Chapter

Hybridogenic Activity of Invasive Species of Asteraceae

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Abstract

We studied taxa from genus *Bidens*, *Solidago*, and *Erigeron*, sect. *Conyza* (Asteraceae). By analyzing the nucleotide sequences of the internal transcribed spacer (ITS1)-ITS2 site, the hybrid origin of the *Bidens* × *decipiens*, previously attributed to the North American alien species *B. connata*, was confirmed. The analysis of trnL-trnF sequences showed that the native *B. cernua* is the maternal species and the invasive *B. frondosa* is the paternal species in all probabilities. Diagnostic morphological features of the three *Solidago* taxa growing together in the vicinity of Pskov have been studied: a native *S. virgaurea*, an invasive species of North American origin *S. canadensis*, and their hybrid *S.* × *niederederi*. *S.* × *niederederi* has an intermediate position between *S. virgaurea* and *S. canadensis*. The hybrid origin of *S.* × *niederederi* is proven by molecular analysis of nuclear DNA nucleotide sequences (ITS1-ITS2 site). It is not yet possible to unambiguously answer the question which parent species is maternal and which is paternal. We also studied invasive species of the genus *Erigeron* sect. *Conyza* in the Mediterranean. Occasionally occurring in Southern Europe, individuals of *E. canadensis* × *E. sumatrensis* with intermediate morphological features, described as "*Conyza* × *rouyana*," are likely unstable and soon "absorbed" by the parent species *E. sumatrensis*. Contrary to the hypothesis by C. Elton explaining the success of plant invasion in a new homeland by strengthening hybridization processes in the secondary distribution range.

Keywords: hybrids, hybridization, invasive species, *Bidens* × *decipiens*, *Solidago* × *niederederi*, *Erigeron canadensis*, *E. sumatrensis*, ITS1-ITS2 site, *rpl32-trnL* intergenic spacer, *trnL-trnF* intergenic spacer

1. Introduction

There is a hypothesis that the strengthening of hybridization processes in the secondary distribution range contributes to more successful existence of plants in their new homeland [1, 2]. Under unusual conditions, alien species can form hybrids with closely related native species, as well as with other alien plants inhabiting a given area. Often, hybrids are better adapted to secondary distribution range conditions than parent taxa [3–5], resulting in landscapes in a new home area. Successful recombination of genetic traits of parent species reduces the lag phase (a period of adaptation of an alien taxon to new conditions during which there is not yet active introduction into natural phytocoenosis and expansion of the secondary distribution range) and leads to the formation of new active "species transformers."

Cross-pollinated plant species are the most predisposed to hybridization. Sympatric species are less likely to be cross-species hybridized than allopatric species or populations [6]. The share of hybrid taxa among invasive species of Middle Russia reaches 10% [7].

For a long time, the most important factor in limiting hybridization was geographical isolation, but nowadays closely related taxa come into contact with each other through a multitude of anthropogenic "corridors" [8]. Thus, inter-regional immigration occurs by means of the introduction of plants, which can be frequent and repetitive, and therefore it significantly increases immigration flow [9]. If we consider the situation where conditions for the hybridization of closely related taxa already exist, there may be several possible developments that coexist: (a) New hybrid taxon may appear, and (b) native taxon disappears. During hybridization, genetic assimilation occurs, and new genes are injected into one or both parent species. Hybrids, even being fertile, can, however, be reproductively isolated from parent plants due to the effect of the selection on reproductive traits (allopolyploidy, heterozygous translocations, recombination, mitochondrial DNA-specific differences) and/or due to factors that predetermine crossing (flowering phenology, separation of ecological niches). Interspecific hybridization may also facilitate the naturalization of rare genotypes and cause an increase in their proportion by inverse crossbreeding with alien parent taxa or hybridization between the hybrids themselves. Greater selection advantages for alien alleles should lead to faster replacement of natural alleles through hybridization and slower replacement without hybridization [10]. The period of displacement (substitution) decreases significantly with increasing immigration flow and selective differentiation. Immigration and selection operate in a variety of ways: increasing immigration levels result in the substitution of native species by suppressing them, while increasing selective differentiation in favor of an alien species results in the substitution of an alien species by genetic assimilation without leaving "pure" native species. At moderate and high immigration rates, the loss of native species can be rapid with or without hybridization. Given the high number of species introduced by humans, the loss of native species can increase only as a result of hybridization [11].

Hybridization increases the threat of extinction of many species due to introgression [12, 13]. High degree of introgression is often manifested by wind-pollinated species such as oaks. Hybridization and introgression can lead to a hybrid complex consisting of many hybrids due to a large number of loci. Thus, multiloci seem to increase the number of hybrid types and genetic complexes and accelerate the reduction of "pure" natural species. In addition, the large number of loci essentially reduces the probability of having a "pure" individual of any parent origin [11]. Without introgression, hybrids, being reproductively isolated, can quickly form a new species. With introgression, speciation slows down as inverse crosses with parent lines occur. The impact of hybridization and introgression on the rate of substitution of native species by closely related ones has been addressed by a mathematical model involving a one-loop bipartite inheritance scheme with different levels of cross-species hybridization [11]. Although the model did not take into account vegetatively propagating hybrids, the results showed that the substitution of natural taxa by alien ones could occur very quickly (in less than five generations). According to the results, hybridization and introgression can increase the degree of substitution of native species by non-native ones. Introgression increases species substitution with low immigration, but prevents substitution when an indigenous species has a significant advantage in selection as well as with higher immigration levels. However, as introductions are associated with increased frequency of hybrids, the impact on the indigenous taxon remains high, and the likelihood of extinction increases significantly [11].

It is known that the highest invasive activity is exhibited by species of Asteraceae family [14], so we have focused our attention on representatives of this group of alien species. It is often impossible to say with certainty whether plants with intermediate morphological features are hybrids (between two species of the same genus). This may also be the case for new ecological forms, resulting from microevolution of species. To confirm or disprove the hypothesis about hybrid origin of certain taxon, it is best to use molecular genetic methods.

2. Materials and methods

DNA was extracted from silica gel dried leaves of *Bidens*, *Solidago*, and *Erigeron* taxa according to the method by Rogers and Bendich [15]. The herbarium specimens are stored in the herbarium of the Tsitsin Main Botanical Garden (MHA). Polymerase chain reaction (PCR) was carried out in a DNA Engine Dyad Peltier Thermal Cycler amplifier (Bio-Rad, United States). For the nuclear ribosomal internal transcribed spacer (ITS) 1-2 (ITS1-ITS2), *nnc18s10* (forward) and *c26A* (reverse) primers with an annealing temperature of 50°C were used. For the chloroplast loci (*rpl32-trnL* and *trnL-trnF* intergenic spacers), primers were used at the annealing temperature from 0.3 to 65°C [16]. For the chloroplast locus *rpl32-trnL*, we used primers *rpl32F* (forward) and *trnL* UAG (reverse), and for the other chloroplast locus *trnL-trnF*, we used primers *c* (forward) and *f* (reverse). Purification of the PCR product for sequencing was carried out in a mixture of ammonium acetate with ethanol. The nucleotide DNA sequences were determined on an automatic sequencer (Syntol). Further processing of the nucleotide sequences was carried out in the BioEdit program. The data were sent to GenBank (2019), in which these nucleotide sequences can be found by the additional numbers assigned to them (**Table 1**). Phylogenetic trees were constructed using SplitsTree4.

3. *Bidens* × *decipiens*

Bidens connata Muhl. ex Willd. is a North American species whose natural area extends from Alaska in the north to Mexico in the south [17]. The species has high polymorphism within native area, and several varieties have been described: *B. connata* var. *ambiversa* Fassett, var. *anomala* Farwell, var. *fallax* (Warnstorf) Sherff, var. *gracilipes* Fernald, var. *inundata* Fernald, var. *petiolata* (Nuttall) Farwell, var. *pinnata S. Watson*, and var. *submutica* Fassett [18, 19]. In the second half of the twentieth century, American botanists made suggestions about the hybrid nature of *B. connata* based on morphological features [20]. This species was indicated as an alien for Europe [21]. However, European plants called "*B. connata*" are morphologically different from American samples. Their outer leaves are clearly leaf-shaped, well-developed, and 3–6 cm long, with no reedy flowers, and the first real leaves are less narrow and with more pronounced petioles than those of *B. connata* and fewer denticles on the leaves, and the denticles, in turn, are usually larger and less regularly located [22]. Plants from European populations have been described as *B.* × *decipiens* Warnst. in 1895. The typical excrement material collected by Carl Warnstorf is stored in the herbaria of Edinburgh (E), Frankfurt (FR), and Charles University in Prague (PRC) [23]. In Europe, the locations of *B.* × *decipiens* are few and far between. A map of the gradual eastward expansion of this species was previously compiled by the authors of this paper [24] and is shown in **Figure 1**. Previously, we studied morphological features of *B.* × *decipiens* in Russia and found that features of this species are intermediate between the North American invasive

Sample no.	Number of ITS/rpl32–trnL/trnL–trnF sequence in GeneBank	Taxon	Date and place of collection, notes
de_1a	MK559763/ – /MK575566	*Bidens* × *decipiens* (= "*B. connata*")	Russia, Kaluga region, Milyatinsky Reservoir, 2013 54.4914° N, 34.3393° E
de _1b	MK559764/ – /MK575567		
de _1c	MK559765/ – / MK575568		
de _1d	MK559766/ – /MK575569		
de _2a	MK559767/ – /MK575570		Russia, Kaliningrad region, 2013 54.95° N, 20.49° E
de _2b	MK559768/ – /MK575571		
de _2c	MK559769/ – /MK575572		
de _2d	MK559770/ – / –		
de _3a	MK559771/ – /MK575573		Russia, Vladimir region, near Tasinsky village, 2014 Formed with dissected lower leaves 55.567° N, 40.172° E
de _3b	MK559772/ – / –		
de _3c	MK559773/ – / MK575574		
de _4a	MK559774/ – /MK575575		Russia, Vladimir region, near Tasinsky village, 2014 Formed with whole lower leaves 55.567° N, 40.172° E
de _4b	MK559775/ – /MK575576		
fr_5a	MK559780/ – /MK575581	*B. frondosa*	Russia, Vladimir region, near Tasinsky village, 2014 55.567° N, 40.172° E
fr_5b	MK559781/ – /MK575582		Russia, Vladimir region, near Tasinsky village, 2018 55.567° N, 40.172° E
fr_5c	MK559782/ – /MK575583		
cr_6a	MK559755/ – /MK575559	*B. cernua*	Russia, Moscow region, near Zvenigorod town, 2014 55.69° N, 36.74° E
cr_6b	MK559756/ – /MK575560		
t_7	MK559754/ – /MK575558	*B. tripartita*	Russia, Vladimir region, near Tasinsky village, 2018 55.567° N, 40.172° E
cr_8a	MK559757/ – /MK575561	*B. cernua*	
cr_8b	MK559758/ – /MK575562		
cr_8c	MK559759/ – /–		
cr_9a	MK559760/ – /MK575563		Belarus, Dzerzhinsk, 2018 53.693° N, 27.165° E
cr_9b	MK559761/ – /MK575564		
cr_9c	MK559762/ – /MK575565		
fr_10a	MK559783/ – /MK575584	*B. frondosa*	
fr_10b	MK559784/ – /MK575585		
de _11a	MK559776/ – /MK575577	*B. decipiens* (= "*B. connata*")	
de _11b	MK559777/ – /MK575578		
de _11c	MK559778/ – /MK575579		
de _13	MK559779/ – /MK575580		Russia, Moscow, park near Sviblovo estate, 2018 55.8639° N, 37.6396° E
v_1a	MK491849/MK474079/ –	*Solidago virgaurea*	Russia, Pskov region, Pskov district, vicinity of Pskov, idle field, 2018 57.80° N, 28.25° E
v_1b	MK491850/MK474080/ –		
v_1c	MK491851/MK474081/ –		
n_2a	MK491852/MK474082/ –	*S.* × *niederederi*	

Sample no.	Number of ITS/rpl32–trnL/trnL–trnF sequence in GeneBank	Taxon	Date and place of collection, notes
n_2b	MK491853/MK474083/ –		
n_2c	– /MK474084/ –		
c_3a	MK491854/MK474085/ –	*S. canadensis*	
c_3b	MK491855/MK474086/ –		
c_3c	MK491856/MK474087/ –		
v_4	– /MK474088/ –	*S. virgaurea*	Russia, Moscow region, Chekhov district, near the village of Chudinovo, idle field, 2018 55.1° N, 37.5° E
c_5a	MK491857/MK474090/ –	*S. canadensis*	
c_5b	– /MK474089/ –		
v_6a	MK491858/– / –	*S. virgaurea*	Russia, Moscow region, "Losiny Ostrov" National Park, Pine forest, 2014 55.89° N, 37.77° E
v_6b	MK491859/– / –		
3	MK397980/– / –	*Erigeron sumatrensis*	Italy, Pompeii, 2016 40.7° N, 14.5° E
5a	MK397981/– / –		Italy, the Island of Ischia, 2016 40.7° N, 13.9° E
5b	MK397982/– / –		
5c	MK397983/– / –		
6	MK397984/– / –		Italy, Herculaneum, 2016 40.8° N, 14.4° E
8a	MK397986/– / –	*E. sumatrensis* × *E. canadensis* (?)	Italy, Naples, 2016 40.8° N, 14.2° E
8b	MK397987/– / –		
10a	MK397988/– / –		Italy, Pompeii, 2016 40.7° N, 14.5° E
10b	MK397989/– / –		
13a	MK397991/– / –	*E. canadensis*	Italy, the Island of Ischia, 2016 40.7° N, 13.9° E
13b	MK397992/– / –		
16	MK397985/– / –		Portugal, Lisbon, 2017 38.7° N, 9.1° W
18	MK397993/– / –		Spain, Madrid, park, 2017 40.4° N, 3.7° W
19	MK397994/– / –	*Erigeron* sp.	
20	MK397995/– / –	*E. canadensis*	
22	MK397990/– / –		

Note: "E. sumatrensis × E. canadensis (?)" – putative hybrids.

Table 1.
Samples of the studied taxa of Asteraceae.

B. frondosa L. and the native *B. cernua* L. *B.* × *decipiens* are covered with two types of hairs—duplex, from two cells (as in *B. frondosa*), and simple multicellular (as in *B. cernua*). In addition, the seeds of *B.* × *decipiens* are quadrilateral and have four axes (as in *B. cernua*) and are covered with warts (as in *B. frondosa*). Heads of *B.* × *decipiens* are similar in size and shape to heads of *B. frondosa*, and the leaves are whole, as in *B. cernua*. On the basis of these data, we hypothesize the hybrid origin of *B.* × *decipiens* [25].

Nucleotide sequence analysis shows that not all individuals defined as *B.* × *decipiens* can be called hybrids. The point is that in cases of nucleotide substitutions

Figure 1.
Map of the secondary distribution range of Bidens × decipiens.

at the ITS1-ITS2 site differentiating *B. frondosa* and *B. cernua*, we encounter heterozygosity of samples of *B.* × *decipiens* (and, accordingly, ambiguity of reading of the sequence) in many cases, but still not in all (as it should be expected for the hybrid F). At the same time, each of our samples of *B.* × *decipiens* is characterized by at least some such ambiguous readings of nucleotides (C or T, A or T, A or C) (**Figure 2**) in the case of substitutions, which, of course, confirms the hypothesis of the hybrid origin of this taxon. Populations of *B.* × *decipiens* from different parts of the range differ in the number of substitutions. Thus, samples from the banks of the Milyatinskoye reservoir in the Kaluga region demonstrate in most cases the presence of ambiguous readings in the case of nucleotide substitutions, with the exception of sample de_1a, in which heterozygosity is not observed in all cases of substitutions. It means that this population is a hybrid one. In addition, both parent species grow on the banks of the Milyatinskoye reservoir in close proximity to the population of *B.* × *decipiens*, which indirectly supports this view [26]. Probably, the differences in the sample de_1a are due to the presence of introgression, i.e., this sample is a backcross resulting from crossing of *B.* × *decipiens* with *B. cernua*, because ITS1-ITS2 of this sample has a stronger DNA area similar to *B. cernua* than others. The same situation is observed with individuals of *B.* × *decipiens* from Belarus. *B.* × *decipiens* specimens from the Kaliningrad region, by contrast, in most cases show similarities with *B. cernua* in the case of substitutions rather than heterozygosity, except for sample de_2a. In this case, there are two possible variants—in the first case, we collected samples of backcrosses and see the result of introgression; in the second case, the parent form is another form of *B. frondosa*, not the widespread *B. frondosa* var. *frondosa*. The second variant is less probable. However, other forms of *B. frondosa* have been recently found [27]. Among plants of *B.* × *decipiens* collected in the Vladimir region, two forms distinct on lower leaves—with a dissected sheet plate (samples de_3a, de_3b, and de_3c) and with a whole sheet plate (de_4a, de_4b)—are clearly distinguished. As it turned out, these forms have genetic differences, but samples 4a and 4b are also not identical in the ITS1-ITS2 section sequences. In this case, it is only possible to estimate which form is closer to *B. cernua* and which one to *B. frondosa* using statistical methods. The plant collected on the territory of Sviblovo Estate in Moscow and based on a set of morphological features defined as *B.* × *decipiens*, in the section ITS1-ITS2, has a very high

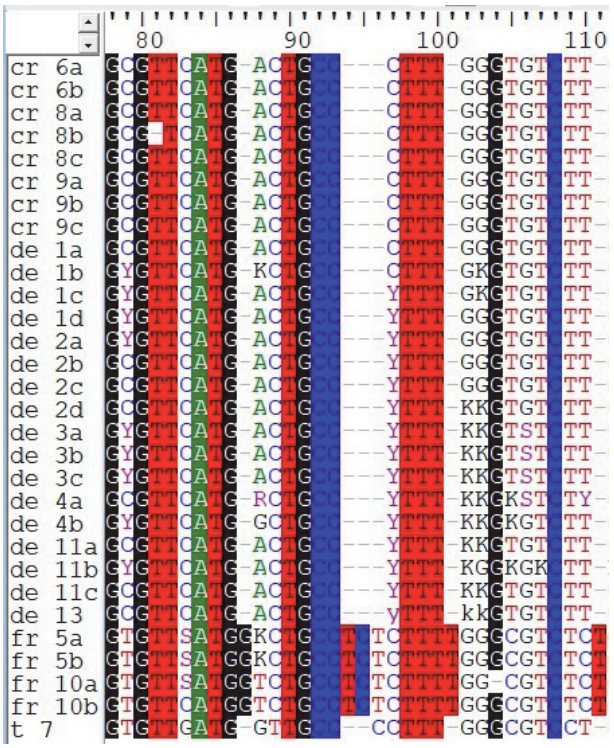

Figure 2.
Fragment of ITS1-ITS2 site of nuclear DNA of various taxa of Bidens *genus. The nucleotides are coded using the International Union of Pure and Applied Chemistry (IUPAC) nomenclature.*

similarity with *B. cernua*. However, in one case this specimen still has heterozygosity in nucleotide substitutions differentiating *B. cernua* and *B. frondosa*, so we cannot say that this specimen is a form of *B. cernua*; most likely, it is the result of introgressive hybridization (**Figure 2**). It is possible that in the case of introgressive hybridization, not only *B.* × *decipiens* × *B. cernua* but also backcrosses are formed (*B.* × *decipiens* × *B. frondosa*). It is interesting that ambiguous readings of a certain nucleotide are also observed for all *B. frondosa* samples in the same position, but they are not related to nucleotide substitutions in other taxa (**Figure 2**). It is not excluded that *B. frondosa* itself is a species of hybrid origin. This is indirectly proved by the high polymorphism of this species in its natural area.

Based on the nucleotide sequences of the ITS1-ITS2 site in the SplitsTree program, the dendrogram is built using the UPGMA method (**Figure 3**). With high probability (with 100% bootstrap support), two clades were separated—sample t_7 (*B. tripartita*). One clade was separated by species, specimens *B. frondosa* (fr_5a, fr_5b, fr_5b, fr_10a, fr_10b), and the other clade included all samples of *B.* × *decipiens* and *B. cernua*, indicating a high similarity.

For trnL-trnF site of chloroplast DNA, samples of *B.* × *decipiens* and *B. cernua* have no differences (this applies to all plants, including those collected in different regions), while *B. frondosa* differs from these taxa by six substitutions of one to two nucleotides and deletion of seven nucleotides (**Figure 4**). *B. tripartita* has another deletion (**Figure 4**), which is absent in other taxa, which once again indirectly confirms its non-participation to the hybrid origin of *B.* × *decipiens*. This means that the aboriginal *B. cernua i*s the maternal species and *B. frondosa* is the paternal species.

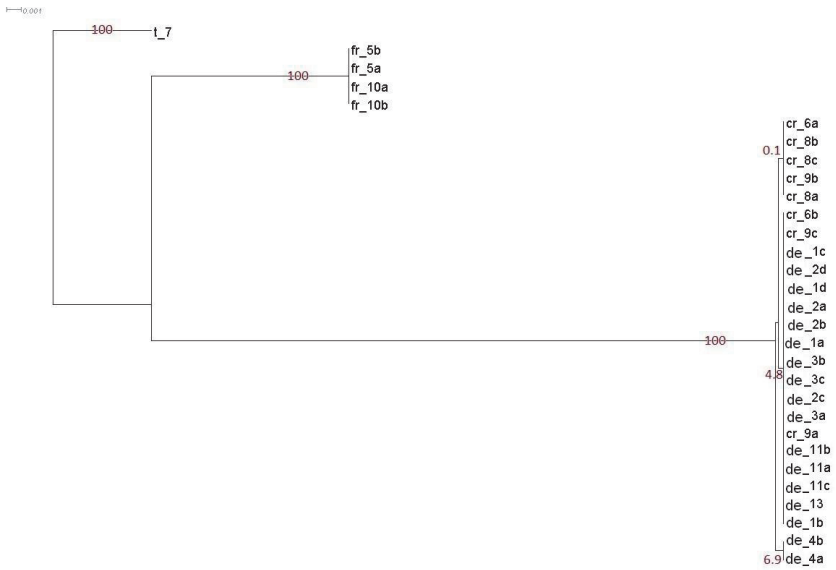

Figure 3.
Dendrogram based on analysis of the ITS region of DNA of various Bidens *taxa with bootstrap support data.*

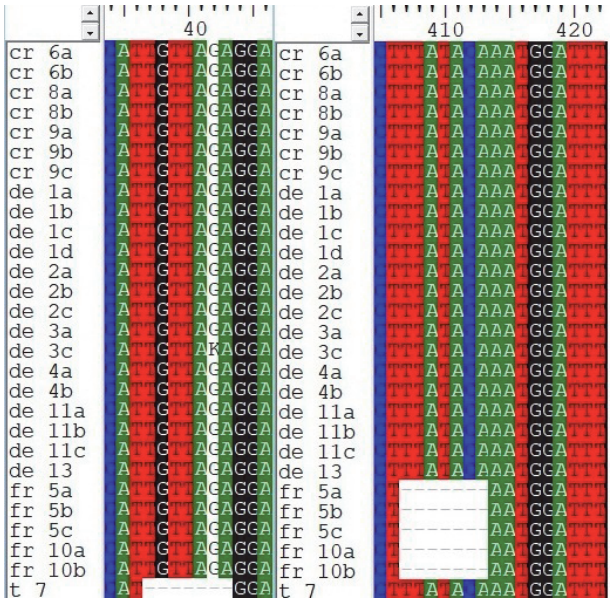

Figure 4.
Fragment of the trnL-trnF intergenic spacer of chloroplast DNA of various taxa of Bidens *genus. The nucleotides are coded using IUPAC nomenclature.*

4. *Solidago* × *niederederi*

We have set ourselves the task to study the features of *Solidago* hybrids in northwest Russia, because in contemporary publications the most numerous references to *S.* × *niederederi* are in the northeastern part of Europe and the Pskov region is the closest region of Russia to it. Previously, on the basis of the analysis of the highly variable noncoding chloroplast region rpl32-trnL by Polish botanists, it was found that hybridization between *S. canadensis* and *S. virgaurea* can occur in both

directions and both species can be both mother and father plants [28]. We aimed to determine the situation with respect to parental taxa in the Pskov populations of these species.

The main difference between the hybrid *S.* × *niederederi* and its parents is the structure of shoot systems (mainly the inflorescence structure, **Figure 5**). In *S. canadensis* numerous heads are collected in a compound raceme, and in *S.* × *niederederi* the number of heads is smaller and is collected in a compressed compound raceme, whereas in *S. virgaurea* the number of heads is smaller, and the branches of the compound raceme are so short that the inflorescence is more like a spike.

The size of the heads themselves also varies (**Figure 6**). *S.* × *niederederi* heads have an oval shape and occupy an intermediate position in diameter between parental species, 2201 ± 45 μm (mean ± error average) with a maximum spread of 1762 to 2728 μm, while for *S. virgaurea* and *S. canadensis*, these values are 3132 ± 30 μm (2874–3548 μm) and 1591 ± 22 μm (1428–1939 μm), respectively [29].

With regard to the length of the head, *S.* × *niederederi* plants in Pskov cannot be clearly distinguished from *S. canadensis* due to the high variability of this indicator in *S. canadensis*. However, in terms of average head lengths, the hybrid also occupies an intermediate position between parent species (**Figure 6**). *S. canadensis* and *S.* × *niederederi* shoots are pubescent, while *S. virgaurea* shoots are glabrous, glossy, and sometimes reddish. The leaves of *S.* × *niederederi* in the middle part of the shoot are linear-lanceolate and dentate along the edge, with three distinct veins (as in *S. canadensis*), while in the basal part of the shoot large, ovate, with reticulate veins (as in *S. virgaurea*). To confirm hybrid origin of S. × *niederederi* population in the vicinity of Pskov, nucleotide sequences of nuclear and chloroplast DNA of Pskov individuals (both parent and hybrid species) as well as individuals of parent species from Moscow region were analyzed. The analysis of the ITS1-ITS2 site showed that in all cases of nucleotide substitutions differentiating *S. virgaurea* and *S. canadensis*, *S.* × *niederederi* has ambiguous readings (**Table 2**), indicating heterozygosity, which confirms the hybrid origin of individuals from this population. One sample of *S. canadensis* (c_3c) showed heterozygosity in three cases of nucleotide substitutions out of four, although morphologically this sample did not differ from other individuals of *S. canadensis,* which indicates the presence of introgressive hybridization

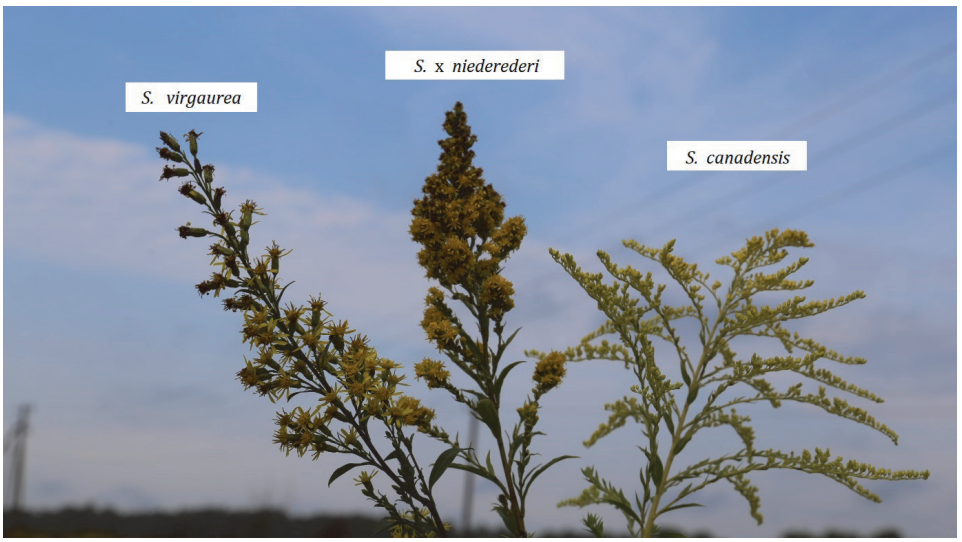

Figure 5.
Panicles of Solidago × niederederi *and its parental species.*

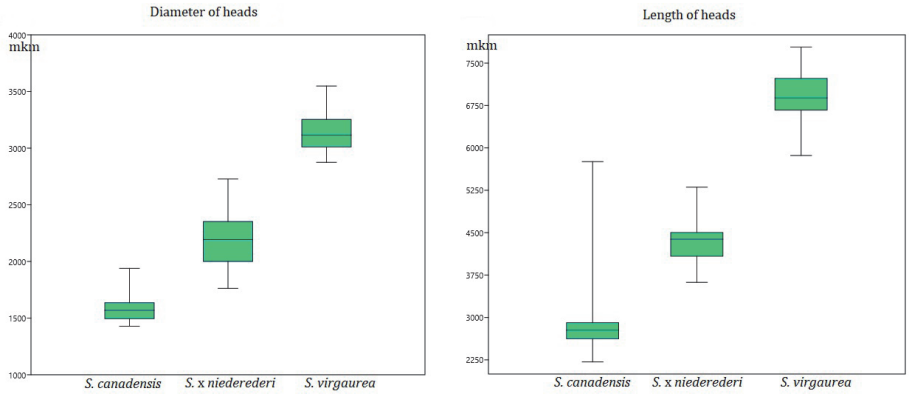

Figure 6.
Parameters of flower heads of Solidago × niederederi *and its parental species: quartiles (first and third), median, maximum, and minimum values are indicated.*

Sample no.	Position in the alignment			
	384	431	508	549
v_1a	T	A	C	A
v_1b	T	A	C	A
v_1c	T	A	C	A
n_2a	Y	M	Y	R
n_2b	Y	M	Y	R
c_3a	C	C	T	G
c_3b	C	C	T	G
c_3c	Y	M	T	R
c_5a	C	C	T	G
v_6a	T	A	C	A
v_6b	T	A	C	A

The nucleotides are coded using IUPAC nomenclature.

Table 2.
ITS1-ITS2 polymorphism for the Solidago × niederederi *hybrid and parental species.*

within the *Solidago* genus. It is likely that this sample is a backcross (result of crossbreeding *S.* × *niederederi* with the parent species *S. canadensis*).

The analysis of the rpl32-trnL high-variable intergenic spacer made it impossible to give an unambiguous answer which species is maternal to *S.* × *niederederi* and which is paternal. In contrast to the results obtained by A. Plizhko and J. Zalevska-Galosh [30], our samples of *S. canadensis* have a higher variability of this section of chloroplast DNA. For example, a sample of s_3a from the Pskov region has a DNA fragment that is absent in other plants of *S. canadensis* not only in the Pskov region but also near Moscow (242–264 nucleotides, **Table 3**). The area occupying positions 271–306 in the alignment of our sequences (292–330 for sample c_3a, **Table 3**) and differentiating parental species in Polish populations [30] may differ not only in *S.* × *niederederi* but also in both parental species. The analysis of another noncoding site of chloroplast DNA, trnL-trnF, also failed to answer this question because all the samples examined were identical in this site. Based on the data obtained, we can only

Sample no.	Position in the alignment				
	191	242–264	271–306 (292–330)	739–741 (746–748, 709–714)	894 (900, 923)
v_1a	A	—	TGTCTAAAAGAATAATTCTTGTATTTCTT	T	C
v_1b	C	—	TGTCTAAAAGAATAATTCTTGTATTTCTTGAATTCT	T	C
v_1c	C	—	TGTCTAAAAGAATAATTCTTGTATTTCTTGAATTCT	—	C
n_2a	C	—	TGTCTAAAAGAATAATTCTTGTATTTCTTGAATTCT	—	C
n_2b	C	—	TGTCTAAAAGAATAATTCTTGTATTTCTTGAATTCT	T	C
n_2c	C	—	TGTCTAAAAGAATAATTCTTGTATTTCTTGAATTCT	T	C
c_3a	A	GAATCTTAATGTTATGTCTAAA	TGTCTAAAAGAATAATTCTTGTATTTCTT	T	A
c_3b	A	—	TGTCTAAAAGAATAATTCTTGTATTTCTTGAATTCT	TTTT	A
c_3c	C	—	—	—	C
v_4	C	—	TGTCTAAAAGAATAATTCTTGTATTTCTTGAATTCT	TT	C
c_5a	C	—	—	T	C
c_5b	A	—	—	TTTT	A

The nucleotides are coded using IUPAC nomenclature.

Table 3.
Polymorphism of the rpl32-trnL region for the Solidago × niederederi hybrid and parental species.

assume that hybridization occurs in both directions in the Pskov population, but there is also a possibility that only one species may be maternal and the other paternal, and it is necessary to search for other, more variable sites of chloroplast DNA.

5. *Erigeron* sect. *Conyza*

Earlier we studied in detail morphological differences between species of the genus *Erigeron* that grow in Eurasia [30], and they are shown in **Table 4**.

Previously a hybrid of *Erigeron canadensis* and *E. sumatrensis*—*Conyza* × *rouyana* Sennen—was described. A typical specimen of this taxon (P04315552), collected by F. Sennen in Catalonia in 1904, is kept in the herbarium of the Museum of Natural History in Paris [P] [7]. Nevertheless, some botanists did not recognize this hybrid and referred *C.* × *royana* to *E. floribundus* [31], which is now treated as synonymous with *E. sumatrensis*. However, it cannot be excluded that morphological differences in several individuals could have been caused not by hybrid processes but by adverse environmental conditions.

The analysis of the **ITS1-ITS2** site of 16 samples of *Erigeron* sect. *Conyza* (supposed hybrids and parent taxa) confirmed our conclusions about the higher polymorphism of *E. sumatrensis* than *E. canadensis*: ITS1-**ITS2** sites of *E. canadensis* samples were identical, while *E. sumatrensis* has substitutions and ambiguous readings (**Table** 5). As for the supposed hybrids (samples 8a, 8b, 10a, 10b, and 22), only in one case an ambiguous reading of the nucleotides coincides with the substitution differentiating *E. canadensis* and *E. sumatrensis*, which indicates that the hybridization has taken place, but since in other cases the substitutions are identical (**Table 5**) and the supposed hybrids have no ambiguous readings, most likely, the reason for their morphological differences is the high polymorphism of *E. sumatrensis* taxon, to which they can be classified.

Features	***E. canadensis***	***E. bonariensis***	***E. sumatrensis***
Number of heads/ generative shoot	500–600	No more 30	less 500
Diameter of heads, mm	4.8 ± 0.1 × 2.4 ± 0.1	6.1 ± 0.1 × 5.2 ± 0.2	6.6 ± 0.1 × 3.2 ± 0.2 At the base, swollen
Structure of shoot systems	The main shoot is barely branched off and ends in a compound raceme occupying the upper third of the escape	The lower lateral deciduous axes of the inflorescence overturn the main shoot axis; the inflorescence covers the upper third of the shoot	The lower lateral deciduous inflorescences are shorter than the main axis of the shoot; the diamond-shaped compound raceme is half the length of the generative shoot
Shape of leaves	Linear-lanceolate with denticle margin	Almost linear with 3–5 denticles	Lanceolate-oval with a serrated margin
Type of pubescence	The leaves are light green, slightly pubescent, the stem is light green, strongly pubescent	The leaves are gray-green, the pubescent leaves and stems are strongly pubescent with long silvery trichomes	The leaves are dark green, softly pubescent, the stems are grayish with abundant soft pubescence

Table 4.
Diagnostic morphological features of Erigeron *species.*

Sample no.	Taxa	Position in the alignment																		
		65, 129, 136, 137, 238, 472/473, 570/571	72, 87, 249, 404	83, 576/577	95	114	130, 567/568, 568/569	211-212	242	412	430	461, 584/585	469	471/472, 530/531, 558/559	499/500	502/503	520/521	535/536	559/560	598-600
3	*Erigeron sumatrensis*	T	C	A	C	C	T	YY	C	C	G	G	—	C	A	C	R	Y	R	TCT
5a		T	C	A	C	C	T	TC	C	C	R	G	—	C	A	C	R	Y	R	TCT
5b		T	C	A	C	C	T	TC	C	Y	R	G	—	C	A	C	R	C	R	TCT
5c		T	C	A	C	C	T	TC	Y	C	R	G	—	C	A	S	R	Y	R	TCT
6		T	C	A	C	S	T	TC	Y	C	R	G	—	C	A	S	R	Y	R	TCT
16		T	C	A	Y	C	T	YY	C	C	G	G	—	C	A	S	R	C	A	TCT
8a	*E. sumatrensis* × *E. canadensis* (?)	T	C	A	Y	C	T	YY	C	C	R	G	—	C	A	S	G	Y	R	TCT
8b		T	C	A	Y	C	T	YY	C	C	R	G	—	C	A	S	R	Y	R	TCT
10a		T	C	A	Y	C	T	YY	C	C	G	G	—	C	A	S	R	Y	R	TCT
10b		T	C	A	C	C	T	TC	C	C	R	G	—	C	A	S	R	Y	R	TCT
22		T	C	A	Y	C	T	YY	C	C	R	G	—	C	A	Y	R	C	R	TCT
13a	*E. canadensis*	C	T	C	C	C	A	CC	C	C	G	G	T	A	T	C	A	C	A	—
13b		C	T	C	C	C	A	CC	C	C	G	A	T	A	T	C	A	C	A	—
18		C	T	C	C	C	A	CC	C	C	G	A	T	A	T	C	A	C	A	—
19	*Erigeron* sp.	C	T	C	C	C	A	CC	C	C	G	A	T	A	T	C	A	C	A	—
20	*E. canadensis*	C	T	C	C	C	A	CC	C	C	G	A	T	A	T	C	A	C	A	—

The nucleotides are coded using IUPAC nomenclature.
Note: "E. sumatrensis × E. canadensis (?)" – putative hybrids.

Table 5.
ITS1-ITS2 polymorphism for different taxa of Erigeron *sect.* Conyza *in the Mediterranean.*

6. Conclusions

Thus, the obtained data on hybrid activity among the Asteraceae family of invasive species are ambiguous.

Hybrid *B.* × *decipiens* has a low polymorphism. *B. cernua* is the most polymorphic taxon, and we can assume the presence of introgressive hybridization of *B.* × *decipiens* with a maternal species. The analysis of ITS1-ITS2 and trnL-trnF sequences showed that *B.* × *decipiens* is of hybrid origin and its maternal form is an aboriginal sequence of *B. cernua* and the paternal one is probably invasive *B. frondosa*. It is possible that *B.* × *decipiens* in its present form has already appeared by introgression, but no morphological differences between supposed hybrids (Belarusian and Kaluga plants) and supposed backcrosses (plants from Moscow and Kaliningrad region) have been revealed. It should be noted that *B. frondosa* itself may be of hybrid origin.

In the northwest of Russia, the populations of three taxa of *Solidago* genus—an invasive species of North American origin of *S. canadensis*, an indigenous species of *S. virgaurea*, and their hybrid *S.* × *niederederi*—grow together in the vicinity of the city of Pskov, which was confirmed by the sequence analysis of the ITS1-ITS2 site. Since both parents, especially *S. canadensis*, are quite polymorphic taxa, it is impossible to answer unambiguously which of the two species is maternal and which is paternal.

In Southern Europe, the hybriogenic activity of representatives of the genus *Erigeron* is close to zero. The low hybriogenic activity can also be explained by differences in the chromosome set: in *E. canadensis* 2n = 18 and in *E. sumatrensis* 2n = 54 [32].

Our data on the rare occurrence of hybrids in comparison with parental species in the Asteraceae family contradict the hypothesis explaining the success of plant growth in the new homeland by strengthening hybridization processes in the secondary distribution range [1, 2], but this situation may change in the coming decades, so the hybriogenic activity of invasive species requires attention of the scientific community.

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Conflict of interest

The authors declare no conflict of interest.

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