2007). Based on the results of interspecies hybridization, DNA sequencing and GISH, it has been suggested that all the *Elymus* species share a common St subgenome originated from the genus *Pseudoroegneria* (Nevski) Á. Löve species and H subgenome from an ancestor of the genus *Hordeum* L. (Dewey, 1984; Sun, Zhang, 2011; Yan et al., 2011; Mason-Gamer, 2013). It was suggested that North American perennial bunchgrass *Pseudoroegneria spicata* (Pursh) A. Löve was most likely donor of the Y subgenome, although Asiatic species *P. cognata* (Hackel) A. Löve (syn.: *Agropyron ferganense* Drobow) and *P. libanotica* (Hack.) D.R. Dewey also could not be excluded (Okito et al., 2009), particularly for the Asiatic *Elymus* species with StY and StHY genome compositions.

C. Yen et al. (2005) divided the genus *Elymus* s. l., strictly in accordance with their genomic constitution, into six genera: *Douglasdeweya* C. Yen, J.L. Yang et B.R. Baum (StStPP); *Roegneria* C. Koch (StStYY); *Anthosachne* Steudel (StSt WWYY); *Kengylia* C. Yen et J.L. Yang (StStPPYY); allohexaploid species with the StStYYHH karyotypes (Yen et al., 2005; Baum et al., 2011) were referred to the genus *Campeiostachys* Drobov, and *Elymus* L. in this treatment included only the species with the StStHH/StStHHHH/StStStStHH karyotypes (Yen et al., 2005; Yen, Yang, 2009). Though the separation of species into genera based on the karyotype constitution is attractive from a genetic point of view (Dewey, 1984; Tzvelev, 1991; Agafonov, 2007), it should be noted that the division species into genera only based on their genome composition does not always correlate with morphological criteria by which species and genera defined and delimited (Jensen, Chen, 1992; Baum et al., 2011). Internal transcribed spacers ITS1 and ITS2 of the nuclear genes 35S rRNA were widely employed in molecular phylogenetic studies of *Elymus* of China and North America (Liu et al., 2006; Wang et al., 2009; Mason-Gamer, 2013; Rabey, 2014; Gao et al., 2015; and others).

The main objective of our study is an assessment of interspecial ITS-polymorphism of *Elymus* of Siberian and the Far Eastern flora. This is interesting from the genetic point of view because, the phenomenon of interspecific and introgressive hybridization is widespread among Siberian and Far Eastern populations/natural races of *Elymus* (Agafonov, 1997; Wu et al., 2015). East Eurasian species of *Elymus* have all the features of a syngameon (Lotsy, 1925). It was necessary to ascertain how this fact effects on genetic distances between the Siberian *Elymus* 'varieties' which taxonomists delimitate as several morphologically discret species. Also, the aim of our study was to study relationships in *Elymus dahuricus* aggr. to which Tzvelev, Probatova (2010) referred four species, also treated as subspecies of species *Campeiostachys dahurica* (Turcz. ex Griseb.) B.R. Baum, J.L. Yang et C.C. Yen (Baum et al., 2011).

Material and methods

Nuclear ribosomal internal transcribed spacer sequences (ITS) were sequenced from 34 accessions belonging to 23 species and subspecies of *Elymus* s. l. (Table 1). The plant samples were collected in the Altai Krai and Altai Republic, Khakassia, the Kemerovo Oblast, Yakutia and the Northern Caucasus from 2004 to 2013. Herbarium specimens are stored in the herbarium of the Laboratory of Biosystematics and Cytology and in the Herbarium LE of the Komarov Botanical Institute.

Total genomic DNA was isolated using the CTAB method (Doyle J.J., Doyle J.L., 1987), with minor modifications described previously (Rodionov et al., 2008). Amplification of the ITS region was performed using primers ITS 1P (Ridgway et al., 2003) and ITS 4 (White et al., 1990). The PCR reaction was carried out in a total volume of 50 μL containing 1× SE-buffer AS (SibEnzyme, Russia), 2.5 mM Mg^{2+}, 2 mM each of dATP, dTTP, dCTP, dGTP) (Helicon, Russia), 0.01 μM of each primer (Beagle, Russia), 1–2 μL total DNA, 5 units of Taq-polymerase (SibEnzyme, Russia) and distilled water to the final volume. PCR amplification was done also using 1× Maxima Hot Start Taq buffer (Thermo Scientific, Sweden), 2.5 mM Mg^{2+} (Thermo Scientific, Sweden), 2 mM dATP, dTTP, dCTP, dGTP (Helicon, Russia), 0.01 μM of each primer, 1–2 μL total DNA, 5 units of Maxima Hot Start Taq polymerase (Thermo Scientific, Sweden), and distilled water. Amplification *parameters*: primary denaturation at 95 °C for 5 min, followed by 30 cycles at 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min, with a final extension step at 72 °C for 10 min. The PCR products were electrophoresed in 1 % agarose gel. The QiaGen Extraction Kit (Qiagen, Germany) was used to extract the DNA from the gel. Sanger sequencing was performed in The Core Facilities Center "Cell and Molecular Technologies in Plant Science" at the Komarov Botanical Institute of the Russian Academy of Sciences. The PCR products were sequenced in both directions on ABI

Table 1. Types of ITS sequences in species of *Elymus* and *Pseudoroegneria*, our data

No.	Species	Genome/Haplome	Origin	GenBank	Ribotype
	Section Turczaninovia (Nevski) Tzvel. (syn. genus *Campeiostachys* Drobov)				
1	*E. dahuricus* Turcz. ex Griseb.	StHY	RF: Khakassia	KJ540222	Southern dahuricus St-rDNA
2	*E. dahuricus* Turcz. ex Griseb.	StHY	RF: Altai Republic	KJ540223	
	Section Goulardia (Husn.) Tzvel.				
3	*E. amurensis* (Drob.) Czer.	StY	RF: Primorsky Krai	KM871828	Southern dahuricus St-rDNA
4	*E. caninus* (L.) L.	StH	RF: Altai Republic	KJ561233	Northern St-rDNA
5	*E. caninus* (L.) L.	StH	RF: North Caucasus	KJ561234	
6	*E. ciliaris* (Trin.) Tzvel.	StY	RF: Primorsky Krai	KM871829	Southern St-rDNA
7	*E. fibrosus* (Schrenk) Tzvel.	StH	RF: Altai Republic	KM363383	Northern St-rDNA
8	*E. fibrosus* (Schrenk) Tzvel.	StH	Finland	KM871830	
9	*E. gmelinii* (Ledeb.) Tzvel.	StY	RF: Kemerovo Oblast	KJ755831	
10	*E. gmelinii* (Ledeb.) Tzvel.	StY	RF: Altai Republic	KM363382	
11	*E. jacutensis* (Drob.) Tzvel.	Unknown	RF: Yakutia	KM363381	
12	*E. jacutensis* (Drob.) Tzvel.	Unknown	RF: Altai Republic	KM575844	
13	*E. komarovii* (Nevski) Tzvel.	StH		KJ561236	
14	*E. macrourus* (Turcz.) Tzvel.	StH		KM379150	
15	*E. macrourus* (Turcz.) Tzvel.	StH	RF: Yakutia	KM502299	
16	*E. mutabilis* (Drob.) Tzvel.	StH	RF: Altai Republic	KM871827	
17	*E. nevskii* Tzvel.	StY	RF: Altai Krai	KJ540224	
18	*E. probatovae* Tzvel.	Unknown	RF: Chukotka	KM871831	
19	*E. sajanensis* (Nevski) Tzvel.	StH	RF: Altai Republic	KM502300	
20	*E. sajanensis* (Nevski) Tzvel.	StH	RF: Tuva	KM871825	
21	*E. scandicus* (Nevski) Tzvel.	StH	RF: Altai Republic	KJ561237	
22	*E. subfibrosus* (Tzvel.) Tzvel.	StH	RF: Yakutia	KM975705	
23	*E. trachycaulus* (Link) Gould et Shinners	StH	RF: Primorsky Krai	KM975706	
24	*E. transbaicalensis* (Nevski) Tzvel.	StH	RF: Altai Republic	KJ561235	
25	*E. transbaicalensis* (Nevski) Tzvel.	StH		KM363385	
26	*E. transbaicalensis* (Nevski) Tzvel.	StH		KM575845	
27	*E. vernicosus* (Nevski ex Grub.) Tzvel.	StY		KJ540221	
28	*E. vernicosus* (Nevski ex Grub.) Tzvel.	StY		KM871821	Northern dahuricus St-rDNA
	Section Elymus				
29	*E. peschkovae* Tzvel.	StH	RF: Yakutia	KM871824	Northern St-rDNA
30	*E. schrenkianus* (Fisch. et C.A. Mey.) Tzvel.	StHY	RF: Altai Republic	KM502297	
31	*E. schrenkianus* (Fisch. et C.A. Mey.) Tzvel.	StHY	RF: Tuva	KM502298	
32	*E. schrenkianus* (Fisch. et C.A. Mey.) Tzvel.	StHY	RF: Altai Republic	KM502301	
33	*E. sibiricus* L.	StH		KJ540220	
	The hybrid				
34	*Elymus* sp.	Unknown	RF: Altai Republic	KJ561239	Northern St-rDNA
	Genus *Pseudoroegneria* (Nevski) A. Löve				
35	*P. geniculata* (Trin.) A. Löve (syn. *Elytrigia geniculata* (Trin.) Nevski)	StSt	RF: Khakassia	KJ561242	Northern St-rDNA

Prism 3130 (Applied Biosystems, USA). All sequences were submitted to the GenBank (NCBI) database. The sequences were aligned with ClustalW using the MEGA 6 (Tamura et al., 2013) software package with subsequent visual verification. The SplitsTree4 algorithm Neighbor-Net (Huson, Bryant, 2006), proposed for the study of network evolution (Bryant, Moulton, 2004; Huson, Bryant, 2006) was used.

Results

We studied variability of ITS-sequences of *E. dahuricus*, *E. excelsus*, *E. franchetii*, *E. tangutorum*, species that constitute *Elymus dahuricus* aggr. (Tzvelev, Probatova, 2010). ITS-sequences of these species, as well as of some other species of *Elymus*, *Elytrigia*. *Pseudoroegneria* and *Hordeum* are provided in Table 1 and Table 2.

Table 2. Types of ITS sequences in species of the genus *Elymus*, *Elytrigia*, *Pseudoroegneria* and *Hordeum*. ITS1-genes 5.8S rRNA-ITS2 sequences from the international database GenBank used in our work

No.	Species	Genome	Origin	GenBank	Ribotype
	Section Turczaninovia (Nevski) Tzvel. (syn. Genus *Campeiostachys* Drobov)				
36	*E. dahuricus* Turcz. ex Griseb.	StHY	South Korea	HQ600520 (Kim Y.D. et al., unpubl.)	Southern dahuricus St-rDNA
37	*E. dahuricus* Turcz. ex Griseb.	StHY	China?	JN009816	Northern St-rDNA
38	*E. dahuricus* Turcz. ex Griseb.	StHY	South Korea: Taeangun, Choongcheongnam-do	KF713222 (Lee J. et al., unpubl.)	Southern dahuricus St-rDNA
39	*E. dahuricus* Turcz. ex Griseb.	StHY	China: Shandan, Gansu	KF905152 (Song et al., 2015)	Northern dahuricus St-rDNA
40	*E. dahuricus* Turcz. ex Griseb.	StHY	China: Yuzhong, Gansu	KF905178 (Song et al., 2015)	
41	*E. dahuricus* Turcz. ex Griseb.	StHY	China	KJ526338 (Gao et al., 2015)	Southern dahuricus St-rDNA
42	*E. excelsus* Turcz. ex Griseb.	StHY	China: Neimenggu	KJ526341	
43	*E. excelsus* Turcz. ex Griseb.	StHY		KJ526342	Northern dahuricus St-rDNA
44	*E. excelsus* Turcz. ex Griseb.	StHY		KJ526343	
45	*E. excelsus* Turcz. ex Griseb.	StHY	China	JN009803 (Li X. et al., unpubl.)	Southern dahuricus St-rDNA
46	*E. excelsus* Turcz. ex Griseb.	StHY		JN009809 (Li X. et al., unpubl.)	
47	*E. franchetii* Kitag. (*E. dahuricus* var. *cylindricus* Franch.)	StHY	China?	JN009805 (Li X. et al., unpubl.)	
48	*E. franchetii* Kitag.	StHY	China: Haiyuan	KF905180 (Song et al., 2015)	Northern dahuricus St-rDNA
49	*E. franchetii* Kitag.	StHY	China: Xinjiang	KJ526336 (Gao et al., 2015)	Southern dahuricus St-rDNA
50	*E. franchetii* Kitag.	StHY		KJ526337 (Gao et al., 2015)	
51	*E. tangutorum* (Nevski) Hand.-Mazz.	StHY		KJ526351 (Gao et al., 2015)	
52	*E. tangutorum* (Nevski) Hand.-Mazz.	StHY		KJ526352 (Gao et al., 2015)	Northern dahuricus St-rDNA
	Section Goulardia (Husn.) Tzvel.				
53	*E. caninus* (L.) L.	StH	China: Nei Monggol, Xilinhot	KJ526335 (Dong et al., 2015)	Northern St-rDNA
54	*E. dolichatherus* (Keng) S.L. Chen	StY	China	EU617242 (Liu Q. et al., unpubl.)	Southern dahuricus St-rDNA
55	*E. dolichatherus* (Keng) S.L. Chen	StY		EU617245 (Liu Q. et al., unpubl.)	Northern St-rDNA
56	*E. fedtschenkoi* Tzvel.	StY	China: Xinjiang, Habahe	AY740838 (Liu et al., 2006)	
57	*E. gmelinii* (Ledeb.) Tzvel.	StY	China: Xinjiang, Altay	AY740842 (Liu et al., 2006)	Northern dahuricus St-rDNA
	Section Clinelymopsis (Nevski) Tzvel.				
58	*E. caucasicus* (K. Koch) Tzvel.	StY	Armenia: Dilidjan	AY740808 (Liu et al., 2006)	Southern St-rDNA
	Section Elymus				
59	*E. confusus* (Roshev.) Tzvelev	StH	Mongolia	FJ040160 (Wang et al., 2009)	Northern St-rDNA
60	*E. sibiricus* L.	StH	China: Gansu, Hezuo	EF396962 (Wang et al., 2009)	
	Gen. *Elytrygia* Desv.				
61	*E. repens* (L.) Nevski	StStH	South Korea: Yungyanggun	KF713228 (Lee J. et al., unpubl.)	Southern St-rDNA
62	*E. repens* (L.) Nevski	StStH	China	MF893161 (Yang et al., 2017)	
	Pseudoroegneria (Nevski) A. Löve				
63	*P. cognata* (Hackel) A. Löve	St	Kyrgyzstan: Osh	EF014226 (Yu et al., 2008)	Northern St-rDNA
64	*P. elytrigioides* (C. Yen & J.L. Yang) B.R. Lu	StSt	China: Tibet, Changdu	AY740798 (Liu et al., 2006)	
65	*P. geniculata* (Trin.) A. Löve	StSt	RF: Altai Republic	EF014228 (Yu et al., 2008)	
66	*P. geniculata* (Trin.) A. Löve	StSt		EU617141 (Liu Q. et al., unpubl.)	
67	*P. kosanini* (Nabelek) A. Löve	Unknown ($2n = 56$)	Turkey	EF014235 (Yu et al., 2008)	Southern St-rDNA

Table 2 (end)

No.	Species	Genome	Origin	GenBank	Ribotype
68	*P. kosanini* (Nabelek) A. Löve	Unknown (2*n* = 56)	Turkey	EF014236 (Yu et al., 2008)	Northern St-rDNA
69	*P. sosnowskyi* (Hack.) A. Löve	St		GQ365150 (Dizkirici et al., 2010)	
70	*P. sosnowskyi* (Hack.) A. Löve	St		GQ365151 (Dizkirici et al., 2010)	
71	*P. spicata* (Pursh) A. Löve	St and StX	USA: Oregon	AY740793 (Liu et al., 2006)	Southern St-rDNA
72	*P. spicata* (Pursh) A. Löve	St and StX	USA: Wyoming, Half Moon Lake	EF014239 (Yu et al., 2008)	Northern St-rDNA
73	*P. stipifolia* (Czern. ex Nevski) A. Löve	St	RF: Stavropol Botanical Garden	EF014240 (Yu et al., 2008)	
74	*P. stipifolia* (Czern. ex Nevski) A. Löve	St		EU617041 (Liu Q. et al., unpubl.)	Southern St-rDNA
75	*P. strigosa* (Bieb.) A. Löve	St? 2*n* = 28	Crimea, Ai-Petri	EF014241 (Yu et al., 2008)	
76	*P. tauri* (Boiss. & Bal.) A. Löve	StP	Iran	EU617155 (Liu Q. et al., unpubl.)	Northern St-rDNA
77	*P. tauri* (Boiss. & Bal.) A. Löve	StP		EU617173 (Liu Q. et al., unpubl.)	Southern St-rDNA
			Gen. *Hordeum*		
78	*H. bogdanii* Wilensky	H	China	AY740876 (Liu et al., 2006)	*Hordeum* spp.
79	*H. murinum* ssp. *leporinum* (Link) Arcang.	HH	Iran: Tehran	KP126672 (Makhoul M.T. et al., unpubl.)	
80	*H. murinum* L. ssp. *murinum* (Hack.) H. Scholz et Raus	HH	Germany	KC193786 (Rabey, 2014)	
81	*H. vulgare* L.	H		FJ593180 (Daniel C. and Knoess W., unpubl.)	
82	*H. vulgare* var. *distichon* (L.) Hook. f.	H	Egypt	KC193783 (Rabey, 2014)	
83	*H. vulgare* subsp. *spontaneum* K. Koch	H	Afghanistan	KM217265 (Georgiev O. et al., unpubl.)	

Traditional evolution models, implying a gradual accumulation of mutations followed by dichotomous branching of phylogenetic trees, are ill-suited for describing species divergence in these taxa (Dobryakova, Nosov, 2015; Rodionov et al., 2017, 2018). Therefore, the results of ITS sequencing results were processed with the Neighbor-Net algorithm by the program SplitsTree4, suggested for reconstruction of reticulate evolution (Huson, Bryant, 2006). The Neighbor-Net algorithm builds a network called a split graph. The split graph (Fig. 1) shows several possible ways of grouping DNA sequences with varying degrees of probability, known as "splits", and reflects the presence of *homoplasy* in the data.

Fig. 1 shows that all species carrying St genomes, *Elymus*, *Pseudoroegneria* and *Elytrigia*, are distributed between two main clusters. We called them according their geographical location, respectively, "Nothern" and "Southern" (Fig. 2). Each of these clusters then split into two separated ribotypes groups called "Core Northern St-ribotype"/"Northern dahuricus St-ribotype" and "Core Southern St-ribotype"/"Southern dahuricus St-ribotype", respectively. The Core Northern Elymus ribotype is widespread among the *Elymus* taxa of Eurasia, including Yakutia, Mountain Altai and Northern and High Mountain China (Tibet, Nei Mongol, Xinjiang, Gansu). It was found also in Finland, the Far East of the Russian Federation, and Mongolia. The Core Southern St-ribotype is typical mostly for more southern populations, including the Caucasus, Primorsky Krai (RF), Pakistan, South Korea, a part of China and Turkey. The Core Southern rDNA was found in *Elytrigia repens* and in diploid *Pseudoroegneria strigosa*, as well as in some other *Pseudoroegneria* species: *P. spicata* (haplome St or StX – Wang et al., 1986), and *P. sosnovskyi* (haplome St – Assadi, 1994). On the other hand, the Core Northern St-ribotype is characteristic feature of *P. cognata* (2*n* = 14 – Lu et al., 1991) and *P. spicata* (PI 232134, 2*n* = 14 – Okino et al., 2009), both carry the haplome St^Y (Okino et al., 2009).

The "Northern dahuricus" St-rDNA and "Southern dahuricus" St-rDNA (ribotypes) are derivatives of these two base types of rDNA, "Core Southern" and "Core Northern". There are 6 SNPs and one deletion that delimited consensus sequences of the "Core Southern" and "Core Northern" ribotypes (Fig. 3). The consensus sequence "Southern dahuricus" St carries 5 SNPs and one deletion that differ from that of "Core Southern" St-ribotype. Differences between consensus "Core Northern" St-ribotype and consensus "Northern dahuricus" St-ribotype consist of 5 SNPs. As results, consensus sequences of the "Northern dahuricus" St-ribotype and the "Southern dahuricus" St-ribotype differ in 11 STPs and two indels.

It should be noted that two different variants of rDNA were found in many species. For example, one of the plants *P. tauri* belongs to the "Core Northern" ribotype, another – to the "Core Southern" ribotype (see Fig. 1, Table 2). One can see the same phenomenon in *P. stipifolia* from Stavropol, *P. spicata* of USA, *P. kosanini* of Turkey, *E. dolichatherus* of China (see Table 2). It appears that this can be correlated with the allopolyploid karyotypes of these tetraploid species.

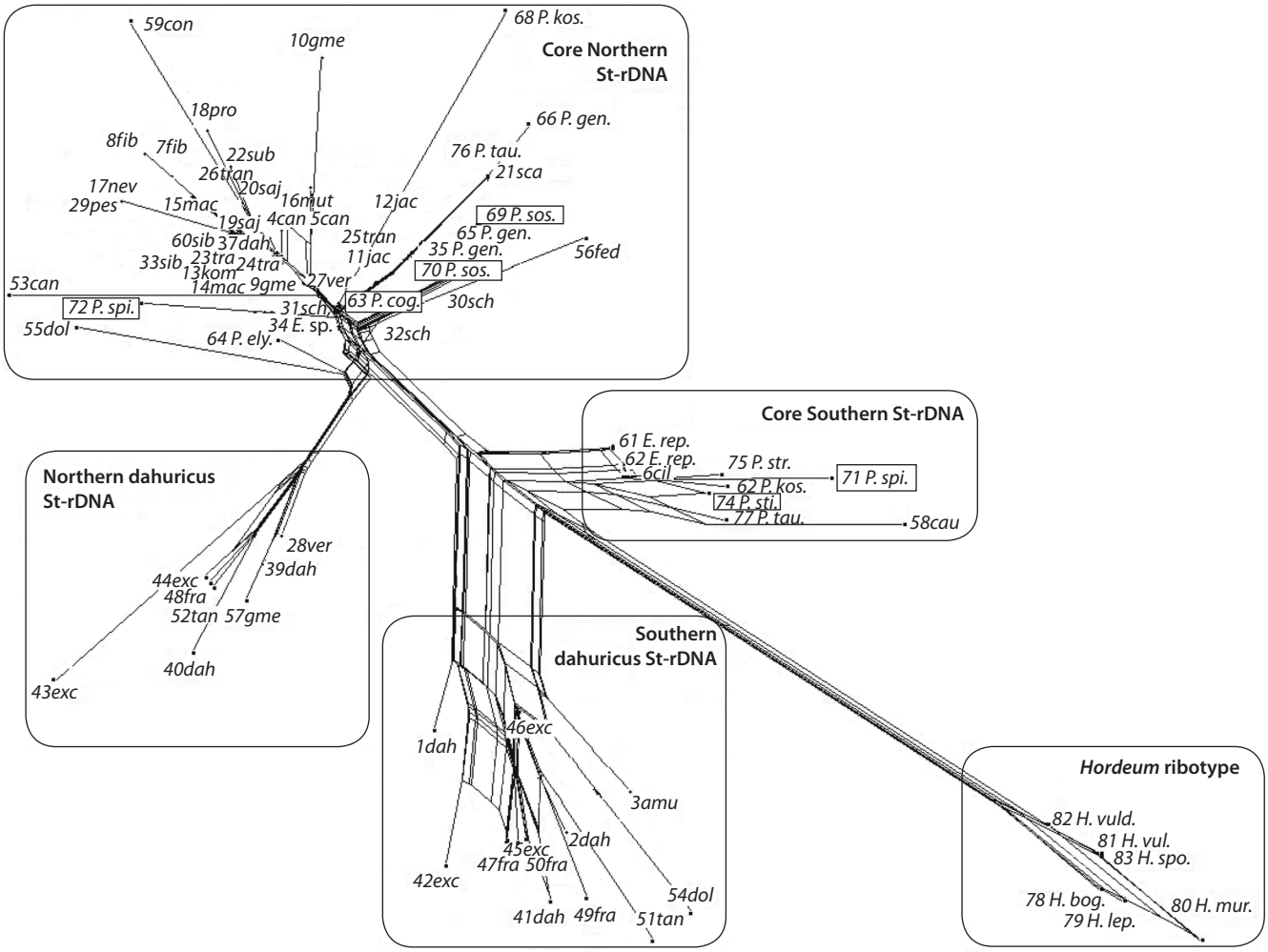

Fig. 1. Split graph for the ITS sequences of *Elymus* species and some other species of the tribe Triticeae generated by SplitsTree4 (Huson, Bryant, 2006).

Species names and numbers of accessions see Tables 1 and 2. Positions of the diploid species with St genome are indicated by boxes. *amu* – *Elymus amurensis*, *can* – *E. caninus*, *cau* – *E. caucasicus*, *cil* – *E. ciliaris*, *con* – *E. confuses*, *dah* – *E. dahuricus*, *dol* – *E. dolichatherus*, *exc* – *E. excelsus*, *fed* – *E. fedtschenkoi*, *fib* – *E. fibrosus*, *fra* – *E. franchetii*, *gme* – *E. gmelinii*, *jac* – *E. jacutensis*, *kom* – *E. komarovii*, *mac* – *E. macrourus*, *mut* – *E. mutabilis*, *nev* – *E. nevskii*, *pes* – *E. peschkovae*, *pro* – *E. probatovae*, *saj* – *E. sajanensis*, *sca* – *E. scandicus*, *sch* – *E. schrenkianus*, *sib* – *E. sibiricus*, *sub* – *E. subfibrosus*, *tan* – *E. tangutorum*, *tra* – *E. trachycaulus*, *tran* – *E. transbaicalensis*, *ver* – *E. vernicosus*, and *E.* sp. – *Elymus* sp., *E. rep.* – *Elytrigia repens*, *H. bog.* – *Hordeum bogdanii*, *H. lep.* – *H. murinum* ssp. *leporinum*, *H. mur.* – *H. murinum* L. ssp. *murinum*, *H. spo.* – *H. vulgare* subsp. *spontaneum*, *H. vul.* – *H. vulgare*, *H. vuld.* – *H. vulgare* var. *distichon*, *P. cog.* – *Pseudoroegneria cognata*, *P. ely.* – *P. elytrigioides*, *P. gen.* – *P. geniculate*, *P. kos.* – *P. kosanini*, *P. sos.* – *P. sosnowskyi*, *P. spi.* – *P. spicata*, *P. sti.* – *P. stipifolia*, *P. str.* – *P. strigose*, *P. tau.* – *P. tauri*.

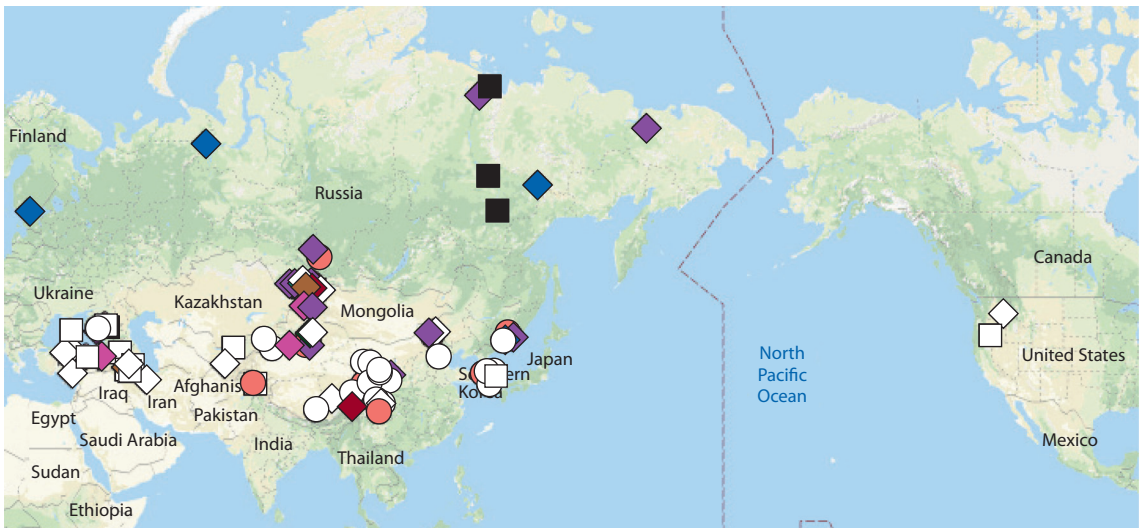

Fig. 2. Distribution of species *Elymus* s. l. with Northern (squares) and Southern (cycles) ribotypes.

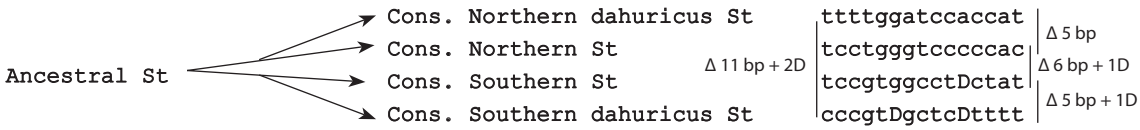

Fig. 3. The origin of St-ribotypes of the genus *Elymus*.

In the figure are shown only positions that are different in the consensus sequences of "Core Northern", "Core Southern", "Northern dahuricus" and "Southern dahuricus" ribotypes. D – deletion.

Discussion

In the present study we have shown that all species of *Elymus* in eastern Eurasia can be divided, according to their ITS sequences, into two families of ribotypes, conventionally called by us as the "Northern" and the "Southern" variants of St-rDNA. Each of these families is reliably divided into two subfamilies, the main, or "Core" variant of St-rDNA, and a modification of the St-ribotype distributed mainly between species *Elymus dahuricus* aggr. that we called "Northern dahuricus" and the "Southern dahuricus" ribotypes. Comparison of ITS sequences of *Elymus* and of *Pseudoroegneria* species showed that the "Core Northern" St-ribotype is close to rDNA of diploid *Pseudoroegneria cognata* with St genomes, accession PI 531720, collected in Kyrgyzstan (Dewey, 1990a; Yu et al., 2008). The "Southern" rDNA variant is closely related to that of *Elytrigia strigosa* PI 531752 (Dewey, 1990b; Yu et al., 2008) of Crimea and of *P. stipifolia* PI 313960 of Stavropol (Hyland, 1969).

The fact that there are *Pseudoroegneria* species with different St genomes have been shown earlier by Yan and co-workers (Yan et al., 2011) that studied nuclear genes *RPB2* and *EF-G*. They shown that *P. libanotica* and *P. tauri* St genomes are separated from the St genome of other *Pseudoroegneria* species, in particular *P. spicata* and *P. strigosa*.

The existence of significant uncertainty in the genome composition of the studied *Pseudoroegneria* species makes it difficult to interpret the results of the comparison between rDNA of *Pseudoroegneria* and *Elymus*. Thus, *P. strigosa* studied by Petrova (1967) was diploid with $2n = 14$. However, Dewey (1990a) observed $2n = 28$ in his sample of this species. In both cases the plants were from Crimea. Later, Khuat and co-workers studied *P. strigosa* from Mongolia and China and showed that they are hexaploids ($2n = 42$) (Khuat et al., 2015). Another *Pseudoroegneria*, *P. spicata* can be diploids ($2n = 14$) and tetraploids ($2n = 28$) (Wang et al., 1996; Khuat et al., 2015). Meiotic analysis and GISH showed that second genome of tetraploid *P. spicata* and second and third genomes of hexaploid *P. strigosa* are not St genomes (Wang et al., 1996; Khuat et al., 2015). So, according to genomic concept of the genus, these tetraploids and hexaploids should not be classified as *Pseudoroegneria*.

The occurrence in eastern Asia of plants of *E. dahuricus* with two different variants of rDNA Northern dahuricus and Southern dahuricus ribotypes implies that these two variants have a common pattern of morphological characters, some *E. dahuricus* syndrome, but they are reproductively isolated. This suggestion can be confirmed by the results of hybridological experiments performed earlier by Agafonov and coauthors (Agafonov et al., 2001; Savchkova et al., 2003). These authors revealed that seed fertility in crossings with various combinations of *E. dahuricus* aggr. parents does not depend primarily on the combination determined by the taxa morphology. It is important that some combinations of seed and pollen parents, delimitated by their morphological characters as the same species, were almost sterile: *E. dahuricus* MES-8709 (Primorye, near Posyet) × *E. dahuricus* CHI-8635 (Siberia, Chitinskij region) – only 4.8 % seed fertility, for comparison: *E. dahuricus* POP-8403 (Primorye, Popov island) × *E. woroschilowii* VLA-8642 (Primorye, Vladivostok) – 69 % seed fertility (Agafonov et al., 2001).

We suggest that there are probably not five different species but only two species in the *E. dahuricus* aggr. in Siberia and Northern China, one of them with the "Northern dahuricus" ribotype and another with the "Southern dahuricus" ribotype. Very likely, they are completely or almost completely genetically isolated from each other. It is unknown if there are any morphological distinctions between plants with different ribotypes or if these are cryptic species. However, it can be said with certainty that if there are differences in morphology, they are not connected with characters that are considered to be taxonomically significant to delimitation of the current species of *E. dahuricus* aggr.

It is appeared that morphological characters currently used for differential diagnosis of *Elymus dahuricus* aggr. species, do not allow to delimit plants with different ribotypes and even current traditional species because the diagnostic characters are weak. For example, various authors indicate curved glumes awns and the thicker stems of *E. excelsus* as diagnostic characters, delimiting *E. dahuricus sensu stricto* from *E. excelsus* (Tzvelev, Probatova, 2010). However, Savchkova et al. (2003) showed that hybrids have an intermediate state between direct and curved awns of lemma (inheritance type is unknown). F2 hybrids are more likely to show curved awns. This character is manifested in varying degrees at the plants' different stages of maturity: as spicules ripen, the awns of lemmas become more curved. Specimens with non-curved awns (an *E. dahuricus* diagnostic character) were collected among the Far Eastern populations, usually considered as *E. excelsus* populations, while examples with curved awns were found among the Altai populations of *E. dahuricus sensu stricto* (Savchkova et al., 2003). Similarly, the differences between *E. franchetii* and *E. excelsus* are insignificant, the first exhibit leaf blade widths of 3–8 mm and the second 8–18 mm (Tzvelev, Probatova, 2010).

Conclusion

In conclusion, we suppose that it is important to determine distribution areas of *Elymus* with the "Northern dahuricus" and the "Southern dahuricus" St-rDNA genomes (ribotypes). After this, it is necessary to reconsider the system of taxonomically

significant characters and try to find unique morphological characteristics appropriate only for plants with the "Northern dahuricus" St-rDNA or with the "Southern dahuricus" St-rDNA ribotypes. There is reason to believe that within this complex, there are at least two different, probably reproductively isolated, cryptic species or two reproductively isolated groups of species and these species (groups of species) may have different origin.

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