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Genetic variability and distance between *Lactuca serriola* L. populations from Sweden and Slovenia assessed by SSR and AFLP markers

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Abstract – The study involved 121 samples of the common weed, *Lactuca serriola* L. (prickly lettuce), representing 53 populations from Sweden and Slovenia. The seed materials, originating from different habitats, were regenerated and taxonomically validated at the Department of Botany, Palacký University in Olomouc, Czech Republic. The morphological characterizations of the collected plant materials classified all 121 samples as *L. serriola* f. *serriola*; one sample was heterogeneous, and also present was *L. serriola* f. *integrifolia*. Differences in the amount and distribution of the genetic variations between the two regions were analyzed using 257 amplified fragment length polymorphism (AFLP) and 7 microsatellite (SSRs) markers. Bayesian clustering and Neighbor-Network were used for visualization of the differences among the samples by country. Under the Bayesian approach, the best partitioning (according to the most frequent signals) was resolved into three groups. While the absence of an admixture or low admixture was detected in the Slovenian samples, and the majority of the Swedish samples, a significant admixture was detected in the profiles of five Swedish samples collected near Malmö, which bore unique morphological features of their rosette leaves. The Neighbor-Network analysis divided the samples into 6 groups, each consisting of samples coming from a particular country. Reflection of morphology and eco-geographical conditions in genetic variation are also discussed.

Key words: biogeography, Dinaric Alps and the Pannonian Plain, DNA polymorphism, ecology, habitats, morphological variation, prickly lettuce, Scandinavia

Introduction

Prickly lettuce (*Lactuca serriola* L., Asteraceae) is the most common species in the genus *Lactuca* L. (Feráková 1977), and has a circumglobal distribution (Lebeda et al. 2004). It is an annual or winter-annual therophyte (Feráková 1977), and an 'r'strategist (Tilman 1988). Its evolution has trended towards a short life cycle, strong self-fertilization ability, good adaptation for wind dispersal, and quick germination (Frietema de Vries 1992, Lebeda et al. 2001). *L. serriola* is a drought-tolerant species (Werk and Ehleringer 1986), mainly growing in sunny microhabitats within anthropogenic habitats such as roadsides, railways, dumps, and urban areas (Feráková 1977, Lebeda et al. 2001, 2004); it is considered a good colonist of a wide spectrum of different habitats with different degrees of invasivity. Prickly lettuce is of Euro-Asian origin, also being native in North Africa (Feráková 1977). It has primarily spread in the Mediterranean and the Near East (de Vries 1997, Lebeda et al. 2007a), and is considered an archaeophyte dependent on a culture from the northern part of central Europe (Meusel and Jäger 1992). The species belongs to a group of Mediterranean ruderal plants that have enlarged their distribution area during the last few centuries (Landolt 2001).

The northern boundary of the European distribution area runs near latitude 65 °N through Finland, and 55 °N through Great Britain (Feráková 1977). The expanding distribution of this species is accomplished by the transport of reproductive propagules, achenes. The ripened achenes with attached pappus are primarily dispersed by the wind, prob-

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ably also by water (Weaver and Downs 2003). The spread of this species is also closely related with human activities, which primarily produce an increase in their transport (Lebeda et al. 2001). Prickly lettuce has drastically increased its geographical range, invading many European, (North-) American, and Australian regions during the last 50-60 years (de Vries 1996, Lebeda et al. 2001, 2004); recently *L. serriola* has spread as an invasive weed throughout Europe (Lebeda et al. 2004, 2007b, D'Andrea et al. 2009), including Scandinavia (Rydberg 2013). Its synanthropic distribution has also been recorded from Australia, including Tasmania and New Zealand (Burbinge and Gray 1970, Webb et al. 1988), as well as Taiwan (Wang and Chen 2010), North America, southern Africa, and Argentina (Strausbaugh and Core 1978, Zohary 1991, Zuloaga and Morrone 1999). The study by Alexander (2010) supported a genetic basis for the differences in the elevation limits of *L. serriola* populations between two parts of its native and introduced ranges.

Two primary morphological forms are recognized within *L. serriola* L. based on cauline leaf-shape variability; the pinnatifid-leaved form *L. serriola* L. f. *serriola*, and the unlobed-leaved form *L. serriola* L. f. *integrifolia* (S.F. Gray) S.D. Prince et R. N. Carter. The *serriola* form is recorded as the most frequent species, occurring at a very high density in Europe; the form *integrifolia* is not so common, and has been recorded in e.g., Switzerland, Italy, France, western Germany, the Netherlands, and is prevalent in the UK (Lebeda et al. 2001, 2004, 2007a, b).

Lactuca serriola is the best known wild species of the genus *Lactuca*, the geographic distribution, morphological, and phenological variations of which have been intensively studied (Lebeda et al. 2004, 2007a, Alexander 2010). *L. serriola* is also an important genetic resource for new resistance to diseases and pests (Lebeda et al. 2014), abiotic factors, as well as for genes responsible for physiological and quality characters (Lebeda et al. 2007a). Prickly lettuce has been used in commercial lettuce breeding for more than 80 years (Lebeda et al. 2007a), especially as a source of race-specific resistance genes against lettuce downy mildew (*Bremia lactucae* Regel) (Parra et al. 2016). It has also been used over the last decade in various molecular studies to characterize genetic variation and diversity in both germplasm collections and natural populations (e.g. Koopman et al. 2001, Kitner et al. 2008, 2015).

The most commonly used methods for the analysis of DNA polymorphism include amplified fragment length polymorphism (AFLP; Vos et al. 1995), and microsatellites (simple sequence repeats, SSRs); Simko (2009) contributed significantly to the development of these for the genus *Lactuca*, and in particular for *L. serriola* Riar et al. (2011). These markers have been successfully applied in *Lactuca* research, addressing e.g., the distribution of the genetic variation of prickly lettuce across Europe (Lebeda et al. 2009a), distribution of genetic variation in natural populations of *L. serriola*, *L. saligna*, and *L. aculeata* in Israel (Kitner et al. 2015), or analyses of gene flow from crops to their wild relatives (Uwimana et al. 2012).

Southern / central Sweden is the northern limit of *L. serriola* distribution in Europe; Slovenia represents an area between the Central European and Mediterranean / Balkan distributions (Feráková 1977). The two areas differ in their climatic, ecogeographic, and ecologic conditions. In Slovenia, prickly lettuce is distributed throughout the entire territory, from the lowlands to the mountain regions (Martinčič and Sušnik 1984), and it most often grows in association with *Stellarietea mediae* – annual weed communities species (Šilc and Košir 2006). In Sweden, *L. serriola* populations are found in southeastern areas, and mostly grow on surfaces and among stones in dry and sunny exposures (Doležalová et al. 2001).

The genetic structure of populations represented by prickly lettuce plants growing at a specific time in a particular site could emerge in at least four different ways: i) achenes can survive in a soil seed bank for 1 to 3 years (Marks and Prince 1982); at the moment of soil disturbance, the seeds can germinate, and these plants bear/represent "old" genotypes for a given population; ii) plants can grow from achenes newly transported to a particular locality by wind, humans, or other transport mechanisms, with such plants bearing "new" genotypes; iii) plants can grow on permanently disturbed soil from generation to generation, and such plants represent a "modified" genotype resulting from continuous evolution under local conditions; iv) "hybrid" plants may appear after natural hybridization between different plant species within the genus *Lactuca*.

The main purpose of this research was to describe the differences in genetic variability and population genetic structures between populations of prickly lettuce (*Lactuca serriola*) coming from two different and distant biogeographic areas of the species' distribution in Europe.

Materials and methods

Plant materials

A set of 121 samples of *L. serriola* L. plants, representing 53 populations, was collected by the authors in Sweden (47 samples) and Slovenia (74 samples) during 2000 (Doležalová et al. 2001). The collected seed samples were regenerated in a greenhouse at the Department of Botany (Palacký University in Olomouc, Czech Republic). During regeneration, the plants were described morphologically according to Doležalová et al. (2002), and the taxonomic status of each sample was verified (Feráková 1977, Doležalová et al. 2002). From each plant two mature leaves were used for DNA extraction (i.e., 121 samples). Data from the individual samples are provided in On-line Suppl. Tab. 1., with the geographic positions of the collection sites given in Fig. 1.

DNA extraction, SSR, and AFLP analyses

Total genomic DNA was extracted from 100 mg of fresh leaf tissue using the CTAB method (Kump and Javornik 1996), with minor modifications. After DNA extraction, the

Tab. 1. Microsatellite (SSR) loci used to assess genetic variability in *Lactuca sativa* L. and *L. serriola* L.; N_A – number of alleles; PIC – allelic polymorphic information content.

Marker	Reference	N_A	Allele size (bp)	PIC (%)
SML-002	Simko (2009)	6	168-207	0.594
SML-019	Simko (2009)	2	163-164	0.599
SML-045	Simko (2009)	4	229-238	0.838
SML-055	Simko (2009)	5	221-240	1.072
WSULs-18	Riar et al. (2011)	4	208-235	0.494
WSULs-75	Riar et al. (2011)	4	161-206	0.684
WSULs-163	Riar et al. (2011)	7	183-197	1.052

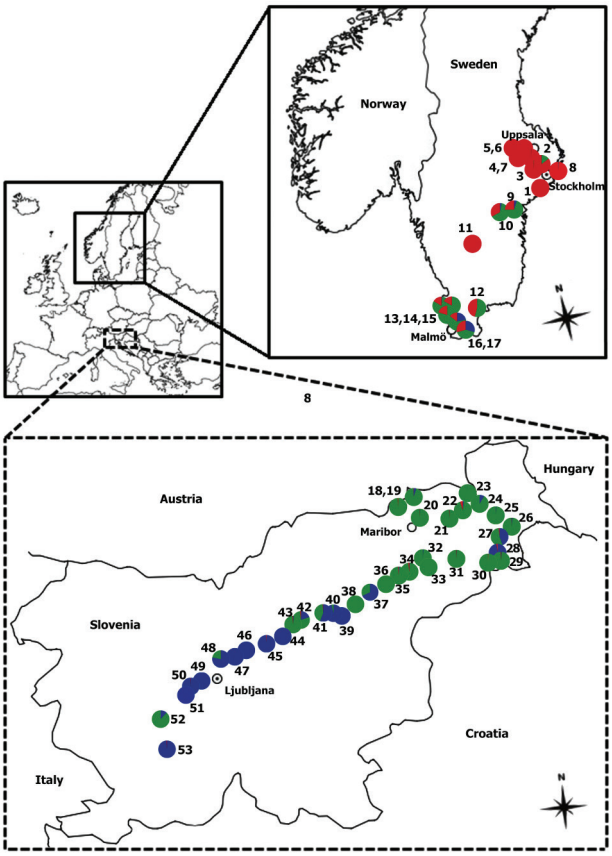

Fig. 1. Collecting sites of the 121 samples *Lactuca serriola* in Sweden and Slovenia. Colors of spots correspond to the results of Bayesian clustering presented in Fig. 2.

quality of the DNA was inspected by 1.5% agarose gel electrophoresis, and the concentration measured on a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Delaware, USA).

For microsatellite genotyping, seven SSR loci were used: SML-002, SML-019, SML-045, SML-055 (Simko 2009), as well as WSULs-18, WSULs-75, and WSULs-163 (Riar et al. 2011). The primer pairs were selected according to their high diversity indices in previously published papers (Simko 2009, Riar et al. 2011); however, randomly without any previous knowledge of their chromosome positions. Amplification of the SSRs was performed according to Jemelková et al. (2015). The length of the SSR alleles was scored based on their migration relative to the molecular weight size markers 30-330bp AFLP® DNA ladder (Invitrogen, Carlsbad, California, USA). The AFLP analyses were carried out according to the protocol of Vos et al. (1995), with modifications, and the AFLP fragment detection according to Kitner et al. (2008, 2012). Five selective primer combinations, with two to three selective nucleotides, were chosen to generate the AFLP profiles (Tab. 2).

The PCR products were separating on a 6%, 0.4 mm thick denaturating polyacrylamide gel using a T-REX sequencing gel electrophoresis apparatus (Thermo Scientific Owl Separation Systems, Rochester, NY, USA).

Tab. 2. Amplified fragment length polymorphism (AFLP) primer sets for amplification reactions with the total number of scored and polymorphic fragments in the *Lactuca serriola* samples; N_F – total number of fragments; N_{POL} – number of polymorphic fragments; P(%) – percentage of polymorphic fragments.

Primer combination	N_F	N_{POL}	P(%)
E - AGC, M - CTG	45	37	82.2
E - AGC, M - CAAC	49	36	73.5
E - AGC, M - CAAT	72	54	75.0
E - ACC, M - CAAC	43	35	81.4
E - ACC, M - CAAT	48	30	62.5
Total	257	192	
Mean	51.4	38.4	74.9

Data scoring

Microsatellite profiles were scored based on the length of the PCR product. The allele frequencies, percentage of polymorphic loci (P%), number of private alleles (PA), observed and expected heterozygosity (H_O and H_E) were all performed using GenAlEx 6 software (Peakall and Smouse 2012). The mean number of alleles per locus (A) was calculated manually. The relative discriminatory value of each microsatellite locus was estimated by the polymorphic information content (PIC), which measures the information content as a function of a marker system´s ability to distinguish between genotypes (Powell et al. 1996). The number of different genotypes (N_G), number of samples with a heterozygous constitution (N_{HET}), and maximal number of heterozygous loci (N_{HETmax}) were calculated manually.

AFLP profiles were checked visually, and only clear and unambiguous bands were scored for their presence (1) or absence (0) across all samples. For AFLP data, the number of private bands (PA), the proportion of polymorphic loci (P%) and gene diversity (H_E) were calculated using GenAlEx 6 software (Peakall and Smouse 2012).

To evaluate the population genetic structure, a Bayesian clustering approach was used as implemented in Structure

2.3.4 (Falush et al. 2007). Structure attempts to assign individuals to clusters/groups/populations on the basis of their genotypes, while simultaneously estimating population allele frequencies. This allows one to compute the likelihood of a given genotype having originated in a predefined number (K) of clusters. In the simplest, 'no-admixture' model, it assumes that each individual belongs to a single cluster. In the more general 'admixture model' it estimates admixture proportions for each individual, allowing one to identify admixed individuals represented by a proportional mixture of two or more signals characteristic for the various clusters. In our analyses, SSR co-dominant data were transferred into binary data based on the presence/absence of a particular allele, and merged with the AFLP binary data; the samples were then ordered according to the increasing latitude of the sampling site within a particular country. An admixture model was used, with correlated allele frequencies. K was set at 1–10, and the highest K value was identified as the run with the highest likelihood value, as recommended by Pritchard et al. (2000). In addition, K values were averaged across 10 replicate runs for each K (100 000 burn-in iteration followed by 1 000 000 MCMC iterations). For the graphical interpretation of clustering for the appropriate K, Structure Harvester (Earl and von Holdt 2012), Clumpp (Jacobsson and Rosenberg 2007), and Distruct (Rosenberg 2004) software packages were used. The optimal K value was selected according to Evanno et al. (2005), who suggested the use of the ΔK value for identifying the correct number of clusters.

To visualize the genetic relationships within and among the analyzed samples, a Neighbor-Network based on Dice´s similarity coefficient (D) was constructed in SplitsTree 4 (Huson and Bryant 2006). The Nexus input file for SplitsTree 4 was exported from GenAlEx. Also, for this purpose, the SSR data were transformed into a binary matrix and merged with the AFLP binary data. The reliability and robustness of the network were tested by bootstrap analysis with 1.000 bootstrap replicates.

Results

Taxonomic verification of *L. serriola*

For all 121 plants, the taxonomic status of *Lactuca serriola* f. *serriola* according to Feráková (1977) was confirmed. Moreover, in one sample (no. 205_00, Bostahusen, Sweden) the plants were morphologically heterogeneous; with divided stem leaves belonging to *L. serriola* f. *serriola*, plants with entire stem leaves that ranged toward *L. serriola* f. *integrifolia*. In our analyses, this sample was split into two subsamples 205_00A (f. *serriola*) and 205_00B (f. *integrifolia*) and treated (analyzed) separately.

Genetic polymorphism

The seven polymorphic SSR loci produced a total of 32 alleles across the 121 individual *L. serriola* plants. The number of alleles per locus ranged from 2 to 7, with an average of 4.57 alleles per locus (Tab. 1). The allele sizes varied from 161 to 240 bp. The mean PIC per SSR polymorphic allele was 0.762, within a range of 0.494 to 1.072. Null alleles only appeared in two accessions from Slovenia (13_00 and 22_00) at the locus SML-055.

Private alleles (PA) were present within both sampled regions (Tab. 3). The *L. serriola* samples from Sweden possessed 5 unique alleles: 193 bp, 204 bp, and 207 bp for locus SML-002, 221 bp for locus SML-055 (i.e., 221 $bp^{SML-055}$), and 188 $bp^{WSULs-163}$. The samples from Slovenia possessed eight unique alleles: 172 bp, 198 bp for locus SML-002, 238 $bp^{SML-045}$, 228 $bp^{SML-055}$; 217 bp and 235 bp for locus WSULs-18, and lastly 183 bp and 195 bp for locus WSULs-163.

The observed and expected heterozygosity (H_O and H_E) ranged from 0.036 to 0.054 (mean 0.045), and from 0.341 to 0.432 (mean 0.387), respectively. The proportion of polymorphic loci (P%) was higher in the Slovenian (84.4%) than in the Swedish samples (75%). Based on SSR data, in all, 51 different genotypes (N_G) were recognized (Sweden = 17; Slovenia = 34) (On-line Suppl. Tabs. 2,3). Genotype G3 was the most common in the samples from Sweden (36.2%), while genotype G29 represented 32.4% of the Slovenian samples (On-line Suppl. Tabs. 2,3). We recorded 17 Slovenian samples that had at least one heterozygous locus (N_{HET} = 17), in contrast to eight samples from Sweden (On-line Suppl. Tabs. 2,3). Three samples from Slovenia and one sample from Sweden bore the maximum number of heterozygous loci (N_{HETmax} = 3) observed from among all analyzed samples.

In total, five primer combinations, with two to three selective bases, were applied for AFLP genotyping (Tab. 2), resulting in 257 unambiguously scored fragments. Detailed overall statistics calculated for each primer combination used are presented in Table 2. The number of private bands (PA) ranged from 19 (Slovenian samples) to 20 (Swedish samples). The expected heterozygosity (H_E) ranged from 0.130 to 0.149 (mean H_E = 0.140) (Tab. 3), and the proportion of polymorphic loci (P%) in the *L. serriola* samples ranged from 44.8% (Swedish population) to 52.9% (Slovenian population). The genetic variability indices for all populations are summarized in Table 3.

Cluster analysis of molecular data

Based on seven microsatellite and 257 AFLP markers, Bayesian clustering and construction of a Neighbor-Network were used for visualization of the putative relationships among the analyzed individuals. Under the Bayesian approach implemented in Structure, the best partition into three clusters (K = 3, Fig. 2) was resolved (ΔK = 214.73; St. dev. LnP(K) = 6.07); they are represented by the green (G-cluster), red (R-cluster), and blue (B-cluster) color signals in Figure 2. In general, a relatively low admixture was detected in the Slovenian samples, which were clearly identified as genotypes from the G- or B-cluster. While the B-cluster can be considered as characteristic for *L. serriola* genotypes

Fig. 2. Results of Bayesian clustering based on the microsatellite (SSR) and amplified fragment length polymorphism (AFLP) data of 121 *Lactuca serriola* samples from Sweden (SWE) and Slovenia (SLO), ordered according to the increasing latitude of the sampling site within a specific country. Each individual is represented by a horizontal line partitioned into segments of different color, the lengths of which indicate the posterior probability of membership in each group as identified by Structure.

from the southern part of Central Europe and the northern Balkans (representing ca. 1/3 of the Slovenian samples), the G-cluster represents the genotype largely dispersed across Europe, contributing significantly to the genotypic composition of the Swedish populations. The signal characteristic for genotypes from the R-cluster was nearly absent in the Slovenian samples, but was recorded in each sample from Sweden; and 48.9% of the Swedish samples fell into the R-cluster with no admixture signal (Fig. 2). For 19 samples, the signal from the R-cluster contributes up to 30% of a particular genotype, and is accompanied with an admixture of the G signal, which prevails in the Slovenian samples (Fig. 2). Further, we observed a nearly equal admixture of signals from all three clusters in five samples collected in southern Sweden near Malmö.

The Neighbor-Network analysis divided the analyzed samples into 6 groups (A-F; Fig. 3), each consisting of samples coming from a separate country. The results fit the results of the Bayesian clustering in terms of assigning individuals from a separate country to the revealed clusters (R-, G-, B-). The samples from Sweden were placed into the A, C, and D groups. While individuals placed in Group C represent the genotype from the R-cluster, Group D is formed by samples with the G-cluster prevailing. Finally, Group A is formed by five samples 215_00, 217_00, 218_00, 219_00, and 220_00, having a strong admixture signal from all three Structure clusters. These samples represent populations no. 16 and 17 from collecting sites close to Malmö (On-line Suppl. Tab. 1). The samples from Slovenia were split into three groups: a majority of the samples fell in groups B and E, both representing the G-cluster in Fig. 2. Samples originating from Slovenian localities below 46°14'34" lat. fell into a separate Group F, which represents genotypes from a unique B-cluster (Fig. 2). It is interesting, that all three

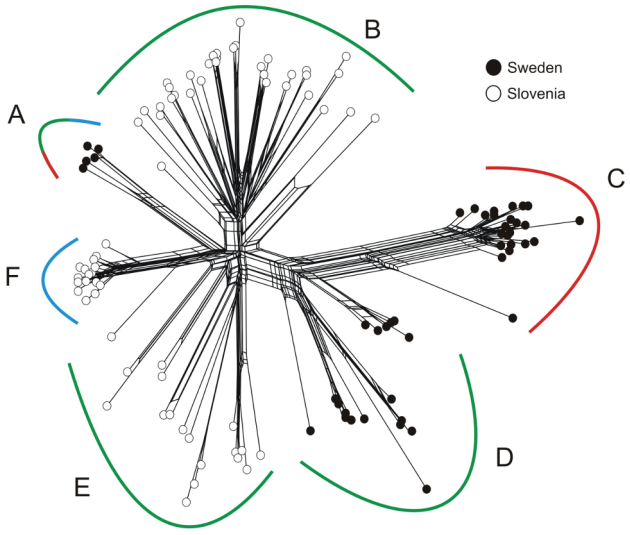

Fig. 3. Neighbor-Network cluster analysis of 121 samples *Lactuca serriola* from Sweden and Slovenia, based on SSR and AFLP analysis. Resulting groups are highlighted by coloring that corresponds to the results of Bayesian clustering presented in Fig. 2.

Tab. 3. Summary data based on 7 SSR and 257 amplified fragment length polymorphism (AFLP) loci of 121 *Lactuca serriola* samples from Sweden and Slovenia in recent study: N – sample size; PA_{SSR} – private microsatellite alleles; PA_{AFLP} – private AFLP bands; A – mean number of alleles per locus; P(%) – percentage of polymorphic loci; observed H_o and expected H_e heterozygosity; SE – standard error.

Country	N	Microsatellite (SSR) data					AFLP data		
		PA_{SSR}	A	P(%)	H_O	$H_E \pm SE$	PA_{AFLP}	P(%)	$H_E \pm SE$
Sweden	47	5	3.42	75.0	0.036	0.341 ± 0.065	20	44.8	0.130 ± 0.011
Slovenia	74	8	3.86	84.4	0.054	0.432 ± 0.049	19	52.9	0.149 ± 0.011

"G-cluster" groups from both countries are in the center of the Neighbor-Network, which resemble their characteristics closely. On the other hand, Group C (SWE, R-cluster) and Group F (SLO, B-cluster) are placed on opposite sides of the network.

Discussion

Verification of the taxonomic status of the plants showed that *Lactuca serriola* f. *serriola* is predominant in both countries. In the entire territory of Slovenia only *L. serriola* f. *serriola* was recorded, which is in agreement with previous observations in Central Europe (Lebeda et al. 2001, 2004, 2007b). Within one sample from southern Sweden (Bostahusen, sample 205_00), apart from *L. serriola* f. *serriola* plants, there were plants identified as *L. serriola* f. *integrifolia*. All remaining samples from Sweden were represented only by *L. serriola* f. *serriola*. It is evident that both populations are very taxonomically homogeneous on the subspecific level. The very rare occurrence of *L. serriola* f. *integrifolia* in southern Sweden could be caused by the repeated introduction (e.g., through truck or ship transportation) of this form from the Netherlands or UK, where it is prevalent (Lebeda et al. 2007a, b). However, from our previous results (Doležalová et al. 2001) it is evident, that this variety is not spreading into northern Scandinavia, where the northern limit of the European distribution for this species is (Feráková 1977). These conclusions are supported by recent observations in Sweden made by Rydberg (2013). Also, in Norway only *L. serriola* f. *serriola* has been recorded (Lebeda 2013, unpubl. results).

The leaf shape (i.e., the division of the leaf blade), can be interpreted as an ecological adaptation of the plant to different factors, including a means of leaf thermoregulation in arid or hot environments, or in reaction to hydraulic constraints (Nicotra et al. 2011). Doležalová et al. (2009) also confirmed the differences in the morphology of rosette and cauline leaves of Swedish and Slovenian *L. serriola* samples. The cauline leaves of Swedish *L. serriola* plants were longer and wider; plants from Slovenia had longer and narrower rosette leaves (divided) (Doležalová et al. 2009). The width and length of cauline leaves (divided) correlate with the latitude, which could be explained as adaptations of the plants to drought. Drier areas of lower latitudes are increasingly represented by plants with smaller leaves. Regarding altitude, a negative correlation with the length and width of the leaves was found (Doležalová et al. 2009), which could mean they are adapting to ecologically worse conditions at higher elevations. The occurrence of *L. serriola* f. *integrifolia* in temperate areas without a dry season (but with a warm summer) in the UK, western part of Germany, Benelux, and France (Peel et al. 2007) supports the theory of the ecological adaptation of leaves presented by Nicotra et al. (2011). The areas in Sweden where lettuce samples were collected belong to the cold climate type, without a dry season or warm summer (Peel et al. 2007). Similarly significant differences in morphological parameters of achenes of *L. serriola* from Slovenia and Sweden were found between populations within countries and between samples within population (Křístková et al. 2014).

The higher phenotypic and genetic variability of the Slovenian samples can be explained by the more favorable climatic and ecological conditions in the country (see Peel et al. 2007). *L. serriola* is distributed throughout the entire country, and movement of diaspores among the surrounding countries is feasible (Lebeda et al. 2004). This is in opposition to Sweden, where the distribution is limited to the southern part (Doležalová et al. 2001), with very limited migration from the surrounding countries. In general, plant species occurring almost in and/or near the center of their diversity, with suitable environmental and ecological conditions, display more genetic/phenotypic variability. Conversely, at the edge of the distribution area, where less favorable conditions exist, the selection prioritizes stable and well-adapted genotypes. Our results on genetic variability are in relationship to the general principles of diversity and allele distribution formulated by Vavilov (1950). Kuang et al. (2008) suggested that eastern Turkey and Armenia, along with the surrounding regions, might be the center of diversity of *L. serriola* (and possibly its center of origin). *L. serriola* might have spread from its center of origin first to the Mediterranean basin and then to Central and Western Europe after the glaciers retreated in the Upper-Pleistocene / Holocene period (Kuang et al. 2008). Recent climatic changes and anthropogenic disturbances contributed substantially to the rapid spread of *L. serriola* into new areas (D´Andrea et al. 2009, Rydberg 2013), as well as increasing the genetic diversity of their populations in the central parts of their natural distribution areas (Lebeda et al. 2009a, van de Wiel et al. 2010, Kitner et al. 2015). This phenomenon

was also clearly demonstrated in the genetic diversity of the Central European population of *L. serriola* (van de Wiel et al. 2010), as well as the resistance of the same population to *Bremia lactucae*. While the Czech Republic has the greatest diversity of resistance phenotypes, the lowest was recorded in the UK (Lebeda et al. 2008, Petrželová and Lebeda 2011).

The results of our study on genetic variability are in good agreement with the different climatic conditions in Sweden and Slovenia. From the viewpoint of genetic variation, the results have proven the existence of *L. serriola* genotypes characteristic for each country. These clearly differ from one another, as is evident from Bayesian clustering and Neighbor-Network analysis, where the R-cluster characteristic for the Swedish samples (Group C), and the B-cluster (Group F) unique for Slovenian samples were distinguished (Figs. 2, 3). A number of samples from both countries were characterized by genotypes characteristic for the G-cluster, which might represent a common genotype resulting from the rapid spread of *L. serriola* in Central Europe (Lebeda et al. 2001, 2007b, D´Andrea et al. 2009). We have not recorded a prevailing microsatellite genotype for the samples representing this G-cluster, and no linkage to the latitude or altitude of the sampled sites. The same phenomenon was described by Lebeda et al. (2009a), demonstrating that some *L. serriola* populations (e.g., Scandinavian, British, some Mediterranean) are quite isolated genetically from the heterogeneous Central and West European populations. Genetic analysis (PCR-RFLP and SSR markers) on 101 populations of *L. serriola* from seventeen countries of Western and Central Europe made by D´Andrea et al. (2017) revealed a strong genetic differentiation between populations, and high inbreeding coefficients within populations. A clear geographical pattern of isolation by increasing distance was found; however, only a weak pattern of correlation between genetic diversity and geographical distance was found on the continental scale. The greatest amount of genetic diversity was characterized in Central Europe, while populations from the western Mediterranean (Spain and Portugal), southern Italy, Great Britain, the Alps, and southern Scandinavia generally possessed lower gene diversities (D´Andrea et al. 2017). Discrepancies were present in Scandinavia with some polymorphic populations, and a monomorphic one. Further, in a recent study, higher genetic variability in the Slovenian samples was observed in terms of the recorded genetic variability indices (Tab. 3) and the higher number of SSR genotypes ($^{SWE}N_G = 17/^{SLO}N_G = 34$) (On-line Suppl. Tabs. 2,3). The level of genetic variation within and between populations can also result from intraspecific crossing. Although autogamy is the predominant breeding system within the genus *Lactuca* L., especially in the marginal parts of the distribution area (Feráková 1977); in the center of the distribution, a higher occurrence of allogamy was estimated (Stebbins 1957). Lindquist (1960) proved experimentally that all species belonging to the "serriola" group were self-fertile. *L. serriola* is primarily a self-pollinated species; however, not only intermediate plants between the two *L. serriola* forms, but also interspecific hybrids of *L. serriola* can be detected in natural populations (Zohary 1990, Křístková et al. 2012). The main differences between the samples from Sweden and Slovenia can be characterized by the presence of genotypes characteristic for the R- or B-cluster, determined by Bayesian clustering (Fig. 2), each unique (with a few exceptions) to a given country. The signal from the R-cluster was present in all Swedish samples and prevails in 48.9% of them. These samples formed Group C on the Neighbor-Network (Fig. 3), 65.2% of them represent the SSR genotype G3, with a completely homozygous character at all loci, and originating from localities at a higher latitude (On-line Suppl. Tab. 2).

A rather interesting characteristic of five *L. serriola* samples was found in a group of plants collected near Malmö. These samples forming Group A on the Neighbor-Network, are represented by a significant admixture signal on the Bayesian diagram, and also bore unique morphological features of their rosette leaves. The apical parts of the rosette leaves in samples 215_00, 217_00, 218_00, 219_00, and 220_00 were not divided, forming a long apex; the remaining two-thirds of the leaves were slightly divided (pinnately lobed). Surprisingly, specific DNA patterns fit better to specific phenotypes of the rosette leaves than to phenotypes of the cauline leaves. This is in contrast to the generally accepted view that morphological traits of the cauline leaves have a more significant taxonomic value than do the rosette leaves. The city of Malmö is an international harbor in the region, and it is possible to explain the exceptional phenotypic characteristics of these samples by the human-moderated introduction of non-indigenous genotypes into the southern parts of northern Europe, with subsequent natural hybridization with indigenous *L. serriola* genotypes. The B-cluster in Slovenian samples showed, with a few exceptions, a continuity with samples from a lower latitude; 96% of these samples are represented by the completely homozygous microsatellite genotype G29 (On-line Suppl. Tab. 3).

This study provides interesting insights into the genetic variability of *L. serriola* populations originating from completely different eco-geographical areas. Specifically those from Slovenia, near the Mediterranean, a world diversity hotspot (Myers et al. 2000), the center of the greatest diversity of the genus *Lactuca* (Lebeda et al. 2009b); additionally, those from Sweden, a region at the northern border of *L. serriola* European distribution (Feráková 1977, Lebeda et al. 2004). This study showed that *L. serriola* populations originating from various eco-geographical conditions differ significantly in their genetic background, which is also reflected in the geographic patterns of their phenotypic features. To obtain more comprehensive information on the genetic structure and variations of this species, it would be interesting to analyze: i) more populations with more individuals from Sweden, and for a comparative study ii) additional samples originating from areas with greater contrasting ecological conditions.

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