



Conference Proceedings

The Ninth Conference in Memory of Alexei K. Skvortsov

Tsitsin Main Botanical Garden, Russian Academy of Sciences, Moscow. February, 2020

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Conference Overview

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The 9th annual Conference to celebrate the Centenary of Professor Alexey K. Skvortsov (1920–2008) was held at Tsitsin Main Botanical Garden of the Russian Academy of Sciences (MBG) in February 2020. The Conference was organized by the Moscow Branch of the Russian Botanical Society and supported by Tsitsin Main Botanical Garden and Moscow State University (MSU). Financial support was provided by the Chimmed Group, Moscow, and the Russian Foundation for Basic Research (grant N° 20-04-20001). Unlike the previous conferences, the topic was widened to encompass several of the areas of interest to Prof. Skvortsov, namely, microevolution, taxonomy, and flora studies. The Conference took the form of a two-day meeting with an additional short lecture course on plant nomenclature given by Irina Belyaeva Royal Botanic Gardens, Kew (RBG, Kew). The 9th Conference gathered botanists from the Russian regions of Moscow, St. Petersburg, Arzamas, Balashov, Borok, Bryansk, Kalach-na-Donu, Magadan, Novosibirsk, Penza, Ryazan, Saratov, Ufa, and Volgograd, as well as Debrecen (Hungary), London (UK), Storrs (Connecticut, US), and Vilnius (Lithuania), 87 in all, who presented their talks and posters, and took part in discussions. The presentations were devoted to the problems of taxonomy, flora studies, studies of rare, endemic, and alien species in their various aspects, from ecology and morphology to molecular diversity, phylogeny and phylogeography in native and alien plants and animals. Altogether, 36 talks and 18 posters were presented and discussed during the conference. Short abstracts of talks and the program of the conference were published before the conference start as a separate brochure in Russian and English, together with a book of A.K. Skvortsov's personal recollections, prepared by Raisa Trochinskaya and edited by Nina Stepanova (MBG).

The sessions were preceded by an introductory welcoming talk by the Director of the Main Botanical Garden Vladimir Upelnik (Upelnik *et al.*, 2020).

During the first plenary session four presentations were given dealing with A.K. Skvortsov's scientific heritage. Irina Belyaeva (RBG, Kew) presented a talk "The Phenomenon of A.K. Skvortsov's heritage" (Belyaeva-Chamberlain, 2020). The presentation by Nina Stepanova and Sergei Poluektov (MBG) was devoted to A.K. Skvortsov's herbarium collections kept at MHA (Herbarium codes are given in all conference proceedings as in Thiers, 2020; Stepanova *et al.*, 2020). It was followed by a talk by Nina Stepanova and Natalya Reshetnikova (MBG) on A.K. Skvortsov's life-long work on the project of the Lower Volga Region Flora. The first plenary session closed with a talk by Larisa Kramarenko (MBG) on Skvortsov's criticisms of language inconsistencies occurring in modern Russian scientific literature. The first sectional session was devoted to problems of Salicology, as Prof. A.K. Skvorsov was a world-renowned specialist in willows and poplars (Salicaceae). The session was opened with a rather debatable talk by Julia Kuzovkina (University of Connecticut, USA) and Alexander Marchenko (Water Plant Nursery, Moscow) about hybrids in *Salix* and their study revealing the importance of the number of ovules in the determination of hybrid formulae. Tatyana Fedorova (MSU) presented her results on leaf micromorphological characters in the diagnostics and taxonomy of black and white poplars. Talks by Alexey Afonin (Bryansk State University) and Vladimir Viktorov (Moscow State Pedagogical University) dealt with shoot morphogenesis and crown architecture in *Salix*.

The next session was devoted to the taxonomy of various groups of plants and animals. It included talks by Alexey A. Borisyyuk (MSU) on sexual dimorphism in populations of *Hippophae rhamnoides* which leads to the formation of a system of populations with different evolutionary histories; Yuri Kopylov-Guskov (MSU) on the taxonomy of feather grasses (*Stipa*, Poaceae) in the light of morphological and molecular data; Michail Serebryany (MBG) on the prospects of taxonomic revision of the genus *Trollius*; Tatyana Kramina with co-authors (MSU) on the geographic trends revealed in *Dorycnium* (Fabaceae) phylogeny; Galina Degtjareva from Botanical Garden MSU (BG MSU) and her colleagues from MSU on the species problem in the genus *Paeonia*; Vladimir Gokhman (BG MSU) on the trends in modern species level taxonomy of parasitoid Hymenoptera where numerous genetically and ecologically differentiated sibling species have been discovered recently; and, finally, by Viktoria Shneer (Komarov Botanical Institute, St. Petersburg) on cryptic hybrids in plants, their detection, frequency, and theoretical and applied significance. During the first conference day evening session, devoted to problems of rare and endemic species, five talks were presented. First, Tamás Malkócs (University of Debrecen, Hungary) presented fascinating results of his morphological, ecological and molecular study of *Salvia austriaca* (Lamiaceae) in Eastern

Europe, which ended up in the discovery of a new species overlooked by previous researchers. Ivan Schanzer's (MBG) talk was devoted to the results of a molecular genetic study of *Potentilla vulgarica* (Rosaceae), an endemic species with a narrow distribution area in the middle part of the Volga River Valley. Maria Ivanova (student at MSU; together with a group from MBG and Papanin Institute for Biology of Inland Waters (IBIW), Borok) presented the results of morphological, caryological and molecular phylogenetic study on endemic species of *Callitriche* (Plantaginaceae) in the Lower Volga Region, which confirmed the species status of *C. fimbriata* and *C. transvolgensis* and revealed their history in connection with the Caspian Sea transgressions. Tatyana Kritskaya (Botanical Garden of Saratov University) together with colleagues from Kazakhstan and Germany, gave a lecture on the genetic diversity of *Tulipa suaveolens* (Liliaceae) and its evolutionary relationship with early cultivars of *T. gesneriana*. The session closed with the talk by Lyudmila Ozerova (MBG) on the genus *Kleinia* (Asteraceae) anatomy, morphology, and taxonomy and its representation in the collection of MBG.

The second day of the conference opened with a plenary session with four lectures. The lecture by Natalya Kovtonyuk (Central Siberian Botanical Garden (CSBG), Novosibirsk) was devoted to A.K. Skvortsov's contribution to the development of herbarium practices with examples from the CSBG collections. Maria Khoreva's (Institute of Biological Problems of the North, Magadan) talk was about the type specimens of vascular plants kept in the Herbarium of the Biological Problems of the North Institute. Michail Ignatov (MBG, MSU) gave a presentation on the problems of moss phylogeny and taxonomy studies under an enigmatic heading "How to rely on the unreliable?" The plenary session was closed with a bright talk by Gabor Sramkó ("Lendulet" Res. Group, Debrecen, Hungary) on the evolutionary history of the genus *Pulsatilla* (Ranunculaceae) which highlighted the history of the appearance and spread of Eurasian grasslands.

The first session of the day continued with talks on rare and endemic species, but also included talks on flora studies. It started with two talks from Hungarian colleagues ("Lendulet" Research Group, University of Debrecen), Sándor Jordán on a phylogenomic study of the Carpathians endemic *Pulsatilla subslavica*, and Lajos Szatmári, who presented a zoological talk on conservation genetics of Pannonian populations of European ground-squirrel, *Spermophilus citellus* (Rodentia, Sciuridae). Next Vladimir Fedosov (MSU), together with colleagues from MBG, presented new data on hidden diversity of mosses in Russia, thus starting discussions on flora studies. Then Anna Shkurko (MBG), together with E. Korolkova of MPSU continued the discussion on moss flora with a talk on the occurrence of *Sphagnum*

medium in Russia. The session closed with the talk by Marina Kazakova (Ryazan State University) on an overview of native flora of the Oka River Basin, the northernmost area of steppe flora.

The last session of the conference was devoted to discussing alien, invasive, weedy, and quarantine plant species. It started with a talk by Natalya Reshetnikova and Andrey Shcherbakov (MBG, MSU) on polemochores. They discussed the distribution of Middle European plant species in Middle Russia at their eastern range limits, which are strongly correlated with the disposition of German forces during the WWII. It was followed by Yulia Vinogradova (MBG) talk on microevolutionary changes in species of the *Solidago* section *Triplinervae* during their secondary range formations and establishing them as invasive aliens. The session closed with the talk by colleagues from Saratov (jointly with MBG) on genetic polymorphism in *Chondrilla* (Asteraceae) species in European Russia and the nature of highly polymorphic facultative apomictic species *Chondrilla juncea* presented by Tatyana Kritskaya. After a general discussion which continued further during a friendly dinner, the conference was closed. The next day conference participants took part in an excursion to the new greenhouse of the Tsisin Main Botanical Garden and, after lunch, attended nomenclature lectures given by Irina Belyaeva (RBG, Kew).

More information about this conference can be found in the paper published by Ivan Schanzer and colleagues (Schanzer *et al.*, 2020).

The following conference abstracts were translated from Russian by Irina Kadis (Boston, US).

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PLENARY SESSION

Collections by A.K. Skvortsov in the Herbarium of Tsitsin Main Botanical Garden

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In the fall of 1966, A.K. Skvortsov was invited by the Director of the Main Botanical Garden (MBG) of the USSR Acad. Sci., N.V. Tsitsin to lead the MBG Herbarium, while keeping his appointment at the Moscow University. From that time on, up until 2003, A.K. Skvortsov was leading the scientific work at our herbarium (MHA). While at the time of his arrival the total herbarium holdings amounted to about 60 thousand sheets, during his tenure the collection grew to 560 thousand. Skvortsov’s own enormous contribution constituted more than 80 thousand specimens (Belyaeva *et al.*, 2008) originating from the entire USSR territory, Europe, North America, India, and China. Most of these samples are filed within pertaining parts of the major collection; others kept separately as A.K. Skvortsov’s Herbarium; a third group of samples are authentic specimens in the type collection.

The major part of the collection bearing Skvortsov’s name are samples from temperate European Russia, the Lower Volga, and also systematic collections of the families Salicaceae, Betulaceae, and Onagraceae – the groups he was most interested in. This collection amounts to a total of more than 40 thousand sheets, its most bulky part (ca. 15.5 thousand) representing the flora of temperate European Russia and the Lower Volga. Among certain taxonomic groups, the genus *Salix* is the one represented the best (more than 15 thousand specimens). The willow collection is further divided in three regional parts. The first one generally matches the region covered in Skvortsov’s monograph of 1968: the territory of the USSR, surrounding European countries, Near East, and Mongolia. The organization of sections within this

collection matches the one accepted in the monograph. This part of the herbarium contains some 9,730 sheets, more than one-third of which are Skvortsov's own collections. The second part of the willow collection constitutes specimens from East and Southeast Asia: India with the Indian Himalayas, China, Korea, and Japan; whereas the third part comprises about 5 thousand specimens from the New World.

The genus *Populus* L. collection contains 4,070 sheets, mostly Skvortsov's own collections from various regions of the USSR, US, and Indian Himalayas. He kept working on systematizing these holdings till his very last days, and this job was left unfinished. The collection for the genus *Betula* (about 5 thousand sheets) comprises nearly all known species. It is organized by section in accordance with Skvortsov's system of the genus described in his works (1997; 2002). Onagraceae are mostly represented by the genus *Epilobium* Dill. ex L. (3,835 sheets).

The Type Collection of MHA includes 120 specimens collected by Skvortsov, of which 90 are type specimens representing 25 taxa described by Skvortsov alone or with co-authors; the rest of his authentic specimens, 11 sheets, have been used by other authors for description of taxa. These are *Aconitum taigicola* Vorosch., *Allium decipiens* Fisch. ex Schult. & Schult. f. subsp. *quercetorum* Seregin, *Festuca pallidula* E.B.Alexeev, *Glyceria acutiuscula* H.Scholz, *Oxytropis baschkiriensis* Knjaz. subsp. *skvortsovii* Knjaz., *Salix acutifolia* Willd. f. *pendula* I.V.Belyaeva, N.Yu.Stepanova & O.V.Epanch., and *Typha valentinii* Mavrodiev. Nineteen more, even though they are not type specimens, are valuable as authentic material (Sokolova, 2018: 6–7).

Ten taxa have been named in honour of Alexei K. Skvortsov. Type material for some of these taxa is preserved at the MHA: *Astragalus skvortsovii* Sytin & L.V.Rjaz., *Circaea* × *skvortsovii* Boufford, *Festuca skvortsovii* E.B.Alexeev, *Legousia skvortsovii* Proskur., *Oxytropis baschkiriensis* Knjaz., subsp. *skvortsovii* Knjaz., *Poa alexejii* Sofeikova & Vorosch., *Potamogeton skvortsovii* Klinkova, *Salix alexii-skvortzovii* A.P.Khokhr.

Additional information can be found in the paper by Stepanova and colleagues (Stepanova *et al.*, 2020).

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A.K. Skvortsov's contribution to the advancement of herbarium collection:

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A.K. Skvortsov is renowned among botanists both in this country and abroad as a high-ranking specialist in plant systematics, the study of floras, and plant introduction and acclimation. Scientific interests and professional activities of A.K. Skvortsov included the flora of Eastern Europe; systematics of the genera *Salix* L., *Betula* L., *Populus* L., and *Epilobium* Dill. ex L.; general problems of the evolutionary theory, intraspecific variability, and microevolution.

A.K. Skvortsov made a large contribution to the development of herbarium collections. Within the span of 65 years (1938–2002) he was collecting during expeditions and field trips across Russia, Europe, North America, India, and China, his collections amounting to more than 50 thousand sheets. From 1966, Skvortsov curated the herbarium collection at the Main Botanic Garden RAN (MHA). Here his personal herbarium collection is preserved along with his specimens from temperate European Russia and Lower Volga and separate collections of willows, poplars, and birches.

The book by Skvortsov *Herbarium methods and techniques. A manual* (1977) has served a handbook for a few generations of botanists. A.K. Skvortsov defined the role of herbaria in the contemporary science as follows: “Full and reliable data on dynamics of the flora in any concrete country during a certain time period can be provided only by the herbarium collection. Versatile application potential, multi-functionality is an essential, immensely important quality of a herbarium specimen. With the advancement of science, it is possible to extract more and more information from the same herbarium specimen. Hence a herbarium specimen is a primary, authentic document that cannot be substituted by any secondary, derived kind of documentation (Skvortsov 1977: 4).

Specimens exchange is an old tradition of botanical institutions. In his description of the Herbarium of the Main Botanical Garden in Moscow (2005), Skvortsov listed the major external sources of its enhancement, which included the Central Siberian Botanical Garden, Siberian Branch RAN (CSBG SB RAN) in Novosibirsk, from where MHA had received more than 13 thousand specimens collected in different parts of Siberia. At the same time, the Central Siberian Botanical Garden was receiving material from the Moscow Main Botanical Garden in exchange.

The herbarium collections of the CSBG SB RAN (NS and NSK) currently amount to about 680 thousand specimens of vascular plants. Of these, 37 500 have been scanned with *ObjectScan* 1600 (Microtek) scanners, in accordance with the international standards: optical resolution of 600 dpi, scans accompanied with the color guide and linear scale ruler, barcoded, and preserved within the online Virtual Herbarium at the open-access site of the CSBG SB RAN (<http://herb.csbg.nsc.ru:8081>). Information extracted from herbarium labels and scans of herbarium specimens are also published as seven datasets on the GBIF (Global Botanical Information Facility) Portal (gbif.org). Among the scanned specimens, there are collections by A.K. Skvortsov from Volgograd, Belgorod, Bryansk, Smolensk, Oryol, Moscow, Sverdlovsk, and Amur regions as well as Crimea and Karachay-Cherkess Republic, Estonia, Armenia, Georgia, Ukraine, Kazakhstan, Sweden, India – a total of 450 sheets received through the exchange between the MHA and NS/NSK. A.K. Skvortsov made an invaluable contribution to the herbarium business development.

**Type specimens of vascular plants in the Herbarium at the Institute of Biological
Problems of the North, Far East Branch RAN, Magadan**

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The Herbarium of the Institute of Biological Problems of the North (IBPN), Far East Branch RAS was founded in 1971 by A.P. Khokhryakov. The collection representing more than 1,700 species of vascular plants comprises more than 110 thousand specimens. Collections from Magadan Region, Chukotka, and other regions of the Russian Northeast constitute the major part of the holdings. At the initiative of A.N. Berkutenko, the international code MAG was assigned to the Herbarium in 1997.

Flora of Magadan Region comprises about 1,450 species and subspecies (*Flora...*, 2010). More than 80 taxa of vascular plants have been described from its territory (excluding Chukotka Autonomous District). Of these, more than 40 species and subspecies were described by A.P. Khokhryakov, either alone or with co-authors. About 40 more taxa were described from this territory by V.N. Vasilyev, P.G. Gorovoy, B.N. Gorodkov, N.S. Probatova, M.T. Mazurenko, A.K. Skvortsov, N.N. Tsvelev, B.A. Yurtsev, and others.

In 2014–2015, the authors undertook a search for type specimens within MAG holdings. Duplicates of type specimens (isotypes) as well as paratypes and collections from loci classici (topotypes) were segregated in dedicated folders. We located type specimens for 32 species and subspecies of vascular plants from Magadan Region described, for the most part, by A.P. Khokhryakov, of which 20 taxa are also represented by isotypes, 10 by isotypes and paratypes, and two more by paratypes (Mochalova, Khoreva, 2015). Photographs of type specimens as well as those of some native species in their natural habitats are displayed at the site of IBPN FEB RAS (www.ibpn.ru).

In course of everyday herbarium work and through comparisons with the Virtual Herbarium of the Moscow State University <https://plant.depo.msu.ru/> holdings, the authors were able to discover additional type material for some 10 taxa, although some cases appear doubtful due to differences with the text of the protologue.

Four nothospecies from the hybridogeneous genus *×Elyhordeum* Mansf. ex Tsitsin & K.A.Petrova have been described from the territory of Magadan Region by D.S. Lysenko (Lysenko, 2010). MAG Herbarium was listed as the depository for the type material. The

author placed his collections in separate folders, a few sheets for each nothospecies. None of the sheets, however, were designated as pertaining holotypes. During our consultation with I.V. Belyaeva, she pointed out that the names, *×Elyhordeum detrinense* Lysenko, *×E. khokhrjakovii* Lysenko, *×E. olaense* Lysenko, and *×E. sinegoricum* Lysenko, were invalidly published as the descriptions were provided only in Russian, hence the necessity of their validation in a publication (Khoreva, 2020). Therefore, four holotypes of *×Elyhordeum* are deposited in MAG.

As of today, within the MAG herbarium holdings, the authors have located type specimens of 42 taxa of vascular plants at the rank of species or subspecies along with types of five nothospecies. We analyzed the listing of taxa described from Magadan Region, which includes 82 names, and the collection was searched for all taxa, which were likely to be represented by duplicate type material.

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Evolutionary history of the genus *Pulsatilla* Mill. (Ranunculaceae) informs us about the history of Eurasian grasslands

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Pasque-flowers (genus *Pulsatilla* Mill.) are typical grassland plants that are distributed in the Holarctic Region. As all species in the genus show pronounced preference to non-arboreal habitats (such as grasslands or rocky outcrops), their evolutionary history should mirror the biogeographic history of grasslands. While relying on the recently published molecular-phylogenetic study (Sramkó *et al.*, 2019; DOI: 10.1016/j.ympev.2019.02.015), I examine the evolutionary history of this grassland genus in connection with the development of the Eurasian steppe zone.

Similar to the situation in other steppic genera, a single lineage, sister to the rest of *Pulsatilla* is confined to Central Asian high mountains (Pamir-Alay). It contains the monotypic subgenus *Kostyczewianae* with the species *P. kostyczewii* (Korsh.) Juz. This hints at the Central Asian origin of the genus, which became separated from the sister genus *Anemone* (*s.str.*) during the Early to Middle Miocene, ca. 23 to 10 million years ago (Mya). This chronology is in line with our current knowledge of the aridification process in northern Eurasia. The development of the Central Asian high mountain ranges and subsequent change of the Asian monsoon regime must have acted as ecological triggers for the evolution of this grassland genus from the ancestors inhabiting arboreal plant communities (i.e., *Anemone s.str.*).

Later on, during the Middle or Late Miocene, another lineage was separated from the rest of the genus, namely, subgenus *Preonanthus*. These plants are now confined to cold habitats, either at high altitudes (the European high mountains and Rocky Mountains of North America) or high latitudes (tundra regions in the Far East). These can be connected with the first expansion of grasslands in the Middle Miocene typified by the ‘Pikermian chronobiome’, an assemblage of grassland-inhabiting ancient mammals. Remarkably, the relicts of this biome are confined to cold climatic conditions, which probably reflects the environment in which this Middle Miocene biome once existed.

The rest of the genus (i.e., subgenus *Pulsatilla*, which contains the majority of species) appears to have originated from an eastern Eurasian centre, as arid conditions probably

persisted there during the Late Miocene. It became more widespread later, during the Quaternary Period, the time of the largest expansion of the grasslands. The high species number in this subgenus must be connected with the periodical climate cycles of the Pleistocene, which drove the speciation throughout the grasslands of Eurasia. Starting from ca. 2.9 Mya on, at least five lineages (ranked as series within the section *Pulsatilla*) diversified from the Far East ancestors represented by section *Tatewakianae*. Of these, only a single lineage, series *Patentes*, reached North America once again, as a second line of colonisers represented by *P. patens* (L.) Mill. subsp. *nuttalliana* (DC.) ined.

Further understanding of evolutionary relationships within section *Pulsatilla* is of utmost importance for a better understanding of the grassland evolution in the Quaternary Period. We will try to gain insight into this relationship using a genomic approach.

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SALICOLOGY SESSION

Determination of hybrid formulae for a few willows (*Salix* L.) using ovule numbers

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Identification of hybrids and species of willows is not always possible. J. Chmelař (1977) stated that the number of ovules in the ovaries of a hybrid is equal to one-half of the sum of ovule numbers characteristic of the parents. The male parent carries and transfers to the progeny the information regarding the number of ovules in the species it belongs to; therefore, for the calculation purpose, one should use the number of ovules characteristic of female individuals in the pertinent species. We have been calling this calculation Chmelař's Method. Chmelař, as well as others, (Valyagina-Malyutina, 2004; Argus, 2010; Marchenko, 2019; He, 2019) determined and listed minimal and maximal

number of ovules in ovaries for each described species. We believe this approach should become a standard for any researcher describing a willow taxon. While using Chmelař's Method (Chmelař 1977) further developed by one of the authors (Marchenko, 2019), we determined or confirmed the origin of a few hybridogenous ornamental cultivars as well as willow hybrids employed as bioenergy crops in the United States.

Salix × cottetii Lager ex A.Kern. was described by A. Kerner in 1864 as a hybrid of *S. myrsinifolia* Salisb. and *S. retusa* L. Kuzovkina *et al.* (2016) suggested that a few different taxa may exist under this name. We determined the number of ovules for *S. myrsinifolia* (10–15) and *S. retusa* (6–9) as well as for the prostrate hybrid *S. × cottetii* (8–12) from the willow collection of the Botanical Garden in Yekaterinburg. Our findings regarding the ovule quantity confirmed the fact that this hybrid was originating from the two listed parents (the plants exhibited morphological characters intermediate between the two). At the same time, the upright shrubs grown in the United States under the name *S. × cottetii* 'The Banker' and studied by us could be clearly sorted into two groups: one with the number of ovules 12–18; the other with 16–23. The former group is approximating *S. eriocephala* Michx. (12–16) both by the ovule numbers and morphological characters. The latter group might include hybrids between *S. eriocephala* (12–16) and *S. tweedyi* (Bebb) C.R.Ball (18–30).

Our determination of the number of ovules in three specimens with normal and branched catkins has confirmed the origin of the cultivar *Salix* 'The Hague' (syn. *S. hagensis*) as the progeny of *S. caprea* L. (12–18) and *S. gracilistyla* Miq. (4–6). While all three specimens belonged to the same hybrid combination, those with branched catkins had the ovule number 6–10, while those with unbranched catkins – 8–12.

We analyzed a confusing group, identified in the United States under the name *Salix pentandra* L., which have been also identified as *S. × meyeriana* Rostk. (*S. pentandra* × *S. euxina* I.V.Belyaeva) (Zinovjev, 2011). Upon completing ovule counts of live and herbarium specimens, we concluded that there are plants belonging to four willow taxa in the United States under the name *S. pentandra*: *S. pentandra* (17–22); *S. serissima* Fernald (15–17); *S. × meyeriana* (11–16), a hybrid of *S. pentandra* and *S. fragilis* L. (6–6); and finally a formerly undescribed hybrid *S. serissima* × *S. fragilis* with the ovule count 8–12.

In the authors' opinion, the number of ovules is a reliable characteristic in addition to traditional morphological and contemporary molecular methods. It has to be employed

for identification of species and verification of hybrid parentage as well as for investigations in willow systematics.

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Population variability of willows from the section *Helix* Dumort. in Eastern Europe

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According to A.K. Skvortsov, the section *Helix* Dumort. (Salicaceae) is the most challenging of willow sections from the taxonomical point of view. Contemporary taxonomists do not have consensus as far as the magnitude of this section. Some believe that three species naturally occur across the European part of the former Soviet Union territory: *Salix purpurea* L. (*s.str.*), *S. vinogradovii* A.K.Skvortsov, and *S. elbrusensis* Boiss. Within the former USSR area, the range of *S. purpurea* (*s.str.*) embraces Lithuania, Latvia, Belarus, West Ukraine, Moldavia, and the Crimea. *S. vinogradovii* inhabits the forest-steppe and steppe zones of Ukraine and Russia, while *S. elbrusensis* is restricted to the Caucasus. However, not all authors agree with the distinctiveness of the three species: some believe that there is only one, *S.*

purpurea (*s.l.*), as the differences between plants placed in the three species refer to just their reproductive organs, while morphological characters of leaves and shoots show no difference.

Over many years, the author collected herbarium specimens of willows from sect. *Helix* within a vast area (from 42.28 to 57.33° N and from 21.00 to 44.08° E) and accumulated a total of 443 samples from 54 natural populations. Altitudes for collection localities varied from 2 to 1,370 meters a.s.l. All samples were taken during the time close to the end of the vegetation period (August to early October) from undisturbed individuals of purple willow. These were normally developed shoots from mid-crowns, at the height of about 1.5 meters from the ground.

In the sampled individuals, morphological characters of vegetative organs (length and diameter of shoots, length and width of leaves, length of petioles, and length of internodes) did not exhibit any statistically significant differences. According to the results of the univariate analysis of variance, none of the studied characters allows discrimination among species within the sect. *Helix*. Yet geographically remote populations appeared different. Plants from Zakarpatye (Trans-Carpathia) had the longest and thickest shoots as well as longest internodes and leaves. Plants from Crimea were found to have the shortest shoots, while their internodes and leaves also turned out significantly shorter than the average for the entire area. The average values for morphological parameters in the populations from the Baltic countries are similar to those in populations from Ukraine, differing only in somewhat smaller leaves.

Floodplain habitats found at altitudes not higher than 300 m a.s.l. and with rather high water flow rates appear to provide the most favorable conditions for the growth of *S. purpurea* (*s.l.*). In mountainous regions and also in habitats with stagnant soil moisture, the correlations of morphological characters grow. The coefficient of determination R^2_m for morphological characters in each population changes from 0.1–0.2 in optimal conditions to 0.4–0.5 and even higher in extreme situations, which is a symptom of the group stress signifying the increase of adaptational pressure on populations.

Significance of leaf morphological characters in diagnostics and taxonomy of black and white poplars (sections *Populus* and *Aigieros* Duby)

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The most widespread Eurasian poplars – *Populus alba* L., *P. tremula* L., and *P. nigra* L. – are included in two of the six sections recognized in the genus: sect. *Populus* and sect. *Aigieros* Duby. When sympatric, species belonging to the same section may freely cross and produce first-generation hybrids, which tend to last for decades due to vegetative reproduction via root suckers or shoot layering. Primary ranges of *P. nigra* and *P. deltoides* W.Bartram ex Marshall do not overlap; however, *P. deltoides* has been introduced to Eurasia and now produces fertile hybrids with *P. nigra*: *P. × canadensis* Moench. These hybrids may, in turn, hybridize with one of the parents, while somewhat altering their genome due to the crossing over during the meiosis. Assessments of extent of hybrids' spread present challenges. Hybrids are often assigned to one of the parent species, as the range of character variability in the species remains undefined.

Major morphological diagnostic characters may be divided in two groups: those of vegetative and reproductive domain. Reproductive characters can be further divided: those of flowers and fruits. Reproductive characters have been deemed most revealing and unambiguous. Vegetative ones are available for the most part of the phenological cycle, yet they are less helpful for identification. The reason for this is our poor understanding of intraspecific variability in poplars and their hybrids due to lack of analyses of representative material. Recommendations for collecting representative material are derived from the study of seasonal and ontogenetic heterophylly, that is, variations of leaf morphology along abbreviated and long shoots during the growing season (Critchfield, 1960; Eckenwalder, 1980c, 1996b, *pro parte*). The key for poplar identification using leaf morphology becomes more effective if one includes characters of leaves preformed in the fall inside buds (preformed, or early leaves) as well as of those newly formed during the late fall, closer to the end of the vegetation (neofomed, or late leaves). Yet lower, shaded branches in old trees often do not develop any late leaves, which may distort one's estimate of the extent of heterophylly within the species.

The authors made an attempt to collect and analyze a representative set of samples, while taking into account all the listed considerations along with some newly discovered facts: at early stages of ontogenesis, leaves may not yet have fully developed marginal glands, while already having fully developed trichomes; at the late ontogenetic stages, they may have glands, yet already lack trichomes (Feodorova, 2019). Attribution of samples to the species *P. nigra/P. deltoides*, *P. alba/P. tremula*, or their respective hybrids *P. × canadensis* or *P. × canescens* (Ait.) Sm. was rigorously controlled by species-specific markers based on NTS 5S ribosomal nucleus DNA for *P. nigra/P. deltoides* (Aleksandrov, Karlov, 2018) and *P. alba/P. tremula* (Aleksandrov, Karlov, 2019).

It has been found that equally important are leaves on both short and long shoots and those belonging to either of the two major categories, that is, “early” and “late.” It’s not only that the latter leaf categories differ in their shape and size, but also in shape and size of their marginal teeth, pubescence density, and presence/absence of basilaminar glands.

We have documented two major types of trichomes in species of *Populus*: glandular and eglandular. Glandular trichomes include marginal glands with epiglandular trichomes and basilaminar nectariferous glands. Eglandular trichomes can be needle-shaped (acicular) and ribbon-like. The needle-shaped (acicular) ones occur mostly on the abaxial midrib or sometimes on the entire abaxial surface. Needle-shaped trichomes can be of two types: long and short; those ribbon-like are flattened.

The pubescence and trichome types are characters of diagnostic significance. Short and long needle-like trichomes are characteristic of *P. nigra*; those short needle-like are typical for *P. deltoides* along with cilia at the margin. In the hybrid of the two, *P. × canadensis*, two types of trichomes occur: short and long needle-like, while marginal cilia are facultative. Basilaminar glands cannot be used for identification of the hybrid, as their occurrence overlaps in the involved species.

The other pair of species and their hybrid exhibit a different set of characters. Long ribbon-like trichomes are characteristic of *P. alba*, while *P. tremula* exhibits acicular ones. Their hybrid, *P. × canescens* is characterized by trichomes of two types: both acicular and ribbon-like. *P. × canescens* also appears to inherit the position of nectaries on the proximal leaves of short shoots from *P. tremula*, and this character may serve a good indicator of hybrid individuals in the field – along with the presence of basilaminar nectaries on the proximal leaves of short shoots.

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Influence of rainfall deficiency on shoot morphogenesis of *Salix alba* L. clones

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White willow, *Salix alba* L. is the type species of the genus *Salix* L. Wild biotypes and cultivars of *S. alba* are employed for establishment of ornamental and recreational as well as environmental plantings. White willow is known for high level of polymorphism in its populations. “Not only morphological traits in willows but also features of their physiology are persistent, genetically determined.” (Skvortsov, 1968: 54). White willow is an allotetraploid ($2n=76$). The elevated amount of genetic information is beneficial for the expansion and preservation of the species in the course of its evolutionary advancement; at the same time, this hampers the eco-genetical analysis of inheritance of economically important characters. In order to reduce the level of genetic variability in the course of an experiment, one can resort to inbreeding with subsequent cloning. White willow is a mesotrophic mesohydrophilous alluvial (floodplain) species with the range of Eurasiatic Type, Boreal-Mediterranean Subtype. In

cultivation, it may grow in versatile edapho-hydrological situations and well outside its primary geographic range.

Due to recent climate changes, atmospheric drought episodes have become more frequent. In order to create white willow plantings that would be capable of steady growth in the situations of precipitation deficit, one must analyze the dynamics of shoot growth in this tree. “Genetic differences in developmental rhythms as well as morphological characters may be also inherited purely individually.” (Skvortsov, 1968: 55).

The work was aimed to investigate the seasonal dynamics of the willow shoot elongation during the brief episode of early summer atmospheric drought in 2019.

Materials and methods

Model clones of *S. alba* produced by the author as a result of inbreeding and grown in the Salicetum of the Bryansk State University served the object of the study. The coordinates of the Salicetum are 53.273180 N, 34.353152 E; the type of forest habitat is determined as D3, that is, mesohygrophilous oak forest, according to the classification traditionally employed in the Russian forestry (Pogrebnyak, 1955). Elongating annotinous shoots on the second-year clones were used in the study. The extent of steady growth was determined through measurement of the metamer formation rate $\Delta N(t)$ and internode length increments $\Delta I(t)$.

Results

The clones of white willow created by the author demonstrated high productivity in an adverse agro-meteorological situation, their shoot length varying from 220 to 290 cm and more. Seasonal dynamics of shoot growth is of cyclical character. It is determined by the endogenous interdependent rhythms $\Delta N(t)$ and $\Delta I(t)$. A short-term atmospheric drought may play a role of external phase synchronizer for growth fluctuations with a period of 27 and 18 days.

Module architecture of crowns in boreal species of *Salix* L.

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In our work we rely on the classification of the genus *Salix* proposed by A.K. Skvortsov (1968). We have analyzed 16 boreal species from 3 subgenera: subg. *Salix* (*S. pentandra* L., *S. triandra* L., *S. euxina* I.V.Belyaeva, *S. alba* L.), *Vetrix* (*S. aurita* L., *S. caprea* L., *S. cinerea*

L., *S. starkeana* Willd., *S. myrsinifolia* Salisb., *S. viminalis* L., *S. gmelinii* Pall., *S. lapponum* L., *S. acutifolia* Willd., *S. rosmarinifolia* L., *S. vinogradovii* A.K.Skvortsov), and *Chamaetia* (*S. myrtilloides* L.).

Within the crowns of the studied species, we recognized the following modular elements: a) in trees and tall shrubs: 1 – metamer, 2 – single-axis shoot, 3 – three-year old shoot system, 4 – limb originating from trunk (in trees and medium-sized to tall shrubs), 5 – crown in its entirety; b) in low shrubs: 1 – metamer, 2 – single-axis shoot, 3 – three-year old shoot system, 4 – crown in its entirety. Each module is represented by variants, which are organized into a hierarchic system.

According to their function, annotinous shoots can be vegetative or reproductive. 12 variants of vegetative shoots and shoot systems have been identified within crowns of the studied species. Trees and tall shrubs exhibit the most diversity of vegetative shoot types (8–10 variants). Minimal diversity in vegetative shoots was observed in low shrubs (5–7 variants). The duration of shoot systems' developmental phases diminishes in shrubs, as compared with that in trees. This happens due to diminished numbers and lengths of shoots and shoot systems in shrubs.

Diversity in vegetative shoots and shoot systems should be attributed to polyvariance in metamers. An annotinous vegetative shoot may consist of a few variants of metamers (synonymous to 'elementary modules' in Savinykh, 2000). The 13 variants of metamers that we have identified differ from each other by the number of internodes, structure of axillary buds, and by presence/absence of sylleptic or proleptic shoots that can develop from these buds.

As to reproductive shoots, we have described 9 variants of these: 3 developing from regeneration buds (those abscising as a whole, those abscising in two parts, and the so-called never abscising); 5 variants of sylleptic shoots, and proleptic ones. The proximal, leafy parts of those reproductive shoots abscising in two steps keep photosynthesizing in the crown together with the two-year-old shoots until the fall. Therefore, according to their function and period of existence, in our opinion, they are identical to sylleptic vegetative shoots.

The major structural unit in the crown of the studied willow species is a three-year old shoot system, which we consider an architectural module. We identified 7 types of architectural modules, while taking into account the following characters: the rate of abscission of the distal metamers of shoots, type of branching, and longevity of vegetative parts in reproductive shoots.

Of the 7 recognized modules, 3 occur in trees and tall shrubs, 2 in medium-sized shrubs, and 2 more in low shrubs.

TAXONOMY AND PHYLOGENY SESSION

Sexual dimorphism in *Hippophae rhamnoides* L.: a system of populations with different evolutionary histories

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Sea buckthorn is a dioecious shrub or small tree exhibiting high morphological, biochemical, and physiological diversity. High economic importance of this plant stimulates constant interest toward studies of various aspects of its biology. One direction is a study of the sexual dimorphism. Differences between male and female individuals have been noticed with regards to the plants' biochemistry and physiology as well as their growth processes. At the same time, data on morphological differences between the sexes appear rather controversial. Another important consideration is that the selection has been, among other directions, aimed toward obtaining less spiny plants. Here the author summarizes own observations on morphological differentiation in native populations of Mongolian sea buckthorn *Hippophae rhamnoides* L. ssp. *mongolica* Rousi.

The author observed statistically significant differences in the length of spines and their density between male and female plants. Besides, there are differences in variability of these characters. It is not just that male plants may produce longer spines, but also the variability range in spine length and density is greater in males. This may be well considered a manifestation of an adaptive compromise.

While within the entire dataset this general trend in morphology is well pronounced, this is not true for some populations. The analysis of spatial distribution of the populations shows that they form two clusters. Juxtaposition of morphological variability data and the results of the molecular-ecological analysis lets one conclude that the morphological sexual dimorphism is most pronounced in the populations found within alleged areas of ice-age refugia and also that the morphological differences between male and female plants are differently pronounced in either of the two major ecotypes of Mongolian buckthorn. Another interesting observation is that the genetic diversity appears to diminish, while morphological diversity, on the contrary, appears to grow within the plant's expansion area.

Taxonomy of East European feather grasses (*Stipa* L.):

molecular data vs. traditional views

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Until very recently, the taxonomic system for feather grasses has been based on exclusively morphological characters. As a result of numerous taxonomic studies of feather grasses undertaken in the later half of the 20th century, the system of the genus has attained more or less actual look, although it was being corrected until 2010s. According to the latest views, within the European part of Russia (excluding the Crimea) there occur 10 species of feather grasses representing 3 sections of the genus: sect. *Stipa* – *Stipa adoxa* Klokov & Osychnyuk, *S. dasyphylla* (Lindem.) Czern. ex Trautv., *S. pennata* L., *S. pulcherrima* K.Koch, *S. tirsia* Steven, *S. ucrainica* P.A.Smirn., and *S. zaleskii* Wilensky ex Grossh.; sect. *Subbarbatae* – *S. lessingiana* Trin. & Rupr.; sect. *Leiostipa* – *S. capillata* L. and *S. sareptana* A.K.Becker.

During the expansion of molecular-genetic methods, there came the turn for studies on feather grasses; however, the methods most often employed with other plant groups – analyses of DNA sequences – so far have not been productive, as they failed to satisfactorily solve the relationships between species distinguished while relying on morphological characters. The chloroplast DNA has been shown to be the most uniform. According to the results of the author's analysis of the loci *trnL-trnF*, *psbA-trnH*, and *ndhC-trnV*, the sequences are completely identical in *S. dasyphylla*, *S. ucrainica*, *S. zaleskii*, and *S. capillata*, that is, even in species from different sections of the genus. The most recent literature data match our results: complete chloroplast genome sequencing of 19 *Stipa* species has demonstrated that the level of similarity in their plastomes is about 99.7%.

As compared with the chloroplast markers, the commonly used locus of nuclear DNA ITS 1–2 yields a better resolution; besides, the clades formed this way well match the division of the genus into sections. As far as the species occurring within the European part of Russia, according to the author's data, a full match is observed. Yet the relationships between species within each clade are not clarified.

A considerable number of works applying molecular-genetic studies to feather grasses have been based on micro-evolutionary techniques (SSR, ISSR, AFLP, etc.). These methods

are sensitive enough to catch differences between closely related species or even between populations, yet due to the high level of homoplasy, they cannot be employed for the phylogenetic reconstruction of such a large genus as *Stipa*.

While considering the existing incongruence between the morphological and molecular data, the author does not see the solution of the problem in merging the morphologically defined species into a few large species-aggregates – the way it has been done for the phylogenetic trees available today. The best approach to further phylogenetic studies in *Stipa* appears to be a search for new markers within nDNA. Judging from the most recently published results, the region IGS may turn out to be the most perspective locus.

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**On the way to the taxonomic revision of *Trollius* L. (Ranunculaceae)
in Asiatic Russia**

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The genus *Trollius* L., globeflower, according to various assessments, includes 30 to 35 species of perennial herbs, which are distributed exclusively in non-tropical areas of the Northern Hemisphere, mostly in mountainous regions within forest-steppe, subalpine, and alpine belts (including mountain tundra and barren heights). On the plain, species of *Trollius* tend to be associated with moist and wet habitats, often growing in floodplain meadows and at streamsides.

The uncertainty regarding the number of species of in the genus *Trollius* can be attributed to:

- *inadequate knowledge regarding variability ranges of a number of species;
- *unsatisfactory understanding of species' ranges within the Asiatic part of Russia;
- *unlimited opportunities for practically all species to hybridize, including plants in the natural habitats;
- *inclusion of *Trollius lilacinus* Bunge and *T. chartosepalus* Schipcz. (sometimes also *T. komarovii* Pachom.) in another genus, *Hegemone* Bunge;

*lack of any authoritative, fundamental monograph of the genus: the work by Alina Doroszewska (1974) is not free of a number of serious drawbacks.

The author has been studying the taxonomy and geography of the genus *Trollius* since 2013. As of today, all *Trollius* authentic specimens preserved at LE and also some type specimens from P, K, and SYKO have been thoroughly examined; herbarium collections at LE, MW, MHA, and MWG have been revised; material has been collected from the Tunka Valley (Buryatia) and the northern macro-slope of the Khamar-Daban Range (Baikal Nature Reserve); observations made back in 1981–1992 in a number of Siberian regions (Altai, Kuznetsk Alatau, Eastern Sayan, etc.) have been analyzed. Molecular-genetic work has been initiated for *Trollius chinensis* Bunge and *T. asiaticus* L. as well as their hybrid populations.

Special attention has been paid to the study of natural populations of *Trollius* species. The author produced geobotanical descriptions of plant communities in which species of the genus *Trollius* occur. Other essential parts of the study include a critical analysis of morphological characters traditionally employed for identification of species; description of variability ranges of some most important species; analysis of geographic ranges of species having the widest distribution within the Asiatic part of Russia; identification of the most important species contact zones and hybridization; and comments on the most current listing of *Trollius* species for the entire Russian territory.

Geographic trends in *Dorycnium* Mill. (Fabaceae) phylogeny

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Sections *Lotus*, *Dorycnium*, and *Bonjeanea* belong to the northern evolutionary branch of the genus *Lotus* L., the latter two often segregated in a separate genus *Dorycnium* Mill. An unambiguous distinction between the genera *Lotus* and *Dorycnium* on the basis of only morphological characters was not possible due to the existence of a number of species whose morphology includes characters typical for both genera. The phylogenesis patterns of this group obtained by studying individual DNA markers in a limited sample were also contradictory. We are presenting the results of a new phylogenetic study of the genus *Lotus* (incl. *Dorycnium*) with an expanded representation of the *Dorycnium* and *Bonjeanea* sections, carried out using ITS-1-2 nDNA and *trnL-F* cpDNA. Specimens from ANK, GAZI, ISTE, LE, MA, MHA, MW, P as well as the authors' own collections have served the material for the

study. Major emphasis was laid on the *Lotus dorycnium* L. (= *Dorycnium pentaphyllum* Scop.) complex, whose maximal taxonomic diversity has been encountered in Asia Minor.

According to the results of phylogenetic analysis based on ITS and *trnL-F* and using the Bayesian approach and the maximum likelihood method, there is a geographical structure of genetic variation among the members of the *Bonjeanea* section in the Mediterranean Region, namely *Lotus hirsutus* L., *L. rectus* L., and *L. strictus* Fisch. & C.A.Mey. Within the range of each species, a geographical subdivision into western and eastern parts was observed, which was most clearly manifested when using the ITS marker.

The *L. dorycnium* complex from the sect. *Dorycnium* forms a separate clade with the ITS marker, yet at the same time cannot be separated from *L. hirsutus* from sect. *Bonjeanea* when the analyses are performed with the plastid marker *trnL-F*. The latter conclusion is confirmed by phylogenetic analysis of a set of four plastid markers (*psbA-trnH*, intron *rps16*, intron *trnL*, and *trnL-F*) in a more limited sample. Separation of taxa within the *L. dorycnium* complex has not been possible, except the segregation of *L. herbaceus* (Vill.) Jauzein with the ITS marker. Certain clades within this complex are separated by geographical and /or taxonomic characteristics: the West European Clade with dominance of *L. dorycnium* ssp. *gracile* (ITS, *trnL-F*), Central European Clade with dominance of *L. germanicus* (Grelli) Peruzzi (ITS), Turkish one with dominance of *L. dorycnium* ssp. *anatolicum* (*trnL-F*), and the clade embracing the two Turkish subspecies of *L. dorycnium*: ssp. *anatolicum* and ssp. *haussknechtii* (ITS). The tree built with *trnL-F* is characterized by low resolution capacity – the difficulty that might be overcome in the future by using more loci of cpDNA. Discriminant analysis of *L. dorycnium* complex according to 16 quantitative morphological characters has resulted in segregating three groups: 1. *L. dorycnium* ssp. *anatolicum*, 2. *L. germanicus* and *L. dorycnium* ssp. *haussknechtii*, 3. *L. herbaceus*, *L. dorycnium*, and most of *D. intermedium* Ledeb. Inclusion of additional qualitative characters in the analysis would allow us to better characterize the taxa within the complex and potentially divide any mixed groups.

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The problem of species in the genus *Paeonia* L.

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The growing interest towards applications of molecular-phylogenetic data for the purpose of determination of the rate and specifics of diversification within various plant groups can be readily attributed to the urgent need for understanding those processes which have resulted in the observed plant diversity, as this is critical for making decisions regarding the size and rank of any taxa. One of the taxonomic groups for which the task of determination of species limits has been an urgent necessity is the genus *Paeonia* L.

Despite the relatively modest number of recognized species (35), there are still many gaps in our understanding of the species' limits. One explanation for this is that the morphological characters analyzed by taxonomists, such as the plant size, shape of leaf segments, extent of leaf blade division, type of leaf pubescence, perianth color, length of peduncle, extent of follicle pubescence, or angle at which follicles deviate, are not quite discrete. In addition to that, the high morphological diversity is exacerbated by the presence of polyploid forms and hybridization processes. Along with such distinct species as, for example, *P. lactiflora* Pall., there exist a number of species-complexes, such as *P. anomala* L., *P. obovata* Maxim., or *P. tenuifolia* L.

According to our previous results of analyses of ITS and ETS nucleotide sequences of nrDNA, which were based on large population samples of species (*P. lactiflora*) and species-complexes (*P. anomala*, *P. obovata*, *P. tenuifolia*), the genetic structure in the genus *Paeonia* appears to be highly versatile. The variability is due to mutations rather than substitutions. These mutations are pronounced as polymorphic sites that include two types of nucleotides. On many occasions, the presence of additional nucleotides cannot be attributed to hybridization processes. Clusters segregated using the ITS and ETS data constitute species (*P. intermedia* C.A.Mey., *P. hybrida* Pall., *P. anomala*, and *P. lactiflora*), groups of species (species-complexes *P. obovata*, *P. tenuifolia*, *P. intermedia* + *P. hybrida*), and also divisions within species-complexes *P. obovata* and *P. tenuifolia* that correspond more to the geographic distribution than to any morphological data. Results of complete plastid genome sequencing undertaken by us were similar to those obtained by Dong et al. (2018). They demonstrated a

low level of nucleotide sequences divergence among *Paeonia* species. Presumably, the observed species diversity in the genus *Paeonia* constitutes a result of contemporary diversification, so that the species limits are still in the process of formation at the molecular as well as morphological level. We may naturally expect some indistinctness and uncertainty when defining limits of young species. In this situation, understanding species in a wide sense appears well justified, while extremes of phenotypic variability could be treated in the rank of a form, so that the information regarding species' biology is not lost.

Modern species systematics of parasitoid Hymenoptera: the beginning of a taxonomic revolution?

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Harboring more than a million potentially recognizable species, parasitoid Hymenoptera are one of the most diverse, taxonomically complicated, and economically important groups of insects. However, high morphological similarity of related forms along with strong host-induced variation and sexual dimorphism of many parasitic wasps make species recognition and delimitation in this group extremely difficult. This apparently means that most obvious morphological differences between closely related species of parasitoid Hymenoptera usually can be attributed to genetic variation, whereas the main part of the intraspecific variation observed in these insects is caused by environmental modification of morphological traits.

If the above-mentioned assumptions are true, the taxonomic study of parasitic wasps at the species level must necessarily be conducted within the framework of the so-called integrative taxonomy, i.e., a combination of approaches and techniques aimed at detection, delimitation, and description of closely related species. Specifically, molecular, chromosomal, and other analogous approaches become highly significant in this case, because they can differentiate between the genetic variation and that induced by the environment. Moreover, the combined use of these techniques often has a synergistic effect on detection and discrimination of cryptic parasitoid taxa. The morphological study of parasitic wasps is therefore considered an important, although not decisive stage of analysis of their taxonomic diversity. In particular,

each known morphospecies of parasitoid Hymenoptera can be subjected to a thorough study aiming at detection of cryptic taxa using modern techniques.

During recent years, successful application of this approach to certain taxa of parasitic wasps has already led to a dramatic increase in species numbers. In fact, this process can be defined as a kind of ‘taxonomic revolution’ currently taking place in this group. This is especially true for the faunistic research of greatly understudied territories, e.g., tropical regions, where the so-called turbo-taxonomic approach appears to be extremely effective. At the same time, recent studies showed that apparently well-known faunas of temperate zones also contain many cryptic species of parasitic wasps. For example, several groups of synanthropic parasitoids of stored product pests harbor previously unknown cosmopolitan species, e.g., *Anisopteromalus quinarius* Gokhman & Baur, 2014 (Pteromalidae).

All accumulated data therefore strongly suggest that the presence of cryptic taxa within widespread morphospecies of parasitic wasps is a universal rule. In other words, when adequate material for a given group is collected and analyzed by relevant methods, detection of cryptic species within this group becomes almost inevitable. In turn, the above-mentioned ‘taxonomic revolution’ also leads to a serious reconsideration of ecological characteristics of many parasitoid species.

RARE AND ENDEMIC SPECIES AND FLORAE STUDIES SESSION

Hiding in plain sight: a new taxon within the mega-genus *Salvia* L. in Eastern Europe

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Salvia L. (*Lamiaceae*) is an evolutionarily successful genus, as reflected by its worldwide distribution and high diversity (it encompasses over 900 species). The genus is characterised by having two stamens instead of four found in other members of the tribe *Mentheae*. Each of the two stamens has an elongated connective that separates the two thecae

from each other. The upper (abaxial) connective arm has two fertile pollen sacs while the lower (adaxial) arm is often sterile. In the typical form of the lever, the filament tip has a narrow attachment point to the connective, so that it is allowed to swing around this joint in a reversible manner when moved by an insect, placing pollen onto the pollinator's body. Generally, in the Old World *Salvia*, the stamens move vertically, in such a way that pollen is placed on the back of the pollinating insect (usually a bee). This is the so-called nototribic pollination. One exception is the Series *Austriacae* (Sect. *Plethiosphace*) containing two species, *S. austriaca* Jacq. and *S. armeniaca* (Bordz.) Grossh., in which stamens move horizontally, placing pollen on the pollinator's body sides (pleurotribic pollination).

Contrary to these observations, we discovered populations of *S. austriaca* in the eastern part of the species' range in which flower morphology is remarkably different from that in the western populations. In these populations, stamens move vertically, consequently placing pollen on the pollinator's back similarly to the majority of Old World *Salvia* species. In order to reveal potential reproductive isolation of these populations of *S. austriaca* from western populations, we sampled *S. austriaca* from seven populations in Hungary, Romania, and Ukraine and performed phylogenomic analyses.

Here we present results of our phylogenomic analyses based on 2911 unlinked SNP markers derived from a RADseq approach, which we employed for investigating the origin of the discovered eastern populations of *S. austriaca* and their relationship to the western populations as well as to other, closely related species: *S. nemorosa* L., *S. nutans* L., and *S. pratensis* L. Our results indicate that the phylogenetic distance between populations with the altered flower morphology and the western populations of *S. austriaca* is comparable to that observed between *S. nemorosa* and *S. pratensis*. Our results also reveal incomplete lineage sorting within chloroplast sequences among the studied taxa. Overall, these results indicate that the investigated populations belong to a new, yet undescribed taxon whose rank has not yet been determined. In order to reveal the evolutionary processes leading to the emergence of this peculiar taxon and determine its proper taxonomic rank, we aim to sample more populations with a better geographic coverage in the near future, including those of *S. armeniaca*.

***Potentilla volgarica* Juz. – a narrow endemic in the midst of the Russian plain**

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The molecular genetic study of two critically endangered endemic species *Potentilla volgarica* Juz. and *P. evermanniana* Fisch. ex Ledeb. together with several related species of *Potentilla* sect. *Multifida* from the territory of Russia revealed extremely high polymorphism of the former species restricted to a small area of a few hundred square kilometers, which constitutes a sharp contrast to genetically poor populations of *P. evermanniana* and *P. arctica* Rouy. We believe *P. volgarica* is a relic species preserved for a long time within its small range on the right bank of the Volga River. The genealogy of plastid markers indicates it may be ancestral to *P. evermanniana* from the Southern Urals.

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Endemic species of *Callitriche* L. (Plantaginaceae) in the Lower Volga Region

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Tzvelev (1975) described two endemic *Callitriche* L. species from the Lower Volga: *C. fimbriata* (Schotsman) Tzvelev and *C. transvolgensis* Tzvelev, attributing them to section *Pseudocallitriche* Hegelm. in accordance with their morphological characters. *Callitriche fimbriata* differs from the closely related *C. truncata* Guss. by its shorter pedicels and fruit dimorphism (some mericarps are nearly wingless, others narrow-winged and minutely fringed). *C. transvolgensis* differs from the closely related *C. hermaphroditica* L. by the obovate fruit shape. Yet the taxonomic rank has remained uncertain for both *C. fimbriata* and *C.*

transvolgensis (Lansdown, 2011; 2014). For example, *C. fimbriata* has been often considered a subspecies of *C. truncata*: *C. truncata* Guss. subsp. *fimbriata* Schotsman (Lansdown, 2006).

We have analyzed genetic variability of *C. fimbriata* (Volgograd Oblast, Saratov Oblast, and Kalmyk Republic) and *C. transvolgensis* (Volgograd Oblast and Astrakhan Oblast) in comparison to that of *C. hermaphroditica* from its entire range. *C. palustris* L. from the section *Callitriche* was used as an outgroup. We analyzed a region of nDNA (ITS) and three regions of chloroplast DNA (trnH-psbA, petL-psbE, and trnS-trnG). We also used the *C. truncata* s.l. ITS sequences from GenBank. We collected root tips and fixed them right in the habitat for the subsequent chromosome count with employment of the standard squashing method.

Through phylogenetic analyses, while using either ITS sequences or chloroplast markers, we have been able to demonstrate that *C. fimbriata*, *C. hermaphroditica*, *C. truncata*, and *C. transvolgensis* form a single clade, where the latter species occupies the basal position. With respect to ITS, the identical basal ribotype has been found in all individuals of *C. transvolgensis*. Within the *C. truncata* s.l. clade, one can segregate the following groups: *C. truncata* subsp. *truncata*, *C. truncata* subsp. *occidentalis* (Rouy) Schotsman (represented by a few ribotypes), and *C. fimbriata* (a single ribotype). The analyses of the chloroplast DNA produce similar results (although no data are available for *C. truncata* s.str.), yet some weak genetic variation without definite spatial structure (at 1 to 3 positions) has been observed within *C. fimbriata* and *C. transvolgensis*.

We have succeeded in pinpointing chromosome numbers for *C. fimbriata* and *C. transvolgensis* ($2n=12$). Chromosome numbers for *C. hermaphroditica* and *C. truncata* Guss. subsp. *occidentalis* (Rouy) Schotsman ($2n=6$) were published by Prančl *et al.* (2014). *C. fimbriata* and *C. transvolgensis* are reproductively isolated from the closely related taxa, for it has been shown that hybrids between *Callitriche* species remain sterile even in cases when the species have identical chromosome numbers (Martisson, 1991; Prančl *et al.*, 2014). Therefore, *C. transvolgensis* and *C. fimbriata* are differentiated from the closely related species by their karyotype, genetical, and morphological characters. The establishment of these endemic species must have been connected with the Caspian Sea transgressions. The segregation of *C. fimbriata* from the Mediterranean *C. truncata* s.str. could take place during the period when the Caspian, Mediterranean, and Black Sea were included in a common basin.

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Genetic diversity of *Tulipa suaveolens* Roth (Liliaceae) and its evolutionary relationship with early cultivars of *T. gesneriana* L.

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Polychromatic *Tulipa suaveolens* Roth occurs over a vast territory ranging from the Crimea to eastern Kazakhstan. Its phylogenetic relationship to cultivated *T. gesneriana* L. is still under discussion. We used sequences from the *psbE-petL* region of chloroplast DNA and the complete internal transcribed spacer (ITS) of nuclear ribosomal DNA to examine the genetic variability of *T. suaveolens* specimens from European Russia and the adjoining regions. Our dataset also included 8 varieties of cultivated *T. gesneriana*. The research on biogeographic patterns of plastid haplotype and nuclear ribotype distribution provides evidence that their origin and dispersal over the Lower and Middle Volga Region are linked to the Khazar or Early Khvalynian Transgression of the Caspian Sea and subsequent events. In the Crimea and the adjacent regions, the pattern of haplotype distribution indicates that the origin and expansion of these haplotypes may be linked to the Karangat Transgression of the Black Sea and the subsequent New Euxinian Regression. Research has shown the complete identity of DNA sequences in early *T. gesneriana* cultivars and *T. suaveolens* wild plants. Therefore, we conclude that *T. suaveolens* is the likeliest wild ancestor of the early *T. gesneriana*.

The genus *Kleinia* Jacq. in the collection of the Main Botanical Garden RAS:

anatomy, morphology, taxonomy

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Species of *Kleinia* Jacq. occur in arid and semi-arid areas of Northern, Eastern, and Southern Africa as well as on the Arabian Peninsula, Canary Islands, and Madagascar. Only a

handful of species of *Kleinia* have penetrated the arid territories of the southwestern Africa. A few have spread into Namibia (*K. longiflora* DC.) and South Africa (*K. stapeliiformis* Stapf, *K. longiflora*, and *K. fulgens* Hook.f.

Just like *Senecio* L. (s.ampl.) and *Othonna* L. (Senecioneae, Asteraceae), the genus *Kleinia* contains some succulent species. Stem succulent kleinias are rather diverse morphologically: in our collection there are deciduous shrubs with hemi-succulent (*K. decoingsii* C.Jeffrey) and non-succulent leaves (*K. neriifolia* Haw., *K. longiflora*) as well as those whose leaves are reduced to scales (*K. stapeliiformis*). *K. fulgens* and *K. amaniensis* (Engl.) A.Berger are frequently cultivated for their ornamental glaucous leaves. Differently from *Senecio*, none of *Kleinia* representatives have true succulent leaves, neither there are any with terete leaves or bearing densely tomentose pubescence (Timonin *et al.*, 2015). Studies of leaf apices under the scanning electron microscope have demonstrated that *Kleinia* do not have unifacial leaves. There is only a single species, *K. picticaulis* (P.R.O.Bally) C.Jeffrey, a deciduous shrub from Tanzania and Ethiopia, featuring cylindrical succulent leaves, yet even in this species the author has observed open rings of vascular bundles on the leaf cross-sections—the evidence of bifacial leaf structure.

Morphologically, kleinias appear rather heterogeneous, so attempts have been made to divide them into a few genera: *Kleinia s.str.*, *Notonia* DC., whereas *Notoniopsis* B.Nord. was further segregated from the latter (Nordenstam, 1978). Molecular-genetic data support the existence of two clades corresponding to *Kleinia s.str.* and *Notonia s.l.* (Stäheli, 2006; Pelsner *et al.*, 2007), yet only 10% of the total species number in the genus have been studied so far, so it is much too early to speak about any reconstructions of *Kleinia* phylogenetic tree.

The author and colleagues have demonstrated that *Kleinia* species are monophyletic and form a sister group with regards to the genus *Solanecio* (Sch.Bip.) Walp., producing a common clade with it (Timonin *et al.*, 2015).

The most recent, additional series of molecular-genetic studies was triggered by the arrival of a new item in our *Kleinia* collection: *K. grandiflora* (Wall. ex DC.) N.Rani was received from India in 2019. So far it is only possible to confirm the distinctiveness of *Notonia*, though with a different composition: excluding *N. picticaulis* (P.R.O.Bally) Cufod., *N. pendula* Chiov., *N. schweinfurthii* Oliv. & Hiern, *N. abyssinica* A.Rich., *N. amanuensis* Engl., and at the same time, very unexpectedly, including *Kleinia neriifolia*, *K. longiflora*, and *Senecio anteuphorbium* (L.) Sch.Bip., which have never ever been included in *Notonia*. We have not been able to separate the genera *Notoniopsis* and *Kleinia*. As to the phylogenetic network, it also clearly demonstrates the distinctiveness of just two groups: *Kleinia* and *Notonia*.

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Phylogenomic study of intermediate pasque-flower, *Pulsatilla subslavica* Futák ex Goliašová

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Series *Pulsatilla* of the genus *Pulsatilla* Mill. (pasque-flower) is represented in the Northern Carpathians by three closely related tetraploid species: great pasque-flower *Pulsatilla grandis* Wender., Slovak pasque-flower, *P. slavica* G.Reuss (G.Reuss), and intermediate pasque-flower *P. subslavica* Futák ex Goliašová. The species identification can be based on morphological differences of fully developed leaves (structure of the compound leaf and leaf segment width) as well as differences in the species’ ecological requirements. Due to intermediate state of several characters in *P. subslavica*, some authors suggested that this taxon could be an introgressive hybrid, a cross between *P. grandis* and *P. slavica* (Goliašová, 1985) or *P. grandis* and *P. vulgaris* Mill. (Virók *et al.*, 2016). Intensive introgressive hybridization among the three pasque-flower species (*P. grandis*, *P. slavica*, and *P. subslavica*) has been

suggested by other authors (Futák, 1982, Mered'a and Hodálová, 2011), though without providing any further detail on hybrid combinations.

We conducted a phylogenomic study to clarify the phylogenetic relationship between the three Northern Carpathian pasque-flower species by using RAD-seq as a genomic approach. By using the rare-cutting SbfI enzyme, we generated a single-digest (classic) RAD-seq library that was sequenced on an Illumina NextSeq platform using a single-end mid-output kit that produced approximately 70 million 150bp long reads. This way we obtained an amount of genomic data large enough to get phylogenetic resolution between the closely related species. We collected samples from the entire area of *P. slavica* and *P. subslavica* as well as from some Hungarian *P. grandis* populations. The results of the analysis of genomic data support the genetic differentiation of the three examined taxa. We did not detect any evidence of *P. subslavica* hybrid origin. At the same time, in some Slovakian *P. slavica* and *P. subslavica* populations, we observed hybridization between these species, the resulting hybrids never exhibiting any significant morphological differences from the parents, which is partially consistent with the earlier results. We also found genetic evidence of the presence of *P. subslavica* in Hungary, a new species for the Hungarian flora.

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Conservation genetics of Pannonian populations of European ground squirrel,

Spermophilus citellus L. (Rodentia: Sciuridae)

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In the Pannonian Region, the population size and stability of the European ground squirrel (EGS), *Spermophilus citellus* L. used to play a major role in the survival of strictly steppic carnivores, such as steppe polecat (*Mustela eversmannii* Lesson), saker falcon (*Falco cherrug* J.E.Gray), or eastern imperial eagle (*Aquila heliaca* Savigny). Due to the degradation and fragmentation of the Pannonian Steppes, the number of known EGS colonies decreased by 71% during the last six decades. The results of former attempts to stabilize EGS population by ad hoc relocation remain unknown.

In order to study the conservation genetics and reveal phylogeographic structure of contemporary Hungarian populations, we analyzed 129 mitochondrial CytB sequences from 42 populations (pop. mean±sd: 3.0±0.8), and eight microsatellite loci (described by Řičanová *et al.*, 2011) of 428 specimens from 32 populations.

On the whole, the Pannonian populations show a homogeneous genetic pattern. The CytB haplotype diversity is low; in most populations only the most dominant alleles are present; alternative haplotypes are rare. This means that no significant isolation recently (on the evolutionary timescale) occurred among the populations. The haplotype network shows a typical star-shaped pattern, which, together with the unimodal mismatch distribution, suggests a recent arrival to the Pannonian Region. Most probably, the entire Pannonian Region was colonized in one recent colonization event. This is somehow surprising, as paleontological records testify for the existence of ground squirrel populations here since mid-Pleistocene. However, the species identification of the paleontological remains is uncertain.

Judging upon the population genetic analyses of microsatellite loci, the heterozygosity (i.e., genetic diversity) is high; yet when we take the report by Ben Slimen *et al.* (2012) into account, the heterozygosity exceeds the value typical for “challenged” populations only in a few cases. Moreover, half of the populations show signs of inbreeding. The Bayesian clustering (“structure analysis”) suggests the most probable grouping value of three (K=3). Geographic boundaries of these three groups neatly coincide with the course of the two large rivers in

Hungary, river barriers apparently isolating parts of the once-continuous Pannonian meta-population. However, the isolating effect is rather moderate within the more or less homogeneous meta-population in the area with moderately isolated groups. At the same time, we may eventually notice the effect of anthropogenic relocations, which could have somewhat counter-acted this isolation by mixing the populations to a certain extent.

On *Sphagnum medium* Limpr. in Russia

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Known for its ecological plasticity and morphological variability, *Sphagnum magellanicum* Brid. was until recently considered one of the most widespread and common species of sphagnum mosses in the Northern Hemisphere. However, recent studies (Kyrkjeeide *et al.*, 2016; Yousefi *et al.*, 2017) have demonstrated significant genetic differences among morphologically and ecologically distinct plants attributed to this species. As a result of this, *S. magellanicum* was divided in three species, its original name applied to only South American plants. Specimens from the Northern Hemisphere have been attributed to two other species, one of which—the one with the wide circumpolar distribution—was newly described under the name *S. divinum* Flatberg & K.Hassel, while the other – the one with the relatively narrow ampho-Atlantic range – has had its previously used name *S. medium* Limpr. restored (Hassel *et al.*, 2018).

In Russia, *Sphagnum divinum* is a common species widespread across the entire country, while *S. medium* (*s.str.*) has not been reported until now. Our findings of the latter species in Pskov Province constitute the easternmost occurrences currently known for the species, while its southern limit has been marked by the recent findings in Turkey (Ellis *et al.*, 2019). However, the distribution of *S. medium* outside Central, Northern, and Eastern Europe is still poorly understood.

To predict the species distribution and assess the climatic factors contribution, we applied the Maximum Entropy Method (Phillips *et al.*, 2006) modeling. According to our models, conditions suitable for *S. medium* may exist in the Tatra Mts. and Caucasus, in

Kaliningrad and Leningrad Provinces and southern part of Karelia Republic as well as along the oceanic coasts of the northern Pacific and the Atlantic Coast of Canada. Remarkably, the climatic factors that explain the distribution of *S. medium* are different from those explaining *S. divinum* geographic range, while the species' distribution along the axes of climatic factors contributing to the models also differs.

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An overview of the native flora in the Oka River Basin

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In the previous communication (Kazakova, Sobolev, 2019) we considered the position of the Oka River Basin (ORB) with regards to the phytogeographic and floristic zoning as well as against the outline of major Russian biomes. A certain similarity between the ORB shape and contours of natural zones in various zonation systems speaks about the most general physico-geographic patterns within this territory.

Grouped in complexes, many zonal forest-steppe species reach the Oka River Valley, often penetrating down to nearly the river mouth. Occasionally, one can find similar habitats

on the left bank, in Moscow, Ryazan, Vladimir, and Nizhniy Novgorod oblasts. The following are examples of species constituting the core of the so-called Oka Flora: *Melica altissima* L., *Stipa pennata* L., *Carex montana* L., *Iris aphylla* L., *Dianthus andrzejowskianus* (Zapal.) Kulcz., *Adonis vernalis* L., *Delphinium cuneatum* Steven ex DC., *Lathyrus pisiformis* L., *Oxytropis Pilosa* (L.) DC., *Vicia pisiformis* L., *Hypericum elegans* Stephan ex Willd., *Prunella grandiflora* (L.) Turra, *Scabiosa ochroleuca* L., *Campanula altaica* Ledeb., *Serratula coronata* L.

The zonal steppe species occasionally enter ORB from the south and southeast. The following species belong in this group: *Stipa dasiphylla* (Lindem.) Czern. ex Trautv., *S. pulcherrima* K.Koch, *Carex melanostachya* M.Bieb. ex Willd., *Lathyrus pallescens* (M.Bieb.) K.Koch, *Linum nervosum* Waldst., *Euphorbia sareptana* A.K.Becker, *Phlomis pungens* Willd., *Salvia nutans* L., and others. This phenomenon was noticed by A.K. Skvortsov (1949, 1951).

A number of zonal forest-steppe yet halophilous species just barely enter the southernmost ORB. These are *Juncus inflexus* L., *Sium sisarum* L., *Cirsium canum* Mill., *Galatella villosa* (L.) Rchb.f., *Inula helenium* L., *Senecio erucifolius* Ledeb. The location of *Fritillaria meleagris* L. in the ORB is most probably relict.

Species belonging strictly to the taiga biome are sometimes encountered in the northern and northeastern extremities of the ORB. They almost never occur on the right river-bank (except for Nizhniy Novgorod Oblast). These are *Goodyera repens* (L.) R.Br., *Pyrola media* Sw., *Arctostaphylos uva-ursi* (L.) Spreng., *Galium trifidum* L.

Species of the Oka Valley and the lower reaches of the Oka tributaries are of special interest. *Salvinia natans* (L.) All., *Iris sibirica* L., *Trapa natans* L., *Gnaphalium rossicum* Kirp. and others belong to this group.

The typical native species of Central European Russia are fairly well represented in the flora of ORB, which makes it possible to consider the latter the “core” containing most species diagnostic for the natural flora of Central European Russia.

ALIEN, INVASIVE, WEEDY AND QUARANTINE SPECIES SESSION

Microevolutionary changes in species of section *Triplinervae* of the genus

Solidago L. during the secondary range formation

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Two species of goldenrods, *Solidago* L., sect. *Triplinervae* appear to be the most distinctive within their secondary European range (especially when grown in homogeneous conditions of experimental plots). These are *S. canadensis* L. and *S. gigantea* Aiton. Either species is distinguished by a combination of morphological characters. *S. canadensis* features pubescent stems (A), short rhizomes (B), spreading inflorescences (C), small heads (D), and dentate leaf blades (E). *S. gigantea* has glabrous stems (except for the axis of the inflorescence) (a), long rhizomes (b), compact inflorescence (c), large heads (d), and either serrulate or entire leaf blades (e).

Yet within their native range in North America, the taxa from the sect. *Triplinervae* appear to be much more variable. Either of them actually constitutes a complex of small species, which may be sympatric in a single habitat. During the expeditions of 2017–2018 across the northern United States (Wyoming, Idaho, Montana, Minnesota, Wisconsin), I encountered only a single specimen of *S. canadensis* featuring the ABCDE combination of characters, the one analogous to that exhibited by this species in Europe. The overwhelming majority of American populations are represented by plants with long rhizomes (AbCDE). As to *S. gigantea*, not a single European-type (abcde) plant was observed anywhere in natural habitats. This combination of characters instead was found in plants growing at disturbed, anthropogenic habitats: around gas stations or near supermarkets.

In 2017, the author planted rhizome fragments of two sampled North American goldenrods, both of them with the AbCDE combination of characters, to an experimental plot at the Main Botanical Garden (MBG RAS). In 2018, the author collected seed from seven more variable specimens of North American goldenrods from the sect. *Triplinervae* with the following character combinations: ABCDE, abCDe, aBcdE, ABcdE, aBcdE, ABCdE, and abcde. These seed were germinated in the greenhouse of MBG RAS during the winter 2018/19. In May, the seedlings were planted outside, at the experimental plot. During the summer of

2019, the plants grown from the rhizome fragments were flowering profusely and appeared different from the European plants not only by their longer rhizomes, but also by earlier flowering (starting from early July vs. early August), smaller height (101.4 ± 1.5 vs. 136.1 ± 1.5 cm) and more petite heads (3.1 ± 0.1 vs. 4.4 ± 0.1 mm) with a larger number of staminate florets in the head. Some of plants originating from seed also formed inflorescences, even though normally goldenrods flower only on the second year. One of these prematurely flowering plants had heads nearly entirely consisting of just staminate florets.

Summarizing these observations, one can conclude that of multiple morphotypes encountered within the native range of *S. canadensis*, only a single one (remarkably, the one the least common in the country of origin) has become widespread and invasive within the secondary range in Europe. This is a textbook example of the so-called bottleneck effect well-known in other invasive plants (*Epilobium* Dill. ex L., *Bidens* L., etc.). Yet it remains unknown which exactly competitive advantages are linked with the genotype that enables a plant to actively expand its range and conquer new territories. The hope is that a complex comparative morphological and molecular-genetic study of goldenrods with variable morphotypes cultivated at the experimental plot will bring us closer to the solution of this puzzle.

**Growing exotic perennials (*Miscanthus* × *giganteus* J.M.Greef & Deuter ex
Hodk. & Renvoize, *Sida hermaphrodita* (L.) Rusby, and *Artemisia dubia* Wall. ex Besser)
in Lithuania**

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The goal of the experiment was to determine the growth dynamics of three perennial plants exotic in Lithuania: Virginia mallow (*Sida hermaphrodita* (L.) Rusby), dubious sagebrush (*Artemisia dubia* Wall. ex Besser), and giant miscanthus (*Miscanthus* × *giganteus* J.M.Greef & Deuter ex Hodk. & Renvoize) in response to fertilizer applications.

The experiment took place on the experimental plots at the Voke Branch of the National Research Centre for Agriculture and Forestry and lasted from 2012 to 2019. The plots were located in southeastern Lithuania, at 54°37'N, 25°06'E. The soil type within the plots was sandy loam. In accordance with FAO UNESCO classification, the soil in this area is known as Haplic

Luvisol. Plants were installed as seedlings with the following densities: 4 individuals per sq. meter for dubious sagebrush and 2 per sq. m for both Virginia mallow and giant miscanthus.

Starting from 2012, plants were twice fertilized with the City of Vilnius communal sewage sludge compost (SSC). Organic fertilizer was first applied for the 3-year period (2012–2016) and another time for the 4-year period (2016–2019). The variants of the treatment in 2012 were as follows. 1) no fertilizer, 2) N₉₀P₆₀K₉₀, 3) 20 tons/ha 4) 40 tons/ha, and 5) 80 tons/ha of dry-matter (DM) SSC. In 2016, the organic fertilizer was applied in the following way: 1) no fertilizer, 2) N₉₀P₆₀K₉₀, 3) 30 tons/ha 4) 55 tons/ha, and 5) 110 tons/ha of DM SSC. The mineral fertilizer was applied annually, every spring.

During the first and second year of the experiment, *Artemisia dubia* yielded the highest biomass; however, starting from the third year, the largest crop was produced by *Miscanthus × giganteus*.

Over time, *Artemisia dubia* produced crops ranging from 3.3 to 7.1 tons ha⁻¹ of DM per year; *Miscanthus × giganteus* – from 2.4 до 8.7 tons ha⁻¹; whereas *Sida hermaphrodita* did the most poorly in the infertile soil of the experimental plots and produced only 0.7–1.6 tons ha⁻¹ of DM per year. The mineral fertilizer produced a statistically significant (p<0.05) positive effect on the DM biomass yield in the studied perennials.

Only the highest norm of the SSC fertilizer (110 tons/ha) could produce a statistically significant (p<0.05) improvement of the productivity in *Artemisia dubia*, and only on the third, sixth, and eighth year of the experiment. The SSC fertilizer never produced any significant effect on the biomass yield of the other two observed plants (p>0.05).

Genetic polymorphism of *Chondrilla* L. (Asteraceae) in European Russia and the nature of *Chondrilla juncea* L.

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Genetic diversity was studied in 54 populations of sexual and apomictic taxa of the genus *Chondrilla* L. (*C. acantholepis* Boiss., *C. ambigua* Fisch. ex Kar. & Kir., *C. brevirostris* Fisch. & C.A.Mey., *C. canescens* Kar. & Kir, *C. graminea* M.Bieb., *C. juncea* L., *C. laticoronata* Leonova, *C. latifolia* M.Bieb., and *C. pauciflora* Ledeb.) in European Russia and the adjacent territories of northwestern Kazakhstan. We analyzed the trnT-trnF region of

plastid DNA and the internal transcribed spacer of ribosomal DNA (ITS1-5.8 S-ITS2), while using the method of economic statistical design, maximum likelihood estimation, and the NeighborNet algorithm. The two detected general evolutionary lines approximately correspond to the two subgenera traditionally recognized within the genus. One line (subg. *Brachyrhynchus*) is represented by the sexual diploid *C. ambigua* and its apomictic derivatives *C. brevirostris*, *C. laticoronata*, and *C. pauciflora*. The former two taxa are supposedly hybrid derivatives, while the latter is a triploid cytotype of *C. ambigua*. Their similarity was also confirmed through analyses of ISSR markers. The other evolutionary line (subg. *Chondrilla*) includes *C. juncea*, *C. acantholepis*, *C. canescens*, *C. graminea*, and *C. latifolia*; however, the results of the analysis of morphological variability and the plastid and nuclear marker genealogy speak in favor of treating these taxa as a sole, facultatively apomictic species *C. juncea*. The obtained data demonstrate that the apomictic way of reproduction does not necessarily result in formation of genetically isolated microspecies.

**Problems in the standardization of plant diagnostic methods in phytosanitary
laboratories as exemplified by the genus *Xanthium* L. (Cocklebur)**

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Identification of plants for their possible attribution to the category of regulated harmful organisms is one important practical application of plant systematics. The results of the identification are used within the legal framework of plant quarantine with the purpose of finding whether the phytosanitary condition of the contaminated material or territory meets the legal requirements. The correct identification is critical, since it may inflict major economic consequences. In Russia, this task is delegated to specialized testing laboratories, accredited in accordance with the National Standard ISO/IEC 17025 and assigned an area of expertise. The accreditation is based on the documents that determine the methods of identification. These documents must meet a number of requirements and include a detailed step-by-step description of all actions, comparisons, interpretation of results, and derivation of conclusions with the final goal of providing the correct plant ID. Unfortunately, illustrated manuals, identification

guides, and monographs produced by the scientific community often lack many necessary components and thus are not suitable for developing accreditation documents.

The All-Russia Center for Plant Quarantine has been developing documents suitable for this purpose. The specifics of producing such documentation have been analyzed, while using as an example the production of an identification key for the species of the genus *Xanthium* L. (cocklebur) present within the Russian territory. The identification key was developed in order to meet the requirements of the countries-importers of agricultural products from Russia.

The initial challenging step to be considered is the choice of the taxonomic approach. While the major goal of contemporary systematics is the construction of the natural, phylogenetic system, it is not always plausible from the practical standpoint, as for the purpose of plant quarantine it is often more productive to resort to an artificial system, which can be more convenient for lab identification and better linked to critical characters, first of all, to the extent of plant harmfulness. As to the genus *Xanthium*, so far no universally approved system has been developed. According to different treatments, the genus contains from 2 to 40 species. The last published monographic treatment was dated 1923, and later on the genus was considered only within floristic context. When developing his key, the author resorted to the artificial system of the genus based on the requirements of the countries-importers.

Another concern is an idealized or statistically averaged depiction of plant characters in identification keys. For the practical laboratory work, one needs keys that can be applied in situations when there is only a small, meager sample available for investigation (there may be just a single infructescence in the product sample); besides, the available material may be in poor condition due to product treatments. Before any taxonomic key can be used for identifications, it must pass approbation and validation stage. The author has produced an original key for identification of *Xanthium* species.

One more obstacle on the way to the correct plant identification is the fact that agricultural products are frequently contaminated with seed (or fruits/infructescences), while the majority of identification keys are based on the characters of mature plants. The key developed by the author for *Xanthium* is based on characters of the infructescences.

It is also important to ensure reproducibility of the identification method in all kinds of laboratories whose staff may have only the very basic level of botanical education and skills. This can be resolved through detailed descriptions and illustrations. The author has used the method of combined layers for photographic illustrations with high resolution.

POSTER SESSION

Population structure and dynamics of some species in the tribe *Neottieae* (Orchidaceae)

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The tribe *Neottieae* contains mycoheterotrophic species along with mixotrophic. In mycoheterotrophic species, the ontogenesis is largely subterranean, so that even during the flowering period plants not necessarily produce above-ground shoots. We have studied populations of the following mycoheterotrophic representatives: *Epipactis palustris* (L.) Crantz in Bryansk (2004–2005) and Moscow Oblast (2000–2019) and in Moscow (2019); *E. papillosa* Franch. & Sav. and *Cephalanthera longibracteata* Blume in Maritime Province (2016–2019). We have to note that both *Epipactis* Zinn and *Cephalanthera* Rich. are capable of producing albino forms, in which cases they become mycoheterotrophic (Selosse *et al.*, 2004; Shefferson *et al.*, 2016). This presents particular interest for studies on the evolution of mycoheterotrophy. *E. palustris* has a long-rhizomatous habit, while both *E. papillosa* and *C. longibracteata* are short rhizomatous. During the study of the population structure, we considered a partial shoot (annual growth increment) as a unit of account.

Virginile and reproductive plants dominated the ontogenetic spectrum of the *Cephalanthera longibracteata* population in the mixed Korean pine (*Pinus koraiensis* Sieb. & Zucc.) broadleaf deciduous forest with sparsely populated forest floor on a slope in the Sikhotealin Nature Reserve (Maritime Province). Juvenile plants and protocorms were not encountered. The number of *Cephalanthera longibracteata* plants has shrunk since 2017 due to forest damage during Lionrock Typhoon—a sever cyclone of late August / early September of 2016.

The newly open areas of bare forest floor where pines (*P. koraiensis*) had been downed by the hurricane underwent a drastic change of the microclimate, day temperatures going up, air humidity and soil moisture diminishing. Reacting to this event, some plants did not produce any above-ground shoots. Over the next three years, the population has been remaining stable.

The study of *Epipactis papillosa* coeno-populations was undertaken in a low-slope mixed *Pinus koraiensis* broadleaf forest, an oak (*Quercus mongolica* Fisch. ex Ledeb.) forest with sparsely populated forest floor, and also in a *Q. mongolica* forest completely devoid of

floor vegetation and situated in a gorge adjacent to a brook. In the bare-floor oak forests of the Sikhote-Alin Reserve, some plants are buried under a 10–15-cm layer of oak litter. The sparse populations consisted mostly of solitary plants at the reproductive stage. Typically, a plant has a shortened rhizome, either broken or deteriorated, bearing no more than one scar of the previous-year shoot and a single regeneration bud. Attempts of identification of virginile or senile plants were challenging. In 2018, we never found any above-ground shoots in the oak forest populations; however, in 2019 the plants terminated their dormancy and re-appeared on the day surface. Upon making observations of the same plant on the Kamchatka Peninsula, T.N. Vinogradova (2012) concluded that the traditional recognition of age categories based on shoot and leaf parameters and the number of leaves is not possible for this species. Juvenile plants with preserved protocorms could have 5 leaves, while those at the reproductive stage had from 3 to 8.

Coeno-populations of *Epipactis palustris* were studied in wet meadows and birch woods of Bryansk Oblast; in a wet meadow, palustrine pine forest, abandoned quarry, and melioration ditch in Moscow Oblast; and also in a birch grove in the Losinyy Ostrov (Moose Island) National Park, Moscow. All coeno-populations were dominated by mature shoots, either vegetative or reproductive, while juvenile plants of seed origin were found only once. The population in the wet meadow in Moscow Oblast has shrunk drastically due to expansion of woody vegetation (the species is known to attain maximum density during early successional stages when the total vegetation cover of competing herbaceous plants is not high and the tree and shrub layer is either sparse or absent). The population observed in the palustrine pine forest with *Phragmites australis* (Cav.) Trin. ex Steud. and sphagnum mosses has showed a fluctuating pattern in its dynamics.

According to the results of Kruskal–Wallis Test (Hammer *et al.*, 2001), plants in the reproductive stage from different populations exhibited significant differences in their shoot length, number of leaves, leaf length and width, length of inflorescence, and number of fruits, which was mostly attributed to the differences in soil moisture and sunlight availability in different habitats.

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Calciphilous flora of the Bolshaya Golubaya River floodplain within Kalachevsky District of Volgograd Province

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Formed in the Malaya Golubaya (Lesser Blue) River Basin, Golubinskiy Cretaceous Landscape is located within the lesser bend of the Don River. The Bolshaya Golubaya (Greater Blue) River valley is incised in the surrounding Cretaceous plateaus to 150 meters deep and even more. It has an appearance of a low mountain valley whose slopes are marked with ledges and chalk cliffs and much divided by a dense network of gullies and small depressions. Surface runoff has resulted in chalk outcrops across a large area here.

The most valuable part of the chalk outcrops flora of the Golubaya Valley and the right bank of the Don is an entire group of calciphilous species which were first described from this exact area. The goal of the current survey was the exploration of the calciphilous flora of the Bolshaya Golubaya River within the borders of Kalachevsky District (Volgograd Province). The project included the following tasks: survey and describe key areas of the river floodplain with special emphasis on the calciphilous plants; produce a listing of calciphilous species; survey locations of rare plant species named in the Red Book of Volgograd Province; explore areas of chalk outcrops that are depicted as “white spots” on the map of rare species distribution. The method of the survey was collecting herbarium specimens along the expedition route.

On the left bank of the Bolshaya Golubaya River, the chalk outcrops are not continuous but rather intermingled with sodded areas, so that zones of more or less “open chalk” constitute about one-fourth of the entire area. Vegetation cover at the chalk outcrops is about 60%. Many species occur only sporadically, without forming continuous clumps. This type of distribution speaks for harsh habitat conditions for the plants. The species composition is impoverished: at the time of the survey, we were able to list only 24 species adapted to growing on chalk. A number of non-calciphilous steppe plants can tolerate growing on chalk.

Other surveyed areas appeared similar to the one described above. A total of 17 species of calciphilous plants were found on the right river-bank, while 26 species were encountered at the river origin.

The “white spots” turned out to be truly understudied, for we discovered new, never previously known populations and locations for such rare species as intermediate swallow-wort (*Vincetoxicum intermedium* Taliev), saltwort wormwood (*Artemisia salsoloides* Willd.), Tartar breadplant (*Crambe tatarica* Sebeók), fragrant stock (*Matthiola fragrans* (Fisch.) Bunge) as well as new locations for a couple monitored species: chalk milkwort (*Polygala cretacea* Kotov) and chalk toadflax (*Linaria cretacea* Fisch. ex Spreng.).

All in all, we have discovered 24 new populations and locations of rare and monitored species and made a substantial contribution to the study of rare plant species within the territory of the district as well as documentation of the chalk outcrops flora.

Assessment of woody vegetation in Sasha Filippov Park, Volgograd

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City parks are a refuge for many. Visitors here can relax and appreciate nature; at the same time, parks improve air quality and provide habitat for plants and animals. Substantial funds have been allocated for the green industry, requirements for park design revised, and quality and assortment of plant material improved.

The goal of this work was to provide an assessment of woody vegetation in the city park (Sasha Filippov Park), including the tasks of documenting tree and shrub species present in the park; evaluating the health and vitality of the vegetation according to a number of criteria; and also providing an assessment of the overall condition of the park from the ecological,

biological, and recreational standpoint. The method that we used for the survey was field trip observations.

The park is more than 50 years old. A reconstruction was undertaken here about 10 years ago. Today's condition of trees and shrubs speaks about the efficiency of this work.

During our walking trips across the park, we identified the following species and varieties of trees and shrubs. **Trees:** Chinese elm (*Ulmus parvifolia* Jacq.), European white birch (*Betula pendula* Roth), horse chestnut (*Aesculus hippocastanum* L.), Siberian crabapple (*Malus baccata* (L.) Borkh.), Niedzwetzky's apple (*M. niedzwetzkyana* Dieck ex Koehne), Norway maple (*Acer platanoides* L.), littleleaf linden (*Tilia cordata* Mill.), European ash (*Fraxinus excelsior* L.), Babylon weeping willow (*Salix babylonica* L.), Chinese weeping willow (*S. matsudana* Koidz. = *S. babylonica* L. var. *pekinensis* Henry), Colorado blue spruce (*Picea pungens* Engelm. 'Glauca'), Colorado spruce (*Picea pungens* Engelm.), Scots pine (*Pinus sylvestris* L.), Anatolian black pine (*P. nigra* J.F.Arnold ssp. *pallasiana* (Lamb.) Holmboe), Oriental arborvitae (*Platycladus orientalis* (L.) Franco), and western arborvitae (*Thuja occidentalis* L. 'Holmstrup'). **Shrubs:** bridal wreath or Van Houtte spirea (*Spiraea × vanhouttei* (Briot) Carrière), Japanese meadowsweet (*S. japonica* L.f.), black-fruited mountain-ash (*Aronia × mitchurinii* A.K.Skvortsov), an assortment of roses, lilacs, Tatarian honeysuckle (*Lonicera tatarica* L.), Japanese barberry (*Berberis thunbergii* DC. 'Atropurpurea'), savin juniper (*Juniperus sabina* L. 'Tamariscifolia'), and Chinese juniper (*J. chinensis* L.).

The list is not extensive. There are no tree alleys. Group plantings are located at margins and specimen trees in the center, while western arborvitae is crowning bed compositions. Some of the park trails are highlighted by neatly pruned hedges of black-fruited mountain-ash. The rest of shrubs are present as either specimen plants (lilacs, Van Houtte spirea, Tatarian honeysuckle) or in small groups (Japanese meadowsweet, Japanese barberry). The designers' intention can be clearly traced: there is no congestion, and trees planted within beds and lawns are accenting the beauty of small architectural forms. Distances between trees and shrubs meet the planting standards and are in accordance with plant heights and crown spans.

The results of the vitality survey demonstrate high-quality care for plants: timely staking and pruning, rational watering. Most trees and shrubs, including old ones, can be assigned Category One as far as their vitality. Utilization of deadwood and rejuvenating of plants yield excellent results. Practically every plant produces flowers and fruits.

Summarizing these results, one can speculate that the rational approach is more important for good outcomes in the city landscaping than a chase for exotic plants. Plant

assortment choices are to be dictated by the ability of plants to adapt to the arid climate of Volgograd.

A report on the finding of Jacob's ladder (*Polemonium caeruleum*) in Balashov

District of Saratov Province

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Raw parts of *Polemonium caeruleum* L. (Jacob's ladder) are practically not available for sale in pharmacies. Preparations from this plant are also absent from the pharmaceutical market. A return of Jacob's ladder to the medicinal practice, investigation of a possibility of obtaining compounds that would be stable during the storage of preparations is particularly urgent. *P. caeruleum* has a sedative potential 8–10 times higher than that of *Valeriana officinalis* L.

P. caeruleum is listed in the Red Book of Saratov Province as a species of Category 3 (Rare), and therefore it has not been studied as an economic plant.

The discovered plants grow on a north-facing slope of a gentle depression, at the base of a river valley terrace slope, on the right bank of the Khover River, the largest left tributary of the Don. The population is located on the slope, at an oak forest margin in the vicinity of Repnoye (Balashov District, Saratov Prov.) at 51°37'41"N, 43°12'50"E and 136 meters a.s.l. The soil is typical chernozem of sandy granulometric composition, with relatively low content of macro- and microelements.

All discovered plants were growing within a small (20×20 m) area, scattered at a distance 3–8 m from one another. The total number of found individuals (clumps) was 8. The following co-habiting medicinal plants occurred in the surroundings: five-lobed motherwort, *Leonurus quinquelobatus* Gilib. (= *L. cardiaca* ssp. *villosus* (Desf. ex Spreng.) Hyl.), occupying an area of ca. 50 sq. m; European field pansy, *Viola arvensis* Murray, occupying ca. 200 sq. m; stinging nettle, *Urtica dioica* L. ssp. *dioica* found within about 500 sq. m; live-forever, *Hylotelephium telephium* (L.) H. Ohba (= *Sedum telephium* L.), spreading over 120 sq. m; and dyer's broom, *Genista tinctoria* L., occurring over 500 sq. m. 70-year-old stands of Scots pine, *Pinus sylvestris* L. occupy ca. 15 hectares.

Exploitable reserves for each of these medicinal plants have been estimated as follows: five-lobed motherwort – 9 kg, field pansy – 20 kg, stinging nettle – 33.3 kg, live-forever – 3.6 kg, dyer's broom – 17.2 kg.

True and false *Solidago* L. hybrids in northeastern Europe

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The North American species *Solidago canadensis* L. and *S. gigantea* Aiton are among the one hundred most aggressive invasive species in Russia (Vinogradova *et al.*, 2015). Each time naturalization of an alien species takes place, it involves interaction with representatives of the native flora, which may also include formation of hybrids. In European Russia, hybrids make up 10% of the total number of invasive species (Vinogradova, Mayorov, 2015). Both alien goldenrods, the subjects of the current work, are capable of forming hybrids with the native *S. virgaurea* L. in their secondary geographic range. The following hybridogenous species have been described: *S. × snarskii* Gudž. & Žaln. (= *S. gigantea* × *S. virgaurea*) (Gudžinskas, Žalneravičius, 2016) and *S. × niederederi* Khek (= *S. canadensis* × *S. virgaurea*).

Samples of *Solidago virgaurea*, *S. canadensis*, *S. gigantea*, *S. × snarskii*, and *S. × niederederi* were collected in Lithuania and northeastern Russia (Kaliningrad and Pskov oblasts). DNA was extracted from silica gel-dried leaves.

The analysis of nuclear ribosomal internal transcribed spacer 1–2 (ITS1–2) has demonstrated the ambiguity of these *Solidago* hybrids: in most cases, the nucleotide substitutions differentiating *S. virgaurea* from *S. canadensis* and *S. × niederederi* had ambiguous readings, indicating heterozygosity, which confirms the hybrid origin of the individuals. One of the samples of *S. canadensis* from Pskov Oblast showed heterozygosity in most cases of nucleotide substitutions, although morphologically this sample did not differ from other individuals of *S. canadensis*, which indicates the presence of introgressive hybridization within the genus *Solidago*. This sample is probably a backcross (i.e., the result of crossing of *S. × niederederi* with the parental species *S. canadensis*). One of the samples of *S. × niederederi* from Lithuania (vicinity of Trakai) has fewer informative substitutions, so this sample is probably also a backcross, although in this case it is a result of crossing between *S. × niederederi* and the native parental species *S. virgaurea*. Analysis of the highly

variable intergenic spacer rpl32–trnL did not produce an unambiguous answer to the question which of the species is maternal for *S. × niederederi* and which is paternal. It is likely that hybridization has been going in both directions.

As for samples identified as *S. × snarskisii*, only one of them (that from Kaliningrad Oblast) has turned out to be a true hybrid. Analysis of the highly variable intergenic spacer rpl32–trnL showed that *S. virgaurea* was the maternal species for this sample, while *S. gigantea* was paternal. In the rest of the samples identified as *S. × snarskisii*, the ITS1–2 nucleotide sequences were identical with those of *S. virgaurea*. To all appearances, hybrids are very unstable, promptly absorbed by their maternal species. It is likely that the remaining plants constitute a sustainable ecological form of *S. virgaurea* and that only this form can occasionally cross with *S. gigantea* and produce unstable hybrids.

**Microevolution of invasive shadbush species *Amelanchier* Medik.
in the central part of European Russia**

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Among representatives of the genus *Amelanchier* Medik. (Rosaceae, Maloideae) in the central part of European Russia, there is a widespread invasive species *A. spicata* (Lam.) K.Koch. It is encountered in Yaroslavl, Tver, Moscow, Nizhny Novgorod, Smolensk, Kaluga, Bryansk, and Belgorod regions. In some regions it has turned into a species-transformer, i.e., one capable of forming dense stands completely devoid of any ground-layer vegetation, where all native species are displaced (Vinogradova *et al.*, 2010; Kuklina, 2011).

During the survey of invasive populations of *A. spicata*, the authors identified variants considerably differing from the homogeneous morphotypes. Certain ecological plasticity was manifested in active development of xylorhizomes whenever the seed propagation was suppressed (Kuklina *et al.*, 2018).

The molecular-genetic analyses of the invasive population samples of *Amelanchier* allowed us to detect multiple microevolutionary transformations. All sequences obtained from these samples from Central European Russia have turned out polymorphic, that is, with varying composition of nucleotides in a number of positions, indicating the presence of a few different ITS copies in the genome. Such polymorphic ITS sequences are characteristic of some North

American *Amelanchier* (Campbell *et al.*, 1997), which points to widespread processes of interspecific hybridization.

Upon conducting the analyses, we were able to conclude that the *A. spicata* widespread in Europe and Russia is genetically very close to *A. alnifolia* (Nutt.) Nutt ex M.Roem. and *A. humilis* Wiegand. It is possible therefore that either or both these species constitute parental forms which have produced *A. spicata*. Another investigation using multi-locus marker systems would serve well for confirmation or rejection of this hypothesis.

Microevolutionary transformations resulting in elevated biodiversity within populations of *A. spicata* could have developed either as a result of mutations or repeated backcrosses with a possible participation of another naturalized species *A. alnifolia*. The acquired traits may have remained preserved in the populations due to agamospermy and can constitute an additional source of enrichment of the adaptive potential for the invasive species in its secondary range.

Adaptation of *Salix* L. species to dynamic forest habitat conditions: balance of euri-, post-, and pre-adaptations

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Trees and shrubs of the genus *Salix* L. are characterized by high morphological variability, which accounts for challenges with attribution of plants to species (Skvortsov, 1968). Evaluation of ecological profiles of willow species demonstrates that a number of taxonomic groups (sections) contain species capable of growing within a wide array of ecological situations ranging from riverside habitats in floodplains with sandy/gravelly alluvium to peaty wetlands (e.g., members of Sect. *Vetrix* Dumort.).

Considering the fact that willows as a phylogenetic group have formed in dynamic situations of river floodplains (Skvortsov, 1968), one may emphasize the following ontogenetic aspects of adaptations in willows: chloroembryo (chlorophyllous embryo) and practical absence of the endosperm in seeds; high moisture requirements and low shade tolerance during the germination and seedling stage; high growth rate; simultaneous manifestation of xeromorphic and hygromorphic characters during leaf development; retention of high regeneration ability during the entire life span.

Structural/functional euri-adaptations concerning water metabolism, mineral metabolism, breathing, etc. function in the mode *Factor-Reaction-Effect*, which ensures plant growth and development. At the same time, willows' success stories in technogenic landscapes (outside floodplains) have to do with the realization of adaptive complexes involving post- and preadaptations. Survival of plants in extreme habitat conditions, such as droughts, contamination with industrial waste, or ecological catastrophes is determined by the performance success of certain adaptive complexes. In case of atmospheric contamination, the protective function depends on leaf pubescence and wax coating (providing that less contaminated air reaches the lenticels) as well as on closing the lenticels (that is, formation of a barrier for contaminants, which then cannot reach the leaf mesophyll). In case of defoliation, willows are capable of regeneration of their entire assimilation apparatus (up to 3–4 leaf regeneration events can occur during a single vegetation period).

Species of *Salix* are characterized by a number of unique traits: high intraspecific variability, ability to form hybrids between species, preservation of ancient structural morphological traits along with ability to adapt to dynamic environment. Besides, the balance among euri-, post-, and preadaptations can be adjusted to match the requirements during different ontogenetic stages and in extreme circumstances. Apparently, this is precisely this structural-functional ambiguity and inconsistency based on realization of euri-, post-, and preadaptations during ontogenesis of willow species that determine their success in extreme conditions of technogenic habitats.

Adaptations of conifers to technogenic environment as exemplified by Scots pine (*Pinus sylvestris* L.)

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While growing in a wild range of environmental conditions, woody plants are forced to adapt to their habitats. Realization of the adaptation potential takes place through changes in plants caused by influence of ecological factors, including extreme situations. Steady growth of woody plants is achieved through changes in their vital parameters at every level of organization from molecular to population (Kulagin, 1974). Over a long period of time, woody plants have developed a complex of adaptive reactions allowing for their reliable growth.

During the past century, a new ecological factor has emerged: anthropogenic changes in the environment, such as industrial contamination and formation of new landscapes (tailing dumps, quarries, etc.). Woody plants have not produced specific adaptation mechanisms to technological environment and continue relying on those developed for overcoming natural extreme situations. The current work has been dedicated to the study of adaptive reactions of Scots pine (*Pinus sylvestris* L.) in response to industrial contamination of multi-metal and petrochemical type.

The research was conducted within Ufa (Bashkortostan Rep.) and Lipetsk (Lipetsk Obl.) industrial centers. In 2017, the total value of emissions from stationary industrial sources in Lipetsk was 286.032 thousand tons (Report..., 2017), of which the share of Novolipetsk Metallurgical Plant was 90.19%. The total value of emissions from stationary sources in Ufa in 2017 was 143.5 thousand tons (Federal Report..., 2017), 75.95% of which was the share of ANK BashNeft PAO Oil Refinery Complex.

The objects of the study were even-aged stands of Scots pine in Lipetsk and Ufa industrial centers. The study included the following tasks:

- evaluation of relative vitality of plantings;
- report on radial increment growth dynamics of trunk and root wood;
- a study of vegetative shoot growth;
- a study of formation of assimilation apparatus during the process of growth;
- determination of photosynthetic pigment concentrations in foliage;
- assessment of density of absorbing roots in soil.

The study has resulted in revealing general and specific reactions of Scots pine in response to habitat industrial contamination of multi-metal and petrochemical type. The adaptive changes allow Scots pine to survive and grow in the technogenic landscapes, while fulfilling its sanitary protection functions.

The work within Lipetsk industrial center was supported by the Russian Foundation for the Fundamental Research as well as Lipetsk Oblast Administration (grant No. 13–04–97518 and 19–44–480001).

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**Assessment of sandy everlasting *Helichrysum arenarium* (L.) Moench
resources in Pot'ma (Rtishchevo District, Saratov Region)**

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The District of Rtishchevo is located in the northeastern part of Saratov Region, within the Khoher River watershed on the Don Plain. It belongs to the sub-zone of meadow-influenced steppes. The total area of the district is 2.3 thousand sq. km. Pot'ma Settlement is located at 52°16'00"N, 43°22'60"E, at 177 meters a.s.l. It is surrounded by floodplain English oak forests, Scots pine plantings, and bayou lakes produced by the Khoher.

The population of sandy everlasting is found on sandy soil, stretching along an edge of Scots pine plantation, in close proximity of two small bayou lakes. The habitat of the species can be identified as sagebrush/everlasting (*Artemisia marschalliana* Spreng. / *Helichrysum arenarium* (L.) Moench) sand steppe. The thicket of everlasting extends over the distance of two kilometers, while being up to five meters wide. The biomass of an inflorescence is on average 0.42 g. An estimate of the total biomass of sand everlasting is 1.008 tons.

According to some assessments, as of July 1999, the economic reserves of this species within the region territory were about 8 tons; as of July 2010, the estimate was more than 11 tons. The growth of the exploitable reserve of raw *Helichrysum arenarium* in the province during the past decades can be explained by abandonment of large areas of arable land, which now constitutes long-term fallow land characterized by prolonged plant successions. Apparently, some of these areas have been re-populated by sandy everlasting.

Our survey has revealed that the biological reserves of sandy everlasting in Pot'ma amount to about 1,012 kg of raw mass, while the mean inflorescence mass of a single model *Helichrysum arenarium* plant was 0.4–0.5 g and the average number of reproductive shoots per sq. m was 125±9.5.

Sequencing of NR ITS region in species of the type section of *Peucedanum* L.

(Apiaceae)

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The type section of the genus *Peucedanum* L. embraces 8 to 15 species occurring in temperate Eurasia, including *P. officinale* L., the type species of the genus. These species are very uniform morphologically. The only qualitative character that allows dividing this group into two is the petal color: either white or yellow. Within either group, the species can be separated only by quantitative characters, such as the width and length of the terminal segment of the basal leaf, number of rays in the central umbel, etc., as well as by their geographic ranges.

A morphometric study of more than 400 samples of 12 species of this section undertaken by the authors has demonstrated that none of the diagnostic characters proposed before allow for definite discrimination between the species. The variability ranges of these characters considerably overlap and do not match the ranges described in the literature. The situation is additionally exacerbated by the fact that among the representatives of the section there are diploids ($2n=22$) as well as hexaploids ($2n=66$); besides, the existence of polyploid series (with $2n=22, 44, 66$) has been revealed for two species.

For clarification of relationships among the species in this section, we used the ITS nucleotide sequences of nuclear ribosomal DNA. Twenty-three samples belonging to 12 species were included in the analysis, ploidy level known for 14 of them. On the phylogenetic tree constructed by the Bayesian Method, one can segregate a few clades and also find separate branches whose relationships remain unresolved. The white-flowered species *P. coriaceum* Rchb. and *P. gallicum* Latourr. do not form a separate clade, and their relationship with other species remains vague. One clade, although with a low level of support, is formed by Greek narrow endemics, diploids *P. vourinense* (Leute) Hartvig and *P. gabriellae* R.Frey. The rest of the samples are divided into two groups, both groups with a high level of support. One group includes the hexaploids *P. officinale* L., *P. morisonii* Besser ex Schult., and *P. ruthenicum* M.Bieb.; the other group – the diploids *P. calcareum* Albov ex Maleev, *P. ruthenicum*, and *P. tauricum* M.Bieb., the tetraploid *P. longifolium* Waldst. & Kit., and *P. luxurians* Tamamsch. (ploidy unknown). The species groupings produced in accordance with the ITS data do not correlate with the morphological characters of *Peucedanum* species – neither with the qualitative (petal color) nor quantitative. The only correlations that can be traced are ones with

the species' geographic ranges (the Caucasus and Crimea vs. the rest of the genus area from Corsica to the Altai Mts.) as well as with the ploidy levels (diploids and a single tetraploid vs. hexaploids).

The data obtained through molecular methods as well as morphological data allow one to make a conclusion that the members of the type section of the genus are in need of a taxonomic revision, in particular, *P. ruthenicum*, whose samples have shown up in two different clades. A further study of this group is in the works.

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Application of scientific names to willow cultivars: *Salix* × *simulatrix* F.B.White

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The Botanic Garden of Ural Branch RAS received *Salix* × *simulatrix* F.B.White through exchange from a German private collection in 1997. Upon a period of acclimatization, this ornamental, lushly flowering, quickly growing, easily propagated willow was chosen as a perspective cultivar suitable for small gardens and rockeries. A standard procedure for any material received from other collections is checking the identification and assigning the correct name. In this case, the comparison of a herbarium specimen collected from the plant in question with the type material (that is, the specimen of *S.* × *simulatrix* (= *S. arbuscula* L. × *S. herbacea* L.) from the F.B.White's collection preserved at PTH, demonstrated that the characters of the cultivated plant received from Germany do not match those of the authentic specimen. Further comparisons with samples deposited at K and BM confirmed this conclusion. Literature analysis has shown that the male clone distributed through nurseries under the name *S.* × *simulatrix* originates from the Alps rather than Scotland, from where *S.* × *simulatrix* was described, and that the clone in question was introduced to cultivation nearly a hundred years ago. Remarkably, *S. arbuscula* L., one of the parent species of *S.* × *simulatrix*, according to F.B.White's protologue, does not occur in the Alps; instead, a couple closely related species occur there: *S. foetida* Schleich. ex DC. and *S. waldsteiniana* Willd. During the post-Linnaean era, before these two species even were described, both had been erroneously listed for the flora of the Alps under a single name *S. arbuscula*, which must have been the start of the current confusion.

The morphological analysis has shown that our plant has a set of intermediate characters and thus could be of hybrid origin. Due its ornamental value and ease of propagation from cuttings, the willow has rapidly become widespread in cultivation and now is included in collections of most botanic gardens and nurseries of Europe and North America. It is also often sold grafted on short rootstocks and forming miniature “trees” that have gained popularity among gardeners. Nowadays, this willow appears to be the most widespread cultivated small-stature hybrid of Alpine origin. It can be found in collections of botanic gardens and nurseries under a few other names, yet the name *S. × simulatrix* appears to be used the most, while being incorrect, as it has been shown in this investigation.

In addition to clarifying the geographic origin and checking the correctness of the name, the list of tasks for this study included pointing to possible parent species for this hybrid, judging on its morphological characters. With a high level of confidence, one can claim *Salix retusa* L. as one of probable parents. A number of characters point to this parentage: prostrate habit; short, abundant catkins developing simultaneously with leaves; two nectaries in the flower; the shape and venation of young leaves very similar to those in *S. retusa*. There is a good chance the other parent could be a species from Sect. *Arbuscella* Ser. – perhaps *S. foetida* or *S. waldsteiniana*. For a more precise determination of this willow’s parentage, a study involving molecular markers should be done.

***Cuscuta* L. species in Penza Region**

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A study of dodders (*Cuscuta* L.), obligate parasitic plants quarantined in the Russian Federation, was undertaken in 2017–2019 in Penza Region and included the following directions: 1) revision of the local species composition of the genus; 2) monitoring of status and distribution of species’ coenopopulations; 3) evaluation of dodder gall weevils of the genus *Smicronyx* Schoenherr as potential agents of biological control over populations of dodder.

Of five dodder species ever registered within the Region, the authors have identified and studied three: field dodder (*Cuscuta campestris* Yunck.), European dodder (*C. europaea* L.), and hop dodder (*C. lupuliformis* Krock.). Host plant listings have been assembled for each

of these species. Field dodder was encountered only in anthropogenic communities: along automobile and agricultural roads, in run-down lawns, at margins of abandoned fields, and in fields of wheat or barley, or else at their margins. In contrast to this species, both European and hop dodder were found in natural communities of river and brook floodplains: in black alder and willow forests and in nettle-dominated community.

During our observations, we recorded two species of weevils on *Cuscuta europaea*: *Smicronyx smreczynskii* Solari and *S. coecus* Reich. and just one (*S. smreczynskii*) on *C. campestris*. Galls were observed only on the latter dodder species. The authors collected 420 galls, which produced 223 larvae in the lab. Of these, 155 reached the stage of imago and were identified as *S. smreczynskii*.

Five habitat types suitable for co-habitation of *Cuscuta* and *Smicronyx* species have been identified while analyzing the field data and observations. Assessment of weevil influence on the coenopopulations of dodder allowed us to conclude that neither *S. smreczynskii* nor *S. coecus* produce any significant effect on dodder and thus cannot be considered as means of control over dodder populations.

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Diversity of plant life forms in herbaceous communities of Central Forest Nature Reserve (Tver Province)

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The subject of the study is biomorphological composition of herbaceous communities within the territory of Tsentralno-Lesnoy (Central Forest) Nature Reserve (Tver Province). The spectrum of life forms in each community was presented in accordance with the life form classification by Serebryakov (1962) modified by Zhmylev *et al.* (2017). Hypogeogenous long-rhizomatous herbaceous perennials prevail in the generalized spectrum. While a total of 36 life forms have been encountered, 85% of species belong to nine prevailing life forms. The life form spectra in generalized partial floras were compared within the following types of herbaceous communities: managed mesic meadows, abandoned mesic meadows, tall-herb meadowsweet (*Filipendula ulmaria* (L.) Maxim.) communities, and tall-herb ruderal

communities. Significant differences were revealed in the life form composition of these plant community types.

Hypogeous long-rhizomatous and caespitose herbaceous perennials prevail in mesic meadows; soboliferous perennials dominate in ruderal communities. Meadowsweet communities are characterized by the prevalence of vegetative annuals (herbaceous plants producing annual monocarpic daughter plants from adventitious roots), while hypogeous long-rhizomatous, caespitose, and taproot herbaceous perennials, as well as typical annuals, are less prominent in meadowsweet communities compared with the other studied community types.

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Flora studies in Tobolsk vicinity

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Flora of the vicinity of Tobolsk has always been of special interest to botanists. A number of species, such as *Allium microdictyon* Prokh., *Alchemilla circularis* Juz., *Calamagrostis andrejewii* Litv., *C. obtusata* Trin., and others have been described from here (*Flora of Siberia*, vol. I–XIV, 1988–2003). The earliest information on the flora of this region was contained in works by botanists of the 18th and 19th centuries: (Gmelin, 1747–1769; Pallas, 1784–1788; Ledebour, 1842–1853). The study of the flora around Tobolsk was intensified at the threshold of the 19th/20th centuries. This was the time when the *Flora of Siberia and the Far East* was published under the supervision of S.I. Korzhinsky. The *Flora* listed rare plants collected by N.L. Skalozubov, Tobolsk Government Museum employee.

Credit should go to the Museum staff who, over a long time, managed to assemble a rich herbarium collection, which contains about 20 thousand sheets. At the request of Korzhinsky, the Keeper of the Tobolsk Museum S.N. Mameev was contributing to the Herbarium at the Botanic Garden and Museum of the Russian Academy of Sciences in St. Petersburg over the time period of more than 10 years and thus added 2,744 herbarium sheets with his authentic plant identifications to that depository. Another Museum employee V.A. Ivanovsky collected valuable material depicting the flora of the unique Chistoye Boloto (Clean Bog) and also assembled a useful collection of weeds occurring in and around Tobolsk. The renowned botanist Porfiry N. Krylov also went on multiple collection trips around Tobolsk. His collections from this area constituted the foundation of his *Flora of West Siberia* (1927–1949, 1960–1964).

The interest in the flora of the vicinity of the City of Tobolsk can be explained by the city's location within the Southern Taiga Zone and thus featuring the zonal flora. Yet at the same time, due to the influence of topographic features (Irtysh River Valley, ancient thermokarst phenomena, etc.), extrazonal representatives of biota have been also preserved here: along the bedrock bank of the Irtysh, on slopes of variable steepness and 80–90 meters tall, there are grass steppe areas with *Stipa pennata* L., *Schizonepeta multifida* (L.) Briq., *Carex obtusata* Lilj., and others; shrub steppes with *Spiraea crenata* L. (also including *Stipa capillata* L., *S. tirsia* Steven, *Potentilla nudicaulis* Willd. ex D.F.K.Schltl., etc.); and even sagebrush steppes featuring *Artemisia frigida* Willd., *A. sericea* Weber ex Stechm., *Allium strictum* Schrad., and others. The flora of Tobolsk vicinity has been under the anthropogenic pressure. Some species represented in the collections by P.N. Krylov, such as *Iris glaucescens* Bunge or *Potentilla sericea* L., have not been relocated any time recently.

The problem of vicariant species in *Euonymus* L. of Northern Eurasia

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Vicariant species is a notion employed in microevolutionary theory and for typology of species and their geographic ranges. Unfortunately, it has not been sufficiently developed as of today.

A number of spindle-trees or burning bushes that occur within the territory of Russia and adjacent territories have been considered closely related or even vicariant by some authors, that is, replacing each other in similar habitats within different geographic regions (such as European Russia, the Caucasus, or Far East). Over various time periods, authors have considered vicariant the following pairs of species: *Euonymus verrucosus* L. and *E. pauciflorus* Maxim.; *E. europaeus* L. and *E. maackii* Rupr.; *E. latifolius* (L.) Mill. and *E. sachalinensis* (F.Schmidt) Maxim.; *E. leiophloeus* Steven and *E. macropterus* Rupr. Some of these statements are more than controversial, so some authors (e.g., T.G. Leonova, 1974) avoided using the term ‘vicariant’ altogether, even though they apparently imply this approach in a number of cases. (e.g., for *E. verrucosus* and *E. pauciflorus*; and less obviously for *E. alatus* (Thunb.) Siebold / *E. sacrosanctus* Koidz. and *E. nanus* M.Bieb. / *E. koopmannii* Lauche). I.S. Ma (2001) accepted treatments of many species in a broad sense, particularly of those having disjunct areas, while ignoring their morphological differences (treating these differences as infraspecific variability) and reducing species to synonyms: *E. pauciflorus* = *E. verrucosus*; *E. bungeanus* = *E. maackii*; *E. sieboldianus* Blume = *E. hamiltonianus* Wall.; *E. velutinus* Fisch. & C.A.Mey. = *E. europaeus*; *E. sacrosanctus* = *E. alatus*; *E. leiophloeus* = *E. latifolius*; *E. planipes* (Koehne) Koehne = *E. maximowiczianus* (Prokh.) Vorosch., *E. ×miniatus* Tolm. = *E. sachalinensis*. The same approach to these species has been implemented by him in the *Flora of China* (Ma, Funston, 2008). At the same time, many Japanese and Korean authors (Ohwi, 1984; Ka, 2006) accept practically all of these species as distinct ones. The authors have assembled contemporary data justifying attribution of these species to vicariant pairs – including their own as well as data obtained by others in the fields of morphology, anatomy, chromosome numbers, DNA analyses (Li *et al.*, 2014), and information on geographical and ecological profiles of certain species. The authors analyzed a few anatomical (and particularly caprological) characters that may facilitate the existence of closely related species in different conditions.

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Peculiarities of microgametophyte development in several species and cultivars of poplar

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Sporoderm development has been studied in two species and two hybrids of poplar. All studied plants have passed the same stages of microsporogenesis and sporoderm development. Differences were observed only in duration of certain stages and hence dates. Simultaneous microsporogenesis in poplars takes place during about one or two weeks of cold winter period, in late January and early February. Trembling aspen (*Populus tremula* L.) is a native species in Moscow Region. Pollen development in this species takes place some two weeks earlier than in the introduced white poplar (*P. alba* L.) and cultivated hybrids (*P. × nevensis* Nasim. and *P. × berolinensis* K.Koch). Duration of stages in the sporoderm development considerably differs. Formation of thin callous wall results in the swift development of primexine over the entire surface of the microspore, ultimately ending up with production of thin, though five-layer exine consisting of tectum, bacules, and foot layer of ectexine along with two layers of endexine. During the stage of volume enlargement of pollen grain prior to ripening, the exine keeps thinning across all of its layers, becoming fragmentary in some areas. At the same time, intine is forming at this stage, which also takes a fairly long period. As a result of these processes, tubular elements of the powerful exocytosis are preserved in the outer layer of the intine over the entire surface of the pollen grain. The inner intine layer remains homogeneous. The multi-layer intine is typical for aperture areas, which, in combination with the fragmentation of the exine, makes poplar pollen grains omniaperturate – in terms of both the structure of the sporoderm and mode of its formation.

Biotechnological methods of rare plant species cultivation

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The Gene Bank of the Main Botanical Garden RAS (MBG) obtained through *in vitro* propagation methods currently includes 153 species and 1157 horticultural varieties and selected forms attributed to 183 genera and 61 families. About 70% of the *in vitro* collection are economically important plants.

Special emphasis is laid on rare and vulnerable species. The *in vitro* collection includes 82 rare species, that is, 17.3% of the total number of angiosperms in the Red Book of the Russian Federation. 54% of rare species samples in the biorepository are species assigned Category One or Two of rarity; two more species are Category Zero (Extirpated). The majority of species are represented by samples from a number of different populations.

Methods of clonal micropropagation have been first developed in MBG for *Gladiolus palustris* Gaudin (Category 0, Extirpated), *Aristolochia manshuriensis* Kom., *Dioscorea caucasica* Lipsky, *Sanguisorba magnifica* I.Schischk. & Kom., and *Euonymus nanus* M.Bieb. (Category 1, Endangered).

Best represented among rare and vulnerable plants of Russia in the MBG *in vitro* collection are Liliaceae (10 species), Iridaceae (8 species), Amaryllidaceae (6 species), Paeoniaceae (6 species), Rosaceae (6 species), Araliaceae (5 species), and Fabaceae (5 species). Most preserved species (90.2% of the total number) are herbaceous plants.

Plant material for the Gene Bank was collected in natural habitats during expeditions and field trips or obtained through exchange with other botanical gardens. Botanical gardens play a major role in preservation of species and have been augmenting their collections.

Positive results have been achieved during non-mycorrhizal germination *in vitro* of orchids belonging to the following genera: *Cremastra* Lindl., *Cypripedium* L., *Dactylorhiza* Neck. ex Nevski, *Epipactis* Zinn, *Gymnadenia* R.Br., *Oreorchis* Lindl., and *Platanthera* Rich. More than 50 species of winter-hardy orchids have been introduced in Moscow and its surroundings.

We have developed recommendations for all stages of species preservation, starting from the introduction to cultivation, micropropagation *in vitro* of the initial material, to the

rooting procedure, and depositing. One has to bear in mind that every species has its own specifics when propagated *in vitro*.

Integrated approach to preservation of plants *ex situ* (in seed banks, living collections, and *in vitro* cultures) considerably improves chances for successful preservation of the gene pool. Every case is unique, so that during the selection of the strategic method for the taxon preservation *in vitro*, one must consider potential outcome of possible approaches in connection with the species' biological traits. When assembling *in vitro* collections, it is essential that each species is represented with as many samples originating from various parts of its range as it is possible.

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