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Phlox sibirica L. in South Siberia

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The paper gives the variation data related to some quantitative characters and anatomy of *Phlox sibirica* L. leaf blade. The most stable characteristics of *Phlox sibirica* leaf anatomy are the leaf thickness around the midrib, the length of upper mesophyll layer cells, the number of epidermal cells, and the stoma sizes, which, along with the peculiarities of mesophyll structure, conducting bundles and stomatal type, can serve as additional diagnostic characters for species identification. *Phlox sibirica* is classified as xerophyte-heliophyte morphological type.

Key words: *Phlox sibirica*, leaf blade anatomy, mesophyll, parenchyma, stomatal index.

Conservation of genetic diversity of natural vegetation is a top priority for current advanced research.

Phlox sibirica L. (Polemoniaceae) is the only representative of the North-American *Phlox* L. genus on the territory of Russia. The species is of apparent academic interest as a relic of the Pliocene steppe complex[1]. Some authors refer *Phlox sibirica* to glacial relicts, Late Pleistocene in age[2]. *Phlox sibirica* is included in the regional Red Data Books of the Urals, Siberia and the Far East.

In order to preserve the species in nature and culture, we need to investigate its biological properties and assess its adaptive potential. The data obtained can be used to develop recommendations for species introduction.

The goal of this research was to study the anatomical properties and population variability of the leaf blade of a valuable decorative plant, *Phlox sibirica*.

MATERIALS AND METHODS

Phlox sibirica L. (Polemoniaceae) was described in 1750 by Carl Linnaeus. He used the materials of the Second Expedition to Kamchatka (1733-1741) by Saint Petersburg Academy of Sciences presented to him by Grigoriy Demidov for identification[3].

According to Grant's[4] genus system, *Phlox sibirica* L. belongs to section *Pulvinatae*, subsection *Sibiricae*.

The areal of *Phlox sibirica* covers the Urals, Cis-Urals (Bashkortostan), mountainous and piedmont areas of South Siberia (Altai, Kemerovo Region, the Republics of Tyva and Khakassia, southern part of Krasnoyarsk Region, Transbaikal Siberia), Yakutia, Mongolia, and northern regions of the Far East (Chukotka Autonomous Region, Magadan Region). The areal is disjunctive and the populations are isolated. According to Krylov[5], a number of American authors and some floristic reviews, *Phlox sibirica* is also common in North

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America and Alaska. E. Hultén acknowledged two races: *Phlox sibirica subsp. sibirica* (including *Phlox alaskensis*) and *Phlox sibirica subsp. richardsonii*. E.T. Wherry pointed out three species: *Phlox sibirica*, *Phlox borealis*, and *Phlox richardsonii* with two subspecies: *Phlox sibirica subsp. alaskensis* and *Phlox sibirica subsp. richardsonii*. W.J. Cody reverted to three species for north-western North America: *Phlox alaskensis*, *Phlox hoodii*, and *Phlox richardsonii*. J.H. Locklear viewed Beringian plants as three allopatric subspecies of *Phlox richardsonii* (*subsp. borealis*, *subsp. alaskensis*, *subsp. richardsonii*). Locklear mentions *Phlox sibirica* for Eurasia only; on the territory of Alaska and Yukon, a related species, *Phlox hoodii* Richardson, can be found in relict island steppes with two subspecies described for it[6].

The common ancestor to the *Phlox sibirica* complex of related species growing in Alaska and adapted to the cold climate conditions must have spread along the Bering land bridge onto the territory of Chukotka and descended from the ranges of Baikal Siberia onto the territory of the South Siberian mountains and then, later, to the Urals and Cis-Urals. There was no mass migration of the *Phlox* genus to the territory of the Far East and Siberia, because most species of the genus are spread further south, between the 30th and 50th parallels of North America under relatively mild climate conditions. Furthermore, migrations from Asia to North America prevailed over backward migrations in the Late Pliocene, since the North Asian flora transformation process outran the similar transformations of the North-American flora[7]. Subsequently, Asian and North-American races evolved separately, which led to the occurrence of a group of related taxa. As the species evolved, the anatomical and morphological properties of its vegetative organs were formed as part of habitat-specific adaptation.

Phlox sibirica is a petrophyte and calciphilous xerophyte. The species is a constituent of a floristic complex of rock outcrops, which, according to Barysheva[8], were a migratory corridor on the one hand and, on the other hand, a refugium for relict species in the event of climatic changes.

Currently, *Phlox sibirica* is represented by a number of isolated populations. It grows at foothills and in the mountains, predominantly at medium altitudes, on rock outcrops (limestones, sandstones, and basalts), rocky and rubble hills of various orientations in river valleys, in rocky steppes, on steppe meadows, as well as in larch and pine forests.

The plant samples for the research of leaf blade anatomy were gathered in the Republic of Khakassia, in the suburbs of Yefremkino Village, in the valley of Bely Iyus River.

Temporary leaf samples were prepared by cutting leaves on the MZ-2 freezing microtome. The slice thickness was set at 60-90 µm. Crossing cuts were performed in the central leaf portion. The epidermis was cut by an open razor in the middle third of the leaf blade between the margin and midrib. The sections were performed in quintuplicate on the leaves sampled from five sprouts; at least 25 sections were analyzed for each sample. Stomatal index was calculated by Kästner's formula[9].

The micropreparations of leaves and fresh pollen were photographed and measured by Carl Zeiss Axio Lab A1 light microscope with an AxioCam ERc 5s digital camera connected to a computer, by means of Axio Vision 4.8 software.

Morphological parameters of a leaf as well as generative characteristics were measured by means of MBS-10 stereoscoping binocular microscope with the measuring scale on the eyepiece in 80-fold replication.

The measuring results were processed using Statistica 8.0 software. The following parameters were determined: " (Mean): arithmetical mean; m (SE): arithmetical mean error; CV: coefficient of variation, %.

RESULTS AND DISCUSSION

Phlox sibirica is a perennial polycarpic rhizomatous bunchgrass growing in the suburbs of Yefremkino Village in rocky herb-sedge-grass steppes together with *Carex pediformis* C.A. Mey, *Carex duriuscula* C.A. Mey, *Aster alpinus* L., *Veronica incana* L., *Potentilla acaulis* L., species of genus *Festuca* L., *Pulsatilla flavescens* (Zuss.) Turch., *Dianthus versicolor* Fisch. ex Link et. al. Forms compact clones. The total grass projective cover is 30-40%; rubble 7-10%, bedrock yield 20-25%, total projective cover of the species is 1-2%.

The population is normal, with predominant generative juvenile, middle-aged and mature individuals. Subsenile and virginile plants have also been detected. The plant density within the population is on average 18.3 per 1 sq. m. It blossoms in May-June, with the second blossoming possible in July-August.

Table 1. Variation of some morphometric parameters of *Phlox sibirica*

Morphometric parameters, M ± m	Populations* 1.	2.	3.	4.
Plant height, cm	6.82 ± 0.16	6.53 ± 0.15	4.9 ± 0.11	12.3 ± 0.37
CV, %	22.3	19.7	20.2	21.5
Leaf length, cm	2.85 ± 0.07	2.5 ± 0.05	2.18 ± 0.05	4.3 ± 0.08
CV, %	15.7	12.7	20.6	23.3
Leaf width, mm	1.78 ± 0.03	1.62 ± 0.03	1.35 ± 0.02	2.69 ± 0.05
CV, %	14.2	13.9	23.6	21.3
Corolla diameter, mm	1.91 ± 0.03	1.85 ± 0.03	1.90 ± 0.04	2.1 ± 0.06
CV, %	9.5	12.2	10.8	11.1
Calyx length, cm	9.5 ± 0.02	9.48 ± 0.03	8.1 ± 0.02	1.08 ± 0.02
CV, %	10.2	11.9	9.5	10.3
Number of flowers on a sprout	1.6 ± 0.02	1.97 ± 0.02	1.75 ± 0.03	1.8 ± 0.04
CV, %	29.4	31.0	32.1	35.2

* 1. Khakassia, suburbs of Yefremkino Village, valley of Bely Iyus River, rocky steppe; 2. Khakassia, valley of Bely Iyus River, fescue steppe; 3. Altai, suburbs of Ust-Kan Village, steppe meadow; 4. Krasnoyarsk Region, valley of Ubey River, forest-covered slope.

Table 2. Anatomical properties of *Phlox sibirica* leaf blades

	Cross section area, sq. µm	Leaf thickness, µm		Dimensions of upper mesophyll layer cells, µm		Number of mesophyll layers	
		Midrib	Leaf blade	Length	Width	Pali-sade	Spo-ngy
M ± m	9628.87 ± 417.15	408.32 ± 2.98	454.33 ± 4.93	54.00 ± 1.02	29.03 ± 0.54	2-3	4-5
CV, %	21.7	3.6	5.4	9.4	9.3	-	-

Table 3. Quantitative aspects of *Phlox sibirica* epidermal cells

	Number of epidermal cells per 1 mm^2		Number of stomata per 1 mm^2	
	Upper epidermis	Lower epidermis	Upper epidermis	Lower epidermis
M ± m	234.24 ± 6.05	202.24 ± 4.96	62.72 ± 5.54	66.24 ± 2.59
CV, %	12.9	12.3	44.1	19.6

Table 4. Stoma sizes and stomatal index of *Phlox sibirica*

	Upper epidermis, µm			Lower epidermis, µm		
	Length	Width	Stomatal index, %	Length	Width	Stomatal index, %
M ± m	51.71 ± 1.41	33.86 ± 0.50	20.47 ± 1.35	51.13 ± 0.85	30.40 ± 0.70	24.79 ± 0.98
CV, %	13.6	7.4	32.9	8.4	11.5	19.8

By comparing the key morphometric parameters of two Khakass, one Altai and one Yenisey-Chulym population, we discovered that the sprout height and leaf dimensions were characterized by medium and high character variability (Table 1). The characteristics of intrapopulation flower variation (calyx length and diameter) are more stable. The most variable parameters are those of the number of flowers on a sprout. Quantitative characteristics of vegetative organs, particularly leaf blade as the most flexible organ, depend on the environmental growth conditions: the parameters reach their detected maximum under humid temperate conditions (forest steppe) and their minimum, under insufficiently humid conditions as an adaptive reaction to the moisture saving mode.

Leaves are sessile, small, 2.0-5.5 cm long, 0.9-1.5 cm thick, entire, linear, acute. There are multicellular trichomes along the margin and midrib on the abaxial side of the leaf blade.

The leaf mesophyll is dorsoventral, but the lower layer of the spongy mesophyll forms a semblance of a palisade one, as a result, the mesophyll resembles an isolateral one. The lower layer cells are authentically smaller than those of the upper mesophyll layer. The leaf mesophyll has 6 to 8 cell layers regardless of the leaf thickness, with 1.7-2.0 times more spongy mesophyll layers than palisade mesophyll layers (Figure 1, Table 2). The number of palisade parenchyma layers in *Phlox sibirica* increases up to 3 as compared to 1-2 layers in the mesophyll species of the genus[10].

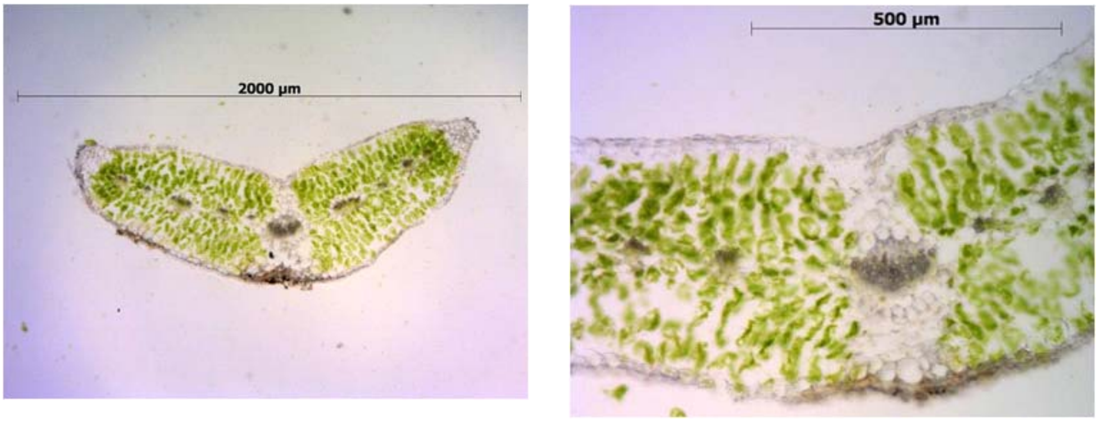

Fig. 1. Transversal cross sections of *Phlox sibirica* leaves

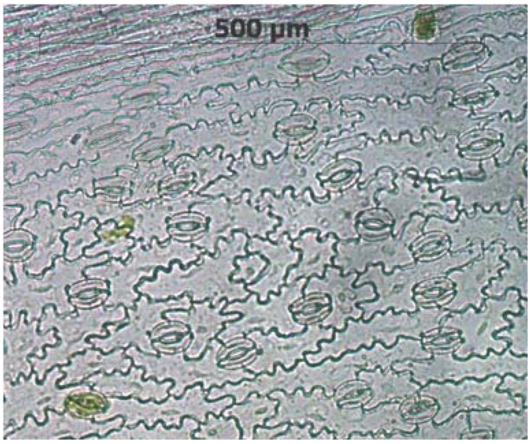

Fig. 2. Epidermis from upper side in surface view

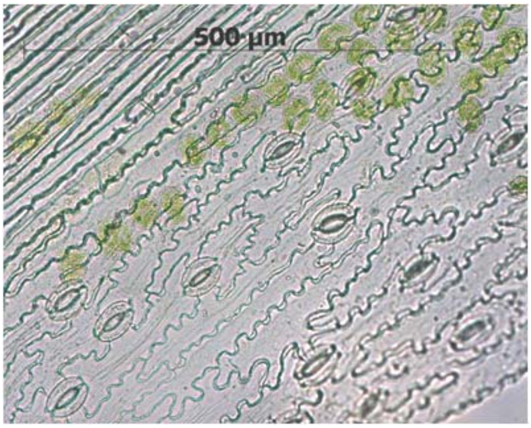

Fig. 3. Epidermis from the lower side in surface view

Collenchyma strands are located along the midrib as well as along the leaf margins. Altogether they form stiffening ribs, which prevent the leaf from crumpling with the loss of turgor.

Phlox sibirica is characterized by closed collateral bundles. The *Phlox sibirica* leaf thickness is smaller around the midrib than in the central part of the leaf between the midrib and the margin, which is due to poorly developed conducting bundle and well developed chlorenchyma.

Species with better developed xylem phloem (ratio of xylem cross-section area to phloem for *Phlox sibirica* is 1.47 ± 0.07, CV = 22.8%) are better adapted to intensive transpiration and passage of more water and, therefore, capable of growing under arid conditions (Table 2).

Phlox sibirica is characterized by a anomocytic stomatal type also attributed to other species of the genus[10]. Leaves of *Phlox sibirica* (Figures 2, 3) are amphistomatous (Table 3).

The stoma sizes on the upper and lower epidermis have no statistically significant differences, their mean values being practically equal. The guide cells have oval shape with the length being more than 1.55 times larger than their width (Table 4). Stoma sizes within the species vary insignificantly (the coefficient of variation is low and does not exceed 13.6%). This index is stable and can be used as an additional character for identification of species. *Phlox sibirica* is notable for large stomata 40.18-62.76 µm long, 26.74-37.17 µm wide.

Stomata take up the whole space between the midrib and the leaf margin and are oriented predominantly parallel. Their deviation angle from the leaf axis does not exceed 30°.

The characteristics reviewed before reflect the adaptation of the species to adverse water conditions and provide fluid balance maintenance.

The side walls of epidermal cells are wavy, and the cells are amoeboid (Figures 2, 3). Epidermal cells are small, axially elongated, with a large number of curves (heliophyte characters) of variable amplitude and incidence. As the species evolved, the anatomical and morphological properties of its vegetative organs were formed as part of habitat-specific adaptation. Thus, xerophytization has been confirmed as the main direction of *Phlox sibirica* evolution.

CONCLUSION

Phlox sibirica is a perennial polycarpic rhizomatous bunchgrass, which forms compact clones. Leaf blades of *Phlox sibirica* are linear, with multicellular trichomes along the margin and midrib on the abaxial side. The dimensions vary significantly depending on the growth conditions. The most stable characteristics of *Phlox sibirica* leaf anatomy are the leaf thickness around the midrib, the length of upper mesophyll layer cells, the number of epidermal cells, and the stoma sizes, which, along with the peculiarities of mesophyll structure, conducting bundles and stomatal type, can serve as additional diagnostic characters for species identification. The research of morphological and anatomical properties of *Phlox sibirica* leaf blade have confirmed the xerophyte-heliophyte nature of the species. The data obtained make it possible to predict the successful introduction of the species in the steppe and forest steppe regions of Siberia.

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