



Conference proceedings

The Eighth Conference in Memory of Alexei K. Skvortsov

Tsitsin Main Botanical Garden, Russian Academy of Sciences, Moscow. February 7, 2019

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Conference Overview

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The 8th annual Conference in Memory of Prof. Alexei Skvortsov was held at the Tsitsin Main Botanical Garden of the Russian Academy of Sciences on February 7, 2019. The Conference was organized by the Moscow Branch of the Russian Botanical Society and



Participants at the 8th Skvortsov Memorial Conference

supported by the Tsitsin Main Botanical Garden and Moscow State University. As with the

previous conference, the topic was narrowed to just one of the areas of interest of Prof. Skvortsov, this time to “Cryptic species and intraspecific variability.” The Conference was held in the already traditional format of a one-day meeting. The 8th Conference gathered scientists from Moscow, St. Petersburg, Rostov-on-Don, London (UK), Paris (France), Debrecen (Hungary), and Shandong (China), 47 in all (see photograph), who presented their talks and took part in discussions. The talks were devoted to the problems of cryptic species, hybridization, and intraspecific variability in their various aspects, from morphology to molecular diversity, as well as to recent evolution and area shifts in native and alien plants and animals. Altogether, 22 oral presentations and 5 posters were presented and discussed in four oral and poster sessions during the conference.

The sessions were preceded by an introductory welcoming talk by the Director of the Main Botanical Garden Dr. Vladimir Upelnik.

The first two lectures by Drs. Michael Ignatov from the Tsitsin Main Botanical Garden (MBG) and Vladimir Fedosov from the Moscow State University (MSU) dealt with cryptic species diversity in mosses revealed by molecular genetic studies and further confirmed by the discovery of new and previously neglected morphological characters. The talk presented by Dr. Olga Yurtseva (MSU) was devoted to a fascinating story of species differentiation in the genus *Atraphaxis* in South Siberia. A detailed morphological and geographical study together with molecular phylogenetic data revealed the structure of this complex composed of several geographically differentiated species with only tiny morphological distinctions. In the next talk, Dr. Tatyana Kramina (MSU) described a similar situation with morphologically poorly yet genetically well differentiated members of the *Lotus corniculatus* s.l. polyploid complex in the Iberian Peninsula. The morning session was completed with the lecture by Dr. Vladimir Gokhman from the Botanical Garden of Moscow State University (BG MSU) on cryptic species among synanthropic parasitoids of the family Pteromalidae (Hymenoptera, Insecta) belonging to genera *Anisopteromalus* and *Lariophagus*. He suggested that the presence of cryptic taxa nearly indistinguishable by morphological characters but reproductively isolated from each other and differentiated by their hosts, karyotypes, and behaviour is rather the rule than an exception in these groups.

The first afternoon session started with another zoological talk by Dr. Sergey Grebelnyi from the Zoological Institute, St. Petersburg, on hybrid speciation and the problem of conservation of some mitochondrial genes as copies in the nuclear genome. The next lecture presented by Dr. Victoria Shneer from the Komarov Botanical Institute, St. Petersburg, dealt with

the problem of truly cryptic species in plants and animals, genetically and geographically differentiated, but lacking morphological differences. The case study of *Milium effusum* was discussed in detail. The talk by Dr. Ivan Schanzer (MBG) dealt with the problems of hybridization and hybridogenic speciation in plants with examples from case studies in *Spartina*, *Cardamine*, *Dactylorhiza*, and *Rosa*. Next, Dr. Irina Belyaeva, from the Royal Botanic Gardens, Kew, UK, discussed in her talk the numerous problems arising in the taxonomy and nomenclature of plants due to the existence of cryptic species. The problems she raised in her lecture were further discussed in talks by Mag. Maria Kolesnikova (MBG), who analyzed the problem of representation of cryptic species in databases, and Dr. Olga Demina from Karachai-Cherkessk State University, Karachayevsk, who spoke on typification of three very close species of *Centaurea* from southern Russia.

The after-lunchbreak session started with two fascinating talks. Dr. Gabor Sramko from Debrecen University, Debrecen, Hungary, presented data from his group on the evolution of Eurasian steppes revealed by phylogeographic patterns of *Pulsatilla patens*, *Adonis vologensis*, and *Salvia nutans*. They all indicate recolonization of East European steppes from the western refugia after the last glacial maximum. The next talk by Dr. Natalya Reshetnikova (MBG) dealt with much more recent plant distribution shifts caused by German troops during WWII. In further lectures, Dr. Marina Kostina from the Moscow State Pedagogical University spoke about hybridization and the species problem in the genus *Populus*; Dr. Galina Degtjareva (BG MSU) discussed problems of taxonomy in the genus *Paeonia* due to intraspecific variability and spontaneous hybridization. Then Dr. Maria Galkina (MBG) presented a talk on the evidence of hybridization between alien and native species of *Solidago* in Eastern Europe. A PhD student Cui Wenqiang from Shandong University, China, presented her results on development of SSR markers for studies in the genus *Rosa*. Finally, in the evening session, five more lectures were presented on various aspects of variability and hybridization in plants by Drs. Ivan Savinov, Larisa Kramarenko, Ekaterina Zheleznaya, Vladimir Sorokopudov, and Lyudmila Ozerova. Dr. Ozerova's lecture dealing with the search for sibling species of *Senecio* s.l. in the Cape flora of South Africa was most dramatically and brightly illustrated by photos of the species taken in nature.

This is the first installment of the conference abstracts translated from Russian by Irina Kadis.

Unknown roommates: Cryptic species of synanthropic parasitoids of the family Pteromalidae (Hymenoptera)

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Parasitoid Hymenoptera, or parasitic wasps, is one of the most species-rich, taxonomically complicated and economically important groups of insects (Godfray, 1994; Quicke, 1997). Although only about 80 thousand parasitoid species are currently described in the world fauna (Huber, 2017), the number of potentially recognized members of this group apparently exceeds one million (Bebber *et al.*, 2014; Forbes *et al.*, 2018). In particular, the parasitoid superfamily Chalcidoidea, with its exceptionally high morphological and ecological diversity, contains about 23 thousand known species (Huber, 2017). Up to now, the family Pteromalidae that included more than 3,500 described species, was considered one of the largest among chalcids (Huber, 2017; Noyes, 2018). This group will soon be split into a number of separate families (see, e.g., Peters *et al.*, 2018), but Pteromalidae s. str. remains the most numerous among them. Pteromalidae can attack many insects and some other arthropods, and these parasitic wasps are therefore extremely diverse in terms of bionomics (Noyes, 2018).

Anisopteromalus Ruschka, 1912 and *Lariophagus* Crawford, 1909 are two genera of the family Pteromalidae whose members mostly develop on a number of stored-product pests of the order Coleoptera (Noyes, 2018). In particular, *Anisopteromalus calandrae* (Howard, 1881) was previously considered a thoroughly studied cosmopolitan synanthropic species (see, e.g., Quicke, 1997). At the end of the 1990s, however, we discovered that different populations which presumably belonged to *A. calandrae* had two different karyotypes with haploid chromosome numbers (n) of 5 and 7 (Gokhman *et al.*, 1998). Further studies showed that this complex actually harbored two cosmopolitan species. One of these species appeared new to science and was later described as *A. quinarius* Gokhman & Baur, 2014 in the course of an integrative taxonomic revision of the genus *Anisopteromalus* (Baur *et al.*, 2014). Moreover, our research also demonstrated full reproductive isolation between these species and found substantial interspecific differences in morphology, ecology, behavior, and all other studied character systems (Gokhman and Timokhov, 2002). In particular, *A. quinarius* and *A. calandrae* appeared

to have alternative life-history strategies which are best interpreted in terms of the r/K -continuum (see, e.g., Pianka, 2000), and these species must be considered as K - and r -strategists, respectively (Gokhman *et al.*, 1999). In turn, this can be explained by the fact that these parasitoids tend to attack two different groups of coleopteran hosts, whose ecological strategies are similar to those of the parasitic wasps (Timokhov and Gokhman, 2003). Specifically, members of the genus *Sitophilus* Schönherr, 1838 (Dryophthoridae), on the one hand, as well as *Stegobium paniceum* (Linnaeus, 1758) and *Lasioderma serricorne* (Fabricius, 1792) (Ptinidae), on the other hand, can be respectively considered as r - and K -strategists. The study of nuclear and mitochondrial DNA sequences (i.e., ITS2 and *cytochrome b*) also showed significant differences between these parasitoid species (Baur *et al.*, 2014).

Interestingly, the family Pteromalidae contains *Lariophagus distinguendus* (Förster, 1841), another cosmopolitan species that attacks various coleopteran stored-product pests. Moreover, different populations of *L. distinguendus* also prefer to develop on different host species, similarly to the members of the *A. calandrae* complex. A preliminary examination of these parasitoids using nuclear and mitochondrial DNA markers demonstrated that they belong to two cryptic species (König *et al.*, 2015), and further chromosome study of this group revealed two different karyotypes with $n = 5$ and 6 . It is highly likely that the chromosome set with $n = 6$ represents an ancestral character state in the *L. distinguendus* complex, and the largest metacentric chromosome in the karyotype with $n = 5$ therefore resulted from a fusion between a medium-sized metacentric and the only acrocentric of the initial chromosome set. It is also possible that a central fusion took place there, i.e., it was preceded by a pericentric inversion that had turned the former smaller metacentric into another acrocentric chromosome. If this is true, then the inverted chromosome segments of both forms of the *L. distinguendus* complex in fact represent the so-called supergene, which can accumulate interspecific genetic differences due to the suppressed crossing-over (Thompson and Jiggins, 2014). It is also noteworthy that a thorough morphometric study did not reveal reliable morphological differences between these cryptic species (Wendt *et al.*, 2014). Moreover, both these taxa were apparently described in the past, but their names are presently considered synonymous. In addition, different synanthropic species of the *L. distinguendus* complex are substantially closer to each other than those of the genus *Anisopteromalus*; they also can interbreed (König *et al.*, 2015) and form F_1 hybrids with $2n = 11$ under certain conditions. In turn, virgin hybrid females produce males with chromosome sets similar to those of the parent forms ($n = 5$ and 6).

The above-mentioned results, together with those provided by a number of similar studies, suggest that detection of cryptic lineages of parasitic wasps has become a routine procedure during recent years. Modern research demonstrates that discovery of these lineages within widely distributed parasitoid morphospecies becomes virtually inevitable, once adequate approaches and techniques are applied (Gokhman, 2018b). The development of molecular and chromosomal techniques therefore led to the origination of a new paradigm in integrative taxonomy of parasitoids (Gokhman, 2018a), which is a combination of approaches and techniques aimed for detection, delimitation, and description of closely related species of these insects. Undoubtedly, this also applies to taxonomic research of many other animal groups that include large numbers of morphologically similar species.

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Hybridogeneous Zoophyta: Species and permanent hybrids among sea anemones, corals, and sponges (Anthozoa and Porifera)

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According to prevailing views, mitochondrial DNA has to be transferred from the maternal organism to its descendants unchanged. It is only after a many-generational lapse that descendants originating from eggs of a certain female (or hermaphroditic organism) show noticeable differences, which may be explained by accumulation of nucleotide substitutions during replication of mtDNA via the division of mitochondria and the cells they are contained in. Nucleotide substitutions thus serve both the time and species divergence measure. However, in addition to true nucleotide substitutions, which are detected during comparisons of sequences working in mitochondria, researchers have also encountered substitutions that occur in the so-called *numts* or nuclear copies of mitochondrial sequences. The latter term was introduced by Lopez *et al.* (1994). As documented through multiple examples accumulated in insect studies, failure to discriminate between *numts* (nuclear mitochondrial sequences) and *cymts* (cytoplasmic mitochondrial sequences) has resulted in barcoding errors and distortions of phylogeny (Leite, 2012). Above everything else, it led to false detection of heteroplasmy and descriptions of superfluous species based on differences in mtDNA.

By no means less treacherous is the complete similarity of mtDNA in forms that clearly differ morphologically. Many species of the genus *Acropora* and other reef-building corals exhibit extreme similarities in mitochondrial markers. For this reason, they have been deemed permanent hybrids. Such hybrids are presumably frequently formed near shorelines of tropical islands located far away from each other. At the same time, reciprocal hybrids of certain forms have been described by earlier researchers as distinct species (Vollmer and Palumbi, 2002; Oppen *et al.*, 2001; Willis *et al.*, 2006).

The most notable examples of situations where species identification by means of their mitochondrial markers would definitely lead to mistakes, can be devised from the study of some vertebrates. Examples of hybridogenesis as a mechanism of reproduction of permanent hybrids and their preservation in natural habitats, beside true hybridogeneous speciation, which is frequently accompanied by polyploidy, are found among green frogs, carp fishes, and parthenogenetic lizards (Borkin and Darevsky, 1980; Borkin *et al.*, 1987; Borkin *et al.*, 2001, 2004; Yakovlev and Slynko, 1997; Yakovlev *et al.*, 2000; Slynko and Slynko, 2010). Within these groups, the origin of hybrid forms has been revealed in many cases, and the maternal ancestor in each case can be traced through the mitochondrial marker analysis (for more detail see Grebelnyi, 2008). It is clear that the mitochondrial haplotype of a hybrid always has to be identical to the haplotype of the female involved in hybridization.

Hybridization and polyploidy among sea anemones and other lower marine invertebrates previously classified as Zoophyta (*Actiniaria*, *Alcyonacea*, *Scleractinia*), probably are as widespread as among vascular plants, though much less studied. For many crustaceans and insects, it has been even possible to build polyploid series similar to those well-known in flowering plants. Remarkably, polyploids in the latter groups at times are treated as karyomorphs, at other times as separate species (Grebelnyi, 2008, Table 2).

Yet another group of lower invertebrates that we have studied as regards similarities or divergences in molecular markers were horn sponges (Demospongiae) belonging to some geographically widespread genera from different families. The comparisons of species pairs were based on nuclear genes coding small and large ribosomal subunits (18S and 28S). In these oldest multi-cellular organisms, the most closely related couples of species proved to be quite distant in space, e.g., tropical and Antarctic species. On the other side, species showing more similarity in markers were not always closely related by their taxonomical position, sometimes even belonging to different families. These results make us assume that in sponges transmission of thermal adaptation genes as well as transfer of nucleotide sequences, used by us as molecular markers, occurs more freely than in higher animals. It is possible that horizontal gene transfer, which is so widespread in prokaryotes and unicellular eukaryotes, is also important in processes of genomic divergence among higher-organized animals (see Golubovsky, 2000).

Preserved as *numts* inside the nuclear genome, copies of mtDNA may probably be moved back to mitochondria and put to work, once they are on demand via the natural selection. This flow, sporadically detected by molecular phylogeneticists, interrupts the beauty of phyletic reconstructions, because it combines both very similar and considerably divergent sequences in

the same mitochondrial haplotype (Grebelnyi *et al.*, 2018). We are talking here not just about technical mistakes in barcoding but rather about co-evolution of the mitochondrial and the “major,” nuclear genome of an eukaryotic cell.

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Cryptic species, interspecific hybridization, and the breakdown of biodiversity

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Since the advent of molecular phylogenetic methods, particularly the polymerase chain reaction (PCR), which provided the key to infraspecific genetic differentiation, there has been an ever increasing avalanche of discoveries of cryptic species in various animal groups (see Bickford *et al.*, 2007). Multitudes of cryptic animal species have been detected, sometimes scores within a single previously recognized species. However, reports of similar discoveries in plants, especially in vascular plants, are much scarcer. This was noted early on by Bickford *et al.* (2007). By 2014, we succeeded in finding about 30 examples in the literature (Shneer and Kotseruba, 2014); after that, we have been able to only add a few cases to this number. Cryptic species in plants continue to produce an impression of special occasions, even though some experts, such as Grant (1984), assumed there must be plenty.

We have detected a pronounced molecular differentiation in nuclear ITS1 and ITS2 of rRNA genes and in some chloroplast genes in a common plant of the European temperate forest, *Milium effusum* L., a species with a wide range in Eurasia extending into North America. The analyses of ITS sequences of a large number of samples originating from Eurasia and North America and additionally some chloroplast gene analyses have demonstrated that samples originating from East Asia (the Russian Far East, Japan, Korea, and China) form a clade isolated from the rest of the samples (ITS differences in 27 positions). The ITS analyses employing the NGS method for representatives of both clades (2 from either clade) and construction of haplotype network demonstrated that there are no duplicate haplotypes in these groups, although there are 4 similar ones. Polymorphism in nucleotide substitutions is more pronounced in Eurasian samples. *M. effusum* is a tetraploid with $2n=28$, which leaves room for the following explanation: upon the formation of the species, as a result of interspecific hybridization of two hypothetical diploids, the populations from the Far East have supposedly lost rDNA of one of the parents, while the rest of the populations lost rDNA of the other parent. Yet intragenomic polymorphisms (single-nucleotide polymorphisms, SNPs, or *snips*) in the genomes of the Far East populations do not overlap with snip combinations found in plant genomes from Kyrgyzstan and Cis-Baikalia. With a 95% probability, these are two different haplotype systems. One

probable cause of this situation could be a prolonged isolation; it can also be explained by the history of advancements and retreats of glaciers (in accordance with M.G.Popov's hypothesis) and the availability of refugia; yet another explanation is in the peculiarities of the monsoon climate in the region and the connected shift to self-fertilization in plants. As per Popov's suggestion, many plants in the Far East that are believed to be conspecific with the European or even Eurasian species could actually constitute distinct species. Our own efforts to detect such plants so far have not succeeded.

The second half of the 20th century was marked by the complete dominance of the Biological Species Concept (BSC) – at least in the field of Zoology. It has been traditionally connected with Ernst Mayr's name, although a considerable contribution was made by Theodosius Dobzhansky, who formulated the notion of reproductive isolation and deemed it a major attribute of species and a crucial factor of speciation, while interpreting the results of his experiments with the fruit fly – *Drosophila*. BSC, however, has never gained much popularity among botanists. In his foreword to the Russian translation of Grant's *Plant Speciation*, Takhtajan noted that the most controversial idea in the book was the acceptance of the BSC by the author. He further remarked that “in Botany, it had a relative success only during the 40's and to some extent the 50's (including the approval from the author of this note)” (Takhtajan, 1984). Many authoritative botanists, including R.Wettstein, J.A.Nannfeldt, V.L.Komarov and S.V.Juzepczuk, were proponents of “smaller” and/or monotypic species.

The major argument of botanists against the BSC was the existence of non-biparental species among plants as well as apomictic species and those with allopatric ranges. Hybridization is a common phenomenon even among biparental species. An attempt to account for all reports on interspecific and even intergeneric hybrids in the flowering plants published before 1970 yielded the staggering number of 23675 (Knobloch, 1971). Hybridization among the contemporary vascular plants has been detected in more than a third of their families and in up to 15% of genera. Differently from zoologists, plant taxonomists would seldom have doubts regarding the distinct species status of the parental organisms.

The existing paradigm started to change with the onset of the new century. One authoritative contemporary author who has been actively expressing and promoting new concepts regarding species is Mallet (2001, 2005, 2006), a specialist on Lepidoptera. Accumulation of new data and observations has made it possible to state that interspecific hybridization is rather widespread in various animal groups including, for example, birds. The hybridization rate among Great Britain's true ducks is known to reach 76% (Mallet, 2005). In

this respect, the genus *Drosophila* constitutes an exception: only about 1% of its 1750 species known to science have produced hybrids, so Mallet believes that the employment of *Drosophila* as a model object in the study of species has resulted in lopsided views, while active research on plants, vertebrates, and large insects has produced more critical information (Mallet, 2005).

The porous nature of the isolating barriers, the crucial role of the ecological differentiation as well as divergence of particular genes, which exhibits mosaic character, and the importance of hybridization and polyploidy in the evolution have been moved to the forefront of the contemporary studies. The rarity of discoveries of cryptic species in the plant world makes it possible to assume that botanists, among whom there have been many “splitters,” actually break down the biodiversity more adequately.

On the typification problem in *Centaurea tanaitica* Klok., *C. triumfettii* All., and *C. czerkessica* Dobroc. et Kotov

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A complicated situation has been encountered with the synonymy of the species in the genus *Centaurea* L. in the Caucasus. According to the *Conspectus of the flora of Caucasus* (2008), *Centaurea tanaitica* Klovov is a recognized species in the section *Protocyanus* Dobroc. Its synonyms include *C. czerkessica* Dobroc. & Kotov, *C. triumfettii* All. subsp. *tanaitica* (Klovov) Dostál, *C. pseudotanaitica* Galushko, *C. stricta* auct. non Waldst. & Kit., and *C. fuscimarginata* auct. non Juz. Separately from these, in the subsection *Fischeriae* Czerep., there is a recognized species *Centaurea triumfettii*. Species that are extremely close to *C. triumfettii* have been often lumped with it and listed as synonyms. These are *C. variegata* Lam., *C. czerkessica*, and *C. tanaitica* (Zernov *et al.*, 2015).

However, *C. triumfettii* occurs only in southern Transcaucasia—even though it is designated as ‘one of the most widespread and polymorphic species of knapweed’ (*Conspectus of the flora of Caucasus*, 2008: 302); *C. tanaitica* is listed for two floristic subregions of the Western Caucasus (Adagum-Pshish and Urup-Teberda), the Central and Eastern Caucasus, and northwestern Transcaucasia (Anapa-Gelendzhik Floristic Region) (*Conspectus of the flora of Caucasus*, 2008).

According to the *Flora of the USSR* (1963), the regions included in the geographic range of *C. tanaitica* are eastern Circum-Pontic Region, the Lower Don, and Northern Caucasus, while

C. czerkessica occurs exclusively in the northwestern Caucasus. In the *Flora of the USSR*, both *C. tanaitica* and *C. czerkessica* are assigned to sect. *Stricticaules* of the series *Alate*, while *C. triumfettii* (= *C. axillaris* Willd.) is treated as a European species belonging to a separate series. At the same time, *C. triumfettii*, when it is treated as a subspecies, is attributed to a completely different section *Variegata*, where this name is listed as a synonym for the Crimean species *C. fuscomarginata* (K.Koch) Juz. (*Flora of the USSR*, 1963).

Regional floras generally tend to treat knapweeds as distinct species rather than subspecies. For example, among those listed for the Western Caucasus, there are *C. fuscomarginata* (rocky outcrops surrounding Anapa), *C. tanaitica* (steppes in the western part of Northern Caucasus: northern Azov-Kuban Plain and the Lower Don), and *C. czerkessica* (dry grassy slopes in northeastern Transcaucasia and the vicinity of Krymsk (Kosenko, 1970). Galushko (1980) lists 4 species for the Northern Caucasus: *C. fuscomarginata*, *C. tanaitica*, *C. pseudotanaitica*, and *C. czerkessica*. The *Flora of the Lower Don* (1985) mentions *C. tanaitica*.

At the same time, according to the *Flora of the European Part of the USSR* (1994), Caucasian plants previously identified as *C. tanaitica* must be placed in *C. czerkessica*. According to yet another note in this *Flora*, samples from Natukhayevskoye Forest Farm, Anapa District, Krasnodar Province, previously identified with *C. fuscomarginata*, must be as well assigned to *C. czerkessica* (*Flora of the European Part of the USSR*, 1994: 272). Yet at the same time, the latter species is characterized as an endemic of the Crimea in the *Field Guide to the Higher Plants of the Crimea* (1972).

These species are quite distinct morphologically. The unique characteristics of *C. fuscomarginata* are as follows: fringe segments on apical appendages of involucre bracts (of phyllaries) are 3–4 times as long as appendage base width; bristles on the inner-series pappi are twice as short as those on the outer pappi series.

Two species of knapweed, *C. tanaitica* and *C. czerkessica*, are compared in the current work as regards their morphology. The material for the study was collected in 2018 and preserved at KCSU (Herbarium of the U.D. Aliev Karachay-Circassian State University).

Centaurea tanaitica is a comparatively tall (up to 60 cm) plant; involucre 16–23 mm tall; heads 1–7; stem narrowly winged, to 3.5 mm thick at points of leaf attachment; leaves elongate lanceolate, long-acuminate at apex; involucre bracts (phyllaries) triangular; apical appendages of the outer and intermediate involucre bracts also triangular, bases of appendages narrow, up to 1 mm broad, predominantly light-colored, sometimes dark; fringe segments of appendages from whitish to brown, up to 2 mm long; outer florets up to 30 mm long. *C. tanaitica*

occurs in the steppe habitats of the Lower Don. This is a typical steppe species; as compared to *C. czerkessica*, it is more mesophytic and of a strikingly different habit. We have encountered *C. tanaitica* on a horse-breeding farmland, Glukhonky Land Parcel between Tselinnyy and Obilnyy settlements, Rostov Oblast, at the northwestern extremity of the Northern Caucasus. The population density was 2–7 plants of various age per m²; population area about 1000 m².

Centaurea czerkessica produces more compact (10–20 cm tall) plants covered with gray-arachnoid (not floccose) indumentum, typically with a solitary large head up to 4.5 cm in diameter; stem broad-winged: all cauline leaves decurrent onto the stem and forming wings up to 8–10 mm in width; leaves broad lanceolate, caudate to cuspidate at apex; outer involucre bracts (phyllaries) broad triangular, intermediate ones elongate elliptic, both with apical appendages decurrent nearly to bract bases, each appendage consisting of black or brown base up to 2 mm broad and light-colored brownish fringe segments up to 5 mm long; outer florets considerably enlarged, to 36 mm long, either dark blue or light blue. *C. czerkessica* occurs on eroded slopes in petrophytic sub-Mediterranean mountain steppes and is a typical petrophyte and euxerophyte. We registered this species in the vicinity of Verkhnebakanskiy Railroad Station, Krasnodar Province. In areas of compact growth, the population density reached 5–12 plants of various age per m²; population covered an area of ca. 400 m².

C. tanaitica and *C. czerkessica* are different from each other both in their ecology and sociology, their geographical ranges and ecological niches never overlapping. Yet a molecular-phylogenetic study is to be undertaken in order to finalize the decision regarding the independence of the two species.

The Caucasian *Scopolia*: *S. caucasica* Kolesn. or *S. carniolica* Jacquin?

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The genus *Scopolia* Jacquin (Solanaceae) was named for Italian-Austrian (Tyrolean) physician and naturalist Giovanni Antonio Scopoli. *Scopolia caucasica* Kolesn. ex Kreyer (1950) is an herbaceous perennial to 80 cm in height, with a stout branched rhizome. Leaves alternate, ranging from scale-like, obtuse (proximal) to ovate-oblong, acuminate (distal), up to 15 cm long. Flowers solitary, nodding, purple, up to 2.5 cm long. Seed kidney-shaped, pitted. The plant

occurs in the Caucasus, in fir-beech, beech, and beech-hornbeam forests, in moist situations, commonly along streams, ascending to 1700 m a.s.l. Rare. This is an ornamental and medicinal plant, whose rhizome contains up to 1% alkaloids, according to the *Flora of the USSR* (Semenova, 1955).

A new location of *S. caucasica* has been discovered within the Karachay-Cherkess Republic territory, in a moist forest on the left bank of the Great Laba River, 3.5 km southwest of Aziatskiy (Asian) Settlement, at Banny Ruchei (Bath Brook), Grushevaya Polyana (Pear Glade), at N 43.89643, E 40.94362, 913 m a.s.l., collected on 04.04.2017.

Galushko listed this taxon in the *Flora of the Northern Caucasus* (Galushko, 1980) in the species rank; however, other experts (Grossheim, 1949; Kosenko, 1970) treated it as a synonym of European scopolia, or henbane bell, *S. carniolica* Jacq., i.e., believed that the plant in the Caucasus should be attributed to *S. carniolica*.

In the description of *S. carniolica* in the *Flora of the USSR*, there is a note listing the following varieties differing in the color and shape of corolla: *S. carniolica* var. *brevifolia* Dun. distinguished by yellow flowers; *S. carniolica* var. *longifolia* Dun. with corolla of subcylindric shape; *S. carniolica* var. *violacea* Semenova with a violet corolla. Plants belonging to the latter variety occur in the Caucasus as well as in the southern part of West Europe (Semenova, 1955: 100–101).

If one accepts the attribution of the Caucasian plant to the European *S. carniolica*, following the recently published *Guide to Vascular Plants of Karachay-Cherkess Republic* (Zernov *et al.*, 2015), then our collection has confirmed the presence of this species in the Great Laba Valley, as previously reported by Vorobieva (1988).

A molecular-phylogenetic study could give a resolute answer to the question about the possible taxonomic independence of the two species. However, it is possible to note right away that the Caucasian plant differs from the *S. carniolica* description provided by Zernov and co-authors: “Corolla brownish on the outside, greenish-yellow inside” (Zernov *et al.*, 2015: 362).

Herbarium samples have been preserved in KCSU, the recently organized Herbarium of the Ilham Aliyev Karachay-Circassian State University.

Paeonia beibersteiniana: is there such a species?

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The International Plant Name Index includes 173 species names for the genus *Paeonia* L. (Paeoniaceae); of these, only about 35 are currently accepted (Stern, 1946; Hong, 2010). High morphological variability, hybridization, and the presence of polyploid forms result in challenges with the species delimitation and explain existing differences in the views of authors regarding the limits of taxa. Hence species identification remains an urgent task for the study of biodiversity in *Paeonia*, while employment of molecular markers appears to be a promising approach.

Paeonia tenuifolia L. occupies an isolated position among the peony species as regards morphological characters, for its leaflets are deeply divided into linear segments. The species range includes the Balkan Peninsula, Southern and Eastern Europe, the Caucasus, and Crimea. The plant occurs in the steppe belt, occasionally en masse. Despite its isolated position in the genus, *P. tenuifolia* is known to be rather polymorphic as far as leaf segment width, extent of leaf dissection, and the overall height. This triggered descriptions of a few species: those with wider segments – *P. beibersteiniana* Rupr., *P. carthalinica* Ketzch., *P. majko* Ketzch. along with one having relatively narrower segments – *P. lithophila* Kotov. Some authors (e.g., Schipczinsky, 1937; Grossheim, 1950; Kemularia-Nathadze, 1961) considered these species to be distinct. More recently, however, *P. beibersteiniana*, *P. carthalinica*, and *P. lithophila* have been treated in the rank of forms or races of *P. tenuifolia* (e.g., Punina and Mordak, 2009; Hong and Zhou, 2003), while *P. majko* is believed to be a nothospecies formed as a result of hybridization of *P. tenuifolia* and *P. caucasica* (Schipcz.) Schipcz. (Punina *et al.*, 2010). The hybridogenic origin of *P. majko* has been further confirmed by molecular data (Punina *et al.*, 2012).

Of the *P. tenuifolia* forms mentioned above, the most widespread is var. *beibersteiniana*, which occurs around Stavropol and in the Greater and Northern Caucasus together with the typical form and is connected with the latter through a very gradual continuum of intermediate forms, differing more consistently only in its later flowering (Busch, 1903; Takhtajan, 1966).

Our goal was to determine if the observed polymorphism in morphological characters of *P. tenuifolia* correlates with the intraspecific nuclear ribosomal DNA divergence. Nucleotide ITS

sequences were obtained using the Sanger sequencing method. We sampled *P. tenuifolia* from herbarium specimens preserved in MHA, MW, and YALT and originating from different parts of the species range: Crimea, Dagestan, Stavropol Krai, as well as Ulyanovsk, Lugansk, and Volgograd oblasts.

ITS sequence analyses showed that specimens with broad leaf segments from the Crimean populations constitute hybrids between *P. tenuifolia* and *P. daurica* Andrews, while samples from other parts of the range did not exhibit any major differences between the typical and broad-leaved forms. *P. daurica* and *P. tenuifolia* co-occur in Crimea, the former being quite different from the latter in its morphology (above everything else, in having twice ternate leaves with rounded segments) as well as ecological preferences: this is a forest species. Hybridization of these two species results in plants whose habit, rather than being intermediate between the parents, is closer to *P. tenuifolia*, differing from it only in wider leaf segments. In other words, plants representing the pure broadleaf form of *P. tenuifolia* are not much different morphologically from its hybrids with *P. daurica*. Remarkably, the cultivars ‘Early Scout,’ ‘Early Bird,’ and ‘Orlenok,’ which have been produced through hybridization of *P. tenuifolia* and *P. lactiflora* Pall. (a species characterized by broad leaf segments, white flowers, and many-flowered inflorescences), also approach *P. tenuifolia* in their morphology.

The results of the study of ITS nrDNA sequences in *P. tenuifolia* justify the treatment of *P. biebersteiniana* in the rank of a form. However, morphological differences between forms of *P. tenuifolia* should not always be attributed to just phenotypic plasticity, as they may also indicate genotypic differences resulting from hybridization. Therefore, molecular data have proven to be illuminating in the course of studies in this group. Morphological similarity of interspecific hybrids to one of the parent species has highlighted a need for another round of detailed morphological studies.

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Major lineages of steppic plants inform us about the last scene in the evolution of the Eurasian steppe

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The Eurasian steppe zone is one of the largest continuous biomes on the planet: stretching from Eastern Asia to Europe, it covers about 12 million km². The Altai Mountains in

Central Asia split this zone into two major parts: the eastern part constitutes the Central Asian steppe zone, whereas the western can be named the Euro-Siberian steppe zone or, according to Lavrenko, the Black Sea–Kazakhstan steppe zone. The Euro-Siberian Steppe forms a continuous belt from the Altai Mountains to the Carpathians. While the Pannonian and Transylvanian basins are not considered as part of this zone by contemporary biogeographers, a large number of species shared between these basins and the Black Sea–Kazakhstan zone speaks in favour of such an inclusion. Moreover, the presence of several species that are restricted to the ‘true’ steppe zone (*Adonis volgensis* DC., *Centaurea ruthenica* Lam., *Salvia nutans* L., *Spiraea crenata* L., etc.) may indicate the presence of the true steppe subsidiary zone within the Pannonian part of the Euro-Siberian steppe zone. As the steppe zone provides the major ground for grain production in Central and Eastern Europe, the territory of steppes has been greatly reduced, and particularly that of Pannonian steppes, which have been documented to have lost 98% of the habitat area. This makes comparisons of floristic composition extremely difficult. However, the connection between the Pannonian and Black Sea–Kazakhstan steppe zone is clear, and therefore members of our research group regard the Pannonian part as the westernmost part of the Euro-Siberian steppe zone.

Despite the importance of the steppe as a major biome and rapidly vanishing habitat type, our knowledge of the evolutionary history of species inhabiting the Eurasian steppe zone and understanding of the history of steppes is very limited. Phylogenetic and palaeobotanical evidence provides a clue regarding the Central Asian origin of the steppes dating back to the Miocene. From there, starting from the end of the Miocene, steppes gradually expanded to the lowlands of Eurasia and reached Europe from the east during the Pliocene, stretching to their largest extent during the glaciations. In accord with this development of the Eurasian steppe biome, several steppe-inhabiting genera (e.g., *Atraphaxis* L., *Lagochilus* Bunge ex Benth., *Calophaca* Fisch., *Caragana* Fabr., *Dontostemon* Andr. Ex C.A.Mey.) have been confirmed to originate from the Central Asian mountains (such as the Tian Shan or Pamir-Alay). However, very little is known about the more recent evolutionary history of steppic plants or their genetic structure throughout their range.

Our research group therefore aimed at understanding the evolutionary history of steppic plants and animals of the Euro-Siberian steppe zone, while focusing on the following species: *Adonis volgensis*, *Crambe tatarica* Sebeók, *Galatella villosa* Rchb.f., *Spiraea crenata*, *Pulsatilla patens* s.s., *Salvia nutans*, *Taraxacum serotinum* (Waldst. & Kit.) Poir., and *Kochia prostrata* (L.) Schrad. This list was augmented with some animal taxa: *Lethrus apterus*, *Kretania pylaon*

agg., *Mustela eversmanni*, and *Sicista subtilis* agg. Our research approach involves studying these species from a phylogenetic perspective by learning about their (i) sister species and (ii) ancestral range; then providing an area-wide phylogeography based on phylogenomic (RAD-seq) and plastid data, while using the sample of the sister species as the outgroup.

According to our preliminary phylogeny, *Adonis volgensis*, a species distributed throughout the Euro-Siberian steppe zone, has a sister species in southern Siberia, *A. villosa*. The origin of the genus traces back to the Tian Shan. When we used rapidly mutating plastid regions to draw a plastid phylogeography outline, the approach revealed the existence of three major geographic groups within *A. volgensis*: the western (from Pannonia to the Dnieper), central (mostly Dnieper to Volga), and eastern (Volga to eastern Kazakhstan). Expectedly, the plastid regions could not provide sufficient resolution for discrimination between the groups. The application of the genomic approach, however, provided nice resolution between the populations sampled throughout the area: in the presence of samples of the sister species, *A. villosa*, we could root the resulting phylogenetic tree for *A. vernalis*. It demonstrated that the species had its basal lineages in the Pannonian part of its range, while the main lineages corresponding to the plastid geographic groups produced phylogenetically younger clades towards the east.

A similar pattern was found for *Pulsatilla patens* s.s., where our genus-level phylogeny has indicated a Central / East Asian origin of the genus and a sister species in the *Pulsatilla* series *Patentes*. When we applied plastid-based phylogeography, it indicated western, more precisely, Transylvanian basal lineages within the species. Similarly, the genomic approach (RAD-seq), where *P. flavescens*(Zucc.) Juz. from Buryatia was used as the outgroup, also indicated western basal lineages. A similar finding was made for *Salvia nutans*, and even though in this case we failed to identify the sister species, the analysis of the population genetic structure across the entire distribution area clearly hints at western refugia.

To sum this up, our results so far indicate the Central Asian origin and a west-to-east direction of migration for the studied Euro-Siberian steppe species. We assume the genomic approach thus reveals the last scene in the evolutionary history of Euro-Siberian steppe plants. During the last glacial maximum (LGM), at least some of them might have retreated to a western refugium (a hypothesis supported by some LGM habitat type reconstructions!); then during the Holocene they colonised the Euro-Siberian steppe in an eastern direction. This finding has an important conservation implication: if the rear edge matters, the Pannonian relict populations of steppe plants (which have been traditionally treated as the vanguard of the steppic biome), have high conservation value.

SSR markers for revealing intraspecific variation in beach rose (*Rosa rugosa* Thunb.) of various provenances

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Study objectives

- Screening *Rosa rugosa* samples of various provenances using SSR markers previously applied to *R. rugosa* and its relatives in Rosaceae
- Selecting the polymorphic SSR markers with steady amplifications among *R. rugosa* of different provenances
- Testing the responses of *R. rugosa* samples of various provenances to the polymorphic SSR markers

A single pooled sample of three *R. rugosa* samples from each of 3 populations (European naturalized population EUR, Chinese native wild population CWR, and Chinese cultivated population CCR) was used to screen and select appropriate SSR primer pairs for intraspecific variation analysis of *R. rugosa*. Samples of EUR group originated from Langeoog, Germany, Dragor, Denmark, and Skanor, Sweden. The SSR primer pairs with polymorphic and steady amplifications will be selected for further analysis. The nine samples of different provenances will be used to test the sensitivity and availability of the selected SSR markers.

Methods

1. Genomic DNA extraction
2. Screening of SSR primers

Genomic identification

- Pre-experimental non-fluorescent PCR amplification
- Pre-experimental fluorescent PCR amplification
- Data Analysis
- Formal experimental fluorescent PCR amplification
- Data Analysis

Results and analysis

A total of 20 primer pairs with polymorphic and steady amplifications were selected from the two rounds of pre-experimental PCR amplifications using common primers and fluorescent primers of 55 loci. The pre-experimental fluorescent PCR amplification of the 20 primer pairs for three *R. rugosa* samples from each of 3 populations with different provenances showed a large intraspecific genetic variation with overall average effective allele number of 2.43, Shannon's Information Index of 0.92, and Nei's expected heterozygosity of 0.51, respectively.

Table 1. Nei's original measures of genetic distance and genetic identity [Nei (1972) Am. Nat. 106:283-292]

Provenances of the tested <i>R. rugosa</i> samples	European introduced wild populations (EWR)	Chinese wild population (CWR)	Chinese cultivated population (CCR)
European introduced wild populations (EWR)	*	0.7956	0.5943
Chinese wild population (CWR)	0.2287	*	0.6405
Chinese cultivated population (CCR)	0.5203	0.4454	*

Nei's original measures of genetic distance (below the diagonal) and genetic identity (above the diagonal) are shown in Table 1. It demonstrates that the samples from the Chinese native population (CWR) were genetically closer to those from the European naturalized population (EWR) than to the Chinese cultivated population. Among them, the samples from the Chinese wild population (CWR) had relatively higher identity with the samples from the Chinese cultivated population (CCR). It means that the selected 20 SSR markers can reveal the genetic divergence among *R. rugosa* samples of different provenances.

Conclusions

The two-step PCR of non-fluorescent primers for pooled sample and fluorescent primers for both pooled and separate samples of *R. rugosa* were effective for the SSR markers selection applied for revealing of intraspecific variation and divergence in *R. rugosa* of different provenances. However, SSR marker selection from previously used primers are still

limited, further SSR marker development based on transcriptome sequencing and genomic DNA sequencing is needed in the future.

Cryptic species in the genus *Senecio* L.

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Tribe Senecioneae is one of the largest in the family Asteraceae. It is distinguished by morphological and ecological diversity of succulent plants from a number of genera: *Senecio* L., *Curio* Heath, *Caputia* Nord. & Pelser, *Othonna* L., *Kleinia* Mill. Among these, there are species with unusual types of leaves: terete subunifacial and unifacial, pseudobifacial, or peltate. *Senecio oxyriifolius* DC. has been often confused in botanic gardens with a look-alike species, *S. tropaeolifolius* MacOwan ex F.Muell. In its native South Africa, *S. tropaeolifolius* was traditionally treated as a subspecies of *S. oxyriifolius*. According to their descriptions, these are geophytes with smooth, weakly succulent stems, peltate long-petioled leaves, and an underground caudex. Initially, the leaf primordia in these species are distinctly bifacial. The subsequent growth results in adaxially enlarged distal part of the primordium, which leads to the formation of a bifacial peltate leaf blade. Due to gradual reduction of the adaxial surface, an unifacial area, the petiole, forms at the base of the primordium. Hence both species have petiolate weakly succulent leaves, which, in the course of their formation, change from bifacial with a rhombic, dentate blade to peltate with a rounded, weakly and sparingly dentate blade and a completely unifacial petiole. We have demonstrated this process with a series of anatomical cross-sections of mature leaves.

According to diagnoses, characters that can be used for differentiation between the two species are mostly differences in the number of phyllaries in the involucre and florets in the head as well as the presence/absence of pseudo-ray peripheral florets and pappi (bristles) on cypselae. Yet generally within groundsels (*Senecio* s. *latiss.*), the stability as well as taxonomic value of these characters is quite variable.

Neither species ever flowers in cultivation. During a prolonged dormancy period, all their vegetative parts die off, except for the underground caudex. The natural habitat for *S. tropaeolifolius* and *S. oxyriifolius* is the frequently burned grassland of the South African Provinces of QuaZulu-Natal, Limpopo, and Mpumalanga, where plants overcome adverse

conditions as dormant underground caudices. Flowering of *S. oxyriifolius* has been observed only infrequently in remote, hardly accessible mountain regions. Flowering of *S. tropaeolifolius* has been known only from herbarium specimens preserved in the Kirstenbosch and Kew Botanical Gardens.

The author has been able to successfully distinguish between the twin species when applying methods of molecular phylogenetics. Both plants had been subject to molecular studies before (Sombra Staheli, 2006; Pelsner *et al.*, 2007); yet only Jeffrey (1986, 1992) regarded *S. tropaeolifolius* and *S. oxyriifolius* as two distinct species in the section *Peltati*. The author studied the relationship between *S. tropaeolifolius* and *S. oxyriifolius* using the structure of the region ITS1–5.8S RNA–ITS2 of the nuclear ribosomal operon in the context of cladistic relationships within the succulent groundsel species group, which has been extended, as compared to the group of species covered in a preceding study (Malenkova *et al.*, 2014). The species are located on the resulting cladogram rather close to each other; however, contrary to the expectations, they were not found to be in a sister relationship. *S. oxyriifolius* is a sister species with *S. junceus* Harv., while *S. tropaeolifolius* is a sister species with *Curio kleiniiformis* Heath. According to the obtained results, *S. oxyriifolius* has little to do with *S. tropaeolifolius*. The data speak not just against including the latter in *S. oxyriifolius* as a subspecies, but even against considering both species within a single monophyletic genus.

Thus, the molecular phylogenetics data justify accepting *S. tropaeolifolius* in the species rank, demonstrating its independence from *S. oxyriifolius*.

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Intraspecific diversity in comet orchids (*Angraecum* Bory)

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Non-nectariferous flowers of orchids attract pollinators in various ways. Many *Angraecum* orchids combine self-pollination with cross-pollination. Although the size of the flower and spur length are frequently mentioned in the identification keys to *Angraecum* species, the micromorphology of the spur observed with a scanning electron microscope has not been studied (Telepova-TeXier, 2017). The author studied the intraspecific variability in three comet orchids: *Angraecum sesquipedale* Thouars, *A. longicalcar* (Bossert) Senghas, and *A. eburneum* Bory. The presence of well-developed spurs of various lengths (4–40 cm) in the *Angraecum* flowers led to comparisons with the length of the proboscis in pollinators from Madagascar.

The most famous of the studied species, *A. sesquipedale* named by Darwin ‘the comet of Madagascar,’ has large white flowers with a diameter of 15–20 cm. The spur length varies greatly: from 25 to 35 cm. The author’s own data on the microstructure of the long-armed *Angraecum* demonstrate that the trichomes of its internal epidermis are involved in maintaining the water balance of the flower. No more than 3 flowers usually form in an inflorescence of this species (4 or 5 in the most favorable conditions).

In contrast to this comet, two other species have 20–30 flowers per inflorescence. A rare endemic of Madagascar, *A. longicalcar*, which has the longest and least constant spur (37 cm in the greenhouse and 40 cm in nature), may not bloom for years waiting for the necessary conditions. As a result of low morphological variability of *A. longicalcar* flower elements, the probability of its extinction increases. In *A. eburneum*, a widespread species whose range

includes Réunion and Madagascar, the diameter of the flower is more or less stable (4–5 cm), but the spur length varies greatly (5–9 cm), although it remains constant within a single inflorescence.

While analyzing the causes of genetic intraspecific variability, which is expressed in the diversity of *Angraecum* morphological characters (in particular, the length and pilosity of the spur), the author came to the conclusion that the study of variability at the population level, the highest level of biodiversity, is exceptionally desirable.

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