Two species of *Leymus* (Poaceae) are artificial as inferred from the molecular-genetic data

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**Abstract.** The results of studying taxonomic relationships between five species of the aggregate *Leymus secalinus* (Georgi) Tzvel. of the genus *Leymus* Hochst. are presented. The research is based on the study of the ITS1-5.8S-ITS2 region obtained by sequencing the nuclear ribosomal DNA internal transcribed spacers, and on the anatomical and morphological data. A molecular-genetic study of the *L. secalinus* aggregate has revealed that the two species *L. ovatus* and *L. jenisseiensis* are artificial.

**1. Introduction**

Plants of the genus *Leymus* Hochst. are perennial grasses, they belong to the tribe *Triticeae* Dum. (Poaceae), and they are both of scientific and practical interest. The taxonomy of the genus *Leymus* is very complicated and there is no consensus between different researchers regarding the number of species within. According to N N Tsvelev [1], the genus comprises 50 species grouped into several sections and distributed in extratropical countries of the Northern Hemisphere and South America. In the genus *Leymus*, it is particularly difficult to delimitate species in the aggregate of *Leymus secalinus* (Georgi) Tzvel. According to N N Tsvelev and N S Probatova [2], the aggregate consists of 10 species, including *L. ovatus* (Trin.) Tzvel. and *L. jenisseiensis* (Turcz.) Tzvel.

The taxon *L. ovatus* was first described by C B Trinius in “Flora Altaica” [3] under the name *Elymus ovatus* Trin., growing on sandy soils in coastal zone of the Chulysyman River, Altai. In the same book C B Trinius also described another species *Elymus dasystachys* Trin., growing on saline soils near the Chuya River, Altai. The range of both species overlaps, so researchers have often documented them as species growing together. Such botanists as P N Krylov, S A Nevsky, and M G Popov attributed plants of *Triticum secalinum* Georgi from the Baikal region to the species of *E. dasystachys* Trin. Later E G Bobrov [4] separated these species and restored the prior species name – “secalinus”, proposed by I I Georgi in 1775. At the same time Bobrov offered the combination – *Elymus secalinus* (Georgi) Bobr., the type of which is the picture drawn by I G Gmelin in “Flora Sibirica” [5], and the "locus classicus” of this species is on Olkhon Island and the sands of the south-western shore of Lake Baikal. The type specimen of *E. dasystachys* Trin. is represented by plants collected in the Chuya River valley and nearby the Altai mountains.

The taxon *E. dasystachys* Trin. was recognized by N N Tsvelev [1] as the synonym of *L. secalinus* (Trin.) Tzvel., and the species *E. ovatus* Trin. was accepted as a subspecies of *L. secalinus* (*L.s*. subsp. *ovatus* (Trin.) Tzvel.). At the same time, Tsvelev expressed the doubt that *E. ovatus* is a separate taxon and assumed that it is only a form of a type subspecies. Previously conducted comparative morphological analysis and SDS-electrophoresis of proteins from individual plant seeds gained from a mixed population of *L. dasystachys* (Trin.) Pilger and *L. ovatus* (Trin.) Tzvel.
have not confirmed the genetic isolation of these species [6]. According to the open on-line taxonomic databases “Catalogue of Life” [7] and “Plants of the World Online” [8], *L. ovatus* is a synonym of *L. secalinus* (Georgi) Tzvel.

The taxon *L. jenisseiensis* (Turcz.) Tzvel. was first described in 1856 by N S Turczaninow [9] at the banks of the Yenisei River in the suburbs of the city of Krasnoyarsk with the name *Elymus jenisseiensis* Turcz. According to the taxonomic databases “Catalogue of Life” [7] and “Plants of the World Online” [8], *L. jenisseiensis* is an accepted species of a hybrid origin (*L. racemosus* x *L. secalinus*).

One of the most difficult tasks in the taxonomy of the genus *Leymus* is the identification of differences between *L. secalinus* (Georgi) Tzvel., *L. littoralis* (Griseb.) Peschkova, *L. dasystachys* (Trin.) Pilger, *L. ovatus* (Trin.) Tzvel., and *L. jenisseiensis* (Turcz.) Tzvel. The significant discrepancies between the data of different taxonomists regarding the volume and range of these species have required additional molecular and genetic research.

The paper presents the study of the phylogenetic relationships of five species of the genus *Leymus*, aimed to understand the taxonomic status of the critical species. Additionally, the species *L. chinensis* (Trin.) Tzvel. from the section *Anisopyrum* (Griseb.) Tzvel. has also been analysed.

2. Materials and Methods

27 samples of 5 species of the genus *Leymus* from places of natural growth of the species were used for molecular-genetic research. The samples were collected during expeditions in various regions of Russia (Republic of Buryatia, Irkutsk Oblast, Republic of Tyva, Republic of Altai, Altai Territory), as well as in Kazakhstan, Mongolia, and China (figure 1). In addition to samples collected in the field, we also used the samples from herbarium collections: V L Komarov Botanical Institute of the RAS (St. Petersburg), M V Lomonosov Moscow State University, N V Tsitsin Main Botanical Garden of the RAS ( Moscow), Central Siberian Botanical Garden SB RAS (Novosibirsk), Tomsk State University, Institute of General and Experimental Biology SB RAS (Ulan-Ude), and Krasnoyarsk State Pedagogical University. So, plants stored in the abovementioned Herbaria from such areas as Tyva, Altai, Krasnoyarsk Krai, and Transbaikalia have also been analysed.

DNA of *L. jenisseiensis* plant was isolated from a sample of herbarium specimen No. 33566 (Krasnoyarsk State Pedagogical University), after being dried in silica gel using NucleoSpin Plant II kit (Macherey-Nagel, Germany), according to the standard protocol. Polymerase chain reaction (PCR) was carried out in a BIO-RAD T100tm Thermal Cycler (BIO-RAD, USA). All parameters, such as the volume, the composition of the reaction mixture, and amplification conditions were described in detail previously [10]. The rDNA ITS1-5.8S-ITS2 section was amplified and sequenced using primers: ITS1 (F) TCCGTAGGTGAACCTGCGG, ITS-B (R) GATATGCTTAAACTCAGCGG.

DNA sequencing was performed using the Sanger method in “Syntol”, LLC (Moscow). The phylogenetic tree was created using WinClada [11] and Nona [12] software, and based on the sequences of rDNA ITS1-5.8S-ITS2 (figure 2). The morphological and anatomical study was conducted and SEM-images were obtained using the scanning electron microscope Hitachi S-800. Voucher herbarium specimens are stored in Herbaria of Institute of General and Experimental Biology SB RAS, and Krasnoyarsk State Pedagogical University.

3. Results and discussion

The phylogenetic tree (figure 2) is divided into four clades, where the two monospecific clades of *L. chinensis* (plants from different regions) and *L. secalinus* (plants from the Baikal region) were formed. The two other clades consist of plants labelled as different species and are of particular interest. Mongolian and Chinese plants labelled as *L. secalinus* are grouped with plants of *L. littoralis* from Buryatia (lower clade on figure 2). In the context of the paper, it is important to emphasize that samples labelled as *L. ovatus* and *L. jenisseiensis* are joined to the 3rd clade formed by samples of *L. dasystachys* collected from different areas (figure 2). This finding gives molecular-genetic evidence that the *L. ovatus* and *L. jenisseiensis* species are artificial since they were grouped with *L. dasystachys* on the phylogenetic tree.
Figure 1. Distribution of the studied populations of *Leymus secalinus* aggregate species involved into molecular-genetic study.

N N Tsvelev [1] assumed that *L. ovatus* is probably only a form of *L. dasystachys* with abnormally developed spikes. Plants with abnormally developed ovate-shaped spikes (teratomorphs) are found throughout the whole range of *L. secalinus* and *L. dasystachys*. Herewith, the distances between the ledges in the spike are shortened, and the spike is developed with very close, convolute spikelets, and an irregularly rounded form (figure 3). The digging up rhizomes of co-growing plants of *L. ovatus* and *L. dasystachys* in natural habitats in the Republic of Altai revealed that generative shoots terminated with ovate-shaped spikes and shoots terminated with normally-shaped spikes are connected by a common rhizome. Besides the shape of spikes was the only differential trait between *L. dasystachys* and *L. ovatus*, as it was revealed in the course of analysis of herbarium specimens, that were always represented by separated aboveground spike-bearing shoots.

It should be noted that *L. dasystachys* populations are widespread in the upper reaches of the Yenisei River (along the banks of Ka-Khem and By-Khem rivers in the Republic of Tyva). Rhizomes and seeds of *L. dasystachys* can migrate downstream along the river and populate the available habitats. Besides, the climate of the upper reaches of the Yenisei River in Tyva is arid and severe extracontinental while Krasnoyarsk Krai has a more humid climate. For this reason, the plants of *L. dasystachys* in Krasnoyarsk Krai are larger by habitus compared to plants from populations in Tyva. Habitual traits were used by N S Turczaninov to describe a new species with the name *L. jenisseiensis*. On the one hand, according to the analysis of herbarium collection, the morphological variability of the two species should be considered simply as a modification with no taxonomical significance. On the other hand, morphological differences have not been supported by the molecular-genetic data to accept the Turczaninov’s species *L. jenisseiensis*.

The morphological and anatomical study of lower lemma and leaf lamina of *L. dasystachys* and *L. jenisseiensis* samples revealed that the pubescence of lower lemma and leaf lamina surface for plants of both species is identical (figure 4).
Figure 2. The phylogenetic tree based on sequence comparison of the ITS1-5.8S-ITS2 region shows the relationship of *L. secalinus*, *L. littoralis*, *L. dasystachys*, *L. jenisseiensis*, *L. ovatus* with the emphasis on the clade of *L. dasystachys* and disposition of *L. jenisseiensis* and *L. ovatus* within.

Figure 3. Fragments of spikes and their axes with spikelet scales in plants of the Tuvan population of *Leymus*. The shape of spikes and different lengths of internodes between the spikelet’s ledges of *L. dasystachys* (1, 2) and *L. ovatus* (3); the left picture shows the spike, the right one shows the spike’s axis with the spikelets removed.
Figure 4. The pubescence character of *L. dasystachys* (1 – lemma, middle part; 2 – lamina surface) and *L. jenisseiensis* (3 – lemma, middle part; 4 – lamina surface).

4. Conclusions
Based on the research of ITS1-5,8S-ITS2 sequences and anatomical and morphological data, we consider it is necessary to reject two species of *Leymus*, namely *L. ovatus* (Trin.) Tzvel. and *L. jenisseiensis* (Turcz.) Tzvel. since they are artificial species. Both taxa should be referred to as synonyms of *L. dasystachys* (Trin.) Pilger.

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