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IDENTIFICATION OF *Populus nigra*, *P. laurifolia* and *P.* × *jrtyschensis* BY LEAF PETIOLE ANATOMY

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Remarkable variability in morphology of the *Populus* genus, as well as widely spread hybridization within the genus complicate taxa identification. Petiole anatomy plays an important role in plant taxonomic attribution and resolution of arguments in systematics. The aim of the study was to examine the petiole anatomy of *P. nigra*, *P. laurifolia* and *P.* × *jrtyschensis* to assess its potential use for taxa identification. The leaf petioles were collected from 252 individual trees in 8 populations of *P. nigra*, *P. laurifolia* and *P.* × *jrtyschensis* in the area of their natural hybridization in the basin of the Tom River in Kemerovo Oblast. Two of those 8 populations, located far from the hybridization centers, were regarded as controls to characterize parent taxa. Cross sections were made in the upper part of petioles. Then the sections were used to examine the shape of petiole cross sections, the contour of the adaxial sides, the vascular system types and lower ring shape. The petioles of trees growing far from hybridization areas were found to differ in their vascular systems: *P. nigra* had a linear, while *P. laurifolia* a high-arc-shaped one. The hybrids were found to belong to several types of vascular systems, namely intermediate, high-intermediate, linear and high-arc-shaped ones. The intermediate type of vascular system is characteristic of the most part of *P.* × *jrtyschensis* trees (78 % of the total samplings). Therefore most of them can be classified as F_1 hybrid and others – as a recombinant species. Examination of *P. nigra* in its natural hybridization center allowed finding an approach to the identification back-crosses. The study confirmed provides a solid basis for the identification of parent species, hybrids and backcrosses in natural hybridization areas of taxa belonging to *Aigeiros* and *Tacamahaca* sections of *Populus* genus.

Keywords: *poplar, differentiation, natural hybridization, backcrosses, basin of Tom River, Kemerovo Oblast, Russia.*

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INTRODUCTION

The main characteristics used for differentiation of *Populus* species are the following: leaf blade morphology, petiole traits, some characteristics of flowers and fruits (Bolshakov, 1992; Eckenwalder, 2010; Koropachinskiy, 2016). However, large morphological variability of leaves makes taxa identification difficult (Bakulin, 2007), and the use of generative organs, although more reliable, is limited to a short period of flowering and fruiting. The widespread hybridization in the *Populus* genus also complicates taxa identification. A few anatomical studies of representatives of the family Salicaceae Mirb. showed the potential of leaf anatomy in solving their complex taxonomic attribution issues (Pautov, 2002; Skvortsov, Belyanina, 2005; Thadeo et al., 2014; Kalouti et al., 2015; Čortan et al., 2017).

The petiole anatomy is of great importance for plant identification and resolution in disputes of systematics. Plant anatomy and topography are stable characteristics that do not depend on environmental conditions, heterophylly, stratification, age and leaf shape (Shabes et al., 2007; Gavrilenko, Novozhilova, 2015). An important feature of petiole anatomy is the specificity of species (Kurkin et al., 2014*a*, *b*), which makes it possible to use it as a reliable criterion for taxa discrimination (Coutinho et al., 2013; Gavrilenko, Novozhilova, 2015, 2017). In addition, anatomy features can also help in identifying species even without generative organs and thus are used for certification and quality control of medicinal plants (Teixeira et al., 2005; Kurkin et al., 2014*a*, *b*, 2015).

In the south of West Siberia the major floodplain forests belong to *Populus nigra* L. (section *Aigeiros* Lunell) and *P. laurifolia* Ledeb. (section *Tacamahaca* Mill.). *P. nigra* is a floodplain species, does not grow at high altitudes in the mountains, preferring wet sandy, sandy loamy and silty soils. It grows along the rivers of West Siberia, the Altai-Sayan region and the southwestern part of Central Siberia (Bakulin, 2007). *P. laurifolia* is a mountain-valley species, preferring gravel-boulder alluvial deposits. Its area embraces the Altai-Sayan mountainous country and the south of Central Siberia. In places of their joint growth, natural hybridization occurs with the resultant hybrid taxon *Populus* × × *jrtyschensis* Chang Y. Yang (Proshkin, Klimov, 2017*a*, *b*). Studies of the *P.* × *jrtyschensis* morphology, a natural hybrid between two distantly related species, showed that in its crown branch differentiation and generative bud location it mostly resembles *P. laurifolia* (Proshkin, Klimov, 2017*b*). At the same time, the morphological features of the *P.* × *jrtyschensis* leaf blades are usually intermediate, which often makes it difficult to make taxonomic attribution.

The objective of this study was to investigate the petiole anatomy of *P. nigra*, *P. laurifolia* and *P.* × *jrtyschensis* for assessing its potential for taxa identification.

MATERIALS AND METHODS

The study was carried out in the basin of the Tom River on the territory of the Kemerovo Oblast. The Tom River is the right tributary of the Ob River, with its mouth located at N 56°50′00″, E 84°29′20″ and 68 m above sea level (a. s. l.). The Tom River starts in the south of the Kuznetsk Alatau Mountains where the latter join the Abakan Ridge at N 53°39′05″, E 89°45′50″ and 903 m a. s. l. The river basin is located at the boundary between the West Siberian Lowland and the Altai-Sayan Mountains in the temperate zone. The black poplar *P. nigra* is the most common poplar species in the area, growing in big stands in the floodplains. The area occupied by *P. laurifolia* is quite disjuncted, the populations being concentrated in the montane sites upstream the Tom River and its right tributaries. The area of combined occurrence of *P. nigra* и *P. laurifolia* embraces the upstream low-montane areas and downstream areas of the major mountain tributaries. In the Tom River basin *P.* × *jrtyschensis* grows usually in mixed stands with its parent species. In some areas the hybrid trees are quite common and numerous, thus marking the hybridization sites.

The leaf petioles were collected from 8 populations of *P. nigra*, *P. laurifolia* and *P.* × *jrtyschensis* in the Tom River basin (Table 1). Two of those 8 populations were used as controls to characterize parent taxa as they were located far from the hybridization centers.

Since our earlier study revealed no variability of anatomic traits at the endogenic level from reproductively mature trees, we collected only one leaf from a short branch mid-crown of each sampled tree (Proshkin, Klimov, 2017*b*).

Dried petioles were separated from the leaf blade and fixed in a mixture of ethyl alcohol 96 %, glycerol and water at the ratio of 1 : 1 : 1. The material was allowed to stay in the mixture for three to four days (Klimov, Proshkin, 2018*a*). Petiole morphology was studied by a comparative morphological method using a stereoscopic microscope

Table 1. Poplar populations studied

Study sites	Geographical coordinates	Number of trees/petioles		
		P. nigra	*P. laurifolia*	*P.* × *jrtyschensis*
SRES	N 53°47′35.52″, E 87°37′17.40″	30/30	30/30	31/31
Karlyk	N 53°49′21.00″, E 87°28′03.00″	30/30	30/30	41/41
Slavino	N 54°02′05.00″, E 87°22′55.00″	30/30	–	–
Srednyaya Maganakova	N 54°19′33.00″, E 87°58′57.00″	–	30/30	–
	Total:	90/90	90/90	72/72

MBS–10 (LZOS, Russia) with a magnification of 16.3×.

Preparation of cross sections for morphological analysis was performed according to the widely used botanical microtechnique (Barykina et al., 2004). The cross sections were made in the upper part of the petiole (at the base of the leaf blade), since its characteristics are used as the keys of the qualifier for the identification of *P. nigra* and *P. laurifolia* (Bolshakov, 1992; Tsvelev, 2000). In addition, the integration of the vascular bundles is observed in the upper part of the petiole, though they are more or less disintegrated in others parts (Kindyakova, Shamrov, 1976). The resulting sections were stained with 2 % aqueous safranin. To reveal the anatomical features with Micromed-1 microscope (Observation Devices Ltd., Russian) a magnification of 100× and 200× was used. A total of 252 microscopic slides were examined.

We measured on each slide with the help of the «Axio Vision 4.8.2» software (ZEISS, 2018) the length (H, μm) of the cross section and the distance between the widest part of the section and its base (B, μm). To discriminate between the cross section shapes, the following gradations of B/H were used: < 0.25 (triangular); 0.25–0.35 (ovate-triangular); 0.35–0.45 (ovoid); 0.45–0.65 (elliptical); and > 0.65 (obovate) (Klimov, Proshkin, 2018*b*).

Since the examined characteristics of petiole anatomy are qualitative, in order to evaluate their variation over the studied territory, anatomy structure types were identified and their frequency was determined using an unbiased estimation (Zhivotovskiy, 1991):

$$p = \frac{n}{N}, \quad (1)$$

where n is the number of cases of detection of the types being investigated in the sample and N is the total sample size.

The statistical error was determined using formula (Zhivotovskiy, 1991):

$$S_p = \sqrt{\frac{p(1-p)}{N}}. \quad (2)$$

RESULTS AND DISCUSSION

Location of vascular system components is the main characteristic of petiole anatomy. The vascular system of poplar species of the studied sections is bundle-type, with the closed collateral vascular bundles visualized on cross sections as rings of a rounded or elliptical shape. In this study we use the term «vascular system type» for relative disposition of rings as they are visualized on cross sections, rather than vascular bundles themselves. The rings are located in tiers of 1–2 rings each (Table 2).

Analysis of the petiole anatomy of *P. nigra* showed that the cross section shape was predominantly ovoid, less often elliptical, with adaxial and abaxial sides being rounded (Fig. 1, *A–D*).

Outside a petiole is covered with epidermis consisting of fine densely packed cells, with thin cuticle layer (up to 5 μm). *P. nigra* in the studied region is characterized by the predominance of individuals with bare leaf blades and petioles. Hairy morphotype of *P. nigra* is confined to hybridization centers (Klimov, Proshkin, 2017). The epidermis of the petioles of such trees' contains few unicellular cover trichomes of a subulate shape.

Under the cover tissue along the entire perimeter of the cross section there was a 4–5 cells thick layer of angular-lamellar collenchyma. On the adaxial and abaxial sides an angular collenchyma was found (Fig. 1, *B*, *D*), reinforcing the rings of the vascular system. In *P. nigra* on the lateral and the adaxial sides slit-like intercellular spaces could be seen at the sites where one collenchyma type was substituted with another. On the sides of the petiole under the sub-epidermal collenchyma there were areas of loose chlorenchyma (Fig. 1, *A*). The vascular system in individual trees of the Slavino population which is remote from hybridization centers was strictly linear with 3–4 linearly arranged round rings, the dimensions of which decreased from 550 μm on the abaxial side to 200 μm on the adaxial one. In the upper part of the petiole the closed collateral bundles forming these rings could hardly be distinguished because of the dense packing (Fig. 1, *C*), while on

Table 2. Vascular system types in the petioles of *P. laurifolia*, *P. nigra* and their hybrids

Vascular system type	Characteristics
Linear	3–5 located above each other of circular or elliptical rings
Intermediate	2 linearly arranged elliptic or rounded rings and 2 parallel rounded or elliptic rings on the adaxial side
High-intermediate	3 linearly arranged elliptic or rounded rings and 2 parallel rounded rings on the adaxial side
Arched	1 elliptical ring on the abaxial side and 2–6 rounded rings on the adaxial side

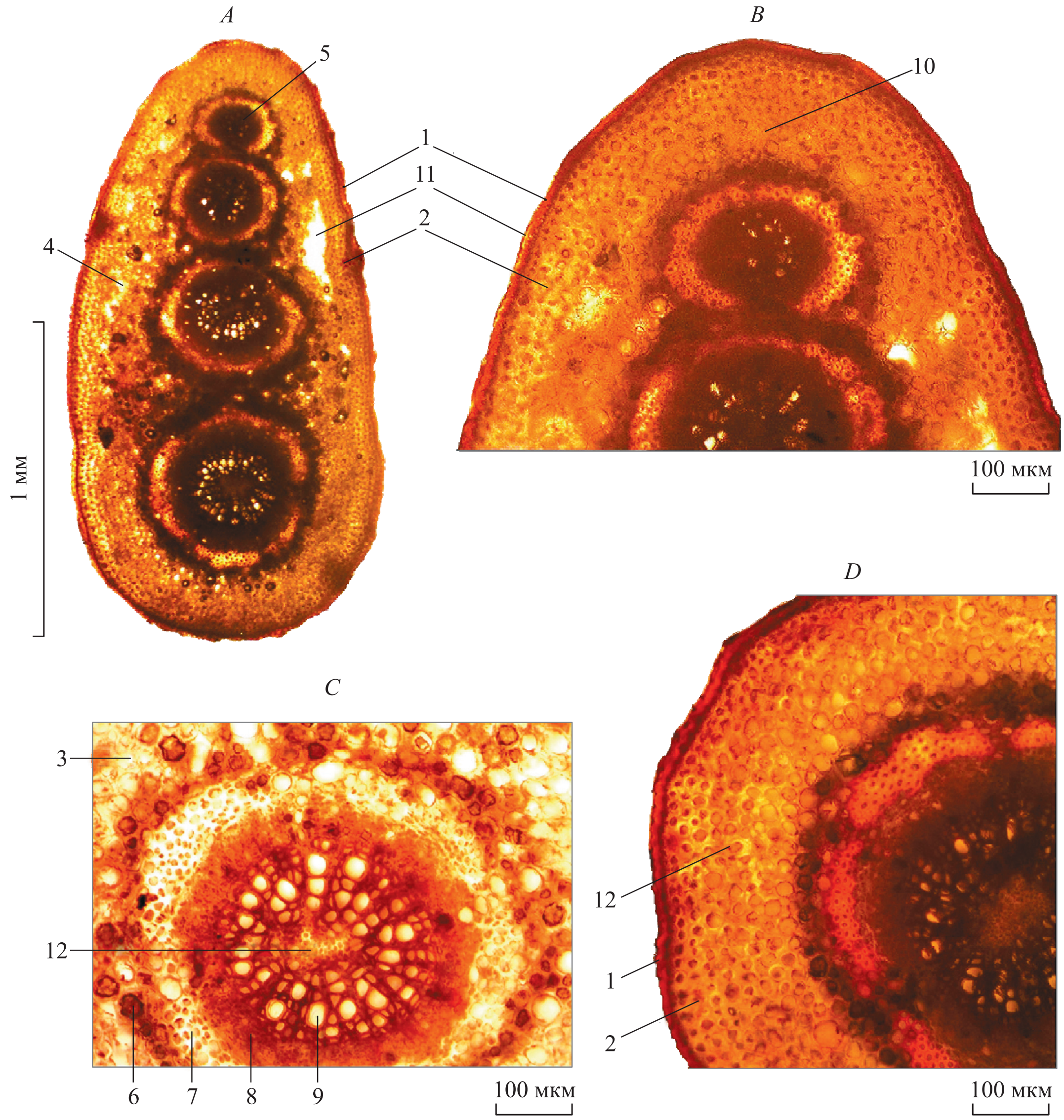

Fig. 1. Fragments of transverse sections of the upper part of *P. nigra* petiole: *A* – general view; *B* – adaxial side; *C* – vascular tissue side. *D* – abaxial side. 1 – epidermis; 2 – angular-lamellar collenchyma; 3 – parenchyma; 4 – chlorenchyma; 5 – vascular ring; 6 – druses of calcium oxalate; 7 – sclerenchyma; 8 – phloem; 9 – xylem; 10 – angular collenchyma; 11 – intercellular spaces; 12 – cordate parenchyma.

the sections of the lower part they were clearly visible (Fig. 2).

The xylem vascular components were radially arranged, the diameter of the vessels ranging from 10 to 30 µm. The phloem cells were small (up to 10 µm). On the periphery the phloem was reinforced with a rather thick layer of sclerenchyma represented by bass fibers. The rings were surrounded by an overlay of big parenchyma cells with a large number of calcium oxalate druses (Fig. 1, *C*).

In the SRES and Karlyk populations of *P. nigra* located in the hybridization centers the tree shoot morphology and leaf shape did not differ from the trees growing outside the hybridization centers. However, they were found to display increased variability of petiole traits (Table 3).

Most of the trees (50.0–76.7 %) had linear type of petiole vascular system, while some showed high-intermediate one (16.7–36.7 %). In the Karlyk population four trees were found belonging to the intermediate type, common for hybrids. Two trees from the SRES population had high-arc-shaped vascular system.

The petiole anatomy of *P. laurifolia* demonstrated different distinctive features. The petiole cross section shape was wide-elliptical, the adaxial side being cordate with a deep groove in the center, with the abaxial side rounded (Fig. 3, *A*–*D*).

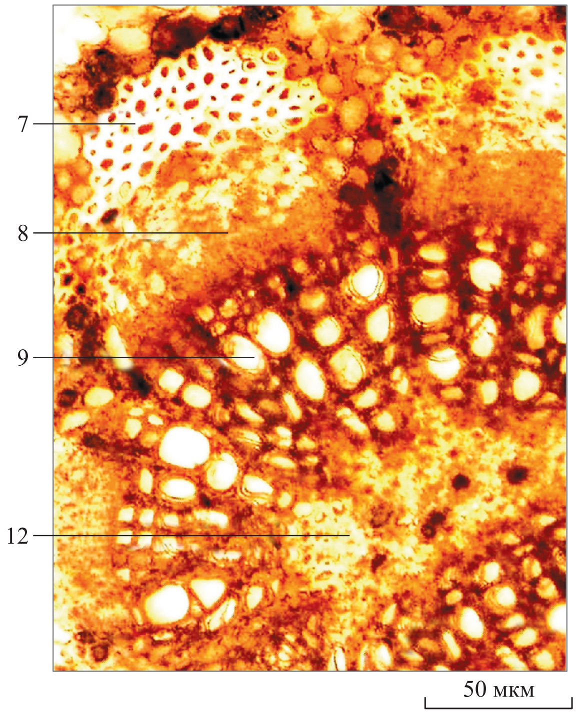

Fig. 2. Closed collateral vascular bundles (cross-section in the lower part of *P. nigra* petiole); for explanations see Fig. 1.

The petiole was covered with an epidermis consisting of densely packed cells stretched to 15 µm. Cuticle thickness was 5–10 µm. Throughout the cross section, there were integumentary unicellular trichomes subulate in shape, 50–130 µm long Along the entire perimeter under the epidermis a layer of angular-lamellar collenchyma of 3–4 cells in thickness was observed. At the adaxial side edges its thickness increased to 6–7 cells (Fig. 3, *B*). The corneal collenchyma was not well expressed and the chlorenchyma was located under the subepidermal layer of the angular-lamellar collenchyma passing from the vascular bundles to the main parenchyma (Fig. 3, *B*, *D*). On the adaxial side its cells were loosely arranged, with large intercellular spaces.

The vascular system in all the studied individuals of all studied populations was represented by rings of closed collateral bundles located as high arcs (Table 4).

On the abaxial side, the vascular bundles were assembled into a large elliptical ring of irregular shape, 800 µm high and 1000 µm wide (Fig. 3, *C*). In its center the heart-shaped parenchyma could be clearly seen. On the adaxial side in the ribs, when a cross-section is cut at a distance of 4–5 mm from the leaf blade, one ring, 300–350 µm in diameter, was located. When the cross section was cut immediately near the leaf blade base, two rings were observed: the upper one was a collateral bundle of 100–140 µm in size, whereas the lower ring (310–350 µm in diameter) consisted of 3–5 bundles.

The location of the xylem vascular components was radial, the diameter of the vessels ranging from 10 to 30 µm. The phloem cells were 10–15 µm. Outside the phloem there was a layer of bass fibers, most of which were lignified. The rings were surrounded by a cover of big and fairly thick-walled parenchyma cells. Calcium oxalate druses in the petioles of the laurel poplar were found not only in the parenchyma around the bundles, but also in the heart-shaped parenchyma and in the chlorenchyma (Fig. 3, *C*). The petiole anatomy of the hybrid taxon *P.* × *jrtyschensis*, as expected, inherited some features from parent species. The petiole cross section shape was oblong-elliptical, the adaxial side truncated, less often slightly sinuate in its outline. The abaxial side was rounded (Fig. 4, *A*–*C*).

Table 3. Petiole anatomy characteristics in the *P. nigra* populations (unbiased estimate of frequency, $p \pm Sp$)

Characteristics		SRES*	Karlyk*	Slavino**
Cross section shape of petioles	Ovate	0.767 ± 0.077	0.567 ± 0.090	0.801 ± 0.072
	Ovate-triangular	0.066 ± 0.045	0.033 ± 0.032	0.066 ± 0.045
	Elliptical	0.167 ± 0.068	0.400 ± 0.089	0.133 ± 0.061
Contour of the adaxial side	Rounded	0.467 ± 0.288	0.500 ± 0.091	0.934 ± 0.045
	Truncated	0.533 ± 0.288	0.500 ± 0.091	0.066 ± 0.045
Vascular system type	Linear	0.767 ± 0.077	0.500 ± 0.091	1.000
	Intermediate	–	0.133 ± 0.061	–
	Arched	0.066 ± 0.045	–	–
	High-intermediate	0.167 ± 0.068	0.367 ± 0.087	–
Lower ring shape	Rounded	0.467 ± 0.288	0.833 ± 0.068	0.934 ± 0.045
	Elliptical	0.533 ± 0.288	0.167 ± 0.068	0.066 ± 0.045

Note. * – Within the hybridization centers, ** – outside the hybridization centers.

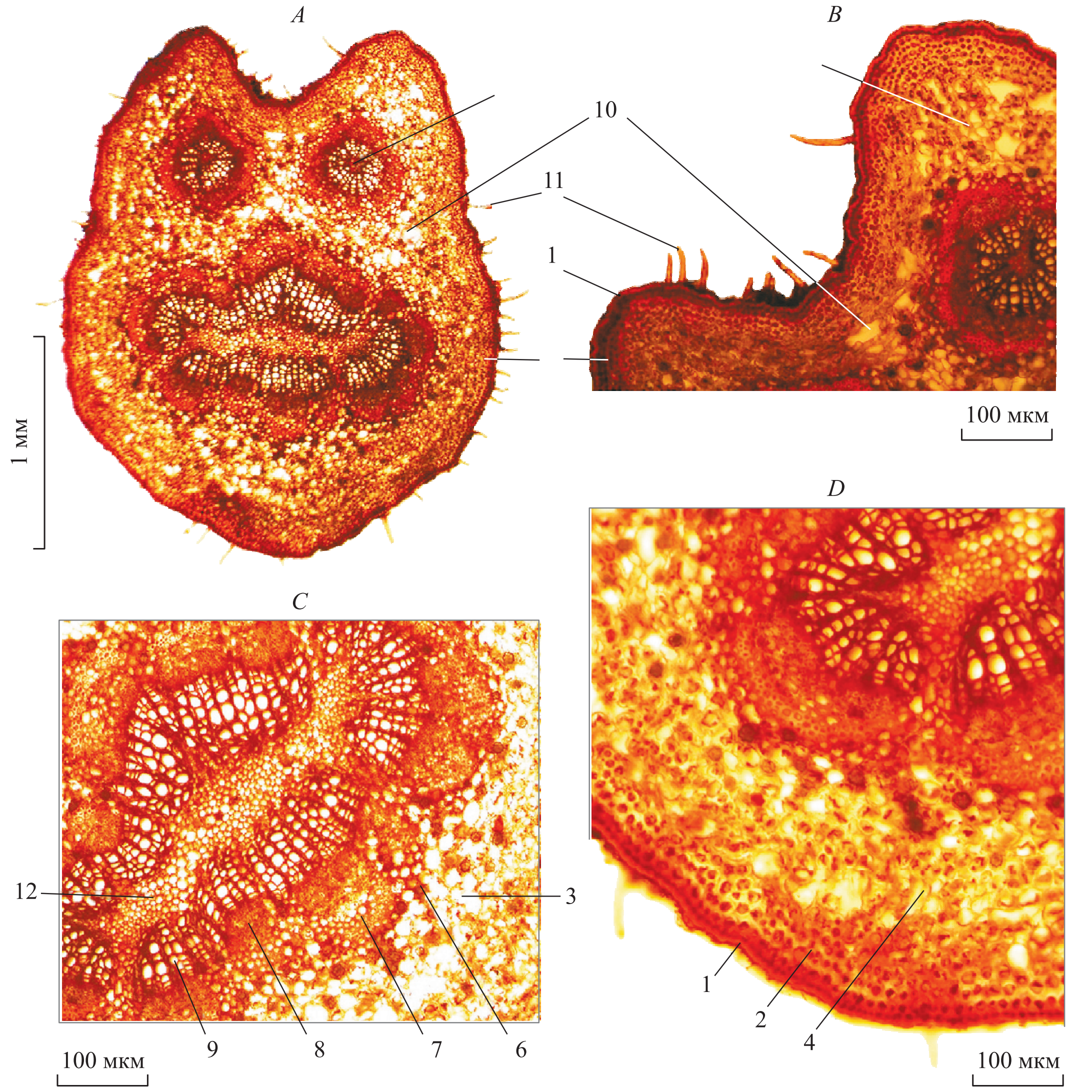

Fig. 3. Fragments of cross-sections of the upper part of the *P. laurifolia* petiole: *A* – general view; *B* – adaxial side; *C* – vascular tissue side; *D* – abaxial side. 1 – epidermis; 2 – angular-lamellar collenchyma; 3 – parenchyma; 4 – chlorenchyma; 5 – vascular ring; 6 – calcium oxalate druses; 7 – sclerenchyma; 8 – phloem; 9 – xylem; 10 – intercellular spaces; 11 – trichomes; 12 – cordate parenchyma.

Table 4. Petiole anatomy characteristics in the *P. laurifolia* populations (unbiased estimate of frequency, $p \pm Sp$)

Characteristics		SRES*	Karlyk*	Srednyaya Maganakova**
Cross-section form of the petiole	Ovate	0.667 ± 0.086	0.633 ± 0.083	0.567 ± 0.090
	Elliptical	0.333 ± 0.086	0.367 ± 0.083	0.433 ± 0.090
Contour of the adaxial side	Auriculate	1.000	1.000	1.000
Form of the vascular system	Arched	1.000	1.000	1.000
Lower ring form	Elliptical	1.000	1.000	1.000

Note. * – Within the hybridization centers, ** – outside the hybridization centers.

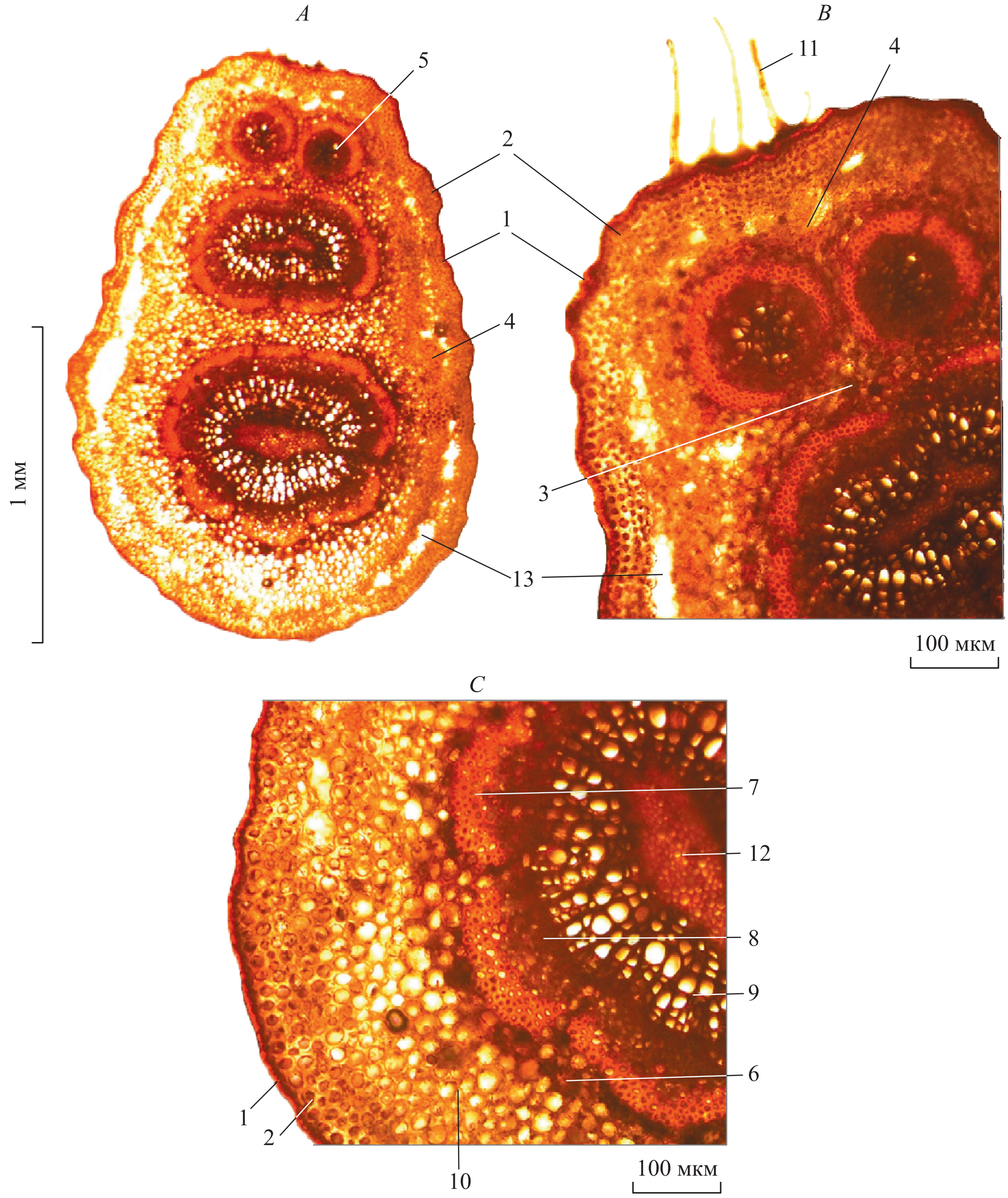

Fig. 4. Fragments of cross-sections of the upper part of the *P. × jrtyschensis* petiole: *A* – general view; *B* – adaxial side; *C* – abaxial side. 1 – epidermis; 2 – angular-lamellar collenchyma; 3 – parenchyma; 4 – chlorenchyma; 5 – vascular ring; 6 – calcium oxalate druses; 7 – sclerenchyma; 8 – phloem; 9 – xylem; 10 – angular collenchyma; 11 – trichomes; 12 – cordate parenchyma; 13 – intercellular spaces.

The petiole was covered with an epidermis consisting of fine densely packed cells, the thickness of the cuticle reaching 5 µm. Throughout the cross sections, there were integumentary unicellular trichomes of the subulate form 90–370 µm in length. Under the epidermis there was a layer of angular-lamellar collenchyma 3–5 cells thick. On the abaxial side it was substituted by angular collenchyma (Fig. 4, *D*), reinforcing a ring of vascular bundles. On the adaxial and lateral sides, the angular-lamellar collenchyma was underlaid by chlorenchyma, the latter in the vicinity of the vascular bundles substituted by the main parenchyma (Fig. 4, *B*–*C*). As with *P. nigra*, there were slit-like intercellular spaces on adaxial and lateral sides. Most hybrids were found to have intermediate vascular system (78 % of the total subset) (Table 5).

On the abaxial side there was a large elliptical ring 700–800 µm wide and 600–650 µm high. On the adaxial side there were two rings 170–250 µm in diameter, consisting of 3–6 collateral bundles (Fig. 4, *B*–*C*).

Table 5. Petiole anatomy characteristics in the *P.* × *jrtyschensis* populations (unbiased estimate of frequency, $p \pm Sp$)

Features		SRES	Karlyk
Petiole cross-section shape	Ovate	0.483 ± 0.089	0.487 ± 0.078
	Elliptical	0.517 ± 0.089	0.513 ± 0.078
Contour of the adaxial side	Truncated	0.258 ± 0.078	0.513 ± 0.078
	Cordate	0.710 ± 0.081	0.487 ± 0.078
	Auriculate	0.032 ± 0.031	–
Valcular system type	Linear	0.096 ± 0.052	–
	Intermediate	0.678 ± 0.083	0.854 ± 0.055
	Arched	0.226 ± 0.075	0.146 ± 0.055
	High-intermediate	–	–
Lower ring shape	Rounded	0.581 ± 0.088	0.317 ± 0.072
	Elliptical	0.419 ± 0.088	0.683 ± 0.072

The medial vascular structure was mainly represented by an elliptical ring 500–600 μm wide and 400–450 μm high. The heart-shaped parenchyma could be clearly seen in the center of the abaxial ring. The vascular components of the xylem were radially located, the vessel diameter ranging from 25 to 35 μm. The phloem cells were 10–15 μm. Outside the phloem a thick layer of bass fibers, mostly lignified, was located. The rings were surrounded by a cover of large and fairly thick-walled parenchyma cells. Calcium oxalate druses were found mostly in the parenchyma around the bundles and less often in the cordial parenchyma and in the chlorenchyma.

Some *P.* × *jrtyschensis* specimens had arcuate (21 %), linear (3 %) and high-intermediate (3 %) vascular systems (Table 5). However, in this leaf morphology these hybrid individuals did not differ from the rest.

The obtained results confirm the usefulness of qualitative traits of petiole anatomy for *Populus* species and hybrids' identification (*Aigeiros* and *Tacamahaca* sections). Leaf petioles from *P. nigra* and *P. laurifolia* trees, growing outside the hybridization centers, can be discriminated very well due to their adaxial side outline and vascular system type.

Petiole anatomy of *P.* × *jrtyschensis* to a certain degree inherits parent traits. In hybrids several types of vascular systems were found to be common, i. e. intermediate, high-intermediate, linear and high-arched types. The intermediate type of vascular system is characteristic of the most part of *P.* × *jrtyschensis* trees (78 % of the total sampling). Therefore most of them can be classified as F_1 hybrid. Other hybrids showed high-intermediate, linear and high-arced types of their vascular systems, but did not differ from the rest in shoot morphology and leaf shape. Taking into account the principle of F_1 hybrids' uniformity, we consider such trees to be recombinant hybrids. Thus petiole anatomy allows not only identifying hybrid plants, but also revealing their heterogeneity. Our results correlate well with the earlier findings in *Populus* natural hybridization centers, where F_1 hybrids predominate, while the next generation hybrids and backcrosses are not numerous due to natural selection pressure (Roe et al., 2014; Zeng et al., 2016; Christe et al., 2016; Chhatre et al., 2018).

In the examined populations of *P. nigra*, located near or in the hybridization centers, the diversity of petiole traits was shown to increase. These trees did not differ from the trees, growing outside hybridization areas. However, they displayed a broad range of vascular system types: linear, high-intermediate, intermediate and high-arched ones. In addition, the populations had drastically increased the abundance of trees with the truncated outline of the upper petiole side and with elliptical shape of the vascular system rings. All these traits, similar to *P. nigra* hairiness, should be regarded as phenotypic manifestation of introgression, once more confirming that the latter can be an important source of genetic variation (Chhatre et al., 2018; Suarez-Gonzalez, 2018*a*, *b*).

The investigation of *P. laurifolia* petiole anatomy both in mixed and remote populations did not reveal the traits, proving the introduction of *P. nigra* genes into its gene pool. The fact is most likely indicative of the asymmetric character of the introgressive hybridization taking place in the Tom River basin, with the gene flow from the donor *P. laurifolia* to the recipient *P. nigra* prevailing.

CONCLUSION

The study confirmed that the use of petiole anatomy provides solid basis for the identification of parent species, hybrids and backcrosses in natural hybridization areas of taxa belonging to *Aigeiros* and *Tacamahaca* sections of *Populus* genus. Petiole anatomy allows not only identifying hybrid plants, but also revealing their heterogeneity. Examination of *P. nigra* in its natural hybridization center allowed to find a new approach to the identification of back-crosses.

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ИДЕНТИФИКАЦИЯ *Populus nigra*, *P. laurifolia* и *P.* × *jrtyschensis* ПО АНАТОМИИ ЛИСТОВЫХ ЧЕРЕШКОВ

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Значительная изменчивость морфологических признаков видов рода *Populus*, а также широкое распространение гибридизации в роде осложняют идентификацию таксонов. Петиолярная анатомия имеет большое значение для диагностики растений и решения спорных вопросов систематики. Целью настоящего исследования являлось изучение анатомического строения черешков *P. nigra, P. laurifolia* и *P.* × *jrtyschensis* для оценки возможностей их применения в идентификации таксонов. Материалами для изучения служили черешки листьев, собранные с 252 особей в 8 популяциях *P. nigra, P. laurifolia* и *P.* × *jrtyschensis* в зоне естественной гибридизации бассейна р. Томи в Кемеровской области. Две из них использовались как контрольные для характеристики родительских таксонов, поскольку они удалены от очагов гибридизации. Поперечные срезы делали в верхней части черешка. На каждом препарате исследовали: форму поперечного сечения черешка, контур адаксиальной стороны, форму проводящей системы и нижнего кольца. Черешки особей, произрастающих вне очагов гибридизации, различаются по форме проводящей системы: у *P. nigra* она линейная, у *P. laurifolia* высокоаркообразная. Для гибридов характерны несколько типов проводящих систем: промежуточная, переходная, линейная и высокоаркообразная. При этом у большинства особей *P.* × *jrtyschensis* она промежуточная (78 % от общей выборки), и, вероятно, их можно рассматривать по большей части как гибриды F_1, а остальные – как рекомбинантные. На примере *P. nigra* в зоне естественной гибридизации показано, как идентифицировать беккроссы. Проведенные исследования подтвердили, что использование признаков петиолярной анатомии позволяет надежно идентифицировать родительские виды, гибриды и беккроссы в зонах естественной гибридизации таксонов секций *Aigeiros* и *Tacamahaca*.

Ключевые слова: *тополь, дифференциация, естественная гибридизация, беккроссы, бассейн р. Томь, Кемеровская область, Россия.*