Relative DNA content differences reliably identify Solidago ×niederederi, a hybrid between native and invasive alien species

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Abstract: Hybridization between native and alien congeners may pose a serious threat to biodiversity and negatively affect native flora. Here we study Solidago ×niederederi, which originated and became established in Europe as a result of a cross between the alien S. canadensis and native S. virgaurea. The recent increase in the number of records of S. ×niederederi in Europe has highlighted the need to monitor its occurrence, spread and behaviour. In the present study, we tested the effectiveness of flow cytometry for detecting hybrid plants of S. ×niederederi. Sequences of the ITS region of nrDNA and the rps15-ycf1 spacer of cpDNA were used to confirm the hybrid origin of analysed plants and to identify the maternal species. Our study included 60 single-species populations of S. canadensis, S. gigantea and S. virgaurea, and 16 mixed populations with the presence of hybrid S. ×niederederi sampled from six countries in central Europe and adjacent areas. All individuals of S. canadensis, S. ×niederederi and S. virgaurea investigated were diploid (2n~2x~18) but differed in their relative DNA content values. The DNA content of S. ×niederederi was intermediate between S. canadensis and S. virgaurea with no overlaps, with the differences between the species being statistically significant. Therefore, we conclude that flow cytometry is a reliable and efficient method for detailed screening for hybrids within mixed Solidago populations and for identifying non-flowering or morphologically ambiguous Solidago plants. Since both parental species varied only negligibly in their DNA content, it may also be applicable across a broader geographic scale. Genetic, flow cytometric and distributional data suggest that the hybrids are to a large extent early generation (likely F1) hybrids as very few cases of supposed introgressants were also inferred. The results from chloroplast rps15-ycf1 spacer showed that hybridization has occurred in both directions.

Keywords: homoploid hybridization, DAPI flow cytometry, nrDNA ITS, plastid DNA sequences, relative DNA content
Introduction

Hybridization, resulting from mating between individuals of different species or genetically divergent individuals within a species, is one of the most important processes in plant evolution and speciation, influencing the evolutionary course of at least 25% of plant species (Mallet 2005, Whitney et al. 2010, Abbott et al. 2013). Besides natural phenomena bringing previously isolated species/lineages into contact (e.g. range shifts due to climate oscillations; Araújo & Luoto 2007), human-induced secondary contacts have become the most influential in this regard since the Age of Discovery (15th century). Related yet different species can meet when crops (Ellstrand et al. 2013) and ornamental (e.g. Lehan et al. 2013, Pergl et al. 2016) plants are purposefully introduced; however, unintentional introductions accompanying human movement between countries are also very common (e.g. Lehan et al. 2013).

Hybridization of native and non-native congeners deserves special consideration; evidence of such cases has increased exponentially over the last few decades (Mooney & Cleland 2001, Largiadèr 2008). Hybridization can have diverse outcomes including serious conservation concerns (Rhymer & Simberloff 1996, Vilà et al. 2000). (i) One of the outcomes may be the establishment of a new (hybridogenous) species that can either remain at its place of origin (Abbott 1992), or, become invasive and adversely affect whole ecosystems and communities [e.g. Sporobolus anglicus (C. E. Hub.) P. M. Peterson & Saarela – CABI 2021a], and, in extreme cases, spread so successfully that it completely displaces its parental species (Hegde et al. 2006). (ii) Gene flow between parental taxa causes ecological and genetic changes in both the introduced and native species (Strauss et al. 2006). Introggression of genes from a native species provides exotic species with preadapted genes for new environments that may enhance its invasiveness (Ellstrand & Schierenbeck 2000). On the other hand, gene flow in the opposite direction may cause erosion of the genetic pool, loss of genetic variability and thus negatively affect locally adapted populations of a native species (especially pronounced in small populations/rare species but even large native populations over the long term; Vilà et al. 2000, Wolf et al. 2001). This may in some cases lead to extinction via hybridization (Todesco et al. 2016, Vallejo-Marín & Hiscock 2016). (iii) In many cases, hybridization may be simply rare with little (long-term) effect on either parental taxon (e.g. Vallejo-Marín & Hiscock 2016, Dormontt et al. 2017).

Considering the above-mentioned threats, preventing the spread of new hybrid lineages requires quick recognition and consequently a rapid management response. Hybrids are often identified in the field based on their intermediate morphology and confirmed by genetic markers or a combination of both (Abbott et al. 2010, Saltonstall et al. 2014, Zaya et al. 2015, Fukatsu et al. 2019). Hybrid origin is also frequently accompanied by decreased fertility or even complete sterility as a result of reproductive isolating barriers (Baack et al. 2015). As hybridization and establishment of a hybrid lineage is often connected to genome duplication (allopolyploidization, e.g. Ainouche et al. 2004, Mandáková et al. 2019), or hybrids arise between congeners of different ploidies (heteroploid hybridization, e.g. Zozomová-Lihová et al. 2014, Musiał et al. 2020), karyological analysis (chromosome counts and/or nuclear DNA content) is another way of identifying hybrids. Although recognition of hybrids at the homoploid level is trickier and requires high quality of analyses (Loureiro et al. 2010), the effectivity of flow
Cytometry (FCM) for detection of homoploid hybrids in natural populations has been repeatedly proven for systems in which genome sizes of the two progenitors differ significantly (e.g. by at least 7%; see Loureiro et al. 2010, Hanušová et al. 2014, Macková et al. 2017, 2018, Agudo et al. 2019). However, the expected intermediate genome size in early-generation hybrids can be disrupted by several evolutionary processes such as genome rearrangements of stabilized hybrids, natural selection of hybrids, or introgression resulting in a continuous variation in genome size linking the distinct values of parental taxa, which often complicate the detection of homoploid hybrids by flow cytometry (cf. Bureš et al. 2004, Loureiro et al. 2010, Hanušová et al. 2014, Pellicer et al. 2021).

Currently, two alien species of the genus *Solidago* L., *S. canadensis* L. and *S. gigantea* Aiton, both of North American origin, are naturalized and invasive in almost all of Europe (Kabuce & Priede 2010, Kowarik 2010, CABI 2021b, c). Here, they share habitats with the European native *S. virgaurea* L., which has led to the origin of two spontaneous hybrids: *S. ×niederederi* Khek and *S. ×snarskisii* Gudžinskas & Žalneravičius. The latter, *Solidago ×snarskisii* (2n = 3x = 27), the result of the heteroploid crossing between diploid *S. virgaurea* (2n = 2x = 18) and tetraploid *S. gigantea* (2n = 4x = 36), the fertility of which is low and is currently only known from several localities in northern and eastern Europe (Gudžinskas & Žalneravičius 2016, Pliszko 2018, Musiał et al. 2020, Vinogradova & Galkina 2020). In contrast, the number of records of *S. ×niederederi* (2n = 2x = 18), which originated from the homoploid hybridization of diploid *S. canadensis* and *S. virgaurea* (both 2n = 2x = 18), has rapidly increased, mainly over the last four decades (Musiał et al. 2020, Skokanová et al. 2020b). *Solidago ×niederederi* was noticed for the first time at the end of the 19th century (Skokanová et al. 2020a) and was recently reported from more than 400 localities in 17 European countries (Skokanová et al. 2020b). *Solidago ×niederederi* seemingly originated through multiple events of hybridization mainly at localities where both parental species grow in close proximity with one another (Pliszko & Zalewska-Gałosz 2016, Galkina & Vinogradova 2019). Further spreading of these hybrids and formation of their own coherent populations cannot be excluded in the near future because plants of *S. ×niederederi* produce viable pollen (< 70%; Migdałek et al. 2014, Karpavičienė & Radušienė 2016). A low percentage of well-developed fruits (6%; Migdałek et al. 2014, Pliszko & Kostrakiewicz-Gierałt 2017) is partially balanced by high seed germinability (91%; Pliszko & Kostrakiewicz-Gierałt 2017). *Solidago ×niederederi* has the potential to successfully combine the astonishing phenotypic plasticity of *S. virgaurea* (Turesson 1925, Takahashi & Matsuki 2016, Hirano et al. 2017, Nardi et al. 2018) with the high invasiveness of *S. canadensis* (CABI 2021b). Assuming that the hybrids and introgressed progeny may benefit from a broader range of environmental conditions than their parental taxa (cf. Bleeker et al. 2007, Currat et al. 2008, Abbott et al. 2013), *S. ×niederederi* may soon pose a serious threat to biodiversity at the species and habitat level. Therefore, monitoring the occurrence and spread of *S. ×niederederi* is an important precaution if its potential negative effect on native European ecosystems is to be mitigated.

Starting from previous reports on genome size differences between *S. canadensis* and *S. virgaurea* (Szymura et al. 2015, Fernandez et al. 2018 and references therein) and on determination of the hybrid origin of *S. ×niederederi* using the internal transcribed spacer region (ITS) of nuclear ribosomal DNA (nrDNA) or non-coding regions of chloroplast DNA (cpDNA; Pliszko & Zalewska-Gałosz 2016, Galkina & Vinogradova
The main goals of the present study are: (i) test whether the hybrid plants of *S. ×niederederi* have intermediate values of the relative DNA content of their parental species, and consequently, if FCM could be unequivocally used for hybrid detection; (ii) test if the *Solidago* species co-occurring with *S. ×niederederi*, i.e. *S. canadensis*, *S. gigantea* and *S. virgaurea*, differ significantly in their relative DNA contents without any overlaps even when more extensive sampling over large areas is taken into account; (iii) verify the hybrid origin of the *S. ×niederederi* plants using the ITS region of nrDNA and, in addition, determine whether there is any evidence of backcrossing or later-generation hybrids in the ITS variation patterns; (iv) identify non-coding regions in cpDNA that are highly polymorphic and could differentiate between the parental species and hence reveal the direction of hybridization. To study the differentiation of genome size in the *Solidago* taxa studied, we employed DAPI flow cytometry, which is accurate and particularly useful for detecting small differences in genome size (Marhold et al. 2010, Suda et al. 2010). Chromosome counting was used for *S. ×niederederi* and its parental species to verify the flow cytometry data.

**Material and methods**

**Studied taxa**

*Solidago canadensis* occurs naturally in the north-eastern part of the United States and southern regions of Canada. Its secondary occurrence is known from Asia, Australia, Europe, and New Zealand (Semple 2021). This species was introduced to England as early as 1645 (Kowarik 2010), afterwards, it spread as it is an attractive ornamental plant to botanical as well as common gardens and nurseries throughout Europe. In Europe, it was recorded in the wild for the first time in 1850, and an exponential increase in the number of its sites started in 1870–1900 (Weber 1998). Today, this species is naturalized and invasive from northern Italy to southern Scandinavia (Kabuce & Priede 2010). *Solidago canadensis* is exclusively diploid (2n = 2x = 18). In its native area, two varieties, *S. canadensis* var. *canadensis* and *S. canadensis* var. *hargeri* Fernald, differing mainly in the type of stem indumentum, are recognized (Melville & Morton 1982, Semple & Cook 2006). The infraspecific classification of European invasive populations of *S. canadensis* is still uncertain (Verloove et al. 2017).

*Solidago virgaurea* is a highly polymorphic complex widespread in temperate and cold climates in Europe and Asia and has a patchy distribution also in the Mediterranean region of southern Europe, the north-western part of Africa and Asia Minor. This complex comprises 14–24 exclusively diploid (2n = 2x = 18) subspecies and microspecies. In Europe, the following subspecies are recognized within *S. virgaurea*: *S. virgaurea* subsp. *virgaurea* widely distributed in Europe; *S. virgaurea* subsp. *minuta* (L.) Arcang. with a patchy distribution in European mountains; *S. virgaurea* subsp. *pineticola* Sennikov from the Baltic region; *S. virgaurea* subsp. *lapponica* (With.) Tzvelev from northern Europe; *S. virgaurea* subsp. *litoralis* (Savi) Briquet et Cavillier; *S. virgaurea* subsp. *macrorrhiza* (Lange) Nyman and *S. virgaurea* subsp. *rupicola* (Rouy) Lambinon from sandy coasts of France and Italy (Yuzepchuk 1959, Slavík 2004, Greuter 2006–2020, Nardi et al. 2018, Semple 2021, Tela Botanica 2021).
*Solidago ×niederederi* is a nothotaxon resulting from the homoploid crossing (at the diploid ploidy level) of alien *S. canadensis* and native *S. virgaurea* (Musiał et al. 2020). It is characterized by having a morphology that is intermediate between that of its parental species (Fig. 1B), especially in respect to the height of the plant and size of the inflorescence and capitulum (Karpavičienė & Radušienė 2016). In addition, plants of *S. ×niederederi* are conspicuous in the frequent presence of vegetative shoots with densely crowded leaves (in the form of a pseudorosette) at the apex (Gudžinskas & Žalneravičius 2016). The hybrid occurs mainly at sites inhabited by both parental species and only sporadically at sites inhabited by one parental species. The known occurrences of *S. ×niederederi* are restricted to a part of Europe between 45.8 and 63.8°N (Skokanová et al. 2020b). Here *S. niederederi* is classified as an established alien (Musiał et al. 2020).

The native range of *Solidago gigantea* is in southern Canada and the central and eastern part of the USA. Nowadays this species is naturalized and invasive in Europe, East Asia, the Azores and New Zealand. In Europe, *S. gigantea* was introduced in 1758 and afterwards distributed as an ornamental to gardens and nurseries (Weber & Jacobs 2005). The first observations of *S. gigantea* in the wild in Europe date back to 1850 (Weber 1998). Since then the number of its localities have exponentially increased and it has spread across almost the whole of Europe (except the north). In its native range, two varieties, var. *gigantea* and var. *shinnersii* Beaudry, both including diploid, tetraploid and hexaploid ploidy levels (only rarely triploid and pentaploid) are reported (Semple 2021). Its invasive populations in Europe belong to var. *gigantea* and are predominantly tetraploid (Schlaepfer et al. 2008a, Semple 2021). *Solidago gigantea* often co-occur with *S. canadensis*, *S. ×niederederi* and/or *S. virgaurea*.

The species studied are long-lived perennials, 0.3–2.5 m tall with 1–40 flowering shoots, capitula are relatively small but numerous (20–2000) (Slavík 2004, Skokanová 2022). All species are obligate outbreeders and are pollinated by a wide range of insects. Plants produce up to 27,700 fruits of small size. Fruits have a pappus and are easily dispersed by wind over long distances. Non-native *S. canadensis* and *S. gigantea* also reproduce asexually via underground rhizomes (Werner 1980, Huang et al. 2007, Moravcová et al. 2010, CABI 2021b, c).

**Sampling**

The sampling was primarily focused on mixed populations of *S. canadensis* and *S. virgaurea* with the presence of hybrid *S. ×niederederi*. Altogether, material from 248 plants originating from 16 mixed populations from Poland, Slovakia, Hungary, and Romania were collected (Fig. 1A, Table 1). The occurrence of *S. ×niederederi* in populations BST, JEL, NSB, BTL, VPA and RZC (Table 1) is reported here for the first time; the remaining localities of *S. ×niederederi* were reported previously (Pliszko et al. 2018, Skokanová et al. 2020b). In cases when *S. gigantea* was present at a site with a mixed population, we also collected plant material of this taxon.

Additional material from 246 plants originating from single-species populations of *S. canadensis*, *S. gigantea* and *S. virgaurea* (20 populations per taxon) from a broader area (Czechia, Slovakia, Hungary, Croatia and Romania; Fig. 1A, Table 1) was collected and analysed to clarify whether the origin of plant material could affect the results due to geographically conditioned variations in genome size.
Table 1. Population (Pop.) code, number (N) of plants included in relative DNA content analyses (FCM), chromosome counts (chrom.), ITS and \textit{rps}15-\textit{ycf}1 analyses, and locality details, including geographical coordinates, altitude, date and collectors of \textit{Solidago} taxa. Relative DNA content, chromosome numbers and cpDNA haplotypes are given for particular taxa within mixed populations as well as from single species populations. *Taxon codes: C – \textit{S. canadensis}; G – \textit{S. gigantea}; N – \textit{S. ×niederederi}, flowering individuals; Nv – \textit{S. ×niederederi}, non-flowering individuals in the vegetative stage; V – \textit{S. virgaurea}; Ic, Iv – supposed introgressants of \textit{S. canadensis} and \textit{S. ×niederederi}. **Collector abbreviations: BŠ – B. Šingliarová, JD – J. Dušek, JK – J. Kochjarová, JO – J. Olšavská, KP – K. Pulíšová, KS – K. Skokanová, PM – P. Mereďa Jr., SM – S. Mikita, SŠ – S. Španiel, VK – V. Kolarčík.

| Pop. code | Taxon code\(^{*}\) | N FCM (RSS mean±SD) | N chrom. N ITS N \textit{rps}15-\textit{ycf}1 (cp DNA haplotypes) | Locality description, date, collector(s) ** |
|-----------|------------------|---------------------|------------------|-----------------------------------------------|
| ABU       | C                | 5 (0.818±0.005)    | 1 (2n = 18) 3 (H9) | Hungary; Abaújvár, W of the village; 48.506699\(^{°}\)N, 21.32746\(^{°}\)E; 212 m; 29.8.2019; KS |
|           | N                | 1 (0.858)          | 1 1 (H1)         |                                               |
|           | V                | 4 (0.911±0.01)     | 2 2 (H1)         |                                               |
|           | G                | 4 (1.464±0.000)    |                 |                                               |
| ARP       | C                | 4 (0.826±0.006)    | 2                | Romania; Arpașu de Sus, S of the village; 45.71156\(^{°}\)N, 24.61177\(^{°}\)E; 553 m; 15.9.2019; KS |
|           | N                | 1 (0.86)           | 1 1 (H9)         |                                               |
|           | V                | 5 (0.918±0.01)     | 2 2 (H1)         |                                               |
|           | G                | 1 (1.473)          | 1 1 (H10)        |                                               |
| BST       | C                | 3 (0.810±0.002)    | 1 1 (H6)         | Slovakia; Banská Štiavnica, W part of the town, Pod Trojickým vrchom Street; 48.4471433\(^{°}\)N, 18.898096\(^{°}\)E; 639 m; 14.8.2020; KS, SŠ |
|           | Ic               | 1 (0.819)          | 1 1 (H9)         |                                               |
|           | N                | 1 (0.856)          | 1 1 (H1)         |                                               |
|           | V                | 4 (0.910±0.004)    | 2 1 (H1)         |                                               |
|           | G                | 1 (1.473)          | 1 1 (H10)        |                                               |
| BTL       | C                | 4 (0.814±0.004)    | 2 2 (H7, H9)     | Slovakia; Betliar, SE of the village; 48.69605\(^{°}\)N, 20.51190\(^{°}\)E; 323 m; 19.8.2020; KS, SŠ |
|           | N                | 1 (0.863)          | 1 1 (H1)         |                                               |
|           | V                | 4 (0.911±0.004)    | 2 2 (H1)         |                                               |
|           | G                | 1 (1.466)          | 1 1 (H9)         |                                               |
| CZA       | C                | 4 (0.807±0.009)    | 3 4 (H9)         | Poland; Czajowice, S of the village; 50.18400\(^{°}\)N, 19.80865\(^{°}\)E; 441 m; 7.9.2019; KS, PM, SŠ, BŠ |
|           | Ic               | 1 (0.799)          | 1 1 (H8)         |                                               |
|           | N                | 3 (0.866±0.006)    | 3 3 (H3, H6)     |                                               |
|           | V                | 4 (0.915±0.004)    | 2 2 (H1)         |                                               |
|           | G                | 1 (1.466)          | 1 1 (H9)         |                                               |
| HAN       | C                | 7 (0.820±0.003)    | 2 2 (H9)         | Slovakia; Handlová, W of the town; 48.71894\(^{°}\)N, 18.7295\(^{°}\)E; 592 m; 18.8.2018; KS |
|           | N                | 2 (0.856±0.003)    | 2 2 (H1)         |                                               |
|           | V                | 6 (0.912±0.005)    | 2                |                                               |
| JAN       | C                | 4 (0.819±0.003)    | 4 4 (H6, H9)     | Poland; Januszowice, W of the village; 50.25505\(^{°}\)N, 20.04258\(^{°}\)E; 312 m; 7.9.2019; KS, PM, SŠ, BŠ |
|           | N/Nv             | 9/1 (0.863±0.007)  | 9/1 9/1 (H1, H9) |                                               |
|           | V                | 4 (0.913±0.005)    | 2 2 (H1)         |                                               |
|           | G                | 4 (1.453±0.006)    | 3 1 (H10)        |                                               |
| JAW       | C                | 6 (0.811±0.011)    | 3 2 (H7, H9)     | Poland; Jachowka, W of the village; 49.75383\(^{°}\)N, 19.67680\(^{°}\)E; 569 m; 6.9.2019; KS, PM, SŠ, BŠ |
|           | N                | 1 (0.857±0.00)    | 1 1 (H1)         |                                               |
|           | V                | 4 (0.917±0.005)    | 2 2 (H3, H4)     |                                               |
|           | G                | 4 (1.455±0.013)    | 2                |                                               |
| Pop. code | Taxon code* | NF C (RSS mean±SD) | N chrom. | N ITS | N *rpSl5-ycf1* (cp DNA haplotypes) | Locality description, date, collector(s) ** |
|-----------|-------------|---------------------|----------|-------|------------------------------------|---------------------------------------------|
| JEL       | C           | 4 (0.811±0.005)     | 2        | 2     | (H9)                               | Slovakia; Jeľava, N of the town; 48.64153°N, 20.24199°E; 294 m; 17.8.2020; KS, SŠ |
| N         | 1           | (0.865)             | 1        | 1     | (H1)                               |                                             |
| I N       | 1           | (0.841)             | 1        | 1     | (H1)                               |                                             |
| V         | 4           | (0.905±0.003)       | 1        | 2     | (H1)                               |                                             |
| MYD       | C           | 7 (0.820±0.01)      | 2        | 2     | (H5, H6)                           | Poland; Mydlniki, Krakow, N of the city district; 50.089781°N, 19.843067°E; 216 m; 7.9.2019; KS, PM, SŠ, BŠ |
| I C       | 1           | (0.824)             | 1        | 1     | (H5)                               |                                             |
| N/Nv      | 4/6 (0.865±0.007) | 4/6 4/6 (H1)       | 2        | 2     | (H1)                               |                                             |
| V         | 4           | (0.915±0.006)       | 2        | 2     | (H1)                               |                                             |
| G         | 4           | (1.449±0.012)       | 2        |       |                                    |                                             |
| NEZ       | C           | 19 (0.815±0.007)    | 3 (2n = 18) | 6     | (H1, H6, H7, H9)                  | Slovakia; Nezbudská Lúčka, loco dicto Za vodou and Hradský potok Creek valley; 49.172162°N, 18.879831°E; 364–400 m; 8.9.2019; KS, PM, SŠ |
| N/Nv      | 3/1 (0.873±0.003) | 1/1 (2n = 18)    | 2/1 2/1 (H1, H9) |       |                                    |                                             |
| V         | 20 (0.919±0.009) |                              | 6        | 6     | (H9)                               |                                             |
| NSB       | C           | 4 (0.812±0.009)     | 2        | 2     | (H6)                               | Slovakia; Nižnosálska Baňa, S part of the village; 48.73687°N, 20.41457°E; 389 m; 19.8.2020; KS, SŠ |
| N         | 4           | (0.858±0.007)       | 4        | 4     | (H1)                               |                                             |
| V         | 4           | (0.915±0.004)       | 2        | 1     | (H1)                               |                                             |
| PLC       | C           | 5 (0.812±0.007)     | 3        | 1     | (H9)                               | Poland; Pálca, N of the village, Dział Polecki settlement; 49.82612°N, 19.73678°E; 472 m; 6.9.2019; KS, PM, SŠ, BŠ |
| N         | 1           | (0.858)             | 1        | 1     | (H1)                               |                                             |
| V         | 4           | (0.916±0.004)       | 2        | 1     | (H3)                               |                                             |
| G         | 4           | (1.474±0.016)       | 1        |       |                                    |                                             |
| PWO       | C           | 4 (0.818±0.013)     | 4        | 4     | (H1, H3)                           | Poland; Podlesna Wola, W of village; 50.41077°N, 20.01547°E; 339 m; 7.9.2019; KS, PM, SŠ, BŠ |
| N         | 3           | (0.870±0.008)       | 3        | 3     | (H1)                               |                                             |
| V         | 4           | (0.912±0.002)       | 2        | 2     | (H1)                               |                                             |
| RZC       | C           | 4 (0.812±0.004)     | 4        | 4     | (H1, H3)                           | Poland; Rozcięta, S of the village; 49.72444°N, 19.32022°E; 468 m; 25.9.2020; KS, SŠ |
| N         | 3           | (0.865±0.006)       | 2        | 2     | (H1)                               |                                             |
| V         | 4           | (0.918±0.006)       | 2        | 2     | (H1)                               |                                             |
| VPA       | C           | 4 (0.816±0.008)     | 2        | 2     | (H9)                               | Slovakia; Hrhov, S of the village, Veľký Paklán hill; 48.58418°N, 20.73478°E; 196 m; 18.8.2020; KS, SŠ |
| N         | 4           | (0.867±0.006)       | 4        | 4     | (H9)                               |                                             |
| V         | 4           | (0.912±0.007)       | 1        | 2     | (H1, H2)                           |                                             |
| G         | 4           | (1.468±0.015)       | 2        | 2     | (H7, H9)                           |                                             |

*Solidago canadensis* – single species populations

| Pop. code | Taxon code* | NF C (RSS mean±SD) | N chrom. | N ITS | N *rpSl5-ycf1* (cp DNA haplotypes) | Locality description, date, collector(s) ** |
|-----------|-------------|---------------------|----------|-------|------------------------------------|---------------------------------------------|
| ABO       | C           | 4 (0.826±0.005)     | 2        | 2     | (H6, H7)                           | Hungary; Abony, N of the town; 47.21983°N, 20.01995°E; 89 m; 26.8.2018; KS |
| BAB       | C           | 10 (0.81±0.006)     | 2        | 2     | (H6, H7)                           | Slovakia; Banská Bystrica, between SC Europa and Belveder; 48.731553°N, 19.134420°E; 370 m; 17.8.2017; KS, JD |
| BRE       | C           | 4 (0.820±0.005)     | 2        | 2     | (H9)                               | Slovakia; Brezníčka, E part of the village; 48.42442°N, 19.74618°E; 219 m; 23.8.2019; KS, JO |
| COR       | C           | 4 (0.818±0.004)     | 2        | 2     | (H9)                               | Slovakia; Cornu de Jos; 45.14794°N, 25.69652°E; 419 m; 16.9.2019; KS, SM |
| DRG       | C           | 4 (0.818±0.004)     | 2        | 2     | (H9)                               | Slovakia; Dargovský priesmyk pass; 48.74341°N, 21.52951°E; 433 m; 1.9.2019; KS |
| DRI       | C           | 4 (0.823±0.003)     | 2        | 2     | (H7, H9)                           | Slovakia; Drietoma, N of the village towards state border; 48.94525°N, 17.92261°E; 292 m; 18.8.2018; KS |
| HEV       | C           | 4 (0.815±0.005)     | 2        | 2     | (H6, H7)                           | Hungary; Heves, SE part of the town; 47.59120°N, 20.29793°E; 88 m; 25.8.2018; KS |
| KRC       | C           | 4 (0.815±0.005)     | 2        | 2     | (H6, H7)                           | Slovakia; Kráľovský Chlmec, NE part of the town; 48.42979°N, 21.98214°E; 101 m; 30.8.2019; KS |
| Pop. code | Taxon code* | N FCM (RSS mean±SD) | N chrom. N ITS N rpS15-ycf1 (cp DNA haplotypes) | Locality description, date, collector(s) ** |
|-----------|-------------|---------------------|---------------------------------------------|---------------------------------------------|
| KRL | C | 4 (0.81±0.006) | 3 2 (H6) | Slovakia; Kráľova Lehota, near railway station; 49.02703°N, 19.78339°E; 657 m; 14.8.2019; KS, JK, KP |
| Ic | C | 1 (0.819) | | |
| LAP | C | 4 (0.809±0.009) | 2 2 (H6) | Romania; between villages of Lăpuș and Rogoz; 47.4722°N, 23.97188°E; 364 m; 21.9.2019; KS, SM |
| LPN | C | 4 (0.815±0.013) | 2 2 (H6) | Slovakia; between villages of Lipany and Kamenica; 49.177508°N, 20.954215°E; 443 m; 17.6.2020; KS, SŠ |
| MOC | C | 4 (0.81±0.009) | | Romania; Mocod, N of the village; 47.26707°N, 24.29732°E; 329 m; 21.9.2019; KS, SM |
| RIC | C | 4 (0.811±0.01) | | Czechia; Říčany, E margin of the village; 49.21356°N, 16.403°E; 324 m; 18.8.2018; KS |
| RVC | C | 4 (0.819±0.006) | 2 2 (H1, H9) | Slovakia; Revúca; 48.67913°N, 20.125706°E; 306 m; 17.5.2020; KS, SŠ |
| SIO | C | 3 (0.813±0.002) | | Hungary; between villages of Siófok and Fokihegy; 46.89348°N, 18.05007°E; 107 m; 29.8.2018; KS |
| SZI | C | 3 (0.808±0.005) | | Hungary; Szikszó, S of the village; 48.17715°N, 20.91450°E; 111 m; 1.10.2017; KS, VK |
| TRE | C | 4 (0.813±0.002) | | Czechia; Třebíč, E part of the town; 49.21661°N, 15.90361°E; 389 m; 19.8.2018; KS, SM |
| VAN | C | 4 (0.825±0.005) | | Romania; Vănilori, W of the village; 46.23738°N, 24.90969°E; 387 m; 20.9.2019; KS, SM |
| VBY | C | 4 (0.81±0.001) | | Slovakia; Bytča, N of the town; 49.24181°N, 18.55254°E; 322 m; 19.8.2019; KS |
| ZBJ | C | 4 (0.812±0.007) | | Slovakia; Zboj, SW of the village; 49.02138°N, 22.472045°E; 340 m; 6.7.2019; KS |
| ALA | V | 4 (0.924±0.005) | | Croatia; Velebit Mts, Alaginac pass; 44.546312°N, 15.168191°E; 1150 m; 18.6.2019; KS, JO |
| BEC | V | 4 (0.900±0.001) | 2 2 (H1) | Slovakia; Beckov, S of the village, Beckovské Skalice nature reserve; 48.77447°N, 17.89199°E; 190 m; 28.8.2020; KS, SŠ |
| BIA | V | 4 (0.912±0.005) | | Poland; Nowa Biała, parking lot near Przelom Bialky; 49.42855°N, 20.12408°E; 625 m; 14.8.2019; KS, JK, KP |
| CRM | V | 4 (0.926±0.015) | | Romania; Bucegi Mts, close to Cabana Caraiman; 45.410478°N, 25.487389°E; 2130 m; 17.9.2019; KS, SM |
| DSP | V | 4 (0.924±0.005) | | Slovakia; Belianske Tatry Mts, Dolina siedmých prameňov valley; 49.22697°N, 20.27944°E; 1520 m; 15.8.2019; KS, JK, KP |
| HUB | V | 4 (0.909±0.004) | | Slovakia; Hubina, SE of the village, valley above Striebornica dam; 49.60794°N, 17.92528°E; 293 m; 21.8.2018; KS, SM |
| JAL | V | 4 (0.912±0.008) | | Slovakia; Banská Bystrica, Jakub quarter, site Jakubské lúky; 48.765893°N, 19.14336°E; 433 m; 7.6.2019; KS |
| JVR | V | 4 (0.917±0.001) | | Slovakia; Uhrovce, E of the village, close to Jankov vřesok hill; 48.739925°N, 18.390209°E; 640 m; 30.5.2020; KS |
| KJS | V | 4 (0.913±0.004) | 3 1 (H1) | Slovakia; Kojšov, E of the village, below Zemničky saddle; 48.824287°N, 21.011266°E; 709 m; 12.5.2020; KS |
| KKO | V | 4 (0.929±0.003) | | Slovakia; Západné Tatry Mts, S slopes of Mt. Kondrátova kopa; 49.23481°N, 19.93175°E; 1957 m; 16.8.2019; KS, JK, KP |
| LSC | V | 4 (0.914±0.005) | | Slovakia; Veľká Fatra Mts, below the top of Mt. Lysec; 49.996177°N, 19.065547°E; 1325 m; 22.6.2016; KS |
| MRK | V | 4 (0.918±0.001) | 1 1 (H1) | Slovakia; Veľký Kolačín, SE of the village, SE slope of Markovnica hill; 48.925081°N, 18.19405°E; 530 m; 12.5.2020; KS |

*Soldago virgaurea – single species populations*
| Pop. code | Taxon code* | N FCM (RSS mean±SD) | N chrom. | N ITS N rpsL5-ycf1 (cp DNA haplotypes) | Locality description, date, collector(s) ** |
|-----------|-------------|----------------------|----------|----------------------------------------|---------------------------------------------|
| MRN V     | 4           | 0.926±0.003          |          |                                        | Slovakia; Muráň, NE of the village, site Šiance; 48.767778°N, 20.079278°E; 683 m; 12.7.2019; KS, JK |
| PLE V     | 4           | 0.906±0.005          |          |                                        | Croatia; Jastrebarsko, Plešivica settlement; 45.725393°N, 15.656632°E; 325 m; 12.8.2018; KS |
| RAB V     | 4           | 0.921±0.003          |          |                                        | Slovakia; Poloniny Mts, Mt. Rabia skala; 49.100927°N, 22.463959°E; 1089 m; 5.7.2019; KS |
| SAN V     | 3           | 0.905±0.006          |          |                                        | Slovakia; Devínska Nová Ves, S of the village, Sandberg nature reserve; 48.201563°N, 16.974144°E; 197 m; 9.5.2019; KS |
| SHO V     | 4           | 0.920±0.004          | 2        | 1 (H1)                                 | Slovakia; Liptovská Teplička, spring of the river Hornád; 48.98089°N, 20.10653°E; 914 m; 31.8.2020; KS, ŠŠ |
| TAT V     | 4           | 0.909±0.003          |          |                                        | Slovakia; Štiavnické vrchy Mts, Tatárska lúka; 48.405194°N, 18.870861°E; 890 m; 1.5.2019; KS |
| TFG V     | 3           | 0.925±0.01           |          |                                        | Romania; Făgărăș Mts, Transfăgărașan; 45.65128°N, 24.60513°E; 1151 m; 15.9.2019; KS |
| TRN V     | 4           | 0.917±0.005          |          |                                        | Slovakia; Krahule, NW of the village, Trnovník hill; 48.7360203°N, 18.92294°E; 964 m; 30.5.2020; KS |

**Solidago gigantea – single species populations**

| ALB G     | 4           | 1.474±0.006          |          |                                        | Hungary; Albertirs, near sports stadium; 47.25215°N, 19.61763°E; 120 m; 26.8.2018; KS |
| BAL G     | 4           | 1.490±0.005          |          |                                        | Hungary; Balatonbény, SE of the village; 46.67373°N, 17.34463°E; 198 m; 29.8.2018; KS |
| DCE G     | 4           | 1.461±0.015          |          |                                        | Croatia; Donja Čemerica, NW of the settlement; 45.33235°N, 15.921917°E; 156 m; 21.6.2019; KS, JO |
| HUM G     | 4           | 1.464±0.008          |          |                                        | Slovakia; Humenné, W of the town; 48.929°N, 21.86554°E; 181 m; 4.7.2019; KS |
| CHA G     | 4           | 1.459±0.015          |          |                                        | Slovakia; Čifába, N of the village; 47.836403°N, 18.8249918°E; 107 m; 18.5.2020; KS, ŠŠ |
| CHO G     | 4           | 1.475±0.011          |          |                                        | Slovakia; Chotín, near the railway station; 47.80647°N, 18.18978°E; 107 m; 21.7.2020; KS |
| KOZ G     | 4           | 1.485±0.011          |          |                                        | Czechia; Kožušnice, N part of the village; 49.15711°N, 17.18747°E; 274 m; 18.8.2018; KS |
| LOP G     | 4           | 1.465±0.007          |          |                                        | Slovakia; Lopai; 48.817572°N, 19.500472°E; 451 m; 12.7.2019; KS, JK |
| MAN G     | 4           | 1.484±0.006          |          |                                        | Romania; Mănerău, E of the village; 46.41198°N, 22.0071°E; 143 m; 14.9.2019; KS, SM |
| OCU G     | 4           | 1.461±0.014          |          |                                        | Croatia; Očura, NE of the village; 46.19688°N, 15.99614°E; 257 m; 16.6.2019; KS, JO |
| OSW G     | 4           | 1.442±0.009          |          |                                        | Poland; Ostrowsko, W of the village, under the bridge; 49.473086°N, 20.110869°E; 567 m; 14.8.2019; KS, JK, KP |
| PAS G     | 4           | 1.470±0.029          |          |                                        | Czechia; Pasohlávky, W of the village; 48.90186°N, 16.55247°E; 161 m; 19.8.2018; KS, SM |
| PES G     | 4           | 1.455±0.008          |          |                                        | Croatia; Plešmo, S of the village; 45.30882°N, 16.83869°E; 104 m; 22.6.2019; KS, JO |
| PEZ G     | 4           | 1.455±0.011          |          |                                        | Slovakia; Pezinok, NW of the town, site Rudné Bane; 48.32°N, 17.23669°E; 235 m; 20.8.2018; KS, SM |
| REC G     | 4           | 1.451±0.005          |          |                                        | Hungary; Recsk, S of the village; 47.91817°N, 20.10032°E; 203 m; 25.8.2018; KS |
| SLP G     | 4           | 1.484±0.019          |          |                                        | Croatia; N of Srednji Lipovec; 45.26524°N, 17.64291°E; 211 m; 2.7.2020; KS, ŠŠ |
| SVI G     | 5           | 1.492±0.013          |          |                                        | Slovakia; Svininá, N margin of the village; 48.79514°N, 18.15519°E; 246 m; 18.8.2018; KS |
| SZE G     | 4           | 1.448±0.008          |          |                                        | Hungary; Székesfehérvár, SE of the town; 47.17173°N, 18.49180°E; 115 m; 29.8.2018; KS |
| VAS G     | 4           | 1.472±0.007          |          |                                        | Hungary; Vaspör, NW of the village, road E86; 46.922698°N, 16.600041°E; 250 m; 4.8.2019; KS |
| VIN G     | 4           | 1.476±0.007          |          |                                        | Slovakia; Viničky, W margin of the village, near the railways; 48.396684°N, 21.73029°E; 98 m; 30.8.2019; KS |

N total 494 8 150 146
The flowering plants were determined in the field using known diagnostic morphological characters (Slavík 2004, Skokanová 2022). For identification of *S. ×niederederi* we also used morphological characters identified by Gudžinskas & Žalneravičius (2016), Karpavičienė & Radušienė (2016) and Galkina & Vinogradova (2019). From four localities with mixed populations (JAN, JEL, MYD, NEZ), nine plants in the vegetative stage, suspected to be of hybrid origin due to the presence of vegetative shoots with densely crowded leaves at the apex typical of *S. ×niederederi* (cf. Gudžinskas & Žalneravičius 2016), were also collected (Table 1, Supplementary Fig. S1).

Plant material was collected in the field during 2019–2020. Selected intact fresh leaves were kept in a cool place (~4–6 °C) until used in the FCM analyses; other leaves, taken from the same individuals, were dried in silica gel for molecular analyses. Some plants were transferred and cultivated in pots for chromosome counting. All collected plants were subjected to FCM analyses. A selection of plants analysed by flow cytometry was subjected to molecular analyses (sequencing of ITS and *rpS15-ycf1* regions) and chromosome counting (see below and Table 1). Voucher specimens were deposited in the

Fig. 1. (A) Map of the sites of the *Solidago* taxa analysed. Mixed populations with the hybrid *S. ×niederederi* are labelled with population codes (see Table 1). Populations marked by asterisk(s) included a supposed introgressant $I_e$ (**) or $I_h$ (***) of *S. canadensis* and *S. ×niederederi* (see Results for more details). (B) *Solidago canadensis*, *S. ×niederederi* and *S. virgaurea* from the Abaújvár locality (Hungary). Photograph: K. Skokanová.
herbarium of the Institute of Botany, Plant Science and Biodiversity Centre, Slovak Academy of Sciences (SAV).

**Molecular analyses (ITS of nrDNA and rpS15-ycf1 of cpDNA)**

Total genomic DNA was extracted from ~3–4 mg of silica gel-dried leaf material using the DNeasy Plant Mini Kit (Qiagen, Düsseldorf, Germany).

The ITS region of nrDNA (ITS1-5.8S-ITS2) was amplified in 150 individuals a priori determined based on morphology. It included one to 10 plants per each taxon from 15 mixed populations (all except population RZC found later), comprising 44 individuals of *S. canadensis*, 47 of *S. ×niederederi*, 28 of *S. virgaurea* and 12 of *S. gigantea*, complemented by one to three plants from single-species populations of *S. canadensis* (5 populations, 11 individuals) and *S. virgaurea* (4 populations, 8 individuals) (Table 1). The PCR reaction mix contained a sample of gDNA, 0.38 U *Pfu* DNA polymerase (Promega, Madison, WI, USA), 1× *Pfu* reaction buffer with MgSO₄, 0.2 mM dNTPs and 0.2 mM forward and reverse primers (ITS5, ITS4; White et al. 1990), in a final volume of 13 µl. Amplification was performed under the following PCR conditions: 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min, followed by 72 °C for 10 min. PCR products were purified enzymatically with a mixture of exonuclease I and FastAP thermo-sensitive alkaline phosphatase, according to the manufacturer’s protocol (Thermo Fisher Scientific Inc., Waltham, MA, USA). The sequencing was carried out at Eurofins Genomics Company (Konstanz, Germany) using both forward and reverse primers. The resulting electropherograms were carefully inspected for the presence of double peaks (intra-individual site polymorphisms, 2ISPs, following Potts et al. 2014), and when confirmed by both forward and reverse sequences, were coded using IUPAC ambiguity codes. The sequences were edited and aligned in Geneious v. R10 (Kearse et al. 2012). The sequence alignment was analysed using NeighbourNet (SplitsTree4 v. 4.14.4; Huson & Bryant 2006) based on uncorrected P-distances and the ‘average’ option for treating ambiguous bases.

Previously employed *trnL-trnF* and *rpl32-trnL*UAG intergenic spacers of cpDNA (e.g. Galkina & Vinogradova 2019) did not yield sufficient resolution to differentiate between *S. virgaurea* and *S. canadensis*, so neither were suitable to determine unequivocably the maternal parent of the hybrids. Therefore, we explored several other cpDNA spacers that are ranked among the most informative (Shaw et al. 2005, 2007, 2014, Prince 2015). The *rpS15-ycf1* spacer turned out to be the most polymorphic one in the initial screening tests. Finally it was amplified in 146 individuals, which were morphologically determined and included: one to 16 plants per each taxon from 15 mixed populations (all except population RZC, found later), comprising 50 individuals of *S. canadensis*, 47 of *S. ×niederederi*, 29 of *S. virgaurea* and 5 of *S. gigantea*, as well as one or two plants from single-species populations of *S. canadensis* (5 populations, 10 individuals) or *S. virgaurea* (4 populations, 5 individuals) (Table 1). The PCR reaction mix contained a sample of gDNA, 0.65 U DreamTaq DNA polymerase (Thermo Fisher Scientific), 1x DreamTaq reaction buffer with MgCl₂, 0.2 mM dNTPs and 0.2 mM forward and reverse primers (rpS15, ycf1; Prince 2015), in a final volume of 13 µl. PCR cycling conditions were the same as used for the ITS region. PCR products were purified enzymatically as specified above. The sequencing was carried out at Eurofins Genomics Company (Konstanz, Germany)
mostly in one direction only (using the forward primer). The sequences were aligned in Geneious v. R10, and five indels (insertion/deletion events) identified in the alignment were coded as additional binary characters, following the simple indel coding approach of Simmons & Ochoterena (2000), and appended to the nucleotide dataset. The final dataset was analysed using the statistical parsimony network implemented in TCS v.1.21 (Clement et al. 2000). This analysis identified different haplotypes in the dataset, revealed haplotype sharing between individuals and species, and determined genetic distances (number of substitutions or indel mutations) among them. Both ITS and rpS15-ycf1 dataset-based analyses were performed with and without sequences of S. gigantea. All sequences were submitted to the GenBank nucleotide database (Supplementary Table S1, MZ005322-MZ0055471 for ITS, MZ020814-MZ020959 for rpS15-ycf1).

**Chromosome counting**

For karyological analyses, root tip meristems from potted plants of S. canadensis (two populations, four individuals), S. ×niederederi (one individual) and S. virgaurea (one population, four individuals) were used (Table 1). The root tips were pre-treated in a 0.002 M water solution of 8-hydroxyquinoline at 4 °C for about 16 h (overnight), fixed in a 1:3 mixture of 98% acetic acid and 96% ethanol for 1–24 h, washed in distilled water, macerated in 1N HCl at 60 °C for 5 min and then washed in distilled water. Tip squashes were made using the cellophane square technique (Murín 1960). Permanent slides were stained with a 7% solution of Giemsa Stain – Modified Solution (Fluka Analytical), in Sörensen phosphate buffer, dried and observed in a drop of immersion oil using a Leica DM 1000 microscope equipped with an HDCE-X5 camera and ScopeImage 9.0 software and the number of chromosomes counted.

**Relative DNA content**

Altogether 494 plants were analysed by flow cytometry using fluorochrome 4’, 6-diamidino-2-phenylindole (DAPI). Based on a priori morphological identification, the following material was included in FCM analyses: one to 20 plants per each taxon from 16 mixed populations (91 individuals of S. canadensis, 51 of S. ×niederederi, 83 of S. virgaurea and 25 of S. gigantea) and one to 10 (mostly 4) plants from single-species populations of S. canadensis (20 populations, 84 individuals), S. virgaurea (20 populations, 78 individuals) and S. gigantea (20 populations, 81 individuals) (Table 1). To ensure the accuracy of the estimates of the relative DNA content, each plant was analysed separately and only fresh plant material was used. Fresh material of Solanum pseudo-capsicum L. (2.59 pg DNA/2C; Temsch et al. 2010a) was added for internal standardization. Nuclei isolation and staining procedure followed the simplified two-step protocol (Doležel et al. 2007) with some modifications. Intact leaf tissue of the analysed plant was chopped together with an internal standard in 1 ml of ice-cold Otto I buffer (0.1M citric acid, 0.5% Tween 20). The crude nuclear suspension was filtered through 42-μm nylon mesh. For staining, 1 ml of a solution containing Otto II buffer (0.4 M Na₂HPO₄·12H₂O), 2-mercaptopoethanol (2 μl/ml) and DAPI (4 μg/ml) was added to the flow-through fraction. Samples were analysed after 10 min incubation at room temperature. Fluorescence of at least 5000 particles was recorded, and only histograms with symmetrical peaks with a coefficient of variance (CV) of the standard and sample G1 peaks below 3% were considered.
Flow cytometric analyses were carried out using a Cyflow ML instrument or Cyflow Space instrument (Partec, Münster, Germany) equipped with a UV-LED as an excitation source. Flow cytometric histograms were evaluated using FloMax software v. 2.7d (Partec, Münster, Germany).

The relative DNA content was calculated as the ratio of G1 peak of standard Solanum pseudocapsicum and G1 peak of the Solidago sample (the ratio standard/sample herein-after referred to as RSS; 2C values presented unless otherwise stated). The relationship between chromosome numbers and relative DNA content of S. canadensis, S. virgaurea and S. ×niederederi was verified using chromosome counts (cf. Table 1).

Box-and-whisker plots and scatter plots were used to depict variation in relative DNA content of the taxa studied; t-tests and the Tukey-Kramer test (Tukey’s test for unequal sample size; Tukey 1977) were used to test for differences in relative DNA content between taxa. The normal distribution was tested a priori using the Kolmogorov-Smirnov test. Analyses were carried out using STATISTICA 12 (StatSoft Inc. 2013).

Results

Molecular analyses (ITS of nrDNA and rpS15-ycf1 of cpDNA)

The overall ITS alignment was 628 bp (base pairs) long and included 46 variable sites (Supplementary Table S2). Intraindividual site polymorphisms (2ISPs), suggesting the presence of multiple ITS copy variants in the genome, were detected in all the taxa examined. Within diploid S. canadensis and S. virgaurea, originating from both pure and mixed populations, such positions were mostly rare and scattered, with up to two 2ISPs per individual. In the tetraploid S. gigantea, 2ISPs were more frequent, with up to six such positions per individual, suggesting higher intragenomic ITS copy variation. The ITS sequences of S. virgaurea and S. canadensis were differentiated by seven substitutions (SNPs, all located in ITS2), which can be treated as species-specific. All individuals identified based on morphology as S. ×niederederi had additive 2ISPs at all these seven positions, clearly supporting the presence of both virgaurea- and canadensis-specific ITS copy variants in their genomes and thus their hybrid origin. The only exception was one non-flowering individual, SOL-N-JEL2, morphologically identified as S. ×niederederi (Supplementary Fig. S1C), which had an ITS sequence lacking any 2ISPs and was identical to the predominant ribotype of S. canadensis (the individual latter denoted as “In”; Supplementary Tables S2, S3). Furthermore, in four individuals classified as S. canadensis based on morphology (SOL-C-KRL13, SOL-C-MYD1, SOL-C-BST1 and SOL-C-CZA1), additive 2ISPs were recorded at four of the seven positions diagnostic for hybrids (these four individuals hereinafter denoted as “Ic”; Supplementary Table S3). The individuals Ic and Is might be a result of backcrossing between S. canadensis and S. ×niederederi and hereinafter are referred to as “supposed introgressants”. In the NeighborNet diagram of ITS alignment (Fig. 2; S. gigantea omitted), individuals of S. canadensis and S. virgaurea formed two well-differentiated, species-specific clusters, whereas individuals of S. ×niederederi were in an intermediate position between them, in accordance with the observed additive 2ISPs patterns. The four supposed introgressants were placed in a separate position, in between S. ×niederederi and S. canadensis. When individuals of S. gigantea were included in the NeighborNet analysis (Supplementary Fig. S2), they appeared in a species-specific cluster, close to S. canadensis.
The overall *rpS15-ycf1* alignment was 495 bp long. It included two variable positions (substitutions) and five indels, coded as additional binary characters, which altogether resulted in ten different haplotypes (with *S. gigantea* included; nine haplotypes without *S. gigantea*; Fig. 3, Supplementary Fig. S3). Two widespread haplotypes were identified, present in 71 individuals (haplotype H1) and 44 individuals (H9). Five haplotypes were present in two to 15 individuals, and the remaining three haplotypes were individual-specific. Samples of *S. canadensis* included six different haplotypes (also including the supposed introgressants with *S. ×niederederi*); three of these appeared to be species-specific (disregarding hybrids; H5, H6, H8), two were shared with *S. gigantea* (H7, H9) and only one was shared with *S. virgaurea* (the most widespread H1). There were four different haplotypes in samples of *S. virgaurea*; three of them were species-specific (disregarding hybrids; H2, H3, H4), and one (H1) was shared with *S. canadensis* (Fig. 3, Supplementary Fig. S3). Samples of *S. ×niederederi* included four different haplotypes; two of which were shared with *S. canadensis* (H6, H9), one with *S. virgaurea* (H3) and one was the widespread one (H1) shared with both parental species. This implies that the hybridization occurred in both directions. The samples of *S. gigantea* contained either a species-specific haplotype (H10) or shared haplotypes with *S. canadensis* (H7, H9). No geographic patterns in the distribution of haplotypes were recorded, which was confirmed by a detailed screening of haplotype variation within population NEZB (Table 1). In *S. canadensis* from NEZB, as many as four different haplotypes (H1, H6, H7 and H9) were recorded. In *S. ×niederederi* from NEZB, two widespread haplotypes were recorded, H1 shared by both parental species, and H9 shared by *S. canadensis* and *S. gigantea*. These findings also indicate that a significant portion of the overall variation in haplotypes recorded here can be found within populations.
Chromosome counts

Chromosome numbers are newly reported for four individuals of *S. canadensis* (from populations ABU, NEZ), four individuals of *S. virgaurea* and one individual of *S. ×niederederi* (both taxa from population NEZ) (Fig. 4A–C, Table 1). Our analyses confirm chromosome counts 2n = 18 for all samples examined.

Relative DNA content

The mean CV values of the G1 peaks of the internal standard *Solanum pseudocapsicum* and *Solidago* estimates were 1.92±0.39% and 2.18±0.44%, respectively. All individuals of *S. canadensis*, *S. ×niederederi* and *S. virgaurea* examined were exclusively diploid with 2n~2x~18 (RSS 0.793–0.938). Relative DNA content estimates of *S. gigantea* corresponded to a tetraploid ploidy level 2n~4x~36 (RSS 1.432–1.507; Supplementary Fig. S4). *Solidago gigantea* significantly differed from the remaining diploid taxa in 2C values as well as in equivalent of Cx values of relative DNA content (Fig. 5A; Tukey-Kramer test, P < 0.001). Based on this finding the involvement of *S. gigantea* in the origin of the studied hybrid can be ruled out.
At the diploid level (Fig. 5B, C), statistically significant differences (Tukey-Kramer, \(P < 0.001\)) were recorded in the relative DNA content of \(S.\ canadensis\) (RSS 0.793–0.838) and \(S.\ virgaurea\) (RSS 0.897–0.938) (Fig. 4D, E, Table 2). However, there were no statistical differences in the relative DNA content of individuals from mixed and single-species populations either of \(S.\ canadensis\) (t-test, \(t = –0.425, n.s.\)) or \(S.\ virgaurea\) (t-test, \(t = –1.257, n.s.\); Fig. 5A, B). In general, variation in the relative DNA content within particular diploid species was low (less than 5.8%; Table 2). The relative DNA content of \(S.\ \times\ niederederi\) (RSS 0.847–0.881) was clearly intermediate between \(S.\ canadensis\) and \(S.\ virgaurea\), as depicted by box-and-whisker plots (Fig. 5, Supplementary Fig. S4). The mean RSS value of \(S.\ \times\ niederederi\) (RSS 0.864) was negligibly lower than the expected one [RSS 0.865; (mean RSS of \(S.\ canadensis\) + mean RSS of \(S.\ virgaurea\))/2]. Differences in relative DNA content between parental and hybrid diploid taxa were relatively low but statistically significant (Tukey-Kramer, \(P < 0.001\); Fig. 4D, E). Values for \(S.\ \times\ niederederi\) were 6% higher on average than those of \(S.\ canadensis\) and 5.6% lower than those of \(S.\ virgaurea\). A histogram of their simultaneous FCM measurements is shown in Fig. 4D. The values of the relative DNA content of \(S.\ \times\ niederederi\)
were clearly distinct from *S. canadensis* and *S. virgaurea* in each of the mixed populations studied (Supplementary Fig. S5).

**Fig. 5.** Boxplots and scatter plots depicting the relative genome size (RSS) – equivalent of Cx values (A) and 2C values (B, C) measured for the *Solidago* taxa studied. Groups not significantly different at P < 0.01 are indicated by the same letters (Tukey-Kramer test; sample I N was not included in the statistical tests as based on a single observation). Boxes define 25th and 75th percentiles, squares show median values, and whiskers extend from the minimum to the maximum. IC, IN – supposed introgressants of *S. canadensis* and *S. ×niederederi*.

**Table 2.** Relative DNA content of the *Solidago* taxa studied. N (ind./pop.) – number of individuals and populations of particular taxa included in relative DNA content analyses; IC, IN – supposed introgressants of *S. canadensis* and *S. ×niederederi*; Var. – variation [calculated as (maximum×100/minimum)-100].

| Taxon (ploidy) | N (ind./pop.) | Ploidy | Relative DNA content (2C values) | Var.  |
|----------------|---------------|--------|---------------------------------|-------|
|                |               | RSS minimum–maximum | RSS mean±SD |
| *S. canadensis*| 171/36        | 2n~2x~18            | 0.793–0.838 | 0.815±0.008 | 5.8% |
| *S. ×niederederi* | 50/16   | 2n~2x~18            | 0.847–0.881 | 0.864±0.008 | 4.0% |
| *S. virgaurea*  | 161/36        | 2n~2x~18            | 0.897–0.938 | 0.916±0.009 | 4.5% |
| IC             | 4/4           | 2n~2x~18            | 0.799–0.824 | 0.813±0.010 | 3.2% |
| IN             | 1/1           | 2n~2x~18            | 0.841       |            |      |
| *S. gigantea*  | 106/28        | 2n~4x~36            | 1.432–1.507 | 1.467±0.018 | 5.3% |
The ITS analyses confirmed the hybrid origin (Fig. 5C, Supplementary Table S3) for almost all flowering and non-flowering individuals identified based on morphology as \textit{S. \texttimes niederederi} (ITS sequences were analysed in 46 individuals out of 50 individuals included in the FCM analyses) and with intermediate relative DNA content values between \textit{S. canadensis} and \textit{S. virgaurea}. In addition, the exceptional non-flowering SOL-N-JEL2 individual identified based on morphology as \textit{S. \texttimes niederederi}, but with an ITS sequence identical to \textit{S. canadensis} (supposed introgressant I_8, see above) had a relative DNA content intermediate between \textit{S. \texttimes niederederi} and \textit{S. canadensis} (RSS 0.841, Fig. 5). The relative DNA content of the other four supposed introgressants I_c, classified based on morphology as \textit{S. canadensis}, but with additive 2ISPs in ITS sequences at four of seven positions diagnostic for hybrids, was in the range of \textit{S. canadensis} (RSS 0.799–0.824; Fig. 5, Table 2). The ITS analyses confirmed the classification of the remaining diploid individuals included in FCM analyses as \textit{S. canadensis} (ITS sequences analysed in 51 individuals out of 171 individuals included in FCM analyses) or \textit{S. virgaurea} (ITS sequences analysed in 36 individuals out of 161 individuals included in FCM analyses) (Fig. 5C, Supplementary Fig. S5).

**Discussion**

**Ploidy levels and chromosome counts for the Solidago species studied**

Diploid chromosome numbers/ploidy levels (2n = 2x = 18, 2n~2x~18) of \textit{S. \texttimes niederederi} detected in the present study agree with the previously published data for 32 populations from Austria, Latvia, Lithuania, and Poland (Karpavičienė & Radušienė 2016, Musiał et al. 2020). Similarly, the diploid level (2n = 2x = 18, 2n~2x~18) here detected for \textit{S. virgaurea} is the same as ~75 previously published chromosome records for this species from Europe (Goldblatt & Johnson 1979–2021, Rice et al. 2015, Szymura et al. 2015, Nardi et al. 2018, Watanabe 2021). The only outstanding reports for \textit{S. virgaurea} in Europe are three decaploid (2n~10x~90) plants reported from a serpentine site in Bosnia and Herzegovina (Pustahija et al. 2013). For \textit{S. canadensis}, 23 chromosome/ploidy level records are available from Europe (cf. Szymura et al. 2015, Watanabe 2021), all of them reporting a diploid ploidy level (2n = 2x = 18) for this species and thus they are in accord with our results. The species is today considered to be exclusively diploid (Semple 2021); a tetraploid chromosome count (2n = 36) was also previously reported for \textit{S. canadensis} (Taylor & Mulligan 1968, Semple & Chmielewski 1987). \textit{Solidago gigantea} comprises in North America more or less spatially segregated diploid, tetraploid and hexaploid populations together with the rare occurrence of triploids and pentaploids (e.g. Semple et al. 1993, 2001, Schlaepfer et al. 2008a, Morton et al. 2019, Semple 2021). But, only tetraploid plants of \textit{S. gigantea} are confirmed by cytogeographic studies within its invasive range in Europe, Russia and Japan (Schlaepfer et al. 2008a) and in south-western Poland (Szymura et al. 2015). Some previous records of diploid and hexaploid plants of \textit{S. gigantea} in Europe (Maurer 1987 as cited in Jurenitsch et al. 1988, Jakobs 2004) are ruled out based on re-examinations of original material or material from the provided localities. These records were attributed to misidentifications, or technical failings (Schlaepfer et al. 2008a). Later, Hull-Sanders et al. (2009) report one diploid and six diploid–tetraploid populations of \textit{S. gigantea} from France, Germany and Switzerland, however, possible
contamination of seed material or confusion with S. canadensis was not considered in their study. In the present study, we recorded only the tetraploid ploidy level for 28 populations (81 plants) of S. gigantea from Slovakia, Poland, Hungary and Croatia, an area poorly represented in previous studies (cf. Schlaepfer et al. 2008a, Szymura et al. 2015 and references therein).

Aneuploidy in the strict sense (the presence of supernumerary A chromosomes) is currently unknown in the Solidago taxa studied, but supernumerary B chromosomes have been reported several times for S. canadensis (Kapoor 1978, Malecka 1989, Albers & Bennert 1998), S. gigantea (Semple et al. 1984) and S. virgaurea (Lövkvist & Hultgården 1999). Although B chromosomes were not detected in the present study, either by chromosome counting or flow cytometry (i.e. by significant increase of relative DNA content), we cannot rule out their presence in some individuals. Studies on other plant groups reveal an effect of B chromosomes on increasing genome size (e.g. Rosato et al. 1998, Chumová et al. 2016, Fourastié et al. 2016). However, considering the generally smaller size of B chromosomes and their unequal distribution in cells of the same individual (D’Ambrosio et al. 2017, Bednáľová et al. 2021), their presence may not always increase genome size enough for it to be detectable by flow cytometry.

The relative DNA content and its reliability for identifying Solidago ×niederederi

Some previous studies used flow cytometry for ploidy level determination to identify Solidago taxa (Verloove et al. 2017) or to map the distribution of cytotypes of polyploid complexes such as S. altissima L. and S. gigantea (Halverson et al. 2008, Schlaepfer et al. 2008a, Etterson et al. 2016). Variation in genome size is rarely used to differentiate Solidago taxa that do not differ in ploidy level (Szymura et al. 2015, Nardi et al. 2018). In the present study, we showed that DAPI flow cytometry is suitable for revealing differences in genome size between diploid Solidago species and, moreover, for detecting their hybrids. The relative values of the DNA content of the diploid taxa studied were very close but did not overlap, and all plants analysed could be unambiguously assigned to either S. canadensis, S. ×niederederi or S. virgaurea, based on these values (Table 2, Fig. 4D, E, 5; Supplementary Fig. S5). Further, the relative DNA content of hybrid plants was clearly intermediate when compared with parental taxa (Fig. 5). Thus, the present study demonstrated that, besides polymorphisms in ITS sequences, relative DNA content can be reliably used to identify S. ×niederederi. In particular, flow cytometry, which combines accuracy with unsurpassed speed and inexpensiveness (Loureiro et al. 2010), can be effectively used to identify Solidago plants with unclear morphology, seedlings and non-flowering or sterile plants, or used for detailed screening of mixed populations and progeny of plants from such populations. Morphology and genome size are highly correlated in several homoploid hybridising complexes (Hanušová et al. 2014, Macková et al. 2017, 2018).

The plants of S. ×niederederi analysed are likely to be putative F1 hybrids with intermediate relative DNA content between the parental species. However, variation in relative DNA content (Fig. 5C) showed that values for a few S. canadensis plants from mixed populations were out of range of the values detected in pure populations, and in fact were very close to the lowest values recorded for S. ×niederederi. This pattern might indicate some level of backcrossing with one of the parental taxa (cf. Bureš et al. 2004, Macková
et al. 2017), but this requires further research. A few discrepancies between morphology, ITS polymorphisms and DNA content values are also recorded, which indicates also cases of backcrossing, but the usage of ITS data may be limited here (see below).

In some groups of plants, genome size is correlated with environmental conditions and/or geographical distribution (Pecinka et al. 2006, Kolář et al. 2009, Dušková et al. 2010, Olsavská et al. 2012). However, we recorded little variation in relative DNA content of *S. virgaurea* samples (5.8%) collected over a substantial part of its distribution area (Fig. 1) and over a wide range of altitudes (190–2130 m a.s.l.; Tables 1, 2). Similarly, Nardi et al. (2018) found no significant differences in absolute DNA content between closely related *S. virgaurea* subsp. *virgaurea* and coastal populations attributed to subsp. *litoralis*. Furthermore, we recorded little variation in the datasets of the European populations of the invasive species *S. canadensis* (5.8%) and *S. gigantea* (5.3%; Table 2) studied. Therefore, it is likely that our results are applicable also in other parts of the non-native range of *S. canadensis*, where it may share habitats with *S. virgaurea* s.l.

Previous studies have shown substantial differences in estimates of DNA content, which may be due to different methodological procedures, different intercalary dyes as well as different plant tissues or internal standards used (cf. Doležel et al. 1998, Loureiro et al. 2006, Bennett et al. 2008, Wang et al. 2015). In the case of *S. canadensis*, previous flow cytometry measurements using intercalating fluorochrome propidium iodide (PI) varied from 1.96 pg (Garcia et al. 2013), 2.1 pg (Bai 2012), 2.04 pg (Kubešová et al. 2010), 2.14 pg (Verloove et al. 2017), 2.03–2.21 pg (Szymura et al. 2015), 2.47 pg (Paniego et al. 2019) to 5.87 pg (Guo et al. 2015). The value of 3.13 pg was recorded using mithramycin dye (Galbraith et al. 1983). Similarly, variation in DNA values was previously revealed by PI flow cytometry for *S. virgaurea*: 2.14 pg (Sliwinska & Thiem 2007), 2.14–2.16 pg (Pustahija et al. 2013), 2.26 pg (Temsch et al. 2010b), 2.31–2.33 pg (Nardi et al. 2018), 2.34–2.36 (Szymura et al. 2015) and 2.35 pg (Garcia et al. 2013). A distinct value of 1.77 pg was revealed using Feulgen densitometry (Vidic et al. 2009).

Differences in previous results indicate the need to use flow cytometry with caution for identifying *S. ×niederederi* as the present study showed that the relative DNA content values of *S. ×niederederi* and parental taxa were very close. Therefore, for the identification of hybrid plants of *S. ×niederederi* either by DAPI or PI flow cytometry, it is advisable to analyse a reasonable number of plants of the parent species at the same time. The value of the DNA content or the standard/sample ratio of the suspected hybrid itself may not always allow unambiguous identification. Instead, it should be verified that the revealed value(s) is/are intermediate between the values for *S. canadensis* and *S. virgaurea*.

**Genetic variation of the Solidago species studied**

The ITS region of nrDNA is a high-copy locus prone to concerted evolution (Álvarez & Wendel 2003), but it is widely known that the process of sequence homogenization is imperfect and intragenomic variation may be common even in diploid species (e.g. Weitemier et al. 2015). Therefore, the presence of 2ISPs among several individuals of both *S. canadensis* and *S. virgaurea*, suggesting the presence of multiple nrDNA copy variants within their genomes, is not surprising. Direct Sanger sequencing does not allow exploration of the full intragenomic ITS diversity; instead, molecular cloning or amplicon high-throughput sequencing would give more detailed insights. Nevertheless,
even with direct sequencing, we were able to identify seven species-specific SNPs that unequivocally differentiated *S. canadensis* and *S. virgaurea* and revealed additive patterns in those sites in *S. ×niederederi*. The same seven SNPs were previously used for the identification of *S. ×niederederi* by Pliszko & Zalewska-Gałosz (2016), while Galkina & Vinogradova (2019) identified only four of them and skipping three additional sites located at the 3’ end of ITS2 region. Perfect additivity in all the individuals of *S. ×niederederi* examined, with only very few exceptions (see below), indicates that they represent an early hybrid generation (likely F1), which is in accordance with their occurrence at sites together with both parental species.

The genus *Solidago* is known for its complex taxonomy (Semple & Cook 2006) and comprehensive phylogenetic reconstructions are still largely lacking. Recent phylogenetic studies have primarily focused on the origin of polyploids within particular (diploid-) polyploid complexes, such as *S. gigantea* (Schlaepfer et al. 2008b), *S. altissima* (Sakata et al. 2015) and *S. houghtonii* Torr. et A. Gray (Laureto & Barkman 2011) or on the origin of ecotypes within the diploid species complex *S. virgaurea* (Sakaguchi et al. 2018). Laureto & Barkman (2011) also resolved the relationships among 26 North American *Solidago* species and it is noteworthy they revealed that the divergence in their nuclear and chloroplast genomes only partially reflected the morphological differentiation of the species studied. Both molecular markers used in the present study (ITS region of nrDNA, *rpS15-ycf1* spacer of cpDNA) indicated a close relationship between invasive populations of *S. canadensis* and *S. gigantea* (Supplementary Fig. S2, S3), which agrees with previous studies (Schlaepfer et al. 2008b, Laureto & Barkman 2011, Sakata et al. 2015). Here we targeted one of the most variable intergenic spacers of cpDNA (Prince 2015), but it still revealed the presence of haplotypes shared among the species analysed, along with some species-specific ones. Explanations of haplotype sharing reported by previous authors (Schlaepfer et al. 2008b, Sakata et al. 2015) also match our results: either the phylogenetic resolution of cpDNA intergenic spacers, despite their high mutation rate, is insufficient to differentiate between these close relatives, or extensive ancestral variation has been maintained due to rapid radiation during early stages of diversification (cf. Maddison & Knowles 2006, Moreno-Letelier et al. 2013). Our European samples of *S. canadensis* shared two haplotypes with *S. gigantea* (H7, H9), but also the most widespread haplotype (H1) with *S. virgaurea*. Because *S. canadensis* has spread secondarily throughout Europe only since 1870–1900 (Weber 1998), the widespread sharing of the haplotype with *S. virgaurea* is probably not due to introgression/hybridization between these two species, but supports the hypothesis concerning the maintenance of ancestral variation among species of *Solidago*.

Haplotype variation was found to be similar in native *S. virgaurea* (four haplotypes) and invasive *S. canadensis* (five haplotypes). There are no geographic patterns in the distribution of haplotypes, which, along with high intrapopulational variation, is apparently due to outcrossing and efficient seed dispersal in these species.

*Who is the mother of Solidago ×niederederi?*

In angiosperms, chloroplasts are predominantly inherited maternally (Corriveau & Coleman 1988). In accord with this general hypothesis, a solely maternal inheritance was confirmed by analyses of the chloroplast haplotypes of the offspring of *Solidago*
gigantea (Schlaepfer et al. 2008b). Building on this presumption, analyses of cpDNA could be useful for revealing the maternal parent of S. ×niederederi. Pliszko & Zalewska-Gałosz (2016), based on an analysis of the chloroplast rpl32–trnL locus, stated that hybridization between S. canadensis and S. virgaurea can happen in both directions. However, Galkina & Vinogradova (2019) later noticed that both parents are relatively polymorphic at this locus, and therefore it is not possible to unambiguously answer the question, which is the maternal parent? The results presented here for the rpS15-ycf1 spacer of cpDNA showed that 10 samples of S. ×niederederi from five populations shared the species-specific haplotypes H6 and H9 of S. canadensis, while two samples of S. ×niederederi from the same population shared the species-specific haplotype H3 of S. virgaurea (Fig. 3, Table 1). Based on these findings, we can infer that hybridization has occurred in both directions, i.e. both S. canadensis and S. virgaurea have been involved as maternal progenitors of the hybrids.

**Does Solidago ×niederederi backcross with parental species?**

While hybrid plants of S. ×niederederi could be determined relatively reliably based on intermediate morphological features (Gudžinskas & Žalneravičius 2016, Karpavičienė & Radušienė 2016, Galkina & Vinogradova 2019), or by cytometric (this study) and ITS analyses (Pliszko & Zalewska-Gałosz 2016, Galkina & Vinogradova 2019, this study), the determination of introgressants resulting from further crossing of hybrids and parental taxa is much more challenging. Within our dataset, we identified two types of putative introgressants based on some discrepancies between morphology, ITS polymorphisms and DNA content values. Four plants (Ic) morphologically resembled S. canadensis but displayed four additive 2ISPs (out of seven diagnostic ones for the hybrid) in ITS sequences, and the relatively variable values for DNA content are still within the range of variation recorded for S. canadensis (Fig. 3, 5, Supplementary Table S2). On the other hand, one non-flowering plant (In) morphologically resembled S. ×niederederi (Supplementary Fig. S1C) but had a homogenized ITS sequence identical to S. canadensis and its relative DNA content was intermediate between S. ×niederederi and S. canadensis (Fig. 5). Thus, both the above-mentioned cases could be attributed to the backcrossing of S. ×niederederi and S. canadensis, but with different extents of concerted evolution in the ITS region and possibly also representing backcrossed individuals of different generations. Nevertheless, it is recognized that concerted evolution acting on ITS of nrDNA may be unpredictable and, for instance, also different evolutionary constraints related to the maintenance of secondary structures may be present and affect mutation rates (Álvarez & Wendel 2003). Therefore, the utility of ITS sequences for detecting introgression may be limited and additional evidence for ongoing introgression or backcrosses is needed.

In any case, multiple polytopic origins of S. ×niederederi (cf. Skokanová et al. 2020b) indicate, no or only a slight reproduction barrier between S. canadensis and S. virgaurea. It is expected that the reproduction barrier would not play an important role in further crossing of S. ×niederederi with any of the parental species. Because plants of S. ×niederederi are visited by many insects, especially Diptera and Hymenoptera, and produce viable pollen and seeds (Migdałek et al. 2014, Karpavičienė & Radušienė 2016, Pliszko & Kostrakiewicz-Gierałt 2017, 2018), they could be successfully involved in further
crosses either with parental species or with other hybrid plants. Therefore, the monitoring of the progressive transition of mixed populations into introgressed populations or hybrid swarms could be difficult, as it seems that at least plants resulting from backcrossing of *S. canadensis* and *S. ×niederederi* are impossible to distinguish from pure *S. canadensis* by means of their morphology and/or genome size.

**Solidago ×niederederi and its potential threats**

The ongoing homoploid hybridization between *S. canadensis* or *S. ×niederederi* and native *S. virgaurea*, regardless of the current extent of this process, represents a potential threat to the *S. virgaurea* complex (Skokanová et al. 2020b). This Eurasian species group includes, besides *S. virgaurea* subsp. *virgaurea*, many taxa and ecotypes adapted to local environments, and they are still undergoing speciation (Sakaguchi et al. 2018). Although the taxonomic and genetic diversity of this species complex is insufficiently explored, subsp. *minuta*, subsp. *pineticola*, subsp. *litoralis*, subsp. *macrorrhiza* and subsp. *rupicola* are currently recognized as nominate subspecies in different parts of Europe (Skokanová et al. 2020b). Hybridization between native and non-indigenous species can imperil native taxa in many ways as documented by dozens of studies. The known scenarios include reproductive interference and decline caused by heterospecific pollen (Suárez-Mariño et al. 2019, Zaya et al. 2021), wasteful production of maladaptive hybrids or demographic swamping (Wolf et al. 2001, Prentis et al. 2007), replacement by viable hybrids or genetic swamping (Ottenburghs 2021), genetic erosion (Johnson et al. 2016), reduced vegetative and sexual fitness of native species in contrast to hybrids (Gallego-Tévar et al. 2019), genetic depletion and reduced fitness of native species (Kellner et al. 2012) and, in some cases, extinction of native populations (Rhymer & Simberloff 1996, Buerkle et al. 2003, Todesco et al. 2016).

Although the degree of the negative effect on native species may differ from case to case depending on the frequency of hybridization and hybrid fitness, hybrid viability and fertility, these circumstances can change over time as hybrids evolve and adapt (Sloop et al. 2009, Li et al. 2021). According to current knowledge, the hybrid *Solidago ×niederederi* does not spread vegetatively as successfully as *S. canadensis* because of the absence of the long rhizomes typical of the invasive parent. But hybrid plants are very viable and form large clumps with many flowering stems (Pliszko & Kostrakiewicz-Gieralt 2019). Although the proportion of well-developed fruits is low, the seed germination rate is high, as is the production of viable pollen (Migdałek et al. 2014, Karpavičienė & Radušienė 2016, Pliszko & Kostrakiewicz-Gieralt 2017). Moreover, seedlings of the hybrid are less affected by allelopathic compounds of *Solidago* species than its invasive parent *S. canadensis* (Karpavičienė et al. 2019), which can facilitate the establishment of the hybrid even in areas densely covered with *S. canadensis*. Despite the low number of hybrid individuals at localities inhabited by both parents (one, rarely two to ten hybrid plants, exceptionally more, Skokanová et al. 2020b), we cannot rule out that the fitness of the hybrid and its interaction with native *S. virgaurea* populations may change over time.

As hybridization between *S. canadensis* and *S. virgaurea* can occur in both directions (Pliszko & Zalewska-Gałosz 2016, and this study) and backcrossing of the fertile hybrid with at least *S. canadensis* seems probable (plants denoted as I<sub>c</sub> and I<sub>s</sub> in our results), another aspect that needs to be considered is the flow of genes from native to invasive
species. It is repeatedly documented that introgression of native fitness-increasing alleles and their phenotypic effects further maintained by natural selection, might promote the invasiveness (by increasing its ecological amplitude and facilitating range expansion) of alien species (Ellstrand & Schierenbeck 2000, Currat et al. 2008, Vekemans 2010, Hall 2016). In our case, it is expected that S. canadensis, which is already highly invasive, could pick up genes adapted to local conditions (adaptive introgression) from the highly variable native S. virgaurea, creating even better-adapted and thus potentially more-invasive phenotypes.

Currently, we can boldly refute the statement that “hybrids … (Solidago ×niederederi)… do not appear to be common nor able to persist” (CABI 2021b). Therefore, it is appropriate to stress the need for monitoring the occurrence, spread and behaviour of alien-to-native hybrid Solidago ×niederederi in order to mitigate its negative effects on the native flora of Europe.

Supplementary materials

Supplementary Fig. S1. – Examples of non-flowering Solidago plants collected in the vegetative stage, identified based on their morphology as S. ×niederederi.

Supplementary Fig. S2. – Genetic variation of the Solidago taxa studied revealed by a NeighborNet network based on the ITS sequences of nrDNA.

Supplementary Fig. S3. – Maximum parsimony network of the cpDNA haplotypes (the rps15-ycf1 spacer) of the Solidago taxa studied.

Supplementary Fig. S4. – Boxplots depicting the relative DNA contents (RSS, 2C values) of the diploid (2n~2x~18) and tetraploid (2n~4x~36) Solidago taxa studied.

Supplementary Fig. S5. – Boxplots and circles depicting the relative DNA contents (RSS, 2C values) of the Solidago plants and taxa studied in particular mixed populations.

Supplementary Table S1. – GenBank accessions numbers for ITS and rps15-ycf1 sequences of the Solidago plants analysed.

Supplementary Table S2. – ITS ribotypes of the Solidago individuals arranged by assignment to taxa.

Supplementary Table S3. – Polymorphisms in ITS sequences of Solidago individuals, arranged by populations.

Supplementary materials are available at www.preslia.cz

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References

Abbott R., Albach D., Ansell S., Arntzen J. W., Baird S. J. E., Bierie N., Boughman J., Brelsford A., Buerkle C. A., Buggs R., Butlin R. K., Dieckmann U., Eroukhmanoff F., Grill A., Cahan S. H., Hermansen J. S., Hewitt G., Hudson A. G., Jiggins C., Jones J., Keller B., Marczewski T., Mallet J., Martinez-Rodriguez P., Möst M., Mullen S., Nichols R., Nolte A. W., Parsons C., Pfennig K., Rice A. M., Ritchie M. G., Seifert B., Smadja C. M., Stelkens R., Szymura J. M., Vainölä R., Wolf J. B. W. & Zinner D. (2013) Hybridization and speciation. – Journal of Evolutionary Biology 26: 229–246.

Abbott R. J. (1992) Plant invasions, interspecific hybridization and the evolution of new plant taxa. – Trends in Ecology & Evolution 7: 401–405.

Abbott R. J., Hegarty M. J., Hiscock S. J. & Brennan A. C. (2010) Homoploid hybrid speciation in action. – Taxon 59: 1375–1386.

Agudo A. B., Torices R., Loureiro J., Castro S., Castro M. & Álvarez I. (2019) Genome size variation in a hybridizing diploid species complex in Anacyclus (Asteraceae: Anthemideae). – International Journal of Plant Sciences 180: 374–385.
Ainouche M. L., Baumel A., Salmon A. & Yannic G. (2004) Hybridization, polyploidy and speciation in *Spartina* (*Poaceae*). – New Phytologist 161: 165–172.

Albers F. & Bennert W. (1998) Chromosomenzahlen der Farn und Blütenpflanzen Deutschlands. – In: Wisskirchen R. & Haeupler H., Standardliste der Farn- und Blütenpflanzen Deutschlands, p. 562–616, Bundesamt für Naturschutz & Verlag Eugen Ulmer, Stuttgart.

Álvarez I. & Wendel J. F. (2003) Ribosomal ITS sequences and plant phylogenetic inference. – Molecular Phylogenetics and Evolution 29: 417–434.

Araújo M. B. & Luoto M. (2007) The importance of biotic interactions for modelling species distributions under climate change. – Global Ecology and Biogeography 16: 743–753.

Baack E., Melo M. C., Rieseberg L. H. & Ortiz-Barrients D. (2015) The origins of reproductive isolation in plants. – New Phytologist 207: 968–984.

Bai C., Alverson W. S., Follansbee A. & Waller D. M. (2012) New reports of nuclear DNA content for 407 vascular plant taxa from the United States. – Annals of Botany 110: 1623–1629.

Bednárová M., Karafiátová M., Hřibová E. & Bartoš J. (2021) B Chromosomes in genus *Sorghum* (*Poaceae*). – Plants 10: 505.

Bennett M. D., Price H. J. & Johnston J. S. (2008) Anthocyanin inhibits propidium iodide DNA fluorescence in *Euphorbia pulcherrima*: implications for genome size variation and flow cytometry. – Annals of Botany 101: 777–790.

Bleeker W., Schmitz U. & Ristow M. (2007) Interspecific hybridisation between alien and native plant species in Germany and its consequences for native biodiversity. – Biological Conservation 137: 248–253.

Buerkle C. A., Wolf D. E. & Rieseberg L. H. (2003) The origin and extinction of species through hybridization. – In: Brigham C. A. & Schwartz M. W. (eds), Population viability in plants: conservation, management, and modelling of rare plants, p. 117–141, Springer Verlag, Heidelberg.

Bureš P., Wang Y. F., Horová L. & Suda J. (2004) Genome size variation in Central European species of *Cirsium* (*Compositae*) and their natural hybrids. – Annals of Botany 94: 353–363.

Bai C., Alverson W. S., Follansbee A. & Waller D. M. (2012) New reports of nuclear DNA content for 407 vascular plant taxa from the United States. – Annals of Botany 110: 1623–1629.

Bednárová M., Karafiátová M., Hřibová E. & Bartoš J. (2021) B Chromosomes in genus *Sorghum* (*Poaceae*). – Plants 10: 505.

CABI (2021a) *Spartina anglica* (common cordgrass). – In: Invasive Species Compendium, CAB International, Wallingford, https://www.cabi.org/isc/datasheet/107739 (accessed 1 May 2021).

CABI (2021b) *Solidago canadensis* (Canadian goldenrod). – In: Invasive Species Compendium, CAB International, Wallingford, https://www.cabi.org/isc/datasheet/50599 (accessed 1 May 2021).

CABI (2021c) *Solidago gigantea* (giant goldenrod). – In: Invasive Species Compendium, CAB International, Wallingford, https://www.cabi.org/isc/datasheet/50575 (accessed 1 May 2021).

Chumová Z., Mandáková T. & Trávníček P. (2016) Are B-chromosomes responsible for the extraordinary genome size variation in selected *Anthoxanthum* annuals? – Plant Systematics and Evolution 302: 731–738.

Clement M., Posada D. & Crandall K. A. (2000) TCS: a computer program to estimate gene genealogies. – Molecular Ecology 9: 1657–1659.

Corriveau J. L. & Coleman A. W. (1988) Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. – American Journal of Botany 75: 1443–1458.

D'Ambrosio U., Alonso-Lifante M. P., Barros K., Kovařík A., Mas de Xaxars G. & Garcia S. (2017) B-chrom: a database on B chromosomes of plants, animals and fungi. – New Phytopathologist 216: 635–642.

Doležel J., Greilhuber J., Lucretti S., Meister A., Lysák M. A., Nardi L. & Obermayer R. (1998) Plant genome size estimation by flow cytometry: inter-laboratory comparison. – Annals of Botany 82 (Suppl. A): 17–26.

Dormontt E. E., Prentis P. J., Gardner M. G. & Lowe A. J. (2017) Occasional hybridization between a native and invasive *Senecio* species in Australia is unlikely to contribute to invasive success. – PeerJ 5: e3630.

Dušková E., Kolář F., Sklenář P., Rauchová J., Kubešová M., Fér T., Suda J. & Marhold K. (2010) Genome size correlates with growth form, habitat and phylogeny in the Andean genus *Lasiocephalus* (*Asteraceae*). – Preslia 82: 127–148.

Ellstrand N. C., Meirmans P., Rong J., Bartsch D., Ghosh A., de Jong T. J., Haccou P., Lu B.-R., Snow A. A., Neal Stewart Jr. C., Strasburg J. L., van Tienderen P. H., Vrielink K. & Hooftman D. (2013) Introgression of crop alleles into wild or weedy populations. – Annual Review of Ecology, Evolution, and Systematics 44: 325–345.

Ellstrand N. C. & Schierenbeck K. A. (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? – Proceedings of the National Academy of Sciences of the United States of America 97: 7043–7050.
Kabuce N. & Priede N. (2010) NOBANIS – Invasive Alien Species Fact Sheet: Solidago canadensis. – Online Database of the European Network on Invasive Alien Species – NOBANIS, https://www.nobanis.org/globalassets/speciesinfo/s/solidago-canadensis/solidago-canadensis.pdf (accessed 1 May 2021).

Kapoor B. M. (1978) Supernumerary chromosomes of some species of Solidago and a related taxon. – Caryologia 31: 315–330.

Karpavičienė B., Danilovienė J. & Vyktaitė R. (2019) Congeneric comparison of allelopathic and autotoxic effects of four Solidago species. – Botanica Serbica 43: 175–186.

Karpavičienė B. & Radušienė J. (2016) Morphological and anatomical characterization of Solidago × niederederi and other sympatric Solidago species. – Weed Science 64: 61–70.

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran Ch., Thierer T., Ashton B., Meintjes P. & Drummond A. (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. – Bioinformatics 28: 1647–1649.

Kellner A., Ritz C. M. & Wissemann V. (2012) Hybridisation with invasive Rosa rugosa threatens the genetic integrity of native Rosa mollis. – Botanical Journal of the Linnean Society 170: 472–484.

Kolář F., Štech M., Trávníček P., Rauchová J., Urfus T., Vit P., Kubešová M. & Suda J (2009) Towards resolving the Knautia arvensis agg. (Dipsacaceae) puzzle: primary and secondary contact zones and ploidy segregation at landscape and micogeographic scales. – Annals of Botany 103: 963–974.

Kowarik I. (2010) Biologische Invasionen: Neophyten und Neozoen in Mitteleuropa. Ed. 2. – Verlag Eugen Ulmer, Stuttgart.

Kubešová M., Moravcová L., Suda J., Jarošík V. & Pyšek P. (2010) Naturalized plants have smaller genomes than their non-invading relatives: a flow cytometric analysis of the Czech alien flora. – Preslia 82: 81–96.

Largiadèr C. R. (2008) Hybridization and introgression between native and alien species. – In: Nentwig W. (eds), Biological invasions, p. 275–292, Springer, Berlin, Heidelberg.

Laureto P. & Barkman T. (2011) Nuclear and chloroplast DNA suggest a complex single origin for the threatened allopolyploid Solidago houghtonii (Asteraceae) involving reticulate evolution and introgression. – Systematic Botany 36: 209–226.

Lehan N. E., Murphy J. R., Thorburn L. P. & Bradley B. A. (2013) Accidental introductions are an important source of invasive plants in the continental United States. – American Journal of Botany 100: 1287–1293.

Li Ch., Ohadi S. & Mesgaran M. B. (2021) Asymmetry in fitness-related traits of later-generation hybrids between two invasive species. – American Journal of Botany 108: 51–62.

Loureiro J., Trávníček P., Rauchová J., Urfus T., Vit P., Štech M., Castro S. & Suda J. (2010) The use of flow cytometry in the biosystematics, ecology and population biology of homoploid plants. – Preslia 82: 3–21.

Lövkvist B. & Hultgården U. M. (1999) Chromosome numbers in south Swedish vascular plants. – Opera Botanica 137: 1–42.

Macková L., Vít P., Ďurišová Ľ., Eliáš Jr. P. & Urfus T. (2017) Hybridization success is largely limited to homoploid Prunus hybrids: a multidisciplinary approach. – Plant Systematics and Evolution 303: 481–495.

Macková L., Vit P. & Urfus T. (2018) Crop-to-wild hybridization in cherries: empirical evidence from Prunus fruticosa. – Evolutionary Applications 11: 1748–1759.

Maddison W. P. & Knowles L. L. (2006) Inferring phylogeny despite incomplete lineage sorting. – Systematic Biology 55: 21–30.

Malecka J. (1989) Studies on the genus Solidago L. I. Karyotype analysis of Solidago canadensis L. s.l. – Acta Biologica Cracoviensia, Series Botanica 30: 137–145.

Mallet J. (2005) Hybridization as an invasion of the genome. – Trends in Ecology & Evolution 20: 229–237.

Mandáková T., Zozomová-Lihová J., Kudoh H., Zhao Y., Lysák M. A. & Marhold K. (2019) The story of promiscuous crucifers: origin and genome evolution of an invasive species, Cardamine occulta (Brassicaceae), and its relatives. – Annals of Botany 124: 209–220.

Marhold K., Kudoh H., Pak J.-H., Watanabe K., Španiel S. & Lihová J. (2010) Cytotype diversity and genome variation in eastern Asia polyploidy Cardamine (Brassicaceae) species. – Annals of Botany 105: 249–264.

Melville M. R. & Morton J. K. (1982) A biosystematic study of the Solidago canadensis (Compositae) complex. 1. The Ontario populations. – Canadian Journal of Botany 60: 976–997.

Migdałek G., Kolczyk J., Pliszko A., Kościńska-Pająk M. & Słomka A. (2014) Reduced pollen viability and achene development in Solidago ×niederederi Khek from Poland. – Acta Societatis Botanicorum Poloniae 83: 251–255.
Mooney H. A. & Cleland E. E. (2001) The evolutionary impact of invasive species. – Proceedings of the National Academy of Sciences of the United States of America 98: 5446–5451.

Moravcová L., Pyšek P., Jarošík V., HavelkoVá V. & Žákravský P. (2010) Reproductive characteristics of neophytes in the Czech Republic: traits of invasive and non-invasive species. – Preslia 82: 365–390.

Moreno-Letelier A., Ortiz-Medrano A. & Piñero D. (2013) Niche divergence versus neutral processes: combined environmental and genetic analyses identify contrasting patterns of differentiation in recently diverged pine species. – PLoS ONE 8: e78228.

Morton J. K., Venn J. & Semple J. C. (2019) [2020] Chromosome number determinations in Solidago gigantea (Asteraceae: Astereae). – Rhodora 121: 347–352.

Murín A. (1960) Substitution of cellophane for glass covers to facilitate preparation of permanent squashes and smears. – Stain Technology 35: 351–353.

Musiał K., Pagitz K., Gudžinskas Z., Łazarski G. & Pliszko A. (2020) Chromosome numbers in hybrids between invasive and native Solidago (Asteraceae) species in Europe. – Phytotaxa 471: 267–275.

Nardi F. D., Pustahija F., Andreucci A., Siljak-Yakovlev S. & Peruzzi L. (2018) Does Solidago litoralis (Asteraceae) merit specific rank? Insights from cytogenetic, molecular and ecological data. – Phytotaxa 346: 121–140.

Olšavská K., Perný M., Španiel S. & Šingliarová B. (2012) Nuclear DNA content variation among perennial taxa of the genus Cyanus (Asteraceae) in Central Europe and adjacent areas. – Plant Systematics and Evolution 298: 1463–1482.

Ottenburghs J. (2021) The genic view of hybridization in the Anthropocene. – Evolutionary Applications 14: 2342–2360.

Paniego A., Panero J. L., Vallés J. & S. Garcia (2019) Contribution to the genome size knowledge of New World species from the Heliantheae alliance (Asteraceae). – Plant Biosystems 153: 559–568.

Pecinka A., Suchánková P., Lysak M. A., Trávníček B. & DoLežel J. (2006) Nuclear DNA content variation among Central European Koeleria taxa. – Annals of Botany 98: 117–122.

Pellicer J., López-Pujol J., Aixarch M., Garnatje T., Vallès J. & Hidalgo O. (2021) Detecting introgressed populations in the Iberian endemic Centaurea podospermifolia through genome size. – Plants 10: 1492.

Pergl J., Sádlo J., Petřík P., Danihelka J., Chrtek J., Hejda M., Moravcová L., Perglová I., Štajerová K. & Pyšek P. (2016) Dark side of the fence: ornamental plants as a source of wild-growing flora in the Czech Republic. – Preslia 88: 163–184.

Pliszko A. (2018) First record of Solidago ×snarskisii (Asteraceae) in Poland. – Botanica Lithuanica 24: 211–213.

Pliszko A. & Kostrakiewicz-Gieralt K. (2017) Resolving the naturalization strategy of Solidago ×niederederi (Asteraceae) by the production of sexual ramets and seedlings. – Plant Ecology 218: 1243–1253.

Pliszko A. & Kostrakiewicz-Gieralt K. (2018) Flower-visiting insects on Solidago ×niederederi (Asteraceae): an observation from a domestic garden. – Botanica 24: 162–171.

Pliszko A. & Kostrakiewicz-Gieralt K. (2019) The importance of sexual, asexual and mixed ramet clusters in production of descendant ramets in populations of Solidago ×niederederi (Asteraceae). – Biologia 74: 953–960.

Pliszko A., Łazarski G., Kalinowski P., Adamowski W., Rutkowski L. & Puchalka R. (2018) An updated distribution of Solidago ×niederederi (Asteraceae) in Poland. – Acta Musei Silesiae, Scientiae Naturales 66: 253–258.

Pliszko A. & Żelewska-Galosz J. (2016) Molecular evidence for hybridization between invasive Solidago canadensis and native S. virgaurea. – Biological Invasions 18: 3103–3108.

Potts A. J., Heddderson T. A. & Grimm G. W. (2014) Constructing phylogenies in the presence of Intra-Individual Site Polymorphisms (2ISPs) with a focus on the nuclear ribosomal cistron. – Systematic Biology 63: 1–16.

Prentis P. J., White E. M., Radford I. J., Lowe A. J. & Clarke A. R. (2007) Can hybridization cause local extinction? a case for demographic swamping of the Australian native Senecio pinnatifolius by the invasive Senecio madagascariensis? – New Phytologist 176: 902–912.

Prince L. M. (2015) Plastid primers for angiosperm phylogenetics and phylogeography. – Applications in Plant Sciences 3: 1400085.

Pustahija F., Brown S. C., Bogunić B., Bašić N., Muratović E., Olshawk J., Hidalgo O., Bourge M., Stevanović V. & Siljak-Yakovlev S. (2013) Small genomes dominate in plants growing on serpentine soils in West Balkans, an exhaustive study of 8 habitats covering 308 taxa. – Plant Soil 373: 427–453.

Rhymer J. M. & Simberloff D. (1996) Extinction by hybridization and introgression. – Annual Review of Ecology and Systematics 27: 83–109.
Rice A., Glick L., Abadi S., Einhorn M., Kopelman N. M., Salman-Minkov A., Mayzel J., Chay O. & Mayrose I. (2015) The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. – New Phytologist 206: 19–26.

Rosato M., Chiavarino A. M., Naranjo C. A., Camara Hernandes J. & Poggio L. (1998) Genome size and numerical polymorphism for the B-chromosome of races of maize (Zea mays ssp. mays, Poaceae). – American Journal of Botany 85: 1068–1174.

Sakaguchi S., Kimura T., Kyan R., Maki M., Nishino T., Ishikawa N., Nagano A. J., Honjo M. N., Yasugi M., Kudoh H., Li P., Choi H. J., Chernyagina O. A. & Ito M. (2018) Phylogeographic analysis of the East Asian goldenrod (Solidago virgaurea complex, Asteraceae) reveals hidden ecological diversification with recurrent formation of ecotypes. – Annals of Botany 121: 489–500.

Sakata Y., Itami J., Isagi Y. & Ohgushi T. (2015) Multiple and mass introductions from limited origins: genetic diversity and structure of Solidago altissima in the native and invaded range. – Journal of Plant Research 128: 909–921.

Saltontstall K., Castillo H. E. & Blossey B. (2014) Confirmed field hybridization of native and introduced Phragmites australis (Poaceae) in North America. – American Journal of Botany 101: 211–215.

Schlaepfer D. R., Edwards P. J., Semple J. C. & Biller J. (2008a) Cytogeography of Solidago gigantea (Asteraceae) and its invasive ploidy level. – Journal of Biogeography 35: 2119–2127.

Schlaepfer D. R., Edwards P. J., Widmer A. & Biller J. (2008b) Phylogeography of native ploidy levels and invasive tetraploids of Solidago gigantea. – Molecular Ecology 17: 5245–5256.

Semple J. C. (2021) Astereae Lab. – University of Waterloo, Waterloo, https://uwaterloo.ca/astereae-lab (accessed 1 May 2021).

Semple J. C. & Chmielewski J. G. (1987) Chromosome number determinations in fam. Compositae, tribe Astereae. II. Additional counts. – Rhodora 89: 319–325.

Semple J. C. & Cook R. E (2006) Solidago L. – In: Flora of North America Editorial Committee (eds), Flora of North America north of Mexico 20: 151–157, Oxford University Press, Oxford, New York.

Semple J. C., Ringius G. S., Leeder C. & Morton G. (1984) Chromosome numbers of goldenrods, Euthamia and Solidago (Compositae: Astereae). II. Additional counts with comments on cytogeography. – Brittonia 36: 280–292.

Semple J. C., Xiang C., Zhang J., Horsburgh M. & Cook R. (2001) Chromosome number determinations in fam. Compositae, tribe Astereae. VI. Western North American taxa and comments on generic treatments of North American asters. – Rhodora 103: 202–218.

Semple J. C., Zhang J. & Xiang C. (1993) Chromosome number determinations in fam. Compositae, tribe Astereae. V. Eastern North American taxa. – Rhodora 95: 234–253.

Shaw J., Licey E. B., Beck J. T., Farmer S. B., Liu W., Miller J., Siripun K. C., Winder C. T., Schilling E. E. & Small R. L. (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. – American Journal of Botany 92: 142–166.

Shaw J., Licey E. B., Schilling E. E. & Small R. L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. – American Journal of Botany 94: 275–288.

Shaw J., Shafer H. L., Leonard O. R., Kovach M. J., Schorr M. & Morris A. B. (2014) Chloroplast DNA sequence utility for the lowest phylogenetic and phylogeographic inferences in angiosperms: the tortoise and the hare IV. – American Journal of Botany 101: 1987–2004.

Simmons M. P. & Ochoterena H. (2000) Gaps as characters in sequence-based phylogenetic analyses. – Systematic Biology 49: 369–381.

Skokanová K. (2022) Solidago L. – zlatobýl. – In: Goliašová K., Hodálová I. & Mereďa P. Jr. (eds), Flóra Slovenska VI/2. Časť 1. [Flora of Slovakia VI/2, Part 1.], Veda, Bratislava (in press).

Skokanová K., Mereďa P. Jr., Šingliarová B. & Španiel S. (2020a) Lectotype of Solidago ×niederederi (Asteraceae) selected from a recently rediscovered original material. – Phytotaxa 438: 62–64.

Skokanová K., Šingliarová B., Španiel S., Hodálová I. & Mereďa P. Jr. (2020b) Tracking the expanding distribution of Solidago ×niederederi (Asteraceae) in Europe and first records from three countries within the Carpathian region. – BioInvasions Records 9: 670–684.

Slavík B. (2004) Solidago L. – zlatobýl. – In: Slavík B. & Štěpáneková J. (eds), Květena České Republiky [Flora of the Czech Republic], 7: 114–123, Academia, Praha.

Sliwinska E. & Thiem B. (2007) Genome size stability in six medicinal plant species propagated in vitro. – Biologia Plantarum 51: 556–558.

Sloop C. M., Ayres D. R. & Strong D. R. (2009) The rapid evolution of self-fertility in Spartina hybrids (S. alterniflora x foliosa) invading San Francisco Bay, CA. – Biological Invasions 11: 1131–1144.
StatSoft Inc. (2013) STATISTICA (data analysis software system), version 12. – StatSoft Inc., Tulsa, http://www.statsoft.com.

Strauss S. Y., Lau J. A. & Carroll S. P. (2006) Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? – Ecology Letters 9: 357–374.

Suárez-Mariño A., Arceo-Gómez G., Sosenksi P. & Parra-Tabla V. (2019) Patterns and effects of heterospecific pollen transfer between an invasive and two native plant species: the importance of pollen arrival time to the stigma. – American Journal of Botany 106: 1308–1315.

Suda J., Trávníček P., Mandák B. & Berchová-Bímová K. (2010) Genome size as a marker for identifying the invasive alien taxa in Fallopia section Reynoutria. – Preslia 82: 97–106.

Szymura M., Szymura T. H. & Kreitschitz A. (2015) Morphological and cytological diversity of goldenrods (Solidago L. and Euthamia Nutt.) from south-western Poland. – Biodiversity Research and Conservation 38: 41–49.

Takahashi K. & Matsuki S. (2016) Morphological variations of the Solidago virgaurea L. complex along an elevational gradient on Mt. Norikura, central Japan. – Plant Species Biology 32: 238–246.

Taylor R. L. & Mulligan G. A. (1968) Flora of the Queen Charlotte Islands. Part 2. Cytological aspects of the vascular plants. – Queen’s Printer, Ottawa

Tela Botanica (2021) Solidago virgaurea subsp. rupicola (Rouy) Lambinon. – https://www.tela-botanica.org/bdf-xx-65123-synthese (accessed 1 May 2021).

Temsch E. M., Greilhuber J. & Krisai R. (2010a) Genome size in liverworts. – Preslia 82: 63–80.

Temsch E. M., Temsch W., Ehrendorfer-Schratt L. & Greilhuber J. (2010b) Heavy metal pollution, selection, and genome size: the species of the Žerjav study revisited with flow cytometry. – Journal of Botany 2010: 596542.

Todesco M., Pascual M. A., Owens G. L., Ostevik K. B., Hübner S., Heredia S. M., Hahn M. A., Cases C., Bock D. G. & Rieseberg L. H. (2016) Hybridization and extinction. – Evolutionary Applications 9: 892–908.

Tukey J. W. (1977) Exploratory data analysis. – Addison-Wesley Publishing Company, Reading etc.

Turesson G. (1925) The plant species in relation to habitat and climate. – Hereditas 6: 147–236.

Vallejo-Marín M. & Hiscock S. J. (2016) Hybridization and hybrid speciation under global change. – New Phytologist 211: 1170–1187.

Vinogradova Yu. K. & Galkina M. A. (2020) Hybridization as a factor of invasive activity of alien species of goldenrods (Solidago). – Biological Bulletin Reviews 10: 57–70.

Wang J., Liu J. & Kang M. (2015) Quantitative testing of the methodology for genome size estimation in plants using flow cytometry: a case study of the Primulina genus. – Frontiers in Plant Sciences 6: 354.

Watanabe K. (2021) Index to Chromosome numbers in Asteraceae. – Kobe University, Kobe University Library, Kobe, http://www.lib.kobe-u.ac.jp/lib/meta_pub-engG0000003asteraceae (accessed 1 May 2021).

Weber E. (1998) The dynamics of plant invasions: a case study of three exotic goldenrod species (Solidago L.) in Europe. – Journal of Biogeography 25: 147–154.

Weber E. & Jakobs G. (2005) Biological flora of central Europe: Solidago gigantea Aiton. – Flora 200: 109–118.

Weitemier K., Straub S. C. K., Fishbein M. & Liston A. (2015) Intragenomic polymorphisms among high-copy loci: a genus-wide study of nuclear ribosomal DNA in Axklepias (Apocynaceae). – PeerJ 3: e718.

Werner P. A., Bradbury I. K. & Gross R. S. (1980) The biology of Canadian weeds. 45. Solidago canadensis L. – Canadian Journal of Plant Science 60: 1393–1409.

White T. J., Bruns T., Lee S. B. & Taylor J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: Innis M. A., Gelfand D. H., Sninsky J. J. & White T. J. (eds), PCR protocols: a guide to methods and applications, p. 315–322, Academic Press, San Diego.

Whitney K. D., Ahern J. R., Campbell L. G., Albert L. P. & King M. S. (2010) Patterns of hybridization in plants. – Perspectives in Plant Ecology, Evolution and Systematics 12: 175–182.
Octomová-Lihová J., Krak K., Mandáková T., Shimizu K. K., Španiel S., Vít P. & Lysak M. A. (2014) Multiple hybridization events in Cardamine (Brassicaceae) during the last 150 years: revisiting a textbook example of neoallopolyploidy. – Annals of Botany 113: 817–830.

Zaya D. N., Leicht-Young S. A., Pavlovic N. B & Ashley M. V. (2021) Heterospecific pollination by an invasive congener threatens the native American bittersweet, Celastrus scandens. – PLoS ONE 16: e0248635.

Zaya D. N., Leicht-Young S. A., Pavlovic N. B., Feldheim K. A. & Ashley M. V. (2015) Genetic characterization of hybridization between native and invasive bittersweet vines (Celastrus spp.). – Biological Invasions 17: 2975–2988.

Yuzepchuk S. V. (1959) Zolotarnik – Solidago L. – In: Shishkin B. K. (ed.), Flora SSSR 25: 31–50, Izdateľstvo Akademii Nauk SSSR, Moskva & Leningrad.

Wolf D. E., Takebayashi N. & Rieseberg L. H. (2001) Predicting the risk of extinction through hybridization. – Conservation Biology 15: 1039–1053.

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