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MORPHOLOGICAL, KARYOLOGICAL AND MOLECULAR CHARACTERISTICS OF *FESTUCA ARIETINA* KLOK. – A NEGLECTED PSAMMOPHILOUS SPECIES OF THE *FESTUCA VALESIACA* AGG. FROM EASTERN EUROPE

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Until recently, *Festuca arietina* was practically an unknown species in the flora of Eastern Europe. Such a situation can be treated as a consequence of insufficient studying of *Festuca valesiaca* group species in Eastern Europe and misinterpretation of the volume of some taxa. As a result of a complex study of *F. arietina* populations from the territory of Ukraine (including the material from *locus classicus*), Belarus and Lithuania, original anatomy, morphology and molecular data were obtained. These data confirmed the taxonomical status of *F. arietina* as a separate species. Eleven morphological and 12 anatomical characters, ITS1-5.8S-ITS2 cluster of nuclear ribosomal genes, as well as the models of secondary structure of ITS1 and ITS2 transcripts were studied in this approach. It was found for the first time that *F. arietina* is hexaploid (6x = 42), which is distinguished from all the other narrow-leaved fescues by specific leaf anatomy as well as in ITS1-5.8S-ITS2 sequences. Molecular data indicating possible hybridogenous origin of *F. arietina*, fall in line with the anatomical-morphological data and explain the tendency toward sclerenchyma strands fusion with formation of a continuous ring in *F. arietina*, as well as *F. arietina* ecological confinement to psammophyte biotopes.

Keywords: *Festuca arietina*, anatomy, morphology, taxonomy, ITS, secondary structure, chromosome number

INTRODUCTION

Festuca arietina Klok. (Poaceae) was reported in 1950 from the territory of Ukraine (Kharkiv region, Zmiiv district) by a Ukrainian botanist, M.V. Klokov (1950). Until recently, all the information about the species was limited to its description both in the protologue and in «Grasses of Ukraine» (Tveretinova, 1977). However, after finding *F. arietina* in the flora of Lithuania and Belarus, data on the species and the peculiarities of its anatomical and morphological differentiation were considerably supplemented (Bednarska, 2014). In particular, it was shown, that this species, according to its anatomical and morphological characters, related to one of the most taxonomically complicated groups of the genus *Festuca* – to narrow-leaved fescues from the compound group *F. valesiaca* agg., where characters of the species essentially overlap within the group.

Despite a considerable interest in *F. valesiaca* agg. in the flora of Europe, discussions around species independence of the majority of the group representatives still do not stop (Pils, 1984; Šmarda, 2006; Šmarda et al., 2009; Arndt, 2008). This causes a constant search for various alternative diagnostic criteria, which in frame of a complex approach, would allow to specify the volume of the species and, in particular cases, the possible ways of their origin.

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At present it is commonly accepted that analysis of non-coding sequences of ribosomal gene cluster allows to evaluate the level of reproductive isolation between certain representatives of related taxa. The latter is achieved by evaluating the distinctions in the secondary structure of internal transcribed spacers ITS, in particular the presence and number of compensatory and hemicompensatory base changes (CBC and hCBC) and the occurrence of mutations, which either change or do not change the secondary structure of particular helices (Coleman and Mai, 1997; Coleman, 2000; Muller et al., 2007; Ruhl et al., 2009). The level of reproductive isolation continuously acquires taxonomic interpretation according to a molecular concept of the species, proposed by A. Coleman (Coleman, 2000, 2007, 2009).

The studies, analyzing the ITS sequences of the European species of narrow-leaved fescues, are scarce (Gaut, 2000; Galli et al., 2006; Inda et al., 2008), whereas the works which include the material from Eastern Europe are practically absent. In some authors' opinion, ITS sequences are low-informative for the resolution of taxonomic problems of narrow-leaved fescues because of their low/weak polymorphism (Galli et al., 2006), which, on the one hand, does not allow to apply this marker as a diagnostic one while identifying the species; and on the other hand, it favors uniting of minor species with one another. However, this opinion is based on the results of a comparison of primary structures of ITS1-ITS2 sequences, and does not take into account a diagnostic value of distinctions between secondary structures of the transcripts.

The present study was aimed at revising data on *Festuca arietina* in Eastern Europe, based on a complex analysis of its anatomical and morphological characters, as well as of secondary structure of ITS1 and ITS2 transcripts and the number of chromosomes.

MATERIALS AND METHODS

Plant material of *F. valesiaca* group deposited in the herbaria CWU, DNZ, KW, KWHA KWU, LE, LW, LWS, LWKS, MSK, MSKU and UU was revised. The basis for the study of anatomical and morphological differentiation of *F. arietina* populations is the authors' herbarium collection, which is kept in a specialized herbarium of the genus *Festuca* in the Institute of Ecology of the Carpathians National Academy of Sciences of Ukraine (LWKS) (Table 1).

To analyze the variability of parameters for *F. arietina* populations, 20-30 specimens from each locality were selected. In total, over 340 plants were investigated.

Eleven morphological and 10 anatomical traits were studied for each specimen. The following morphology characters were measured: height of plants (stem), leaf length, panicle length, spikelet length (measured as the total length according to the tradition of East-European agrostology school), number of florets, length of lemma and awn, length of lower and upper glumes. For measuring lemma and awn length, the second flower of each spikelet was studied.

Quantitative characters for leaf anatomical structure are rather few – these are the diameter of leaves, number of ribs, number of vascular bundles and sclerenchyma strands including the tiniest drops (isolated 2–3 cells). The prevailing majority of anatomical characters and indumentum of different plant parts refer to the category of qualitative. Correspondingly, for their analysis, each character was divided into a certain number of states, which illustrate the range of variation for this character (Table 2). Since asymmetry occurs quite often in the leaf anatomical structure, we examined characters of the right and left halves of the leaf-blade separately; as a result, their arithmetic mean value was calculated.

The length of spikelets, lemmas, awns and glumes was measured using a binocular microscope LOMO MBS-9 fitted with a 0.1–10 mm micrometer. Cross sections were made from the central third of the leaf of a sterile shoot from herbarium sheets. In total, about 950 cross sections were analyzed.

MULTIVARIATE ANALYSIS

A set of 342 specimens of *F. arietina* was chosen for analysis. Three measurements for each specimen were averaged to characterize quantitative morphological characters. Three leaf blade cross sections were made and illustrated for each plant to characterize leaf anatomical structure. Thus, for each specimen in our data set, three measurements were entered. The specimens were recognized as Operational Taxonomic Units (OTU's). The Kaiser-Meyer-Olkin (KMO) test was applied to check the sampling adequacy for the species under study and adequacy of submitting the data set to factor analysis.

The data were analyzed with Principal Component Analysis (PCA) for all variables (12 quantitative and 9 qualitative). The quantitative variables were represented by their mean values. All character values were standardized by subtracting the mean and dividing by the standard deviation (to equalize the weights in the construction of the axes of PCA). We checked for interrelationships between the variables (clustering of variables) by estimating Pearson correlation coefficients to ensure that the multivariate analysis was not distorted. The calculations were done with the STATISTICA 7.0 package (Stat Soft Inc.) and SPSS 10.0 package.

TABLE 1. List of investigated populations. The samplings used as material for karyological analysis are marked by an asterisk.

Code of population	Locality and habitat
IB-1429* 28 plants	Locus classicus. Ukraine, Kharkiv Region, Zmiiv district, Zadonetske village, NNP "Gomilshanski forests", flood plain of the Siversky Donets river. Outskirts of pine forest, sandy loam soil. 08.06.2010 – I. Bednarska, V. Popov
IB-1430 18 plants	Ukraine, Kharkiv region, Balakliia district, southern outskirts of Balakliia town. Communities between road and pine forest. 08.06.2010 – I. Bednarska, V. Popov
IB-0062 25 plants	Ukraine, south-east suburb of Kyiv (former village Bortnychi). Dry grassland on the edge of pine forest on sand. Grows in mix population with *F. polesica*. 15.06.2002 – I. Bednarska, N. Shyjan, V. Goncharenko, A. Levanets
IB-1451 26 plants	Ukraine, Kyiv, Desnianskyi District, interfluve between rivers Desenka and Dnieper. Xeromesophytic oligotrophic grassland. 03.06.2009 – I. Bednarska, V. Melnik
IB-1455 24 plants IB-1668*	Ukraine, Cherkasy region, Kaniv district, Liplyave village, in the vicinity of the Kaniv Nature Reserve, flood plain of the Dnieper river. Outskirts of oak forest, sandy loam soil. 05.06.2009 – I. Bednarska IB-1668 In the same place 07.07.2014 – I. Bednarska, I. Kostikov (material for karyological studies)
IB-1459 25 plants	Ukraine, Cherkasy region, Kaniv district, Liplyave village, in the vicinity of the Kaniv Nature Reserve, Natural landmark "Scythian Settlement". Fragments of xeromesophytic grasslands on the flat slope ("windows") among sparse forest on long-standing overgrown leas. 06.06.2009 – I. Bednarska
IB-1460 16 plants	Ukraine, Cherkasy region, Kaniv district, Liplyave village, in the vicinity of the Kaniv Nature Reserve, Natural landmark "Maria's Hill". Steppe meadow. 06.06.2009 – I. Bednarska
IB-1461 26 plants	Ukraine, Cherkasy region, Kaniv district, Liplyave village, in the vicinity of the Kaniv Nature Reserve, Natural landmark "Hrushky". Psammophyte meadow on dislocated alluvial sands on the top of the hill. 06.06.2009 – I. Bednarska
IB-1506 20 plants	Republic of Belarus, Gomel Region, Kalinkavichy district, Juravichy village, bedrock bank of the Prypyat River. Xeromesophytic meadow on the slope. 19.06.2010 – I. Bednarska
IB-1507 30 plants	Republic of Belarus, Gomel Region, Kalinkavichy district, Juravichy village. Low sandy ridges in the Prypyat River bottomland (with planted pine forest). 19.06.2010 – I. Bednarska
IB-1510 21 plants	Republic of Belarus, Gomel Region, Mazyr district, vil.Skrygalow. Water meadows in bottomland of the Prypyat River. 20.06.2010 – I. Bednarska
IB-1444 25 plants	Republic of Belarus, Gomel Region, Vietka district, Navasiolki village. Upper edge of the Sozh River bedrock bank. Light loess loam. Xeromesophytic grass meadow. 17.06.2010 – I. Bednarska
IB-1445* 28 plants	Republic of Belarus, Gomel Region, Vietka district, Adnapolle village. Barren dry meadows on sandy loam soil (also grows on water meadows in the valley of the Sozh River). 17.06.2010 – I. Bednarska
IB-0230* 30 plants	Lithuania, Varena district municipality, 5 km south-east of Merkine town. Valley of the Merkys river. Psammophilous grassland dominated by *Koeleria delavignei*, *F. polesica*, *F. pseudovina*, *Sedum sexangulare*. 11 plants: 20.06.2006 – I. Bednarska, V. Stukonis; 19 plants: 21.06.2016 – I. Bednarska, V. Stukonis

CYTOLOGICAL ANALYSIS

Chromosome numbers of *Festuca arietina* were determined for populations from Ukraine, Belarus and Lithuania. For each population (indicated by an asterisk in Table 1) we chose 8–15 seeds from 10 plants. Mitotic chromosomes were prepared from root-tip meristematic tissue. Seeds were germinated on a wet filter paper in a Petri dish at 23–24°C. Once the roots reached a length of 0.8–1.0 cm they were dissected and placed on ice with a few drops of ice-cold water. Then, immediately the samples were moved into the fridge and stored at 3–5°C for 24 hours. Next the root samples were transferred to ethanol: acetic acid (3:1) solution and incubated at 3–5°C for 2–3 hours. Later the solution was discarded and the roots were washed with acetic acid (45%). Then the samples were transferred into acetocarmine solution (2% acetocarmine in acetic acid) and incubated at 50°C for one hour. Once the staining had been finished, acetocarmine solution was discarded and a drop of acetic acid (45%) was applied on the root. Only meristematic root tip (0.5 mm) was dissected for microscopy and an additional drop

TABLE 2. Annotated list of qualitative characters and their states.

Characters	States
Thickness of central sclerenchyma strand	(1) thin (non-thickened, 2-3 cells layers); (2) medium (slight thickening of strand occurs); (3) thick (multilayered strand, often with almost horizontal layer of upper cells).
Thickness of marginal left/right sclerenchyma strand	(1) thin (1-2 layers of cells); (2) medium (about 2-3 layers of cells with thickening in angles); (3) thick (4 and more layers of cells, often unevenly thickened).
Type of marginal left/right sclerenchyma strand	(1) short, does not reach large vein; (2) discrete by *F. pseudovina* type (sclerenchyma is poorly developed); (3) discrete by *F. brevipila* type (separate strands formed in presence of additional vein); (4) continuous; (5) confluent with additional strand; (6) as a part of the ring.
Additional left/right sclerenchyma strand	(0) absent; (1) drop-like; (2) moderate; (3) elongated, well developed; (4) confluent with other strands or as a part of the ring.
Outline of sclerenchyma	(1) discrete (separate) strands; (2) marginal and additional strands are confluent; (3) discontinuous ring (possible numerous breaks); (4) continuous ring (possible single slight break).
Prominence of the left/right rib opposite the large vein	(0) absent; (1) poorly developed (the groove is not deep); (2) well developed.
Indumentum of abaxial epidermis (by number of trichomes within sight on the cross section)	(0) absent; (1) one trichome (moderate indumentum); (2) two and more trichomes (intensive indumentum).
Indumentum of lemma	(1) glabrous; (2) slightly scabrous; (3) scabrous; (4) ciliate on the margins; (5) pubescent in upper part; (6) pubescent over the most of surface; (7) very intensive densely pubescent long-pilose.
Indumentum of culms in upper part (under panicle)	(0) glabrous; (1) slightly scabrous; (2) scabrous (rough); (3) pubescent.

of acetic acid (45%) was applied. The root tip was then transferred on a microscopic slide and covered with a cover glass. The cover glass was then gently tapped and moved rotationally to squash the root tip. A Nicon Eclipse E800 microscope was used for cytological examination of the samples.

DNA EXTRACTION, AMPLIFICATION, SEQUENCING AND ANNOTATION

One plant with *F. arietina* distinctive diagnostic characters was chosen from each set of specimens: from populations from *locus classicus* and from Belarus, and subsequently used for the analysis of sequence of ITS1-5.8S-ITS2 of the nuclear ribosomal gene cluster. Leaf fragments of herbarium specimens were used for molecular analysis. Total DNA extraction was performed using a CTAB-method (Doyle, Doyle, 1987) modified for herbarium specimens (Tarieiev et al., 2011). ITS1-5,8S-ITS2 sequence amplification was performed using ITS1 and ITS4 primers (White et al., 1990) on Techne thermocycler. Sequencing of amplicons with forward and reverse primers was done commercially in Macrogen Inc. (http://www.Macrogen.com, The Netherlands). Sequences were edited

manually using BioEdit software (Hall, 1999). The obtained sequences were deposited in NCBI (accession numbers KP796238 (Ukraine, *locus classicus*) and KP796239 (Belarus)).

ITS1 was annotated in correspondence with terminal regions according to Galli et al. (2006). ITS2 annotation was done by modeling of the terminal part of 5.8S rDNA sequence and its complementary part of 28S one in MFold (Zuker, 2003). For *F. arietina* these parts are as follows:

5' (CTGCCTGGGCGTCACG(CCA) end 5.8S)-(ITS2)-(start 28S (GAC)CGCGACCCCAGGTCAG) 3'

ITS1 and ITS2 secondary structure models were constructed by direct folding of transcripts using online service MFold (Zuker, 2003). The obtained structures were visualized in Pseudoviewer 3.0 (Byun et al., 2009).

The data set for comparison of secondary structures included original data on *F. arietina* as well as sequences from NCBI with highest similarity to *F. arietina* (no less than 99% at 100% query coverage). Also the sequences of the European flora representatives, belonging to narrow-leaved fescues and known from the territories of Ukraine, Belarus and Lithuania were added to the data set (Table 3). The species were grouped by maximum similarity into 5 species clusters (*F. ovina* agg., *F. glauca* agg., *F. beckeri* agg., *F. valesiaca* agg. and *F. halleri* agg.). A system of species aggregates, proposed by N. Tzvelev (Tzvelev, 2010) with some additions was used as the basis for such grouping. This system describes species diversity in Eastern Europe in the best way and corresponds well with our own views. *Festuca valesiaca* agg. overlaps with *F. valesiaca* group according to Arndt (2008), while *F. halleri* agg. corresponds to *F. halleri* group according to Kiem (1987). *Festuca* glauca agg. is a relative/aggregate group of taxa, which have glaucous (pruinose), usually smooth leaves with a sclerenchyma ring and strongly long trichomes on the adaxial side of leaves (*F. pallens* Host, *F.* psammophila (Hack. ex Celak) Fritsch, *F. vaginata* Waldst. & Kit. ex Willd. etc.).

RESULTS

ANATOMICAL AND MORPHOLOGICAL PECULIARITIES

Multivariate Analysis. The KMO Test gave a high value of 0.807, indicating an adequate sampling of the species under study, thus allowing the data set to be submitted to factor analysis. The clustering between variables showed no correlation and therefore all 21 variables were retained for the PCA. All OTUs were grouped into one main cluster (Fig. 1) with significant overlapping between the groups. The first two components of the PCA represented about 42.4% of the cumulative variance (26.3% and 16.1%, respectively). The following characters most of all contributed to the division of specimens along the PC1 (character loadings in descending order): length of lemma (0.85), spikelet length (0.84), length of upper and lower glumes (0.79 and 0.76 respectively), height of plant (0.57) and panicle length (0.55). Along the PC2: number of veins (0.72), leaf-blade diameter (0.67), number of ribs (0.66), additional sclerenchyma strands (0.66) and outline of sclerenchyma (0.65).

The results of the analysis of anatomical and morphological parameters in *F. arietina* populations are given in Tables 4–6. There are no significant differences between populations inside Ukraine. The populations from the territory of Belarus were also very similar (Table 4). When comparing the collected samplings from distant regions, only a few differences between the populations can be observed. For example, such distinctions were found for populations in the basin of the Dnieper river (UA, Kyiv and Cherkasy regions) in comparison to populations in the basins of the Pripyat river and the Sozh (BY, Gomel region) or samplings from the basin of the Merkys river (LT, Varena distr.). At the same time the general level of population variability does not allow to explicitly claim any geographical or ecological differentiation inside the species.

As for qualitative traits, the indumentum of different parts of plants, in particular lemma and culms in the upper part (Table 6), were most variable. In fact, in each region we detected different populations with the prevalence of either glabrous or scabrous or pubescent plants.

Since the species is rather polymorphic, within it the morphotypes can be singled out according to the anatomical characters (Table 5). The morphotype, which occurs most frequently in populations of this species (background morphotype, the one to make up approximately 70% of cross sections), is characterized by ovoid and wedge-shaped leaf cross sections with medium thickened central and marginal sclerenchyma strands and moderately developed additional strands. Moreover, all the strands are isolated (Fig. 2: 1). Nevertheless, the most frequent morphotype is not diagnostic, since a similar structural type is characteristic of many other *F. valesiaca* group species as well, in particular of *F. valesiaca* Schleich ex Gaud., *F. rupicola* Heuff., *F. pseudodalmatica* Krajina, *F. macutrensis* Zapał., *F. saxatilis* Schur etc. For the identification of *F. arietina* the availability of cross sections (sometimes not numerous) with sclerenchyma strands, which fuse and form a ring – either continuous or with slight breaks, is crucial.

TABLE 3. Data set for comparison of ITS1 and ITS2 secondary structures and criteria of sequence selection for the data set (columns A, B, C).

Abb.[a]	Taxa in NCBI[b]	Accession numbers	Country, ploidy	Notes	A	B	C
***Festuca ovina* agg. (O)**							
Ov1	*F. ovina*	AF532959	DE 2x	Catalan et al. 2004			+
Ov2	*F. ovina*	HM453194	UA, 2x	Hand et al. 2010			+
Ov3	*F. ovina cultivar* BY 1692	AY327792	NL	Hunter et al. 2004 (NZAgRes. Seed)			+
Of1	*F. filiformis*	AF147155	RO	NCBI data only			+
Of2	*F. filiformis*	AF147160	YU	NCBI data only			+
Of3	*F. filiformis*	AJ240160	FR 2x	Charmet et al. 1997			+
***Festuca glauca* agg. (G)**							
Gp1	*F. pallens*	AY254373b	AT, 2x	Galli et al. 2006	+	GT	+
Gp2	*F. pallens*	HM453197	HU 2x	Hand et al. 2010			+
Gp3	*F. pallens*	AY254373a	HU-Sz 2x	Galli et al. 2006	+	GT	+
Gs	*F. pallens*[c]	AY254373c	HU Buda 4x	Galli et al. 2006 (Hungary-Buda Mt)	+	GT	
Gv	F.vaginata	AY254379	HU 2x	Galli et al. 2006	+		
***Festuca beckeri* agg. (B)**							
Bp1	*F. polesica*	AF171130	SU	Gaut et al. 2000	+		+
Bp2	*F. polesica*	AF171131	SU	Gaut et al. 2000	+	AY	+
Bp3	*F. polesica*	AF171132	SU	Gaut et al. 2000	+		+
***Festuca valesiaca* agg. (V)**							
Vo	*F. pseudovina*	AY254375	HU 2x	Galli et al. 2006	+		+
Va1	*F. valesiaca*	AF147149	RO	NCBI data only			+
Va2	*F. valesiaca*	EF584978	DE 2x	Inda et al. 2008			+
Va3	*F. valesiaca*	HM453198	RU 2x	Hand et al. 2010 (Transcaucasia)			+
Va4	*F. valesiaca*	AJ508377	HU 4x	Galli et al. 2006	+	GY	+
Vw	*F .wagneri*	AY254378	HU 4x	Galli et al. 2006	+	GT	
Vs	*F. stricta*	AY254377	HU 6x	Galli et al.2006	+		
Vj	*F. javorkae*	AY254372	SK, l.cl. 6x	Galli et al. 2006	+		
Vr1	*F. rupicola*	AY254376	HU 6x	Galli et al. 2006	+		+
Vr2	*F. rupicola*	AJ508379	HU 6x	Galli et al. 2006	+	GY	+
Vp	*F. pseudodalmatica*	AY254374	HU 4x	Galli et al. 2006	+		+
Vd1	*F. dalmatica*	AJ508378	HU	Penksza et al. 2003	+	GY	
Vd2	*F. dalmatica*	AY254371	HU 4x and 6x	Galli et al. 2006	+		
***Festuca halleri* agg. (H)**							
Hr1	*F. rupicaprina*	AF171145	SE 2x	Gaut et al. 2000	+	GT	
Hr2	*F. rupicaprina*	AF171146	SE 2x	Gaut et al. 2000	+	GT	

[a] Abb. – abbreviation of operation taxonomic units: first letter – name of the species aggregate (V-*F. valesiaca* agg., O-*F. ovina* agg., B-*F. beckeri* agg., G-*F. glauca* agg., H-*F. halleri* agg.), second letter – species epithet; columns A,B,C – criterion, by which the sequence is included into template: A – similarity ITS1-5.8S-ITS2 no less than 99%; B – similarity by variants of alleles ITS1-H4 (s.198 R>A>G) (first letter) and ITS2-H1 (c.35 Y>C>T) (second letter) = 100% (comparing to the ITS1-5.8S-ITS2 sequence of *F. arietina*); C – European species, which occur in Ukraine, Belarus and Lithuania. Abbreviations of countries: HU – Hungary, NL – Netherlands, DE – Germany, YU – former Yugoslavia, RO – Romania, FR – France, CA – Canada, RU – Russia, IR – Iran, AT – Austria, SK – Slovakia, US – USA, TR – Turkey, SU – former Soviet Union, SE – Sweden.

[b] Species names are given according to NCBI and can be different from modern nomenclatural combinations.

[c] Tetraploid populations of *F. pallens* are currently named *F. scikhegyensis* (Smarda et. al., 2007).

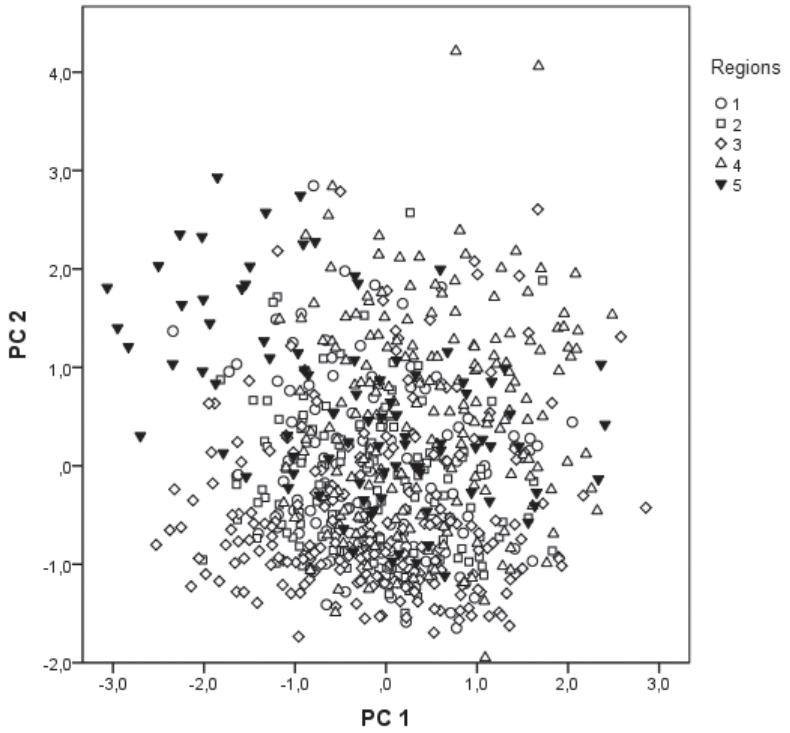

Fig. 1. Two-dimensional scatterplot of principal component analysis (PCA) of *Festuca arietina* populations from different part of the range. (1) Kharkiv reg., (2) Kyiv reg., (3) Cherkasy reg., (4) Gomel reg., (5) Varena distr.

Another key feature of *F. arietina* is the presence of flat ribs on the adaxial side of the leaf. They are not regularly present their frequency on cross-sections from different populations vary from 10 to 30%. This aspect makes species identification more difficult, because there is a need to have at least 5–10 specimens from each locality. It should be noted that morphotype ratio in populations of *F. arietina* in different parts of their distribution are rather distinct (Table 5). Thus, in specimens from *locus classicus* a background morphotype prevails (67% specimens have 5 veins, 31% have – to various extent – confluent sclerenchyma strands, in 15% ribs are prominent), whereas in specimens from Belarus both confluent sclerenchyma strands and flat ribs occur twice more frequently (50–70% specimens have 6–7 veins, approximately 30% are with flat ribs, from 40 to 85% have confluent sclerenchyma strands). Specimens from Lithuania are similar to the ones from Belarus, although they possess rather thinner leaves (Fig. 2: 2a).

CHROMOSOME NUMBER

ABOUT 300 seeds from a total of 30 plants identified as *F. arietina* from Ukraine, Belarus and Lithuania were subjected to cytological analysis. Viability of the seeds was sufficient with 67.3% germinating and developing a radicle. Meristematic radicles were dissected and pooled separately for each mother plant. Multiple (5 to 10) metaphase plates were karyotyped per each pool to determine the number of chromosomes. A total of 122 metaphase plates were karyotyped. All the specimens had a karyotype of 2n = 6x = 42 (Fig. 3).

CHARACTERISTICS OF ITS1 AND ITS2

In *F. arietina* from both *locus classicus* and Belarus fragments of the cluster of nuclear ribosomal genes with the length of 652 and 630 bp, respectively, were sequenced. The obtained sequence fragments included a part of 18S rDNA (41 and 19 bp), complete sequences of ITS1, 5.8S rDNA and ITS2 (217, 166 and 209 bp, respectively) and a part of 28S rDNA sequence (19 bp). In populations from both *locus classicus* and Belarus two sites with single-nucleotide polymorphism (SNP) were detected. These were the sites 198.R in ITS1 (helix 4) and 35.Y in ITS2 (helix 1) (Fig. 4). In the population from *locus classicus* the peaks of allelic nucleotides in these sites were equal (ITS1 198.R: G=A; ITS2 35.Y: T=C); in the specimen from the Belarusian population a signal from one of the allelic nucleotides prevailed over the other (ITS1 198.R: G>A; ITS2 35.Y T>C).

TABLE 4. Comparative table of arithmetical means in *Festuca arietina* populations (full names of localities are given in Table 1).
Upper row: 5% percentile – arithmetic mean (bold) – 95% percentile; lower row: standard deviation.

Code	Locality	Leaf length (cm)	Plant height (cm)	Panicle length (cm)	Total spikelet length (mm)	Number of florets	Lemma length (mm)	Awn length (mm)	Upper glume length (mm)	Lower glume length (mm)	Leaf-blade diameter (mm)
IB-1429	UA, Kharkiv reg., Zmiiv, locus class.	24-**32.3**-43 5.5	39-**57.2**-71 9.2	6.5-**8.6**-11 1.3	5.5-**6.8**-8.2 0.7	4-**4.8**-6 0.7	3.8-**4.3**-4.9 0.3	1.3-**1.7**-2.2 0.3	3.2-**3.8**-4.6 0.4	2.3-**2.8**-3.5 0.3	0.48-**0.60**-0.75 0.08
IB-1430	UA, Kharkiv reg., Balaklia	18-**27.3**-40 5.8	54-**67.6**-80 7.7	8-**9.7**-11.5 1.2	5.5-**6.7**-8.0 0.9	4-**4.8**-6 0.7	3.7-**4.3**-4.8 0.4	0.9-**1.6**-2.2 0.4	2.8-**3.7**-4.5 0.5	1.9-**2.8**-3.5 0.5	0.47-**0.6**-0.78 0.09
IB-0062	UA, Kyiv reg., Bortnyczi	15-**23.4**-30 4.0	46-**60.2**-78 10.2	7-**8.4**-10.5 1.1	6.0-**6.9**-8.0 0.6	4-**5.0**-6 0.6	4.0-**4.5**-4.8 0.2	1.5-**2.1**-2.7 0.4	3.2-**3.9**-4.5 0.4	2.2-**2.8**-3.1 0.3	0.5-**0.58**-0.7 0.055
IB-1451	UA, Kyiv city	14-**20.3**-26 4.2	35-**47.4**-61 7.2	5.3-**6.9**-9.0 1.1	5.3-**6.5**-8.5 0.9	4-**5.1**-6 0.7	3.7-**4.2**-4.9 0.3	1.3-**2.0**-2.9 0.5	3.3-**3.8**-4.7 0.4	2.3-**2.8**-3.6 0.4	0.51-**0.6**-0.73 0.07
IB-1455	UA, Cherkasy reg., Liplyave	10-**17.7**-29 6.1	33-**53.9**-76 13.8	5.5-**8.1**-11.6 1.9	5.2-**6.7**-8.3 0.9	3-**4.1**-6 0.8	3.7-**4.3**-4.7 0.3	1.5-**2.0**-2.7 0.4	3.2-**4.1**-5.0 0.6	2.0-**2.9**-3.7 0.5	0.43-**0.56**-0.75 0.1
IB-1459	UA, Cherkasy reg., Scythian Settlement	21-**30.1**-43 7.2	46-**62.4**-75 8.9	7.0-**8.8**-11.5 1.3	6.0-**7.5**-9.5 1.0	4-**5.5**-7 1.0	3.9-**4.5**-5.2 0.4	1.2-**2.0**-2.6 0.4	3.4- **4.0**-4.6 0.4	2.4-**2.9**-3.4 0.3	0.46-**0.59**-0.79 0.1
IB-1460	UA, Cherkasy reg., Maria's Hill	15-**25.5**-36 5.7	49-**60.7**-73 7.5	6.5-**9.4**-12 1.6	6.3-**7.3**-8.5 0.7	5-**5.5**-7 0.6	3.8-**4.6**-5.2 0.35	1.2-**2.0**-2.6 0.4	3.3-**4.1**-4.6 0.4	2.4-**2.9**-3.6 0.35	0.45-**0.57**-0.71 0.07
IB-1461	UA, Cherkasy reg., Hrushky	13-**22.3**-33 5.4	37-**49.8**-70 10.5	5.7-**8.4**-11.1 1.6	5.3-**6.6**-80 0.8	4-**4.7**-6 0.8	3.8-**4.3**-5.0 0.3	1.7-**2.1**-2.7 0.3	31-**3.8**-4.8 0.5	2.2-**2.7**-3.5 0.4	0.46-**0.56**-0.72 0.07
IB-1506	BY, Gomel reg., Juravichi, Pripyat bank	10-**14.3**-20 2.7	37-**57.3**-79 13	53-**8.0**-10.5 1.5	6.5-**7.9**-9.1 0.9	4-**5.0**-7 0.85	4.0-**4.7**-5.4 0.4	1.6-**2.3**-3.2 0.4	3.4-**4.2**-5.3 0.6	2.2-**2.9**-3.7 0.5	0.56-**0.72**-0.85 0.08
IB-1507	BY, Gomel reg., Juravichi, Pripyat floodplain	12-**18.1**-24 3.5	40-**55.5**-74 10	6.5-**8.7**-11.4 1.4	6.5-**7.5**-9.0 0.8	4-**4.6**-6 0.8	4.3-**4.6**-5.3 0.3	1.4-**1.9**-2.5 0.4	3.4-**4.1**-5.1 0.5	2.2-**2.9**-3.7 0.5	0.54-**0.67**-0.85 0.09
IB-1510	BY, Gomel reg., Skrygalow	15-**21.9**-27 4.9	4.8-**61.2**-78 9.5	6.5-**9.2**-12 1.6	6.7-**8.0**-9.3 0.9	4-**4.9**-6 0.8	4.3-**4.6**-5.2 0.3	1.5-**2.0**-2.7 0.4	3.5-**4.1**-4.7 0.4	2.3-**2.9**-3.6 0.4	0.57-**0.73**-0.93 0.11
IB-1444	BY, Gomel reg., Navasiolki	14-**20.1**-28 4.4	38-**55.6**-74 10.9	6.0-**8.4**-8.6 0.7	6.0-**7.3**-8.6 0.7	4-**4.9**-6 0.8	4.0-**4.5**-4.9 0.3	1.5-**2.1**-2.7 0.3	3.4-4.0-5.1 0.5	2.4-**2.9**-3.7 0.4	0.5-**0.64**-0.76 0.08
IB-1445	BY, Gomel reg., Adnapolle	10-**14.5**-18 2.7	50-**66.0**-85 10.7	6.0-**8.1**-10.0 1.2	5.0-**6.7**-8.5 1.0	3-**4.2**-6 0.8	4.0-**4.5**-4.9 0.3	1.6-**2.1**-2.8 0.4	3.5-**4.2**-4.9 0.45	2.3-**2.9**-3.6 0.4	0.58-**0.71**-0.85 0.08
IB-0230	LT, Varena distr., Merkine	14-**20.1**-30 5.6	29-**49.7**-70 12.7	5.0-**7.6**-11.0 1.7	5.5-**7.1**-8.5 0.9	4-**5.6**-7 0.9	3.5-**4.2**-5.0 0.45	0.9-**1.5**-2.2 0.4	2.8-**3.7**-4.5 0.5	2.0-**2.8**-3.5 0.5	0.51-**0.63**-0.77 0.07
	Arithmetic mean for the species	12-**21.9**-35 7.2	38-**56.8**-76 11.9	5.8-**8.4**-11.2 1.6	5.5-**7.1**-8.8 1.0	4-**4.9**-6 0.9	3.8-**4.4**-5.0 0.4	1.2-**1.9**-2.7 0.4	3.2-**4.0**-4.8 0.5	2.2-**2.9**-3.6 0.4	0.49-**0.63**-0.8 0.1

TABLE 5. Frequencies of anatomical character states of *Festuca arietina* (%) (full transcript states see in "Materials and Methods").

Code	Locality	Number of ribs			Number of veins			Thickness of central scl. strand			Thickness of marginal scl. strand			Additional sclerenchyma strands					Outline of sclerenchyma			
		3	4	5	5	6	7	1	2	3	1	2	3	0	1	2	3	4	1	2	3	4
IB-1429	UA, Kharkiv reg., Zmiiv, locus classicus	85	10	5	67	5	28	20	70	10	27	60	13	4	9	50	12	25	69	4	8	19
IB-1430	UA, Kharkiv reg., Balaklia	90	2	8	54	17	29	17	51	32	25	52	23	0	0	22	21	57	34	3	17	46
IB-0062	UA, Kyiv reg., Bortnyczi	90	7	3	66	15	19	18	68	14	19	63	18	2	1	43	26	28	56	10	19	15
IB-1451	UA, Kyiv city	96	4	0	61	13	26	38	43	19	14	60	26	8	4	35	24	29	63	8	11	18
IB-1455	UA, Cherkasy reg., Liplyave	87	3	10	72	8	20	51	46	3	33	67	0	2	6	50	17	25	69	1	11	18
IB-1459	UA, Cherkasy reg., Scythian Settlement	92	7	1	67	12	21	40	55	5	32	65	3	4	3	53	20	20	71	5	13	11
IB-1460	UA, Cherkasy reg., Maria's Mountain	90	6	4	52	21	27	50	50	0	35	62	3	3	3	59	17	18	69	10	19	2
IB-1461	UA, Cherkasy reg., Hrushky	96	1	3	72	8	20	53	43	4	41	56	3	6	11	61	8	14	81	5	7	7
IB-1506	BY, Gomel reg., Juravichi, Pripyat bank	68	10	22	32	8	60	20	53	27	29	50	21	0	2	7	12	79	15	8	22	55
IB-1507	BY, Gomel reg., Juravichi, Pripyat floodplain	70	16	14	47	11	42	24	66	11	38	42	20	1	1	25	14	59	31	18	18	33
IB-1510	BY, Gomel reg., Skrygalow	65	13	22	25	13	62	32	54	14	24	65	11	0	0	23	25	52	36	16	24	24
IB-1444	BY, Gomel reg., Navasiolki	75	9	16	37	15	48	45	55	0	54	43	3	4	7	39	26	24	64	16	10	9
IB-1445	BY, Gomel reg., Adnapolle	62	17	21	27	9	64	21	64	15	26	56	18	1	0	27	22	50	37	15	20	28
IB-0230	LT, Varena distr., Merkine	70	10	20	36	6	58	30	52	18	22	58	20	1	2	29	36	32	50	3	28	19
	Arithmetic mean for the species	81	8	11	51	12	37	33	55	12	30	57	13	3	4	37	20	36	53	9	16	22

TABLE 6. Frequencies of character states of *Festuca arietina* indumentum (full transcript states see in "Materials and Methods").

Code	Locality	Indumentum of lemma							Indumentum of culms in upper part			
		glabrous	slightly scabrous	scabrous	ciliate	pubescent upper part	pubescent most surface	very intensive pubescent	glabrous	slightly scabrous	rough	pubescent
IB-1429	UA, Kharkiv reg., Zmiiv, locus classicus	11	13	16	37	23	0	0	4	25	49	22
IB-1430	UA, Kharkiv reg., Balaklia	27	17	24	20	12	0	0	10	22	56	12
IB-0062	UA, Kyiv reg., Bortnyczi	7	8	11	44	30	0	0	0	49	51	0
IB-1451	UA, Kyiv city	17	21	15	32	11	4	0	74	15	4	7
IB-1455	UA, Cherkasy reg., Liplyave	26	11	18	32	8	4	0	15	29	35	21
IB-1459	UA, Cherkasy reg., Scythian Settlement	36	24	4	28	4	4	0	58	17	25	0
IB-1460	UA, Cherkasy reg., Maria's Mountain	27	13	29	15	2	15	0	31	33	24	12
IB-1461	UA, Cherkasy reg., Hrushky	34	12	9	25	8	8	4	27	33	27	13
IB-1506	BY, Gomel reg., Juravichi, Pripyat bank	0	2	0	5	45	10	0	41	43	16	0
IB-1507	BY, Gomel reg., Juravichi, Pripyat floodplain	18	27	0	48	7	0	0	20	35	35	10
IB-1510	BY, Gomel reg., Skrygalow	10	42	0	43	5	10	0	25	55	16	4
IB-1444	BY, Gomel reg., Navasiolki	0	36	0	52	12	0	0	14	24	43	19
IB-1445	BY, Gomel reg., Adnapolle	4	70	0	26	0	4	0	4	26	53	17
IB-0230	LT, Varena distr., Merkine	19	40	24	8	9	0	6	10	32	33	25
	Arithmetic mean for the species	17	24	11	30	13	4	1	24	31	33	12

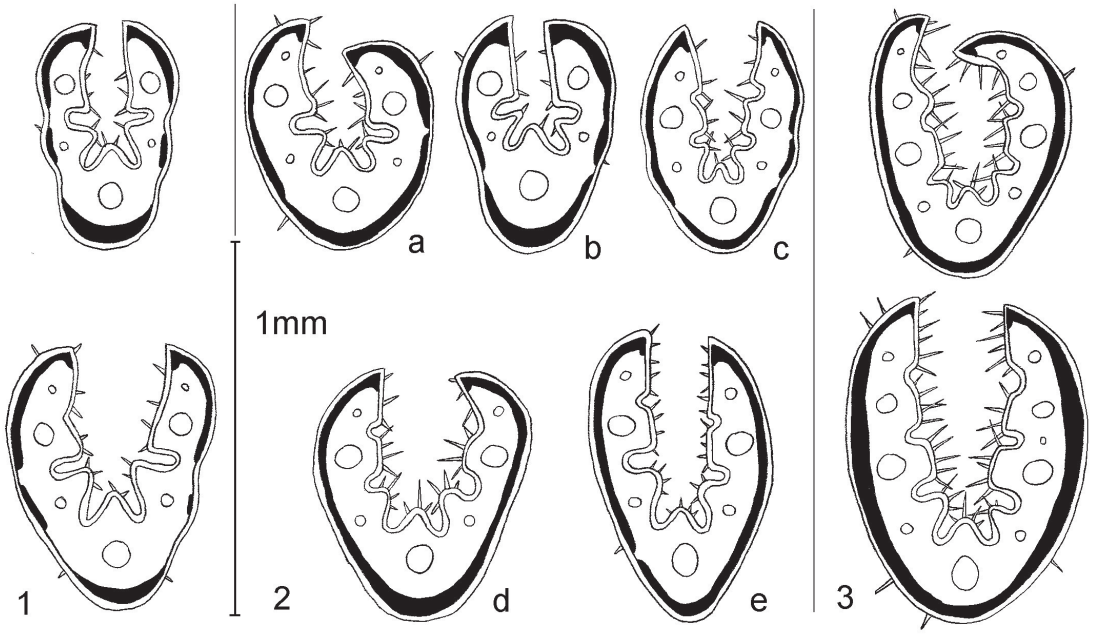

Fig. 2. Patterns of leaf-blade anatomy in cross sections: 1 – background morphotype *Festuca arietina*; 2 – diagnostic morphotype *Festuca arietina* (**a–b**) *Locus classicus*, (**c**) Lithuanian specimens, (**d–e**) Specimens from Belarus); 3 *Festuca polesica*.

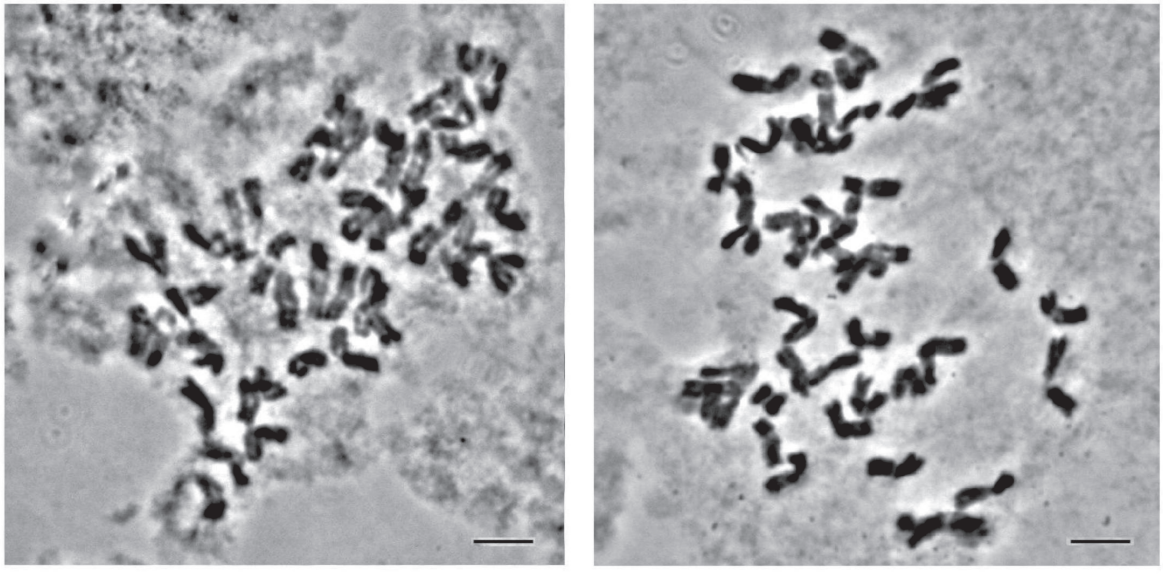

Fig. 3. Metaphase plates of *Festuca arietina* (population IB-1455; Ukraine, Cherkasy reg., Kaniv Nature Reserve). Scale bars = 10 μm.

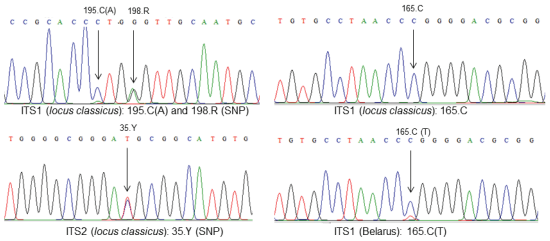

Fig. 4. Single-nucleotide polymorphism in sequences ITS1 (H4) and ITS2 (H1) in *F. arietina* (on example of specimens from *locus classicus*) and minor additional peaks in site 195 of the specimen from *locus classicus* and in site 165 of the specimen from Belarus.

Between ITS1-ITS2 sequences from the two populations two insignificant differences were detected, with respect to the presence/absence of sites with weak single nucleotide polymorphism: the specimen from *locus classicus* in site 195 ITS1 (H4) contained a minor adenine peak apart from a main peak of cytosine – ITS1: 195.C(A); the specimen from Belarus in site 165 ITS1 (H4) contained a minor thymine peak apart from the main peak of cytosine – ITS1: 165.C(T), which was absent in the specimen from the classical locality. Taking into account the weakness of the signal in the sequences deposited in NCBI the presence of minor additional peaks in sites 165 and 195 of ITS1 of the corresponding specimens, was not represented. As for the other sites, ITS1-ITS2 sequences of both populations were identical.

ITS1 secondary structure. The model of ITS1 secondary structure of *F. arietina* contained four main (H1–H4) and two additional helices (Ha and Hb) (Fig. 5). Helix H3, both by its primary and secondary structure, corresponded to the reconstruction of a universal for Chlorobionta motive by Liu and Schardl (1994).

In the analyzed data set, the representatives of *F. ovina* agg. (*F. ovina* L. s.str. AF532959, HM453194, AY327792 and *F. filiformis* Pourr. AF147155, AF147160, AJ240160) differed from the other European narrow-leaved fescues by the ITS1 secondary structure (helix H2). The presence of AC-insertion on 5'-side of the first loop of H2 and of hCBC in site 114 (result of A>G substitution) clearly delimited the representatives of *F. ovina* agg. Thus, according to the secondary structure of H2 of ITS1, *F. arietina* definitely does not belong to *F. ovina* agg.

Within the group of *F. glauca* agg., *F. beckeri* agg., *F. valesiaca* agg. and *F. rupicaprina* (Hack.) A.Kern. ITS1 had eleven variable sites. Five of them (sites 16, 43, 57, 107, 154) were taxonomically low informative, since they were located in the single-stranded parts (loops). In addition, the possible substitutions

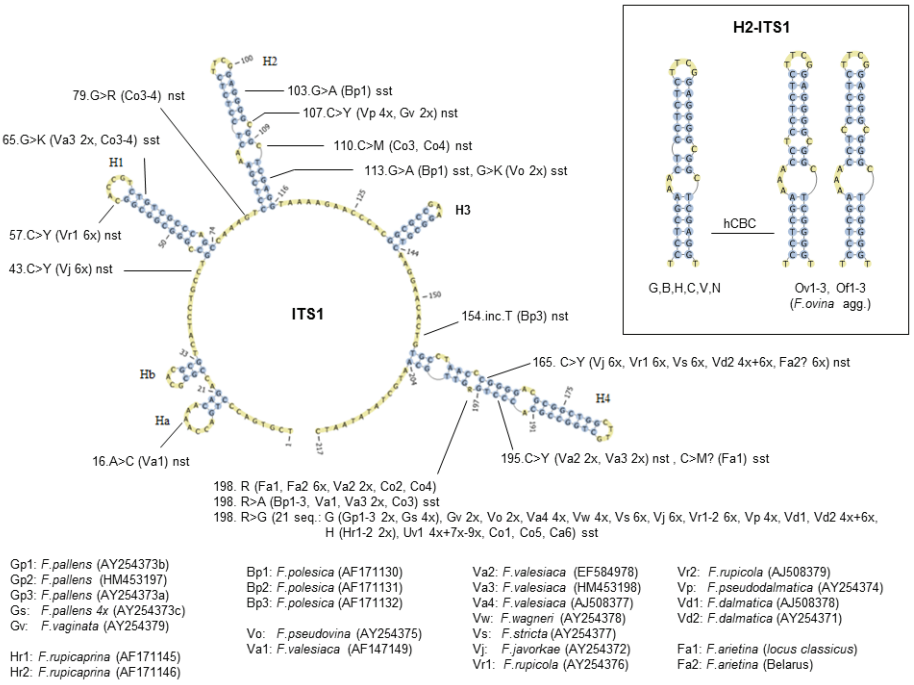

Fig. 5. Model of secondary structure of ITS1 *Festuca arietina* from *locus classicus* and variable sites of sample group of sequences in narrow-leaved fescues *F. glauca* agg., *F. beckeri* agg., *F. valesiaca* agg., *F. rupicaprina* (in the inset – distinctions in secondary structure of the second helix of *F. ovina* s.str. and the rest of narrow-leaved fescues).

or indels in these sites did not lead to changes in ITS1 secondary structure, and thus, they can be considered variants of intragenomic polymorphism.

In the three sites (65, 103, 113) substitutions of one nucleotide by another one caused the changes of ITS1 structure in diploid specimens of *F. valesiaca* (HM453198, Russia), *F. pseudovina* Hack. ex Wiesb. (AY254375, Hungary), *F. polesica* Zapał. (AF171130, former USSR), namely the conversion of double-stranded parts (stems) of H1 and H2 helices into single-stranded ones (loops).

The presence of essential distinctions in ITS1 secondary structure in different specimens of the three above mentioned species provided the evidence for incomplete reproductive compatibility within each of the three species, hence for their genetically determined intraspecific polymorphism.

Three sites with single nucleotide polymorphism (SNP) (sites 165, 195, 198), located between basal and subapical loops of H4 helix, are partially differentiating for the delimitation of operation taxonomic units (OTU) aggregates *F. glauca* agg., *F. beckeri* agg., *F. valesiaca* agg. (Fig. 6). In particular, allele combinations of these sites demonstrated six variants of H4 structures. Furthermore, the fourth variant was represented by *F. arietina* only, the first, second and third variants – solely by diploids of *F. valesiaca* and *F. beckeri* aggregates, whereas the fifth variant – just by tetra- and hexaploids of *F. valesiaca* aggregate. Sequences of the representatives with the first and sixth variants did not contain SNP in the fourth helix, in contrast to the representatives with the second to fifth variants of ITS1-H4. Generally, by its secondary structure the fourth helix of ITS1 of *F. arietina* looked like an intermediate variant between diploids of the first (*F. valesiaca* or *F. polesica*) and tetraploids of the sixth variants.

ITS2 secondary structure of *F. arietina* corresponded to a ring-like model with four helices, where helix H3 was the longest and on its 5'-side in subapical part (sites 121–126) contained a conservative for higher plants motif CGTGGT (Coleman, 2007). Helix H2 was G/C- rich in the basal part and contained a sub basal T-T mismatch (Fig. 7). All sequences of the sample group of the species *F. glauca* agg., *F. beckeri* agg., *F. valesiaca* agg., as well as *F. rupicaprina* had ITS2 secondary structure similar to *F. arietina*. Distinctions within the mentioned sample group were due to the presence of two SNP (sites 35 and 60), located in the first and second helices of ITS2, respectively.

The occurrence of SNP (60.A>W) in a terminal loop of the second helix was a unique feature for one of *F. valesiaca* (Va3: HM453198); nevertheless, alternative alleles at this site did not change ITS2 secondary structure, and therefore, this SNP is taxonomically low informative.

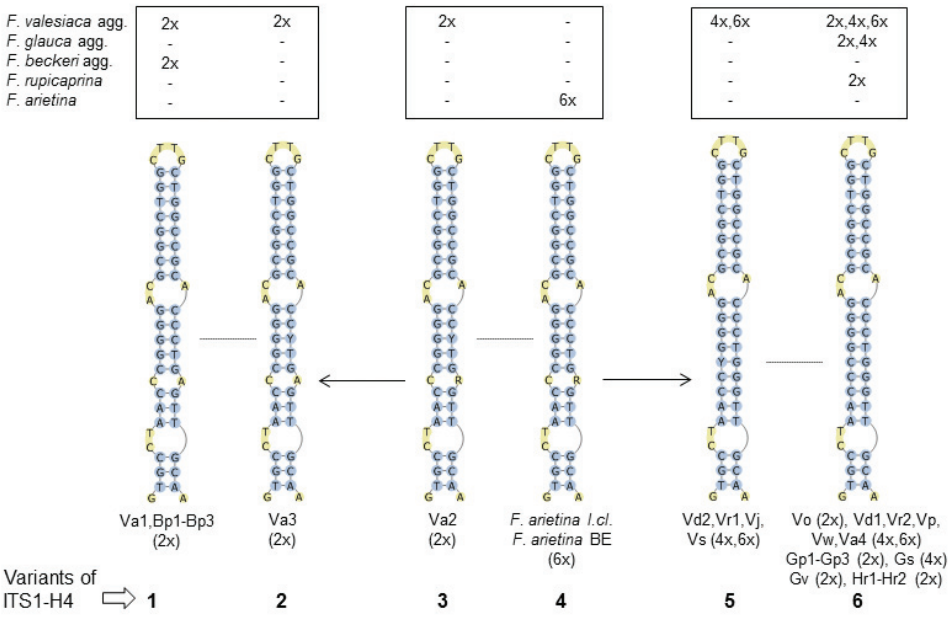

Fig. 6. Variants of secondary structure of the fourth helix ITS1 in representatives of *F. glauca* agg., *F. beckeri* agg., *F. valesiaca* agg., as well as *F. arietina* and *F. rupicaprina* (abbreviation for names of OTU's are provided according to Table 6).

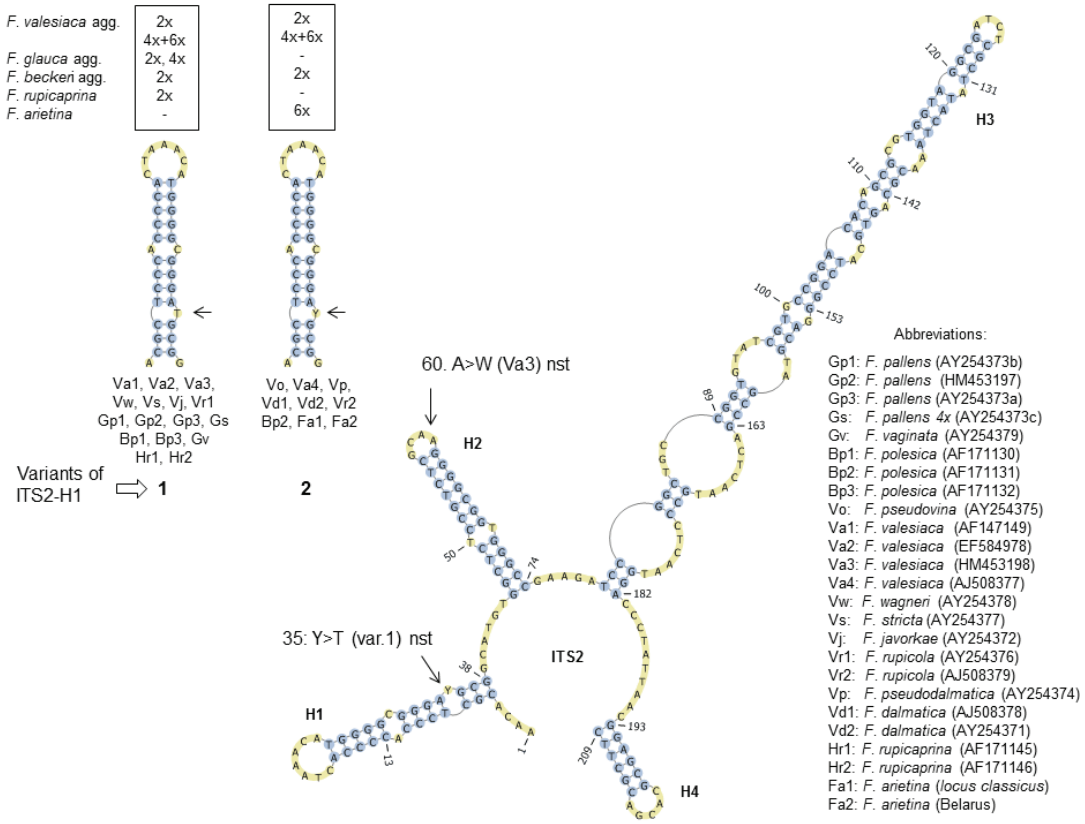

Fig. 7. Model of ITS2 secondary structure for *Festuca arietina* from *locus classicus* and variable sites of sample group of sequences of narrow-leaved fescues *F. glauca* agg., *F. beckeri* agg., *F. valesiaca* agg., *F. rupicaprina* (in the inset – distinctions in secondary structure of the first helix of the narrow-leaved fescues sample group).

By the presence/absence of SNP in site 35 the sample group of narrow-leaved fescues was divided into two subgroups. The first subgroup, in which SNP at site 35 was absent, was represented mostly by diploids, as well as by possible allopolyploid *F. pallens* – *F. csikhegyensis* Simonk., and by some polyploid species, distributed (with the exception of *F. rupicola*) only on the territory of Western Europe (*F. wagneri* (Degen, Thaisz & Flatt) Krajina, *F. stricta* Host, *F. javorkae* Májovský). The second subgroup, characterized by the presence of SNP (35.Y), was represented, first of all, by the polyploid species from the group *F. valesiaca* agg. (*F. pseudodalmatica*, *F. dalmatica* (Hack.) K.Richt., *F. rupicola* Heuff. and tetraploid *F. valesiaca*) and *F. arietina*, the which distributions partly or completely cover the territories of Ukraine, Belarus and Lithuania. In the subgroup 2 there were two diploids as well *F. pseudovina* (*F. valesiaca* agg.) and one of the specimens of *F. polesica* (*F. beckeri* agg.). Substitution of thymine to alternative cytosine in site 35 of ITS2 did not cause changes in ITS2 secondary structure. Therefore, the presence of these substitutions is not – to any extend – the evidence for existence of genetically determined reproductive restrictions for crossing between the representatives of different subgroups.

DISCUSSION

TAXONOMIC RELATIONSHIPS

Habitually (by phenotype) *F. arietina* is – in general – similar to other polyploid species of the group *F. valesiaca* agg., which possess green non-pruinose leaves (Bednarska, 2000, 2011, 2014). The most prominent distinctions of the species refer to the leaf-blade anatomical structure, namely, the tendency toward sclerenchyma strands fusion with formation of a continuous ring. Among representatives of *F. valesiaca* agg. such morphotypes are quite rare and apart from *F. arietina*, are typical of *F. macutrensis*, a species that prefers carbonate soils of North-West Podolia, as well as to *F. taurica* (Hack.) A.Kern. ex Hack. that prefers a kind of granite outcrop of southeastern Ukraine. *Festuca arietina*, associated with sands and sandy loam soils is unique in this list.

It is reasonable to mention *F. brevipila* Tracey, which is similar to *F. arietina*, because it is also a psammophyte, hexaploid and able to form a sclerenchyme ring. In addition, the range of their habitats are partially overlapped. However, a number of significant differences can be observed in morphological features as well as in molecular ones. In such a way, *F. brevipila* has mainly dove-colored (glaucous) waxed leaves 0.85–0.95 mm in diameter, whereas *F. arietina's* leaves are always green and much thinner (0.45–0.75 mm). As for molecular data, ITS1 and ITS2 sequences of *F. brevipila* (NCBI accession numbers AF147165, AF147161, AF147157, AF147179, AF147180, AF147153, AF147152, AF147151) differ significantly not only from *F. arietina*, but also from all other species of *F. valesiaca* group by primary and secondary structures.

Another species which is frequently misinterpreted as *F. arietina* is *F. wolgensis* P. Smirn. This species was described as an endemic plant of the Zhiguli Mountains located in Russia on the right bank of the Volga River in the Samara region. As opposed to *F. arietina*, *F. wolgensis* has glaucous leaves and grows on chalkstone outcrops in rocky steppes. In the issue "Grasses of the USSR" ("Zlaki SSSR") Tzvelev (1976) considered these two species to be subspecies (*F. wolgensis* P. Smirn. subsp. *wolgensis* and *F. wolgensis* subsp. *arietina* (Klokov) Tzvelev) just as the majority of representatives of *F. valesiaca* group. When he switched to a narrow species concept, all taxa mentioned above became treated as separate species (Tzvelev, 2010). However, some researchers from Western Europe started to treat *F. arietina* as a synonym of *F. wolgensis* or *F. illyrica* Markgr.-Dann. (Markgraf-Dannenberg, 1980; Clayton et al., 2016; http://www.gbif.org/species/4122573). We think that such reduction is incorrect and most probably happened due to lack of information.

Not less special diacritical character which distinguishes *F. arietina* from the majority of the species mentioned above, is the abundance of cross sections with flat ribs (Fig. 2: 2). In the case of a developed sclerenchyma ring, which makes leaves rigid, and in the case of flat ribs, specimens of *F. arietina* become highly similar to *F. polesica* (Fig. 2: 3) – the species of another compound group *F. beckeri* agg. (Tzvelev, 1976, 2010), which belongs to psammophytes as well.

Besides, ecological optima of both species significantly coincide – they are not demanding, they both grow on poor turf-podzolic sandy soils or on sand (more typical of *F. polesica*). They often occur both in communities with stable vegetation (for example, on river terraces, outskirts of pine woods), and in pioneer ones (for example, in spots with dug out sand, along roads), frequently they are dominant or codominant in plant communities. Geographical overlap is also characteristic of these species. Although general distribution of *F. arietina* is not determined definitely, in the region of the study its distribution completely fits within the range of *F. polesica*. Moreover, these species can be observed in one biotope because their ecological optima partially overlapped. We consider such «neigborhood» to be evidence of immediate connection between *F. arietina* and *F. polesica* during speciation process.

Most of species in *F. valesiaca* group, which are poliploids and tend to form a sclerenchime ring, are treated as species with hybridogenous origin by scientists from Eastern Europe (Alexeev et al., 1988; Tzvelev, 1976; Tveretinova, 1977; Kulikov, 2001; Probatova, 2007). In such a situation, on the one hand we have species which always have a schlerenchyme ring (*F. ovina* agg., *F. glauca* agg., *F. beckeri* agg.), and on the other hand *F. valesiaca* agg. representatives, which are characterized by 3(-5) isolated bundles. It should be accentuated that these species have hybridogenous origin, but are not recent hybrids. Nowadays they are fully separated and stabilized species. For *F. wolgensis* and *F. arietina* most probable hybrid parents are *F. valesiaca* s.l. and *F. beckeri* s.l. (*F. beckeri*, *F. polesica*, *F. sabulosa* (Anderss.) Lindb.) (Alexeev et al., 1988; Tzvelev, 1976; Tveretinova, 1977).

MOLECULAR DATA

Hexaploidy and presence of SNP in diagnostic sites both of ITS1 (site 198) and ITS2 (site 35) indicate a possible allopolyploid origin of *F. arietina*. On the one hand, formation of a polycopy sequence of ITS1 with SNP 189.R could have been a result of hybridization between individuals possessing the first and sixth variants of ribotypes H4-ITS1. Furthermore, SNP ITS2 35.Y could be inherited solely from the representative with the second variant of the first helix of ITS2, while among narrow-leaved fescue species there are no known representatives with ITS1 35.C allele, though a number of species possess a 35.T allele. On the other hand, hexaploidy of *F. arietina* suggests hybridization of diploid gametes with tetraploid ones with subsequent stabilization of a triploid zygote by means of genome duplication.

The sequence of ITS1-5.8S-ITS2 of allele *F. arietina* ITS1 198.A in the case of 100% overlapping shares 100% similarity with *F. polesica* AF171131 (Bp2, SU). According to the literature data, this species is represented by diploids (Tveretinova, 1977; Böcher, 1974; Markgraf-Dannenberg, 1980; Pawlus, 1983; Šmarda et al., 2007). Earlier data suggesting that in *F.polesica* 2n=28 (Tzvelev, 1976) are most likely mistaken.

Thus, *F. polesica* is one of the possible parental forms for allopolyploid *F. arietina*.

However, allele *F. arietina* ITS1 198.G in the case of 100% overlapping is identical to three sequences from different polyploid species belonging to *F. valesiaca* agg.: *F. rupicola* AJ508379 (Vr2, HU, 6x), *F. dalmatica* AJ508378 (Vd1, HU), *F. valesiaca* AJ508377 (Va4, HU, 4x). Among these three species *F. rupicola* does not qualify as the second parental species of *F. arietina* completely, as it is hexaploid. The two remaining species, *F. dalmatica* and tetraploid (probably of autopolyploid origin) *F. valesiaca*, are more suitable candidates for the role of the second parental form of *F. arietina*.

Currently, the question about the second parental species of *F. arietina* remains open. Over 10 species of the group *F. valesiaca* agg. are known in the flora of Ukraine. About as many other «sulcata type» species are found in the flora of Western and Eastern (Russia) Europe (Tzvelev, 1976; Markgraf-Dannenberg, 1980). This group of species is one of the most numerous, yet at the same time, the least studied among all narrow-leaved fescues. The lack of data about the sequences of ITS1-5.8S-ITS2 for the absolute majority of the species of *F. valesiaca* agg., as well as lack of reliable data on their chromosomal numbers make it impossible to unambiguously define the second parental species. To summarize, it can be affirmed, that the hypothesis about hybridogenous origin of *F. arietina* as a result of hybridization of diploid *F. polesica* (*F. beckeri* agg.) with tetraploid representative of *F. valesiaca* agg. is strongly supported by the molecular data.

Assumptions that *F. arietina* may be of a hybrid origin have been previously voiced in the literature, where *F. polesica* was considered a possible parental species (Tzvelev, 1976; Tveretinova, 1977). The original results of both anatomical-morphological and molecular-genetic analysis, as well as the data on the number of the chromosomes, on the one hand, prove separation of *F. arietina* as an independent species; on the other hand, they prove its hybridogenous origin. In particular, a diagnostic morphotype of *F. arietina* and *F. polesica* are similar by the presence of a sclerenchyma ring, flat ribs and intense indumentum on an adaxial leaf surface.

Festuca arietina. Specimina visa.
Ukraine. Kharkiv region. Zmiiv district: Biological Station neighborhood. Pinery, site No. 28 (Kindjak 1941 KW ISOTYPUS, 2 sheets; LE TYPUS); between Zmiiv and Merefa, sandy terrace of the Mza River (Tzvelev 1964 LE; Tzvelev 1982 LE); v. Skrypai (Klokov 1976 KW). Dergachi district, Dergachi city [?] (Lavrenko 1912 KW); Zadonetske village, NNP "Gomilshanski forests", flood plain of the Siversky Donets river (Bednarska, Popov 2010 LWKS IB-1429). Balakliia district: southern outskirts of Balakliia city (Bednarska, Popov 2010 LWKS IB-1430); Vovchansk district: Vovchansk city, in a pine forest (Bednarska, Popov 2010 LWKS IB-1439). **Kyiv region.** The southeastern part of Kyiv city (former village Bortnyczi), dry meadow on the edge of a pine forest (Bednarska, Shyjan, Goncharenko, Levanets 2002 LWKS IB-62, IB-125); Kyiv city, the area between the Desenka and Dnieper rivers (Bednarska, Melnik 2009 LWKS IB-1451); Kyiv city, historic district "Obolon", flood plain of the Dnieper river (Bednarska, Melnik 2009 LWKS IB-1454). **Cherkasy region.** Kaniv district: v. Liplyave, flood plain of the Dnieper river (Bednarska 2009 LWKS IB-1455; Bednarska, Kostikov 2014 LWKS IB-1668); Liplyave neighborhood, "Maria's Mountain", step grassland (Bednarska 2009 LWKS IB-1460); Liplyave neighborhood, "Scythian Settlement", dry grassland on the slopes (Bednarska 2009 LWKS IB-1459); Liplyave neighborhood, "Hrushky", psammophyte meadow (Bednarska 2009 LWKS IB-1461).

Belarus. Gomel region. Chachersk district: v. Zagorie, valley of the river Sozh (Skuratovich, Morozova 2004 MSK); v. Podluzhje, valley of the river Sozh (Skuratovich, Morozova, 2004 MSK). Dobrush district: Dobrush city (Kozlovska, Simonovich, Bulat 1977 MSK). Kalinkavichy district: v. Grjada, left bank of the Pripyat River (Tretjakov 1998 MSK); v. Juravichi (Savich 1927 MSK; Dodoleva 1949 MSK; Vynaev, Tretjakov 1978 MSK; Dubovik 1999 MSK; Skuratovich 2000 MSK; Dubovik 2008 MSK; Bednarska 2010 LWKS IB-1506, IB-1507); v. Ignato-Fabianovka (Dubovik 1999 MSK). Khoiniki district: v. Krasnoselje (Dubovik 1999 MSK); v. Orevichi, left bank of the Pripyat river Dubovik 1999 MSK); v. Tulgovichi, left bank of the Pripyat river (Burtys, Busko 1981 MSK). Mazyr district: Mazyr city, (Vynaev 1988 MSK); v. Pkhov (Tretjakov, Vynaev 1976 MSK); v. Skrygalow, floodplain of the Pripyat river (Skuratovich, Morozova, Kostenevich 1998 MSK; Bednarska 2010 LWKS IB-1510); v. Strelsk, left bank of the Pripyat river (Kozlovska, Blazevich 1973 MSK; Dubovik 1998 MSK); v. Akulinka, left bank of the Pripyat river (Dubovik 1998 MSK); v. Niznij Mlynok (Dubovik 1998 MSK). Narowlya district: v. Dernovichi, floodplain of the Pripyat river (Kruganova, Burtys, Petrunchuk 1968 MSK); v. Vepry (Tretjakov 1986 MSK). Pietrykaw district, railroad station Ptich, railway embankment (Dubovik, Skuratovich 2005 MSK). Vietka district: v. Navasiolki, right bank of the Sozh river (Tretjakov 2001 MSK; Bednarska 2010 IB-1444);

Adnapolle village, barren dry meadows on sandy loam soil, valley of the Sozh river (Bednarska 2010 IB-1445). **Minsk region.** Minsk district: Minsk city (Tretjakov 1987 MSK). **Mogilev region.** Asipovichy district: Asipovichy city, railway embankment (Tretjakov 1994 MSK). Babruysk district: v. Stupeni (Dubovik 1996 MSK). Slawharad district, v. Kremianka, left bank of the Sozh river (Dubovik 1995 MSK). **Brest region.** Brest district: Brest city, railway embankment (Tretjakov 1997 MSK). Byaroza district: railroad station Bronnaja Gora (Tretjakov 2002 MSK).

Lithuania. Varena district municipality, 5 km south-east of Merkine town, valley of the Merkys river, psammophyte meadow (Bednarska, Stukonis 2006 LWKS IB-230; Bednarska, Stukonis 2016 LWKS IB-1734).

Distribution of *F. arietina* is shown in Supplementary Fig. S1.

CONCLUSIONS

The analysis of species variability and population differentiation in different parts of the natural habitat were conducted on the basis of studying 11 morphological and 10 anatomical characters in 14 *F. arietina* populations. The most important diagnostic features of this taxon are green, non-pruinose, scabrid leaves 0.55–0.65 mm in diameter, sclerenchyma strands which are well developed in broad bands or forming an interrupted or continuous, unevenly thickened ring; 7 veins and flat ribs are often present. According to the results of the revision of the herbarium collections, the species distribution was determined in the north and north-east of Ukraine, the south of Belarus and Lithuania. Ecologically, this species tends to coincide with psammophyte communities in river bottomlands and terraces.

It was found that *F. arietina* is hexaploid, which – by the sequence of ITS1-5.8S-ITS2 of the cluster of nuclear ribosomal genes – differs from all the other allied species of narrow-leaved fescues. The principal distinctive character is the presence of SNP in site 198 of ITS1 (198.R) and in site 35 of ITS2 (35.Y). It was shown that *F. arietina* demonstrates characters of the species of allopolyploid origin, that probably arose as a result of hybridization of diploid *F. polesica* with tetraploid representative of *F. valesiaca* group.

Molecular data, that indicate possible hybridogenic origin of *F. arietina*, conform well with anatomical and morphological data. This explains the existence in the populations of background morphotype specimens with isolated sclerenchyma strands, typical of the species of *F. valesiaca* agg., and of diagnostic morphotype with a continuous ring of sclerenchyma and flat ribs, which is similar to *F. polesica*, as well as the ecological confinement of *F. arietina* to psammophyte biotopes.

The examined complex of anatomical, morphological and molecular-genetic markers argues that *F. arietina* is a separate independent species, which is clearly isolated from other related taxa of *F. valesiaca* group.

AUTHOR'S CONTRIBUTIONS

IB – original idea, data collection, anatomical and morphological analyses, statistical analysis, chorological analysis, writing and editing the manuscript; IK – analysis of molecular data, interpretation of data concerning ITS1 and ITS2, drafting of manuscript; AT – DNA extraction, amplification, analyses of molecular data; VS – data collection, cytological analysis. The authors declare that they have no conflicts of interests.

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