Трансформация экосистем
Ecosystem Transformation
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Invasive taxa of the genus *Solidago* L. in the vicinity of the city of Pskov

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Received: 07.02.2019
Accepted: 24.02.2019
Published online: 20.05.2019

DOI: 10.23859/estr-190207
UDC 575.858:582.998.1(470)
URL: http://www.ecosysttrans.com/publikatsii/detail_page.php?ID=116

ISSN 2619-094X Print
ISSN 2619-0931 Online

Translated by S.V. Nikolaeva

Three taxa of the genus *Solidago* occur in the vicinity of Pskov: *S. virgaurea*, an aboriginal species, *S. canadensis*, an invasive species of North American origin, and their hybrid *S.* × *niederederi*. We studied the morphology of the hybrid and parental species. The diameter of the S. × *niederederi* flower heads is intermediate between those in *S. virgaurea* and *S. canadensis.* The hybridogenic origin of *S.* × *niederederi* was demonstrated using molecular analysis of nucleotide sequences of nuclear DNA (ITS region). At present, it is not possible to unequivocally determine which of the parental species was maternal and which was paternal. By analyzing the highly variable noncoding loci of chloroplast DNA (rpl32–trnL), it can be assumed that hybridization proceeds in both directions.

Keywords: invasions, hybridization, *Solidago canadensis*, *S.* × *niederederi*, *S. virgaurea*, flower heads diameter, ITS region, chloroplast loci rpl32–trnL.

Galkina, M.A., Vinogradova, Yu.K., 2019. Invasive taxa of the genus *Solidago* L. in the vicinity of the city of Pskov. *Ecosystem Transformation* **2** (2), 12–18.

Introduction

Invasions of alien species lead to the depletion of the flora of vast territories and may cause economic damage (McGeoch et al., 2010; Vinogradova et al., 2010). The North American species *Solidago canadensis* (Linnaeus, 1753) is among the top one hundred most aggressive invasive species in Russia (Vinogradova et al., 2015). Indeed, when a foreign species is becoming naturalized, there is an interaction with representatives of the native flora, which can also lead to the formation of hybrids. There is a hypothesis that in secondary ranges, the intensity of hybridization processes increases (Ellstrand and Schierenbeck, 2000; Elton, 1958). Moreover, hybrids may be more adapted to the conditions of the new environment than their parental taxa (Abbott et al., 2003; Bleeker et al., 2007; Zalapa et al., 2009). In European Russia, hybrids make up 10% of the total number of invasive species (Vinogradova and Mayorov, 2015). Parent species of the European hybrid *S.* × *niederederi* (Khek, 1905) are *S. canadensis* and *S. virgaurea* (Linnaeus, 1753). Populations of *S.* × *niederederi* have been recorded in Austria, Great Britain, Lithuania and Poland (Pagitz, 2016), and over the past decades it has significantly expanded its range (Karpavičiené and Radušiené, 2016; Pliszko and Zając, 2016). In our previous studies using the ISSR method, it was found that plants collected in European Russia, identified using morphological features as *S.* × *niederederi*, are highly likely to be of hybrid origin (Vinogradova and Galkina, 2019), however that can only be confirmed by analyzing the nucleotide DNA sequences of the presumed hybrids and parents. We aimed to study the features of *Solidago* hybrids in northwestern Russia, since the most numerous recent records of *S.* × *niederederi* are from northeastern Europe, to which the Pskov region is the most closely located region of Russia.

Previously, based on an analysis of the highly variable non-coding chloroplast rpl32–trnL locus, Pliszko and Zalewska-Gałosh (2016) found that hybridization between *S. canadensis* and *S. virgaurea* can go both ways, and both species can be either maternal or paternal plants. We aim to determine which situation, with respect to the parental taxa, is observed in the Pskov populations of these species.

Materials and methods

The diameter and length of the flower heads were measured using a Keyence VHX–1000 digital microscope. For each of the three taxa, we measured 30 flower heads from plants collected in the Pskov region (Pskov district, vicinity of Pskov, an unused field near a highway). Statistical data were processed in the PAST 3 program.

DNA extraction was carried out using the CTAB method (Rogers and Bendich, 1985) from herbarium material collected in the Pskov and Moscow regions (Table 1). In the Moscow region, representatives of the parental species were collected at two sites, in the Losiny Ostrov National Park (in a pine forest with spruce and birch) and in the vicinity of the village of Chudinovo in the Chekhov district (an unused field). These additional samples were used to more accurately assess the polymorphism of the invasive species *S. canadensis* and the native *S. virgaurea* in European Russia, as well as to control for incorrect interpretation of the results of the analysis of chloroplast DNA loci to determine the maternal species.

Polymerase chain reaction (PCR) was performed in a DNA Engine Dyad Peltier Thermal Cycler amplifier (Biorad, USA). For the nuclear region of ITS, the

Table 1. Samples of various taxa of the genus *Solidago* L. (presumed *S.* × *niederederi* hybrid and parental species) used for molecular genetic analysis.

Sample number	The number of the ITS sequence in GenBank	The number of the rpl32–trnL locus in GenBank	Taxon	Collection site and habitat	Collection year
v_1a	MK491849	MK474079			
v_1b	MK491850	MK474080	*S. virgaurea*		
v_1c	MK491851	MK474081			
n_2a	MK491852	MK474082			
n_2b	MK491853	MK474083	*S.* × *niederederi*	Pskov region, Pskov district, vicinity of Pskov, idle field, N 57.80°, E 28.25°	2018
n_2c	–	MK474084			
c_3a	MK491854	MK474085			
c_3b	MK491855	MK474086	*S. canadensis*		
c_3c	MK491856	MK474087			
v_4	–	MK474088	*S. virgaurea*		
c_5a	MK491857	MK474090		Moscow region, Chekhov district, near the village of Chudinovo, idle field, N 55.1°, E 37.5°	2018
c_5b	–	MK474089	*S. canadensis*		
v_6a	MK491858	–			
v_6b	MK491859	–	*S. virgaurea*	Moscow region, “Losiny Ostrov” National Park, pine forest, N 55.89°, E 37.77°	2014

primers nnc18s10 (forward) and c26A (reverse) were used at an annealing temperature of 50 °C. For the chloroplast locus rpl32–trnL, primers rpl32F (forward) and trnL UAG (reverse) were used at an annealing temperature of 0.3 to 65 °C according to J. Shaw et al.'s (2007) method. The same PCR conditions were adopted for another chloroplast locus, trnL–trnF (primers c and f). The PCR products were purified for sequencing using the ammonium/Ethanol mixture protocol. Determination of nucleotide DNA sequences was carried out using an automatic sequencer in ZAO "Syntol". The nucleotide sequences were further processed using the BioEdit software. The data obtained were deposited into the GenBank database (https://www.ncbi.nlm.nih.gov/nuccore), where these nucleotide sequences can be found using their assigned numbers (Table 1).

Results and discussion

The main difference between *S.* × *niederederi* and his parents is the structure of the shoot systems (mainly the structure of the inflorescence, Fig. 1). In

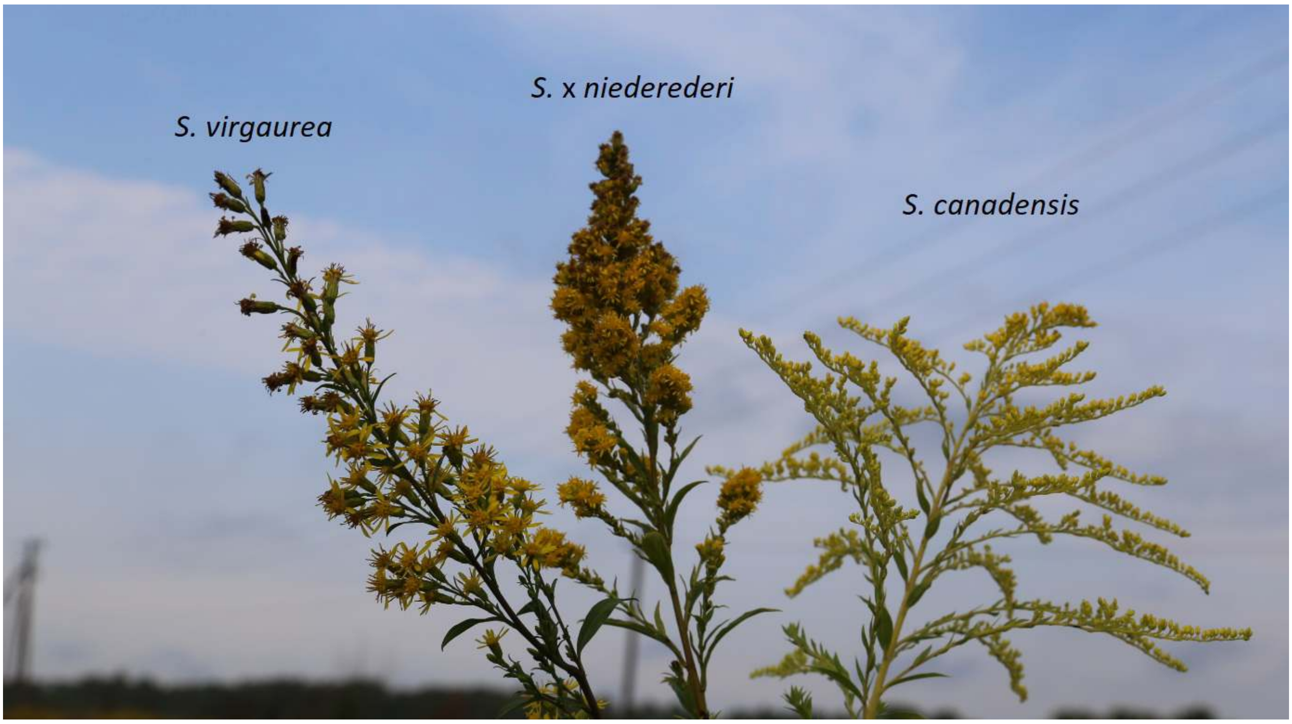

Fig. 1. Flower head morphology of *Solidago* × *niederederi* and its parental species.

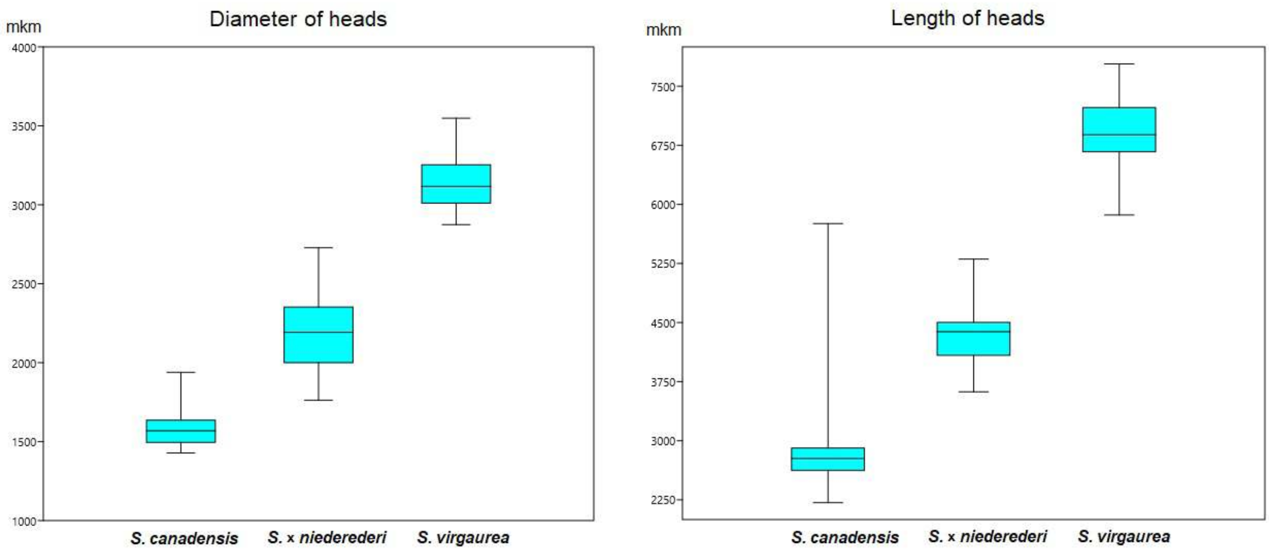

Fig. 2. Parameters of flower heads of *Solidago* × *niederederi* and its parental species: quartiles (1st and 3rd), median, maximum and minimum values are indicated.

Fig. 3. Flower heads of *Solidago* × *niederederi* with a measurement scheme.

S. canadensis, numerous flower heads are assembled in loose panicles; in *S.* × *niederederi* flower heads are fewer and they are compressed in a compressed panicle, whereas in *S. virgaurea* the number of flower heads is the lowest, and the branches of the panicle are so short that the inflorescence resembles a spike. The sizes of the flower heads are also different (Fig. 2). The flower heads of *S.* × *niederederi* (Fig. 3) are oval in shape and are intermediate in diameter between the parental species – 2201 ± 45 μm (mean ± standard error) with a maximum spread from 1762 to 2728 μm, whereas for *S virgaurea* and *S. canadensis* these values are 3132 ± 30 μm (2874–3548 μm) and 1591 ± 22 μm (1428–1939 μm), respectively. As for the length of the flower heads, the Pskov representatives of *S.* × *niederederi* cannot be clearly distinguished from *S. canadensis* using this character, due to its high variability in *S. canadensis*. However, according to mean lengths of flower heads, the hybrid also occupies an intermediate position between the parental species (Fig. 2). The shoots of *S. canadensis* and *S.* × *niederederi* are pubescent, whereas the shoots of *S. virgaurea* are bare, shiny, and sometimes reddish in color. The leaves of *S.* × *niederederi* in the middle part of the shoot are

Table 2. ITS polymorphism for the *Solidago* × *niederederi* hybrid and parental species. The nucleotides are coded using IUPAC nomenclature.

Sample no.	Position in 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

Tabe 3. Polymorphism of the rpl32–trnL region for the *Solidago* × *niederederi* hybrid and parental species. The nucleotides are coded using IUPAC nomenclature.

Sample no.	Position in 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

linear-lanceolate, dentate along the edge, with three distinct veins (as in *S. canadensis*), and in the basal part of the shoot are large, ovate, with a reticulated venation (as in *S. virgaurea*).

To confirm the hybridogenic origin of the *S.* × *niederederi* population in the vicinity of Pskov, the nucleotide sequences of the nuclear and chloroplast DNA of Pskov individuals (both parental and hybridogenic species), as well as individuals of the parental species from the Moscow region, were analyzed. The analysis of the ITS region showed that in all cases of nucleotide substitutions differentiating *S. virgaurea* and *S. canadensis*, *S.* × *niederederi* had ambiguous readings (Table 2), indicating heterozygosity, which confirms the hybrid origin of individuals from this population. One of the samples of *S. canadensis* (c_3c) in three cases of four of the nucleotide substitutions showed heterozygosity, 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 *S.* × *niederederi* with the parental species *S. canadensis*).

Analysis of the highly variable intergenic spacer rpl32–trnL did not produce an unambiguous answer of which of the species is maternal for *S.* × *niederederi*, and which is paternal. Unlike the results obtained by A. Pliszko and Z. Zalewska-Galosh (2016), our *S. canadensis* samples show higher variability of this locus of chloroplast DNA. For instance, sample c_3a from the Pskov region has a DNA fragment, which is absent in other representatives of *S. canadensis* not only from Pskov, but also from the Moscow region (242–264 nucleotides, Table 3). The locus in positions 271–306 in the alignment of our sequences (292–330 for sample c_3a, Table 3) and differentiating the parental species in Polish populations (Pliszko and Zalewska-Gałosh, 2016) may differ not only in *S.* × *niederederi* but in both parental species. The analysis of another noncoding region of the chloroplast DNA (trnL–trnF), also did provide us with an answer, as all the samples under study turned out to be identical in this region. Based on the data obtained, we can only assume that in the Pskov population hybridization proceeds in both directions, but there is also the probability that only one species can be maternal and the other paternal, and a search for other, more prominently variable regions of chloroplast DNA is required. This is a subject for further study.

Conclusions

In the unused field studied in the vicinity of Pskov, three taxa occur together: *S. canadensis* (an invasive species of North American origin), *S. virgaurea* (a native species) and their hybrid *S.* × *niederederi*. This was confirmed by analyzing the ITS sequences of nuclear DNA. As both parents, especially *S. canadensis*, are relatively polymorphic taxa, it is not possible to unequivocally determine which of the two species is maternal, and which is paternal. The presence of introgressive hybridization within the genus *Solidago* is highly probable.

Acknowledgments

The study was performed within the framework of the State Contract of the GBS of the Russian Academy of Sciences (no. 19–119012390082–6) with partial support from the Russian Foundation for Basic Research (project no. 18–04–00411).

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