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Using petiole anatomy to identify hybrids between and species of *Populus* sections *Aigeiros* and *Tacamahaca*

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Keywords: hybridization, identification, petiole anatomy, *Populus*, taxa.

Summary. The article presents the results of the study of the petiole anatomy peculiarities of the hybrids between *Aigeiros* and *Tacamahaca* sections. Petiole anatomic structure was found to be helpful in assigning taxa to a section and to find intersectional hybrids, which is actual for studying populations in natural and anthropogenic hybridization zones. Cross sections made in the upper part of petioles were used for analyzing anatomic traits by light microscopy. All representatives of the *Aigeiros* section have linear form of the vascular system, consisting of 3–5 rings, with a rounded contour of the petiole adaxial side. In the *Tacamahaca* section taxa the vascular system is highly arched, and the adaxial side is cordate. The study of the hybrids between species of the same section revealed that such hybrids inherit anatomy features common for the section. We can consider such traits as adaxial side shape and vascular system type to be the most important markers for intersectional hybrids. Truncated or notched shape of the adaxial contour and vascular system type were found to be characteristic features of hybrids, as small notches in their petioles' upper part are common for all hybrids. Most of hybrids have small notches, rather than grooves, in the upper part of their petioles. Most of the intersectional hybrids have transitional shape of vascular system. The anatomy of *Populus* × *sibirica* petioles confirmed earlier results that it is a hybrid cultivar, that originated as a result of crossbreeding between *Aigeiros* and *Tacamahaca* section species.

Использование признаков петиолярной анатомии для идентификации гибридов и видов секции *Aigeiros* и *Tacamahaca* рода *Populus*

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Ключевые слова: гибридизация, идентификация, петиолярная анатомия, таксоны, *Populus*.

Аннотация. Представлены результаты изучения петиолярной анатомии видов секций *Aigeiros* и *Tacamahaca* и их гибридов. Установлено, что анатомическое строение черешков позволяет диагностировать принадлежность таксона к секции и выявлять межсекционные гибриды; последнее актуально при исследовании зон естественной и антропогенной гибридизации. Листья для работы отбирались со средней части кроны

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репродуктивно зрелых деревьев с укороченных побегов. Для анализа использовались поперечные срезы, выполненные в верхней части черешка. Материалы исследовались с помощью методов световой микроскопии. Для всех представителей секции *Aigeiros* характерна линейная форма проводящей системы из 3–5 округлых колец и округлый контур адаксиальной стороны черешка. У таксонов секции *Tacamahaca* она высокоаркообразная, а контур адаксиальной стороны сердцевидный. Изучение гибридов между видами одной секции показало, что они полностью наследуют специфику строения характерную для группы. Наиболее важными анатомическими признаками, маркирующими межсекционные гибриды, следует считать: контур адаксиальной стороны и форму проводящей системы. Характерной особенностью гибридов является усеченный или выемчатый контур адаксиальной стороны черешка, поскольку у большинства из них в верхней части черешка наблюдается не желобок, а только выемка. Форма проводящей системы у большинства межсекционных гибридов промежуточная. Анализ строения черешков *Populus* × *sibirica* подтвердил данные ряда авторов о том, что это гибридный культивар, возникший в результате скрещивания видов секций *Aigeiros* и *Tacamahaca*.

Introduction

Natural hybridization between species from different sections is quite common in the *Populus* L. genus.

In the North America spontaneous crossing between *P. deltoides* W. Bartram ex Marshall (*Aigeiros* Lunell section) and *P. heterophylla* L. (*Leucoides* Spach section) is widely spread (Eckenwalder, 2010). But hybridization between representatives of *Aigeiros* and *Tacamahaca* Mill. sections is even more common, as they can cross relatively freely despite the presence of mechanisms for reproductive isolation (Proshkin, Klimov, 2017a). Spontaneous hybridization between *Aigeiros* section species (black poplars) and *Tacamahaca* section (balsamic poplars) occurs in contact sites of taxa areas and is poorly limited by prezygotic barriers. Genetic composition of trees in hybridization zones is determined by natural selection, with two parental groups and one hybrid group easily distinguished. F_1 often predominate among hybrid offspring (Proshkin, Klimov, 2017a). However, hybrids of the next generations and backcrosses are not always discarded by natural selection and can be quite abundant in populations (Hersch-Green et al., 2014; Roe et al., 2014; Chhatre et al., 2018). Numerous studies in hybridization zones showed that backcrossing between hybrid plants and those of parental species are common (Christe et al., 2016; Hu et al., 2016; Jiang et al., 2016; Zeng et al., 2016; Vasilyeva et al., 2018). Therefore, the observed hybridization is retrogressive, being accompanied by the gradual transfer of genetic material from one taxon to another across inter-species isolation barriers. Introgression is considered to be an important source of genetic variation (Chhatre et al., 2018; Suarez-Gonzalez et al., 2018a, b, c). Alongside with the natural one, anthropogenic hybridization between the cultivated and native species is currently widely spread. Broad and often unjustified use of adventive and numerous cultivars of hybrid origin results in their contact with native species populations, thus providing the possibility for the exotic genes transfer into the native gene pools and hence threatening their preservation (Proshkin, Klimov, 2017b). Moreover, increased invasive ability of hybrids can facilitate their introduction into indigenous plant communities, and not only in the floodplains (Kostina et al., 2016). All these necessitate development of reliable methods of identifying hybrid plants. The use of molecular biomarkers is currently the best approach, but its high cost has been so far prohibitive for the wide use. Besides that, the method is most effective in comprehensive studies employing other techniques, like phenetics etc. (Udalov, Benkovskaya, 2011). Thus, investigation of the initial stages of hybridization processes should be based on plant anatomy and morphology.

Our earlier research showed that morphology studies are quite reliable in identifying poplar hybrids between *Aigeiros* and *Tacamahaca* sections by differentiation patterns of the shortened branches in their crowns. The *Tacamahaca* section trees have specialized shortened branches, so called discoblasts, while *Aigeiros* section trees do not have such, their shortened branches being represented only by leptoblasts. Discoblasts are always inherited by both native and cultural hybrids, being preserved even in the backcrosses. Unfortunately, the trait has a serious shortcoming: well developed shortened branches can be found only on rather mature trees. The use of generative organs morphology also has certain limitations: a) the method does not allow indentifying trees that have not reached the reproductive age, b) poplars are strictly dioecious plants, c) poplars have rather short flowering and fruiting periods, and d) their reproductive organs, except for fruits, so far have been poorly studied (Klimov, Proshkin, 2019).

In zones of natural hybridization between *Aigeiros* and *Tacamahaca* section species, where the parent species are reliably known, morphology methods overcome such shortcomings. However, while investigating the hotspots of anthropogenic hybridization, morphology methods cannot be relied on as the primary ones for species identification, as in such zones the parent involvement of certain species is not obvious, and hybrid cultivars often cross.

Thus, systematic assignment of the hybrid trees at the section level often becomes the primary goal of research. The situation is actualized by the wide spreading in culture of poplar cultivars and clones of the unknown descendance. The latter results not only from the lost data about the assortment of planted trees, but also from breeding methods and practices, which were widely used in the past: breeding, based on selecting in nurseries spontaneous hybrids with economically valuable traits but unknown origin; pollinating with pollen mixture and the absence of detailed description of the obtained cultivars (Klimov et al., 2018). A telling example is provided by the wide use for urban green landscaping and protective agriculture in the south of West Siberia of the hybrid *P.* × *sibirica* G. V. Krylov et G. V. Grig. ex A. K. Skvortsov, the exact origin of which is not known (Kostina et al., 2016, 2018; Proshkin, Klimov, 2017b). For a long time, the taxon was believed to be *P. balsamifera* L. (Bakulin, 1990, 2005; Kuklina, Merzlyakova, 2013). V. T. Bakulin (1990) noted that in Siberia the species is not homogenous, being represented by different clones of unknown origin. Several researchers indicated its hybrid nature, basing their conclusion on fruits and vegetative organs' morphology (Skvortsov, 2007; Maiorov et al., 2012). However, up till now the unequivocal evidence for the hybridization origin was not found. Few studies of the anatomy of the Salicaceae Mirb. family showed the importance of such approach for solving complicated taxonomic problems (Thadeo et al., 2014; Kalouti et al., 2015; Čortan et al., 2017). Thus, petiole anatomy, i.e. peculiarities of petiole structure, can be considered one of the most promising methods for poplar taxonomic attribution (Skvortsov, Belyanina, 2005; Kurkin, 2014a, b; Gavrilenko, Novozhilova, 2015, 2017).

The aim of the study was to examine petiole anatomy of *Aigeiros* and *Tacamahaca* section species and their hybrids with the purpose of assessing the potential of the method for taxonomic identification.

Materials and Methods

Our earlier studies showed that to reveal the variability of petiole traits in hybrid and parental taxa in the natural hybridization zones one should not focus research efforts solely on model trees. Only comprehensive efforts to study population variability can be effective in analyzing hybridization and introgression processes. At the same time, research on model trees is quite justified for studying cultivars, which have limited variability (Klimov et al., 2018).

The following taxa were the objects of the study:

Aigeiros section: *P. nigra* L. and *P. deltoides* Marshall.

Tacamahaca section: *P. balsamifera* L., *P. trichocarpa* Torrey et A. Gray, *P. laurifolia* Ledeb. and *P. suaveolens* Fisch.

Natural and cultural hybrids were studied (Table 1).

Table 1

The studied *Populus* genus hybrids

Name	Parent taxa	Provenance
P. × *moskoviensis* Schroeder	*P. laurifolia* × *P. suaveolens* (probably)	Moscow, spontaneous hybrid
P. × *canadensis* Moench	*P. nigra* × *P. deltoides*	Europe, spontaneous hybrid
Hybrid no.14	*P. laurifolia* × *P. nigra* var. *italica*;	Bred by V. T. Bakulin
P. × *jrtyschensis* C. Y. Yang in C. Wang & S. L.	*P. laurifolia* × *P. nigra*	The Altai-Sayan Mountain Country, natural hybridization
P. × *leningradensis* P. Bogdan.	*P.* × *canadensis* × *P. suaveolens*	Bred by P. L. Bogdanov
P. × *sibirica* G. V. Krylov et G. V. Grig. ex A. K. Skvortsov	*(P. laurifolia* × *P. nigra)* × *P. balsamifera* (probably)	?

Besides that, materials obtained from dendrarium of the Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences (Novosibirsk), Educational Botanical Garden of the Novokuznetsk Institute of the Kemerovo State University (Novokuznetsk) and herbarium of species and hybrids of the Moscow Region. The leaves of *P. nigra, P. laurifolia* and *P.* × *jrtyschensis* were collected by the authors in 2016–2017 in poplar populations in the basin areas of the Tom, Biya and Katun Rivers.

To account for the possible heterogeneity of *P.* × *sibirica* we used five trees collected in several areas, namely in Kemerovo and Novokuznetsk (Kemerovo Region), Novosibirsk and Berdsk (Novosibirsk Region) and Altai Territory (Biisk). Leaves were sampled mid-crown from the short branches of the reproductively mature trees. Petioles were separated from the blades and placed into the mixture of 96% ethanol, glycerol and water (1:1:1 v/v). The petioles and leaves from herbaria were incubated in the similar mixture for three or four days. Cross-sections were made from the upper part of a petiole, i.e. at the base of the leaf blade, as their characteristics were used as keys to discriminate between the species of the *Aigeiros* and *Tacamahaca* sections (Menitsky, 1989; Molganova, Ovesnov, 2016). Besides that, in the upper part of a petiole conducting bundles joined together, whereas in other parts they were separated to different degrees (Kindyakova, Shamrov, 1976). The cross-section slides were colored using 2 % aqueous safranine solution. Petiole anatomy was examined by using light microscopy (Micromed-1, Nabludatelniye pribory LLC, Russia) at 40 and 100 magnifications.

The following features were recorded while studying microscopic slides: the shape of the petiole cross-section, the contours of petiole adaxial and abaxial sides, the shape of the closed collateral rings, and the type of conducting system. The length of the petiole cross section (H, µm) and the distance between the widest part of the section and its base (B, µm) were measured on each slide by using Axio Vision 4.8.2 (ZEISS, 2018) software. The following ratios were used to categorize cross-section and ring shape: $B/H < 0.25$ (triangular); 0.25–0.35 (ovate-triangular); 0.35–0.45 (ovoid); 0.45–0.65 (elliptic); and >0.65 (obovate) (Klimov, Proshkin, 2018a).

Results and discussion

The type of vascular system was determined according to the relative position of closed conducting collateral vascular bundles, seen as rings on the petiole cross-section microscopic slides, rather than individual bundles. The rings are located in tiers. Earlier we distinguished four types of the vascular system of the studied poplar taxa: linear, consisting of the 3–5 rounded or elliptic rings, located one above another; transitional, consisting of two linearly positioned rounded or elliptic rings and two rounded or elliptic rings positioned in parallel on the adaxial side; highly transitional, consisting of three linearly positioned rounded or elliptic rings; and high arched type, consisting of one elliptical ring on the abaxial side and 2–6 parallel rounded rings on the abaxial side (Fig. 1).

The study showed that petiole anatomy in *Populus* genus trees can attribute a taxon to a section, which is actual for studying zones of the natural and anthropogenic hybridization (Table 2). All the representatives of the *Aigeiros* section have linear vascular system of three-five rounded rings and the rounded contour of the adaxial side. The taxa from the *Tacamahaca* section have high-arched vascular system, while the adaxial side is cordate (Fig. 2) (Klimov, Proshkin, 2018b).

Table 2.

Diagnostic traits of petioles of the poplar species from the *Aigeiros* and *Tacamahaca* sections and their intersectional hybrids

Traits	Peculiarities of traits		
	Aigeiros	*Aigeiros* × *Tacamahaca* hybrids	*Tacamahaca*
The shape of the petiole upper part cross-section	egg-shaped triangular, elliptic	ovoid, elliptic	Egg-shaped, elliptic
Adaxial side contour	rounded	Truncated, seldom v	cordate
Vascular system shape	linear	Predominantly transitional	High-arched

Overall one-tier epidermis, consisting of small densely packed parenchyma cells, is a characteristic feature of *Aigeiros* and *Tacamahaca* species. Only *P. laurifolia* has epidermis of elongated up to 15 µm cells. The width of its cuticle ranges 5–10 µm.

All studied *Tacamahaca* section had unicellular subulate trichomes 50–130 µm long. It should be noted that the degree of trichome development and their length may vary between individuals in populations of the same species (Klimov, Proshkin, 2017, 2018b).

The *P. nigra* populations in the basin areas of the Tom, Biya and Katun Rivers are characterized by the prevalence of trees with naked leaf blades and petioles, whereas the haired morphotype of the species grows manly in sites of its hybridization with *P. laurifolia* (Klimov, Proshkin, 2017, 2019; Proshkin, Klimov, 2019). The morphotype is characterized by unicellular cover trichomes that are not abundant. Under the cover tissue and along the entire perimeter of the cross-section there is usually a 4–5 cells thick layer of the angular-lamellar collenchyma. In the ribs of the adaxial side of the balsamic poplar leaves the layer is 6–7 cells thick.

Below the layer of the angular-lamellar collenchyma there is a layer of the angular one, forming a transition to the basic and assimilating parenchyma (chlorenchyma) almost along the entire perimeter.

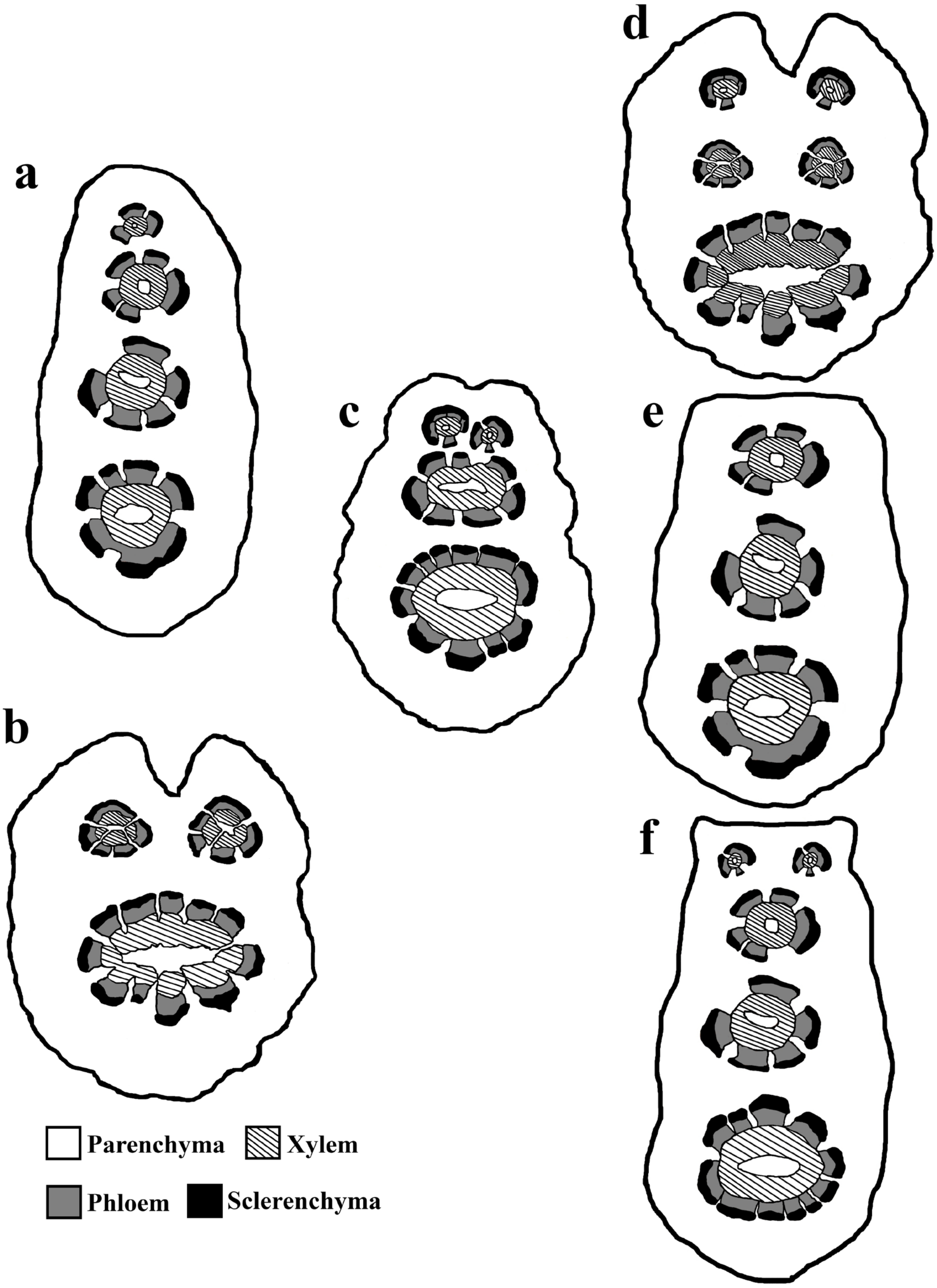

Fig. 1. The types of vascular systems: a – *Aigeiros* section taxa (linear type); b – *Tacamahaca* section taxa (high-arched type); c – hybrid (intermediate); d – hybrid (high-arched type); e – hybrid (linear type); f – hybrid (high-intermediate) type.

The *Aigeiros* section species have slit-like intercellular spacers on the adaxial and lateral sides in sites where one type of collenchyma substitutes another. The petiole sides have loose chlorenchyma patches under the sub-epidermal collenchyma layer. The angular collenchyma of balsamic poplars can not be seen on the adaxial side, where large intercellular spacers reaching the upper side of the abaxial conducting bundle ring through the assimilating tissue layer. Smaller intercellular spacers can be found on lateral and abaxial sides.

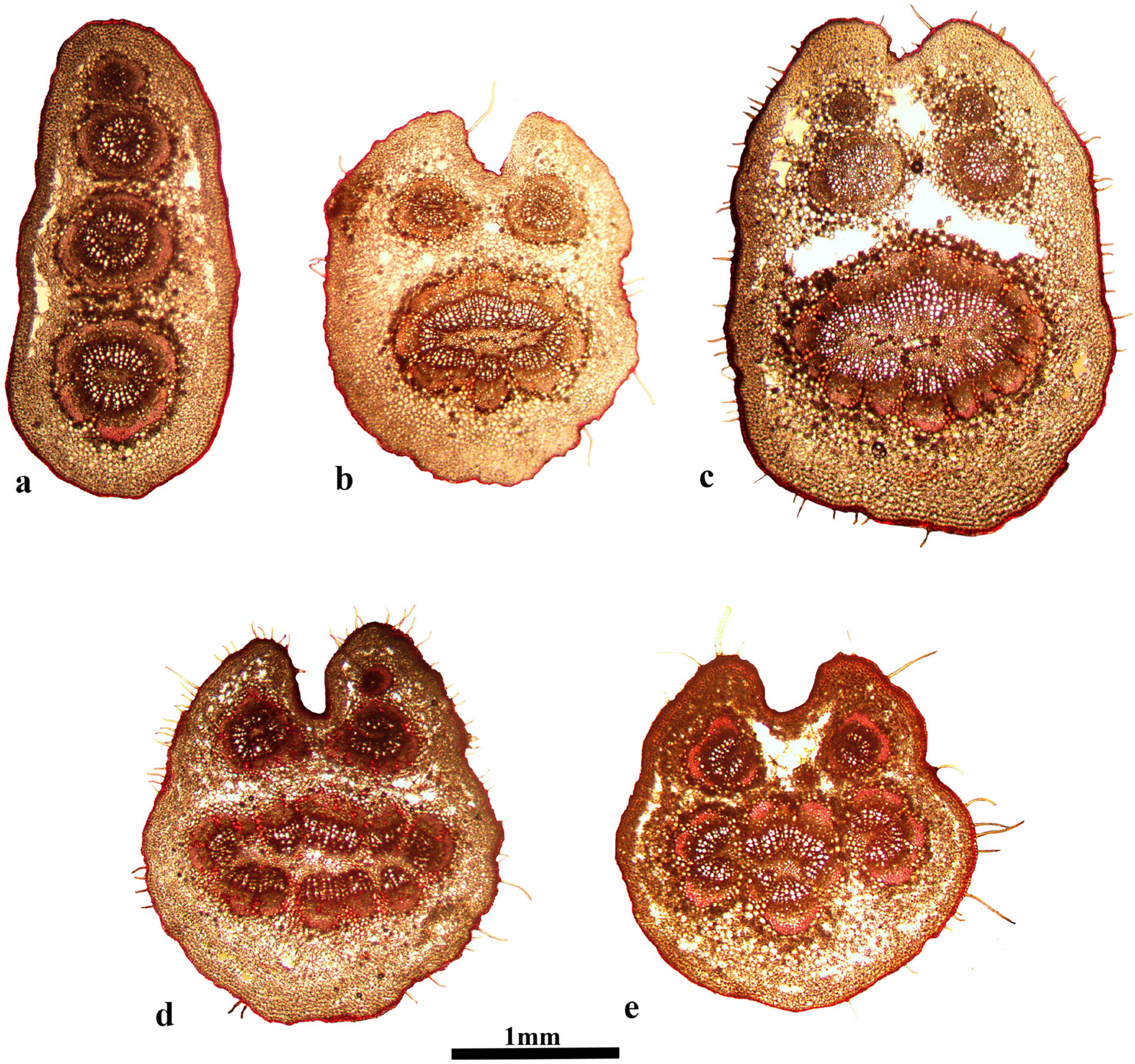

Fig. 2. Petiole cross sections: a – *Populus nigra*; b – *P. balsamifera*; c – *P. trichocarpa*; d – *P. laurifolia*; e – *P. suaveolens*.

The presence of numerous calcium oxalate druses is common for all studied poplar species. The black poplars have them mainly in cells surrounding the conducting bundles, while balsamic poplars can have the druses also in chlorenchyma (Klimov, Proshkin, 2018b).

Examination of intra-section hybrids showed that they fully inherit the anatomy features specific for the section, which allows to discriminate such hybrids from the inter-section ones (Fig. 3).

The following traits can be considered the most important anatomic features, marking inter-section hybrids: the petiole cross-section shape in the upper part, the contour of the adaxial side and the type of the vascular system (Fig. 4). In their petiole cross-section shape the hybrids are close to *Aigeiros* species, which is flattened in the petiole upper part. Additionally, some of the hybrids have slit-like intercellular spacers on adaxial and lateral sides.

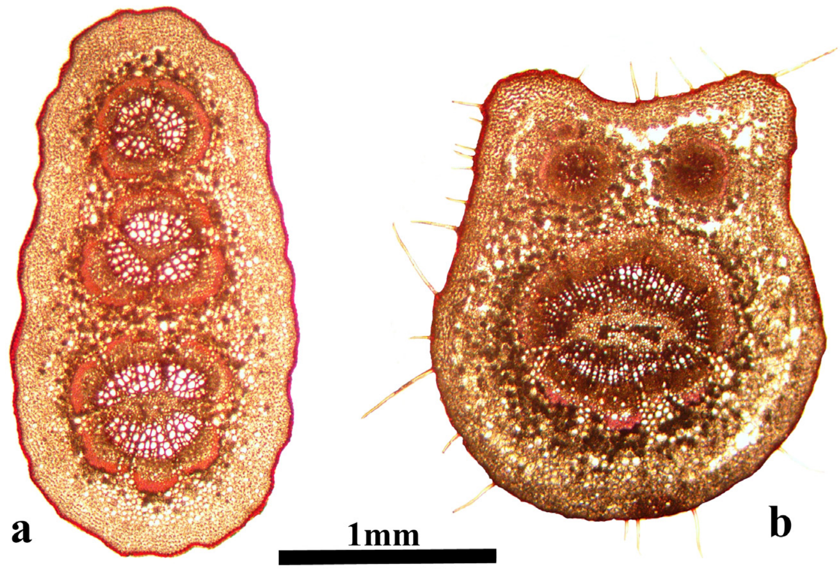

Fig. 3. Petiole cross sections: a – *Populus* × *canadensis*; b – *P.* × *moskoviensis*.

Truncated or notched contour of the petiole adaxial side is a characteristic feature of hybrids (Fig. 1). It reflects the degree of groove development on the petiole upper side. According to some researchers, the presence or absence of the groove can be considered one of the major traits, discriminating species of *Aigeiros* и *Tacamahaca* sections (Pautov, 2002; Mayorov et al., 2012). Many authors noted that in hybrid trees the groove in the petiole upper part is poorly developed (Mayorov et al., 2012; Molganova, Ovesnov, 2016; Kostina et al., 2016). Our findings comply with this conclusion, as most of the hybrids in our study had only the groove in their petiole upper part.

The type of the vascular system in hybrids may be the most obvious qualitative trait for their identification. Examination of petiole anatomy of *P.* × *jrtyschensis* from the Tom River basin populations showed that most of the hybrids (73.0 % of the set) had intermediate type of conducting system (Fig. 1c, 3a). The trees with such type of conducting system are likely to be F_1 hybrids, which is partially confirmed by examining petiole anatomy in the first generation hybrids obtained in culture between *Aigeiros* and *Tacamahaca* section species: *P.* × *leningradensis* (*P.* ×*canadensis* × *P. suaveolens*; bred by P. L. Bogdanov) and hybrid No.14 (*P. laurifolia* × *P. nigra* var. *italica*; bred by V. T. Bakulin). The studied cultivars of this hybrid generation also had intermediate conducting system (Fig. 3c, d). Some of the *P.* × *jrtyschensis* trees are characterized with high-arched (21.0 %), linear (3.0 %) and high-intermediate (3.0 %) vascular systems, their leaves morphology not differing statistically from the trees with intermediate type of vascular system. Petiole structure analysis confirmed earlier findings that it is a hybrid cultivar, resultednt from the crossing between *Aigeiros* and *Tacamahaca* section species (Fig. 3b). It should be noted that already in 1956 G. V. Krylov (Albensky et al., 1956) analyzed the use of poplars for green landscaping and protective sylviculture in Siberia and concluded that it is Siberian poplar, rather than *P. balsamifera*, that was spread in West Siberia. The latter was used in the Kazakh Soviet Socialist Republic (Albensky, Dyachenko, 1940; Albensky et al., 1956). In 1957 G. V. Krylov described (in Russian) Siberian poplar as a taxon at the species level (Gureeva et al., 2016), regarding it as a separate local Altai-Sayan species. The latter conclusion was certainly wrong, as the species cannot be found in natural ecosystems of the region. G. V. Krylov may have not distinguished it from the natural hybrid *P.* × *jrtyschensis*, which is quite common in river valleys and the foothills of the Altai and the Sayan Mountains in areas where the parent species *P. laurifolia* and *P. nigra* grow together. However, so far the presence of the latter species has not been recorded in any native dendroflora inventory of Siberia. *P.* × *sibirica* и *P.* × *jrtyschensis*, as hybrid taxa, look very similar indeed: both have well developed discoblasts, the ribbed in the upper part and cylindrical in the lower part young shoots prevailing, mostly bivalve, less often tricuspid fruit boxes, similar leaf blade shapes. At the same time,

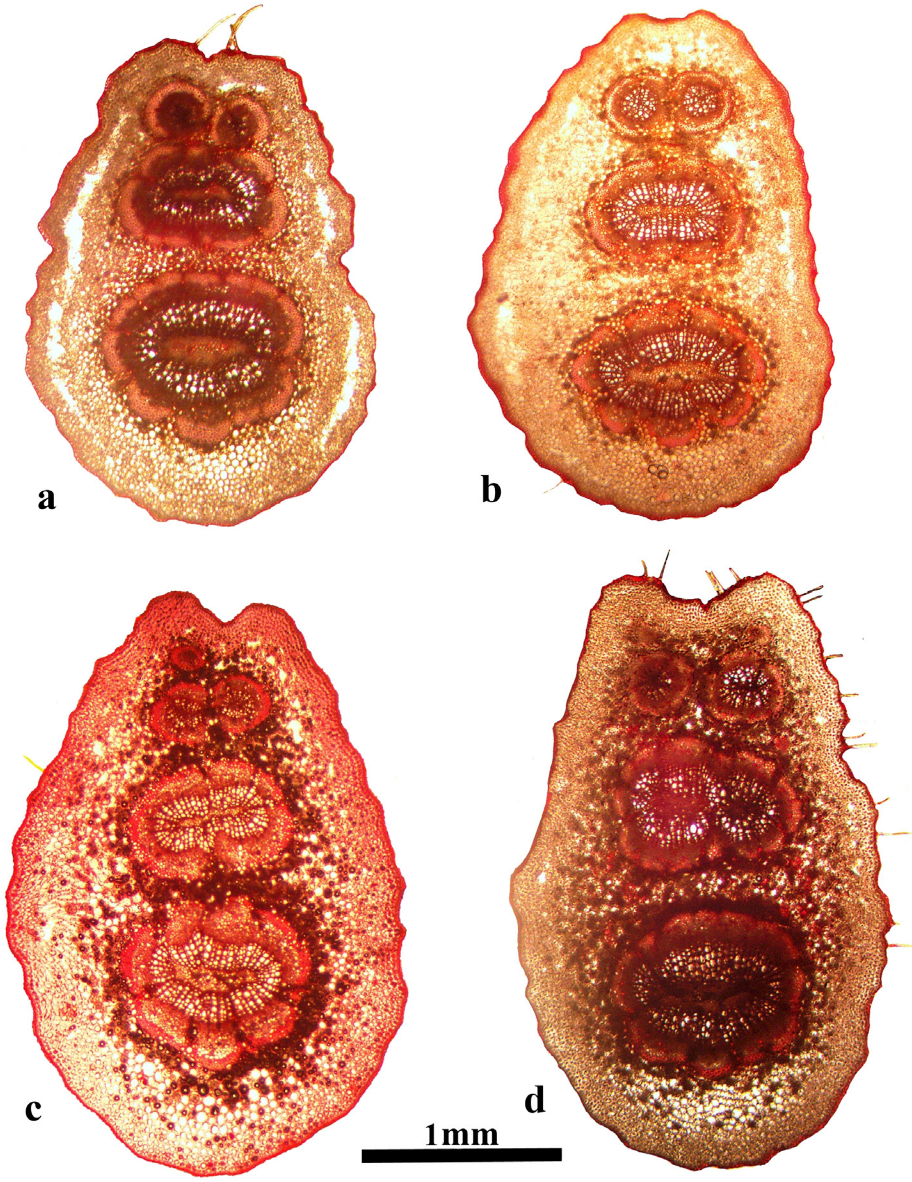

Fig. 4. Petiole cross sections: a – *Populus* × *jrtyschensis*; b – *P.* × *sibirica*; c – *P.* × *leningradensis*; d – hybrid No. 14.

they differ in some morphological traits: *P.* × *sibirica* has glistening grey-orange peridermis of the crown shoots, whereas *P.* × *jrtyschensis* has grey one. The Siberian poplar has ovoid leaves on its shortened branches (ca. 60 %) and ovoid-triangular leaves (ca. 40 %) with 1–2 small glands in the site where the blade joins the petiole, although not on all leaves. The Irtysh poplar has predominantly ovate-triangular leaf blades (60–80 %), albeit triangular, ovoid and elliptical can also be found; no glands. Despite its native origin, the Irtysh poplar, in contrast to *P.* × *sibirica*, is not widely used for urban green landscaping in Siberia. Its decorative form was bred by Z. I. Luchnik and is known as 'Gornoaltaisky 2'. *P.* × *jrtyschensis* was found in small numbers on plantations in Biysk, Novokuznetsk, Tomsk according to T. E. Kuklina and I. Ye. Merzlyakova (2013).

Conclusions

The poplar species of the *Aigeiros* and *Tacamahaca* sections can be discriminated on the basis of their petiole anatomic structure. As expected, hybrid taxa, originated as a result of the crossing between the species, to a certain degree inherit the traits of the parent species, but can be easily identified according to the adaxial side contour and vascular system type. The intra-section hybrids do not differ from parent species in their petiole anatomy. The petiole structure of *Populus* × *sibirica* confirms earlier results that it is a hybrid cultivar, originated due to the crossbreeding between *Aigeiros* and *Tacamahaca* section species.

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