INTRODUCTION

Phylogeographic studies performed on the European high mountain flora provided evidences for the Asian origin of many species that evolved and diversified on the highlands of the Qinghai-Tibetian Plateau (QTP) and adjacent areas from where they have colonized the Northern Hemisphere (NH). Species’ migration route from the QTP toward Europe reaching the European Alpine System (EAS, the biogeographic region covering the Pyrenees, the Alps, the Carpathians and the Northern Balkans) and the nordic subarctic, arctic areas was proposed to follow the so-called Central Asiatic highland corridor with the subsequently intervening high mountain ranges between Asia and Europe (Kadereit, Licht, & Uhink, 2008; Ohba, 1989). However, an expansion toward the northern Siberian region from the QTP was also mentioned for some species like Saxifraga oppositifolia (Abbott, Smith, & Milne, 2000) that by reaching Siberia and the Taymyr area—which might also served as a refugial territory—followed both an eastward and a westward migration route. Northern glacial survival and extensive postglacial migration in the Arctic region was also reported in Vaccinium uliginosum (Alsos, 1989).
Engelskjaer, Gielly, Taberlet, & Brochmann, 2005). Moreover, some Scandinavian populations of Dryas octopetala provided evidence for the existence of a contact zone in the area where the colonizing European lineage most probably was intermingled with immigrants of the Northeast lineage (Alsos et al., 2007; Skrede, Eidesen, Pinheiro Portela, & Brochmann, 2006). The complex biogeographic connection of the QTP and the NH involves also intercontinental disjunction reaching North America, which was demonstrated in some herbaceous species inhabiting the arctic regions and high mountain elevations (Alsos et al., 2015). The radiation toward the new habitats, the evolution of genetic lineages and the different migration and colonization potential of species in response to the historical climatic oscillations and abiotic changes provided a complex biogeographic pattern in most taxa originating from the QTP (Zhang, Meng, Allen, Wen, & Rao, 2014). Moreover, studies on population level of single species across the entire range provided further insights into species’ phylogeography and evolution in relatively recent historical times involving the climate oscillations of the Quaternary (Christe et al., 2014; Schönswetter, Stehlik, Holderegger, & Tribsch, 2005; Taberlet, Fumagalli, & Wust-Saucy, 1998).

The number of species in the Rhodiola genus varies according to different authors between 60 (Ohba, 2005) to 200 (Germano & Ramazonov, 1999). This genus (fam. Crassulaceae) is one of the most studied plant genera of QTP origin (Mayuzumi & Ohba, 2004; Zhang et al., 2014). Phylogenetic studies have revealed a relatively rapid diversification and radiation with high species diversity on the QTP and adjacent regions. Only five species are distributed in northeast Asia and three species of Rhodiola exhibit intercontinental distribution. These are Rhodiola integrifolia Raf. that occurs on both sides of the Bering Strait, Rhodiola rhodantha (A. Gray) H. Jacobsen that is distributed only in the western part of North America (Ohba, 1989) and R. rosea. L. with the broadest range among all Rhodiola species, ranging from East Asia, toward the Arctic regions, Europe as well as eastern North America. In Europe R. rosea is distributed in Iceland, the British Isles, the European Alpine System, and Scandinavia (Hegi, 1963). It is a dioecious, cold-adapted perennial, occupying a narrow range of the arctic-alpine habitats (Figure 1). In eastern North America, it is restricted to the eastern coastal areas (Cuerrier, Archambault, Rapinski, & Bruneau, 2015; Ohba, 1989; Olfelt & Freyman, 2014).

Due to its extremely high morphological variability as described by Ohba (1981), there are several taxonomic interpretations within R. rosea. Recently, the Plant List reincudes this taxa in the genus Sedum, the valid name considered to be Sedum roseum (L.) Scop. (Plant List https://www.theplantlist.org). The Flora of China differentiates two varieties, R. rosea var. rosea with bigger leaves, longer flowering stem and almost entire leaf margin while R. rosea var. microphylla has smaller leaves, shorter flowering stem and the leaves are serrate (Fu & Ohba, 2001). Gontcharova, Gontcharov, Yakubov, and Kondo (2009) recognize three subspecies: R. rosea subsp. rosea, R. rosea subsp. Arctica, and R. rosea subsp. sachalinensis based on seed surface morphology. Yanbaev, Baimagulov, Redkina, and Mullagulov (2007) reported Rhodiola iremelfica Boriss. as an endemic species to the southern Urals, but based on molecular evidences, using the internal transcribed spacer György, Szabó, Bacharov, and Pedryc (2012) could not differentiate this species proposing this taxa only at subspecies rank of R. rosea. Several other molecular studies also demonstrate the high genetic variability within this taxon (Elameen, Klemsdal, Dragland, Fjellheim, & Rognli, 2008; György, Fjelldal, Szabo, Ashholm, & Pedryc, 2013; György, Vouillamoz, Ladányi, & Pedryc, 2014; Kozyrenko, Gontcharova, & Gontcharov, 2011).

Zhang et al. (2014) stated that Rhodiola species of disjunct intercontinental distribution have reached North America at least twice, via Beringia and via the amphi-atlantic route. Latter route seems to fit well for R. rosea with its extremely small, winged seeds compared to other species. This particular seed morphology makes R. rosea a candidate species for long-distance dispersal that most probably dates to the period when the NALB (Northern Atlantic Land Bridge) was not available anymore for species’ migration.

Molecular study applied on the European populations of R. rosea by microsatellite markers has revealed distinct clusters of populations within the EAS and suggested at least two glacial refugia for the species, one in the area of the Eastern Alps and the Carpathians and another one in the Dolomites (György, Vouillamoz, & Höhn, 2016).

With its wide and disjunct circumboreal distribution and high genetic variation, R. rosea seems to be a model plant species to study spatial diversification and migration from the QTP toward the Northern Hemisphere. Kozyrenko et al. (2011) based on ISSR markers, have come to the conclusion that at least two distinct evolutionary-ary lines exist within R. rosea. The species migrated from its center of origin westward along the Ural Mts. and eastward by the mountain ridges of eastern Siberia. In a recent study on the chloroplast trn L-F region, Cuerrier et al. (2015) reported the existence of two inraspecific variants. Coastal and Alpine populations were found to differ in sharing two indels (duplication of 23 and 19 bp) suggesting for coastal population a common North American and Scandinavian origin, while the Alpine populations seem to have originated from Eurasia via the Central Asian highland corridor.
Accordingly, we assumed that along the westward expansion of the species, R. rosea has diversified within the area of Europe (EAS), which resulted in the two infraspecific variations observed by Cuerrier et al. (2015). These genetic lineages potentially contributed to the colonization of the Northern American continent. In the present study, chloroplast sequence data were used to gain further insights into the species’ genetic diversity along its distribution range, especially in Europe and by using a phylogeographic approach to propose a historical biogeographic scenario for the present-day distribution of the species, focusing on the genetic lineages and evolution within the EAS.

2 | MATERIALS AND METHODS

2.1 | Sampling material

Altogether 44 populations of R. rosea were sampled, which are listed in Table 1. Mostly leaves were collected and stored at -20°C. In some cases, seeds were obtained from Pharmaplast Ltd., germinated, and the plantlets were used as source of DNA. Vouchers of the samples were deposited at Szent István University. DNA was extracted with SP Plant Mini Kit (Omega, VWR International Kft, Budapest). DNA concentration and quality was assessed using NanoDrop (BioScience, Hungary) and visually checked on 1% agarose gel.

2.2 | PCR amplification of the studied chloroplast regions

After preliminary screening of twelve regions of the chloroplast genome, the following six regions proved to amplify satisfactorily: trnL-trnF, psbA-3′trnK-matK, psbA-trnH, psbB-psbH, 5′ps12-rpL,20, and trnCycF6 (Shaw et al., 2005).

Amplifications were performed in 25 µl reaction volume containing 20–80 ng DNA, 10× PCR reaction buffer, 2.5 mM MgCl₂, 0.02 mM dNTP mix, 2.5 µmol of each 5′ and 3′ end primers, 1 unit of DreamTaq DNA polymerase (Fermentas, Waltham, MA, USA), 2% BSA and 1% DMSO and sterile distilled water. PCR was carried out in a Swift MaxPro thermocycler (Esco Healthcare Pte, Singapore) as described by Taberlet, Gielly, Pautou, and Bouvet (1991), annealing temperature of each primer pair is shown in Table 2. The PCR products were loaded on a 1% (w/v) ethidium bromide-stained agarose gel in 1TBE buffer with xylene cyanol loading buffer to verify the amplification. Fragment sizes were estimated by comparison with the 1 kb DNA ladder (Fermentas, Kft, Budapest). DNA concentration and quality was assessed using NanoDrop (BioScience, Hungary) and visually checked on 1% agarose gel.

with the EZ-10 Spin Column Plasmid DNA Minipreps Kit (Bio Basic Canada Inc., Markham, ON, Canada). Sequencing was performed in an automated sequencer ABI PRISM 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). For each fragment, the nucleotide sequences were determined in both directions. Forward and reverse sequences were edited and assembled using MEGA7 (Kumar, Stecher, & Tamura, 2016). DNA sequences were compared using BLASTN at NCBI and alignments were built with ClustalW in Bioedit 7.2.5 (Hall, 1999; Thomson, Higgins, & Gibson, 1994). All sequences have been deposited in GenBank (KX078522–64, KX611154, and MG938064–938283).

In some samples, not one, but two, (Triglav, Passo Gavia, Val Fredda, Zirbitzkogel, Prábičh) or even three fragments (Hochkar) were detected on the agarose gel in case of the trnL-F locus. After cloning and sequencing, these fragments all turned out to represent the same sequence. Duplication has happened in the primer binding site and due to this one (or two) of the fragments was an artifact.

2.3 | Diversity and differentiation analysis

Genetic diversity for each loci, that is, the number of haplotypes (h), haplotype diversity (Hd) (Nei & Tajima, 1981), and nucleotide diversity (p) (Nei, 1987) were calculated using the software DnaSP v5.1.0.1 (Librado & Rozas, 2009). Genetic distances (p-uncorrected) within and between lineages were calculated with MEGA7 (Kumar et al., 2016). Polymorphic information content (PIC) values were calculated for each chloroplast locus based on the formula: PIC = 1 − ∑(Pj)^2, where Pj is the frequency of the jth allele revealed by the jth marker summed across all alleles amplified (Botstein, White, Skolnick, & Davis, 1980). The marker is highly informative if PIC value is >0.5 but only reasonably informative if PIC value is ranging from 0.25 to 0.5.

PopART (Leigh & Bryant, 2015) with implemented Templeton-Crandall-Singh (TCS) statistical parsimony network analysis (Clement, Snell, Walker, Posada, & Crandall, 2002) was used to evaluate genealogical relationships among sequences. Each insertion/deletion (indel) was considered as a single mutation event, and all indels were therefore coded as single positions in the final alignments. The connection limit for the TCS analysis was 95% and gaps were treated as a fifth state.

The Bayesian clustering approach implemented in STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) was used to infer groups or subpopulations in the sequence dataset. The analysis was performed with an admixture linkage model with correlated allele frequencies. K value (the number of genetic groups) was set to 1–10 with a burn-in period of 105 steps followed by 106 repetitions of Markov chain Monte Carlo (MCMC), which is capable to deal with linked markers. Twenty repetitions were set for each run. The web-based STRUCTURE HARVESTER (Earl & von Holdt, 2012) was used to apply the Evanno method (Evanno, Regnaut, & Goudet, 2005) to detect the value of the optimal K that best fit the sequence data. The 20 simulations were averaged using CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007) and represented in the form of bar graphs using POPHELPER (Francis, 2017).
| Site                  | Country          | Origin or GPS coordinates                      | GenBank accession nr. | GenBank accession nr. | GenBank accession nr. | GenBank accession nr. | GenBank accession nr. | GenBank accession nr. | GenBank accession nr. | GenBank accession nr. | GenBank accession nr. | GenBank accession nr. | GenBank accession nr. |
|----------------------|------------------|------------------------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Akureyri             | Iceland          | Botanical garden of Univ. of Oulu               | KX07852               | MG98169               | MG98209               | MG98222               | MG98233               | MG98234               | MG98256               | MG98283               | MG98302               | MG98311               | MG98318               |
| Kilpisjarvi          | Finland          | Bertalan Galambosi, MTT, Finland                 | KX07853               | MG98171               | MG98211               | MG98224               | MG98235               | MG98236               | MG98258               | MG98285               | MG98304               | MG98313               | MG98320               |
| Halti                | Finland          | Bertalan Galambosi, MTT, Finland                 | KX07854               | MG98173               | MG98213               | MG98225               | MG98236               | MG98237               | MG98259               | MG98286               | MG98305               | MG98314               | MG98321               |
| Varanger fjord       | Norway           | Paul Erik Aspholm, Bioforsk, Norway              | KX07855               | MG98175               | MG98215               | MG98227               | MG98238               | MG98239               | MG98261               | MG98288               | MG98306               | MG98315               | MG98322               |
| Kvaloya              | Norway           | Paul Erik Aspholm, Bioforsk, Norway              | KX07856               | MG98177               | MG98217               | MG98229               | MG98240               | MG98241               | MG98262               | MG98289               | MG98307               | MG98316               | MG98323               |
| Sligo                | Ireland          | Andreas Plescher, Pharmaplant, Germany           | KX07857               | MG98179               | MG98219               | MG98231               | MG98242               | MG98243               | MG98263               | MG98290               | MG98308               | MG98317               | MG98324               |
| Kola peninsula       | Russia           | Dmitry Bacherov, Komi Science Center.           | KX07858               | MG98201               | MG98223               | MG98235               | MG98234               | MG98236               | MG98258               | MG98282               | MG98300               | MG98315               | MG98321               |
| Cadar Urs           | Russia           | Russian Botanical garden of Univ. of Oulu       | KX07859               | MG98181               | MG98221               | MG98233               | MG98244               | MG98245               | MG98265               | MG98291               | MG98309               | MG98318               | MG98324               |
| Tskoks peninsula     | Russia           | Russian Botanical garden of Univ. of Oulu       | KX07860               | MG98183               | MG98223               | MG98235               | MG98234               | MG98236               | MG98258               | MG98282               | MG98300               | MG98315               | MG98321               |
| North-Ural          | Russia           | Russian Botanical garden of Univ. of Oulu       | KX07861               | MG98185               | MG98225               | MG98237               | MG98236               | MG98238               | MG98260               | MG98284               | MG98302               | MG98317               | MG98323               |
| Yakutia              | Russia           | Russian Botanical garden of Univ. of Oulu       | KX07862               | MG98187               | MG98227               | MG98239               | MG98238               | MG98239               | MG98261               | MG98285               | MG98303               | MG98318               | MG98324               |
| Kockej Altaia       | Italy            | Italian Botanical garden of Univ. of Oulu       | KX07863               | MG98189               | MG98229               | MG98241               | MG98240               | MG98242               | MG98262               | MG98286               | MG98304               | MG98319               | MG98325               |
| Montecarlo           | Italy            | Italian Botanical garden of Univ. of Oulu       | KX07864               | MG98191               | MG98231               | MG98243               | MG98242               | MG98243               | MG98263               | MG98287               | MG98305               | MG98320               | MG98326               |
| Kola                  | Russia           | Russian Botanical garden of Univ. of Oulu       | KX07865               | MG98193               | MG98233               | MG98245               | MG98244               | MG98245               | MG98265               | MG98289               | MG98307               | MG98322               | MG98328               |
| Kola                  | Russia           | Russian Botanical garden of Univ. of Oulu       | KX07866               | MG98195               | MG98235               | MG98247               | MG98246               | MG98247               | MG98267               | MG98291               | MG98308               | MG98323               | MG98329               |
| Zirbitzkogel         | Austria          | Austrian Botanical garden of Univ. of Oulu      | KX07867               | MG98197               | MG98237               | MG98249               | MG98248               | MG98249               | MG98269               | MG98293               | MG98309               | MG98324               | MG98330               |
| Hochkar              | Austria          | Austrian Botanical garden of Univ. of Oulu      | KX07868               | MG98199               | MG98239               | MG98251               | MG98250               | MG98251               | MG98271               | MG98297               | MG98310               | MG98325               | MG98331               |
| Dürrenstein          | Austria          | Austrian Botanical garden of Univ. of Oulu      | KX07869               | MG98201               | MG98241               | MG98253               | MG98252               | MG98253               | MG98273               | MG98298               | MG98311               | MG98326               | MG98332               |

### Footnotes

(Continues)
| Site              | Country | Origin or GPS co-ordinates                      | GenBank accession nr. trnL-F | GenBank accession nr. psbA5'R-matk8F | GenBank accession nr. psbA-trnH | GenBank accession nr. psbB-psbH | GenBank accession nr. 5'rpS12-rpL20 | GenBank accession nr. trnCF-ycf6R |
|------------------|---------|------------------------------------------------|-----------------------------|-------------------------------------|-------------------------------|-------------------------------|-----------------------------------|----------------------------------|
| Triglav          | Slovenia| 46°18′51.29″N, 13°47′10.37″E                     | KX078556                    | MG938094                           | MG938125                      | MG938192                      | MG938220                          | MG938274                          |
| Binntal          | Switzerland | José Vouillamoz, Agroscope, Switzerland        | KX078525                    | MG938095                           | MG938132                      | MG938152                      | MG938228                          | MG938268                          |
| Unteralp         | Switzerland | José Vouillamoz, Agroscope, Switzerland        | KX078558                    | MG938104                           | MG938133                      | MG938155                      | MG938233                          | MG938265                          |
| Mattmark         | Switzerland | José Vouillamoz, Agroscope, Switzerland        | KX078544                    | MG938098                           | MG938126                      | MG938186                      | MG938230                          | MG938269                          |
| Val de Nomnom    | Switzerland | José Vouillamoz, Agroscope, Switzerland        | KX078560                    | MG938096                           | MG938127                      | MG938154                      | MG938235                          | MG938266                          |
| Piano dei Canali | Switzerland | José Vouillamoz, Agroscope, Switzerland        | KX078549                    | MG938099                           | MG938128                      | MG938188                      | MG938229                          | MG938267                          |
| Mengusovska dolina | Slovakia               | 49°10′28.23″N, 20°03′27.24″E                    | KX078545                    | MG938083                           | MG938140                      | MG938187                      | MG938198                          | MG938264                          |
| Chopok           | Slovakia | 48°56′43.27″N, 19°36′45.70″E                     | KX078527                    | MG938082                           | MG938138                      | MG938157                      | MG938197                          | MG938247                          |
| Lomnicky stit    | Slovakia | 49°12′20″N, 20°13′31″E                           | KX078543                    | MG938105                           | MG938139                      | MG938158                      | MG938196                          | MG938248                          |
| Fagaras          | Romania | 45°36′6.09″N, 24°36′59.33″E                       | KX078533                    | MG938107                           | MG938117                      | MG938161                      | MG938201                          | MG938252                          |
| Cindrel          | Romania | 45°34′32.58″N, 23°45′48.10″E                      | KX078528                    | MG938085                           | MG938120                      | MG938163                      | MG938218                          | MG938240                          |
| Calimani         | Romania | 47°07′13″N, 25°10′17″E                           | KX078526                    | MG938087                           | MG938111                      | MG938164                      | MG938217                          | MG938250                          |
| Grau Roig        | Andorra | 42°31′26.81″N, 1°41′19.92″E                       | KX078535                    | MG938079                           | MG938114                      | MG938160                      | MG938210                          | MG938241                          |
| Juclar           | Andorra | 42°35′54.11″N, 1°41′57.16″E                       | KX078538                    | MG938080                           | MG938116                      | MG938159                      | MG938211                          | MG938258                          |
| Tavargatai       | Mongolia | Andreas Plescher, Pharmaplant, Germany          | KX078554                    | MG938093                           | MG938110                      | MG938180                      | MG938199                          | MG938282                          |
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2.4 | Phylogenetic analysis

The trnL-F region was evaluated for phylogenetic construction, since GenBank (NCBI) sequences for other geographical regions, including Greenland and North America, were only available for this locus. Prior to the analysis, FastGap 1.2 (Borchsenius, 2009) was used to code the phylogenetic information of gaps (indels) as binary characters within the sequence matrix by applying the “simple indel coding” algorithm of Simmons and Ochoterena (2000). The refined alignment of sequences and the indel binary matrix was incorporated in the concatenated dataset. The maximum likelihood (ML) approach (Felsenstein, 1981) implemented in RaxMLGUI 1.5b2 (Silvestro & Michalak, 2012) was used for tree construction, which is based on explicit models of sequence evolution and proved to be statistically robust. RaxMLGUI was run under the GTR nucleotide substitution model with gamma-distributed rate heterogeneity (GTR+Γ). Bootstrap support of branches was calculated with 1,000 data resamples. Rhodiola semenovii, Rhodiola kirilowii, and R. integrifolia voucher specimens were used as outgroups in the phylogenetic analyses.

3 | RESULTS

We sequenced six loci of the chloroplast genome of R. rosea in a collection of 44 sites from Eurasia. The six regions covered 4,943 bases. Within our alignment, we observed 111 nucleotide substitutions (of which 52 were singletons) and 37 indels of which 25 were duplications. Two of the six loci were earlier used in other studies with Rhodiola species (trnL-F by Cuerrier et al., 2015 and psbA-trnH by Zhang et al., 2014). The mean nucleotide diversity estimates for these was π = 7.688 × 10⁻³. Tajima’s D values for all loci studied did not show a significant deviation from neutrality (Table 2). We assigned the studied genotypes to five geographic regions: Asia, the Carpathians (including the Rila Mt. from the mountain region of the Balkan), the Alps (including the Pyrenees), the British Isles, and Scandinavia. Among the chloroplast markers, psbA-trnH had the highest levels of nucleotide diversity across Eurasia. The mean nucleotide diversity level was 27.810 × 10⁻³, which is a magnitude higher than for the rest of the markers. PIC value, nucleotide
diversity, number of haplotypes and haplotype diversity are compiled for all cpDNA regions in Table 2.

3.1 | Analysis of the studied loci

In case of the *trnL-F* locus, the amplified fragment length was between 921 and 1,055 bp resulting in an alignment of 1,109 bp length.

Altogether five indels were identified at four sites of the *trnL-F* region and ten SNP, of which seven are singletons. Indels of 23 and 19 bp were already known from this region (Cuerrier et al., 2015), which were also detected in this study. Both are duplications. Furthermore, an indel of 12 bp was found close to the 3‘ end, which is also a duplication. At the 5‘ end, an insertion of 67 bp was detected alone or duplicated (Figure 2).

In case of the *psbA5′R-matk8F* locus, the amplified fragment length was between 845 and 852 bp resulting in an alignment of 863 bp length. This region contained six indels of short sizes at four sites and 23 SNPs of which more than 50% were singleton. The region of *psbA-trnHGUG* was found to be extremely variable. The amplified fragment length was between 320 and 361 bp resulting in an alignment of 393 bp length. Compared to the small fragment size, 12 indels were detected of varying sizes at nine sites. Also many, 30 SNPs were found of which only six were singletons. In case of the *psbB-psbh* locus, the amplified fragment length was between 634 and 653 bp resulting in an alignment of 682 bp length. The region contained five indels at four sites ranging between four and 19 bp and eight SNPs of which six were singletons. The amplified fragment length of the *5′rpS12-rpl20* locus was between 911 and 930 bp and the aligned length was 937 bp. The region contained six indels of small size at four sites and 18 SNPs most of which were singletons. Finally, in case of the *trnC*GCAFycf6R locus, the amplified fragment length was between 892 and 900 bp resulting in an alignment of 900 bp length. The region contained just two small sized indels at two sites and 22 SNPs of which seven were singletons. All sequences are deposited in the NCBI GenBank under the accession number of KX078522–64, KX611154, and MG938064–MG938283 (Table 1).

3.2 | Haplotype network analysis

There are between seven and 27 haplotypes for each loci, separately in our samples, and we constructed the haplotype networks for each of these loci. Unrooted haplotype genealogies were estimated from the substitution polymorphisms (including indels coded as single characters) observed at each of the six loci (Figure 3), and each analysis yielded one most parsimonious arrangement.

There is a phylogeographic pattern in the distribution of *R. rosea* haplotypes, which provides clues about the spread of roseroot across Eurasia. For all six loci studied, the samples from Asia are in the center of the network, representing the origin of the species. Samples from the Alps are in a clearly separate haplotype cluster, with a single link to Asian samples. The samples from the Carpathians are clustered together with the Asian samples or are in direct connection with those. Scandinavian and British Isles samples are connected to each other and are connected to the Asian samples, sometimes sharing the haplotype. For *trnL-trnf, psbB-psbh*, and *5′rpS12-rpl20*, a rather simple network structure outlined, while for *psbA5′R-matk8F, psbA-trnHGUG*, and *trnC*GCAFycf6R, the structure is more complex, but still biogeographically interpretable. A concatenated haplotype network (Figure 4) was also built, but since using all six loci resulted in a very complex network, in which almost all samples represented a separate haplotype, we decided to omit the two most polymorphic loci (*psbA5′R-matk8F, psbA-trnHGUG*).

We used Bayesian Structure analysis to see whether the samples from the same geographic region are clustered together in the same group. Even though we assigned the samples into five geographical groups, the Evanno method indicates that a *K* = 3 model fits best the data (Figure 5) or less likely a *K* = 4 model. When three groups are formed, Atlantic and Scandinavian samples (Finnland, Norway, Iceland, British Isles, Kola Peninsula) clustered together. Another obvious group is formed by samples from the Alps and northern parts of the Carpathians. The rest of the samples, forming the third group, are more admixed including samples from Asia, the Pyrenees and some part of the Carpathians. When *K* = 4 is considered, besides the group of the Atlantic and Scandinavian samples and the Alpine samples two more admixed groups are formed both including Asian and Carpathian samples.

Since there are data available in the literature only for *trnL-F* and *psbA-trnHGUG* loci in case of *R. rosea*, these results provide the opportunity of a much more comprehensive evaluation. In case of the *trnL-F* locus, seven haplotypes were distinguished, which are in accordance with the presence or absence of the different indel. Cluster A (Figure 2; including also one SNP) contains Eurasian samples including samples from the EAS, all Asian samples and one of the Irish samples. None of these sequences contain any indels. Cluster B contains one single sample from the Calimani Mt. (Eastern-Carpathians), which is characterized by a duplication of 12 bp close to the 3‘ end of the *trnL-trnf* region. Cluster C includes one sample from Wales and one from Ireland both bearing only one of the indels that was previously described by Cuerrier et al. (2015). Cluster D contains samples from the Atlantic Coast, Northern Finland, Northern Norway, Kola-peninsula, Iceland, and also an Irish sample. These samples contain both indels that were previously described by Cuerrier et al. (2015). Cluster E includes a single sample from Hochkar (Göstling Alps) and F includes some more samples from the Austrian Alps, Julian Alps, and the Dolomites. These latter two groups are closely related, not including the two earlier described indels by Cuerrier et al. (2015), but another in the 5‘ end of the locus. They both have a 67 bp duplication, which generated the double band on the agarose gel and cluster E has besides this duplication a further 54 bp insertion, which is partly a duplication of the other 67 bp insertion, which generated the triple band on the agarose gel. Blast analysis revealed that this 67 bps have not been detected earlier in *R. rosea*. 53 bases out of these 67 are present in the *trnL-trnf* region of *Rhodiola dumulosa*, *Rhodiola yunnanensis*, and *Rhodiola cretinii*. The unmatched bases are 5‘-AAAAAAAAAGGGGGG-3‘.
**FIGURE 3** Haplotype networks obtained from the Templeton-Crandall-Singh analysis of the studied 44 *Rhodiola rosea* samples. The size of the circle represents the frequency of each haplotype. (a) A: trnL-F, B: psbA5'R−matk8F, C: psbA−trnHGUG, (b) D: psbB−psbH, E: 5′rpS12−rpl20, F: trnCGCAF−ycf6R. Different colors correspond to the different samples. Black dots indicate missing intermediate haplotypes that were unobserved in the analyzed sample set. Hash marks on branches represent mutation steps (number of base pair changes) between haplotypes.

**FIGURE 4** Concatenated haplotype network obtained from the Templeton-Crandall-Singh analysis of the studied 44 samples based on four chloroplast regions: trnL-F, psbB−psbH, 5′rpS12−rpl20, trnC−ycf6. The size of the circle represents the frequency of each haplotype. Different colors correspond to the different haplotypes. Black dots indicate missing intermediate haplotypes that were unobserved in the analyzed sample set. Hash marks on branches represent mutation steps (number of base pair changes) between haplotypes. Colored frames represent the five geographical regions we assigned the samples in.
In case of the trnL-F loci, sequences from other geographical regions are available in the GenBank, like Greenland, Canada, China, and also some European countries. Figure 6 shows a maximum likelihood tree derived from the combined matrix of our sequences supplemented with other *R. rosea* and also *R. integrifolia*, *R. kirilowii*, *R. semenovii* trnL-trnF sequences mined from the GenBank. In these analyses, sequences were modified, gaps (indels) were coded as single mutations in order to weight SNPs and indels equally. Most of the samples from the Alps, from the Carpathians and from Asia are clustered together including also other *Rhodiola* species. Based on the presence of the long indels, six samples from the Alps are distinguished and also based on the presence of the two previously known indels the Scandinavian, North American, and British Isle samples are forming a separate clade.

A few sequences are also available for the *psbA-trn* region in the GenBank. However, this locus proved to be the most variable region, so even though an attempt was made to generate a maximum parsimony tree also for these sequences a very complex tree was obtained without any clear clustering.

**FIGURE 5**  Estimated structure for $K = 2$, $K = 3$ and $K = 4$ of assignment analysis performed in STRUCTURE (Pritchard et al., 2000). The most likely number of clusters ($K$) detected by Evanno et al. (2005) method implemented in STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Each individual is represented by a vertical stacked column indicating the proportions of $K$ groups.

### 4 | DISCUSSION

The noncoding regions of the chloroplast are widely used to study genetic lineages and relationships among taxa, and to describe complex historical biogeography of species and genera in relation to their present distribution. The main advantages of these chloroplast regions are the universality of the primers and the robustness of the amplification process (Taberlet et al., 2007).

In the study of DNA barcoding of *Rhodiola* species by Zhang et al. (2014, five frequently used sequences (*rbcL*, *matK*, *trnH-psbA*, *ITS* and *trnL-F*) were tested for their utility. Even though ITS sequences were found to be the most powerful, *trnL-F* region showed to be also a promising alternative marker for barcoding *Rhodiola* species. Regarding phylogeographical patterns, the study by Cuerrier et al. (2015) has revealed two intraspecific variants of *R. rosea* based on this chloroplast region. Coastal and Alpine populations were found to differ in the presence or absence of two indels (duplications of 23 and 19 bp). Based on this, the authors have concluded that coastal populations of North America...
and Scandinavia are genetically separated from the Alpine populations. Our Structure analysis (Figure 5) and the extended maximum likelihood tree (Figure 6) confirm their findings, but on the other hand, our results revealed a more elaborated pattern within Europe. Both the Structure analysis (Figure 5) and the haplotype networks (Figures 3 and 4) identify the samples from the Central-European mountains as a distinct group. In the QTP, which is considered to be the center of origin and in adjacent regions, no insertions are present in the trnF-L region of ros-root. Neither were these insertions detected anywhere in Asia nor in most of the European samples. However, since there are some coastal samples in Europe like Dingel in Ireland, or Novaya Zemlya, Kamchatka in Asia which do not exhibit these two characteristic “coastal” indels (Cuerrier et al., 2015) and there are samples containing the two indels being not exactly nearby the coastal areas like Halti or Kilpisjärvi in Finland the term amphi-Atlantic would be more accurate to be used instead of coastal.

The lineage harboring the two insertions, which is distributed along the coastal parts of Scandinavia, the British Isles and in the eastern parts of North America suggest common origin of these populations. We interpret this pattern as evidence for the large periglacial distribution of *R. rosea* during the time of the Pleistocene from where it could have colonized the North Atlantic costs, Iceland, and North America postglacially (Abbott & Brochmann, 2003; Brochmann, Gabrielsen, Nordal, Landvik, & Elven, 2003; Schmitt, 2007). However, in the British Isles, both types are present (containing and missing the two insertions), while in Scandinavia only those with the two insertions were found. Moreover, we also observed individuals in Wales and in the Inismore island (Ireland) bearing only one insertion (Figure 3A). Accordingly, the earlier described two indels might have had their origin on the British Isles from where they have moved toward the north (Scandinavia) and west (North America; Figure 7B). Alternatively, individuals exhibiting the two insertions have expanded from an ancient, Taymir-Siberian Northeastern lineage (Figure 7A) (Alsos et al., 2007) even before the onset of the glaciations (Taberlet et al., 1998) as it has been reported in some...
According to Zhang et al. (2014), ancestors of *R. rhodantha* have been present in the species at least since the Middle Pleistocene (Alsos et al., 2015). Molecular analysis of distribution of *Rhodiola* by long-distance dispersal resulted in the eastern North American region. This is supported by our results as well. *R. rosea* arrived more likely via the amphi-Atlantic route, which is suggested by the existence of only one insertion present in Wales and in Inishmore island. Accordingly, the British Isles proved to be even contact zones between the European and Eastern lineages (Skrede et al., 2006).

In *R. rosea*, the lineage without any insertion of the British Isles most probably originates from the EAS and underwent stepping stone mutation process on the British Isles. This is indicated by the existence of only one insertion present in Wales and in Inishmore island. Accordingly, the British Isles proved to be a major diversification center in case of *R. rosea*. Moreover, two other loci studied in our work, *psbA-trnH* and *trnGCA-ycf6R*, support this hypothesis (Figure 3C,F).

Coastal and intercontinental colonization toward the northwest by long-distance dispersal resulted in the eastern North American distribution of *R. rosea* as it has been earlier reported for other amphi-arctic species (Alsos et al., 2015). Molecular analysis of *Rhodiola* species extending toward North America have reported the presence of the species at least since the Middle Pleistocene and suggested its entry into the American continent at least twice. According to Zhang et al. (2014), ancestors of *R. rhodantha* and *R. integrifolia* have reached the continent from the east via Beringia while *R. rosea* arrived more likely via the amphi-Atlantic route, which is supported by our results as well.

Our *trnL-F* results confirm the two distinct ancient evolutionary lineages from the QTP: an eastward and a westward expanding lineage, as proposed by Kozyrenko et al. (2011). After reaching the EAS most probably before the glacial cycles or at the beginning of the glacial cycles of the Pleistocene (Taberlet et al., 1998), *R. rosea* underwent a considerable diversification that resulted in a higher diversity in the Alpine-Carpathian mountain ranges. In the Eastern Alps, (Julian Alps and the Austrian Alps) individuals forming the group E and F (based on *trnL-F* haplotypes) do not have the insertions described formerly by Cuerrier et al. (2015) differentiating these samples from those distributed along the Atlantic coastal and intercontinental area. However, they do have a duplication of 67 bp in the 5′ end of the *trnL-F* region. Group F includes beside the samples from the Eastern Alps also the samples from the Dolomites. Moreover, at the Hochark (Austrian Alps) two insertions are present (beside the duplication a further 54 bp insertion, group E from Figure 2). This diversification might have dated back to the time of the glacial cycles of the Pleistocene when species were forced to migrate and underwent withdraw- als and expansion events (Schönhütt et al., 2005; Taberlet et al., 1998). Also all other loci studied confirm diversification in the region of the EAS (Figures 3 and 4). Our former study based on nuclear microsatellites has already revealed that the Eastern Alps and the Dolomites exhibit a distinct genetic pattern compared to other Alpine regions and might have served as possible refugia for *R. rosea* (György et al., 2016) in the EAS. Indeed, several phylo-geographical studies on alpine perennial plant species have mentioned the Eastern Alps and the surrounding lower mountainous regions as refugial territories, where species probably survived during the glacial cycles of the Pleistocene and could have recol- onized the Alps in the postglacial (Mráz et al., 2007; Schönhütt et al., 2005; Tribsch & Schönhütt, 2003). The insertion detected in the *trnL-F* region in the Hochkar sample (forming the group E, Figure 2) might represent an ancient lineage as it is also present in the northeastern and eastern Asian species, like *R. du- mulosa, R. yunnanensis, R. cretinii, and might date back to the time of the intensive diversification period of *Rhodiola* genus (Zhang et al., 2014). We can presume that the strong climate fluctuation of the Pleistocene might result also in the loss of some genetic ma- terial reaching the EAS formerly from the Central Asian highland corridor and have been only maintained in the refugial territory of the Eastern Alps.
Interestingly, in case of the trnL-F region another, earlier not known duplication has been revealed within the EAS, namely in the sample of the Eastern Carpathians, the Calimani Mts. (Figure 3A). Although only one population was included in the study, we could detect the signs of a different gene stock only persisting in the Eastern Carpathians. Samples from the neighboring Southern Carpathians proved to be different as they belong to the large group without any insertions in the trnL-F region (Figure 2, group A). The sample from the Calimani Mts. indeed represents a distinguished haplotype based on psbA-trnL-GUG, and 5'ypS12-tnLL20 loci also. We consider it would be interesting to analyze more samples from this region to highlight the characteristics of the gene stock preserved along the Carpathians. Earlier, genetic lineages different from those of the Alps have been reported from the Northern Carpathians, the Tatra Mts.; in case of D. octopetala, an eastern genetic lineage was detected being more closely related to the northeast Russian ones than to the Alpine (Skrede et al., 2006). The available case studies reported also phylogeographical structuring of populations from the Eastern and Southern Carpathians (Mráz et al., 2007; Ronikier, 2011).

5 | CONCLUSION

Our study explores high resolution in the genetic pattern of R. rosea a widely distributed arctic-alpine perennial species of the Northern Hemisphere based on six chloroplast regions. As it has been already stated this species originates from the QTP (Figure 3A). Migration and diversification toward the east were documented by several studies (Hou & Lou, 2014; Kozyrenko et al., 2011). Based on the sequence alignment of the trnL-F region, northern expansion toward Siberia and the colonization of western Eurasia have started most probably at the same historical time (Zhang et al., 2014). Our results support the migration of the species into Europe via the Central Asian highland corridor, reaching the EAS and also the western European edge, the British Isles. As it has been documented in earlier studies that glacial cycles of the Pleistocene have had strong influence on the genetic pattern of the arctic-alpine species. In case of R. rosea, the EAS proved to be an important center of high genetic variation, especially the region of the Eastern Alps and the Dolomites where glacial refugia might have had existed. Although in many arctic-alpine species postglacial colonization of northern Europe, Scandinavia was supposed to be from the EAS in case of R. rosea we only could detect a strong relation between the northern amphi-Atlantic coastal parts and the British Isles. However, our former study based on microsatellite markers has already revealed the genetic differentiation of the Scandinavian populations from that of the EAS (György et al., 2016). The high variation and distinct genetic pattern preserved in the Alpine and Carpathian populations emphasizes the role of the EAS in the diversification of R. rosea most probably dating back to the glacial cycles of the Pleistocene and supporting the existence of long-standing refugia. Apart from those of the EAS, a common lineage was detected along the Atlantic coast from the British Isles toward Scandinavia as well as Iceland and the Eastern parts of North America. Accordingly, the British Isles seems to represent the main link between the northern Atlantic and southern EAS lineages.

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AUTHORS’ CONTRIBUTIONS

ZG designed the research, collected the plant material, performed the molecular work, analyzed the sequence data, and wrote the manuscript, EGT participated in the collection of plant material, data interpretation and analysis and prepared the illustrations, NI performed part of the molecular work and analyzed the sequence data, BM participated in the collection of plant material and performed part of the molecular work, MH participated in the collection of plant material, discussed the results, and wrote the manuscript. All authors read and approved the final manuscript.

DATA ACCESSIBILITY

DNA sequences: GenBank accessions (NCBI): KX078522–64, KX611154, and MG938064–MG938283.

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