Evaluating SSR marker transferability and plastid barcode variation in native *Populus* and *Salix* species of Türkiye

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ABSTRACT

Populus and *Salix* species are key components of forest and riparian ecosystems and hold substantial economic value in forestry, bioenergy, and restoration practices. In Türkiye, these genera are widely distributed across diverse ecological zones, yet regional molecular data remain limited. In this study, I examined genetic relationships among six native species—*Populus nigra* L., *Populus alba* L., *Populus tremula* L., *Populus euphratica* Olivier, *Salix alba* L., and *Salix caprea* L.—using an integrative molecular approach. Twelve nuclear microsatellite (simple sequence repeats; SSR) markers and three plastid DNA barcode regions (*matK, rbcL, trnH–psbA)* were employed to assess marker performance, genetic variation, and species-level relationships. The analyses revealed clear genetic differentiation between *Populus* and *Salix* and substantial variation among *Populus species*. SSR loci detected both conserved and lineage-specific alleles, with limited allele sharing between genera, while plastid barcodes showed marker-specific patterns of sequence variation and phylogenetic resolution. Among the plastid regions, *matK* provided the strongest discriminatory signal, whereas *rbcL* was more conservative and *trnH–psbA* exhibited higher variability. *P. tremula and P. nigra* displayed higher levels of sequence and allelic variation compared with the other taxa, whereas *P. euphratica* showed a more distinct genetic profile. The combined use of nuclear SSRs and plastid barcodes provided complementary insights into genetic structure and evolutionary relationships among selected *Populus* and *Salix* species. Although limited in taxon and marker coverage, this study contributes regionally focused molecular data from a biogeographically underrepresented area and provides a foundation for future multilocus and genome-wide investigations of Salicaceae diversity in Türkiye.

Subjects Biodiversity, Genetics, Molecular Biology, Plant Science, Population Biology
Keywords Populus, Salix, DNA barcode, Nuclear microsatellite, Phylogenetic

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INTRODUCTION

The family Salicaceae, including the genera *Populus* and *Salix*, comprises fast-growing, predominantly dioecious trees and shrubs that play key ecological roles in temperate and boreal ecosystems (*Dickmann & Kuzovkina, 2014*). Species within this family contribute to soil stabilization, carbon sequestration, hydrological regulation, and habitat formation, particularly in riparian and floodplain environments (*Karrenberg, Edwards & Kollmann, 2002*; *Kuzovkina & Volk, 2009*). Owing to their rapid growth, high biomass production,

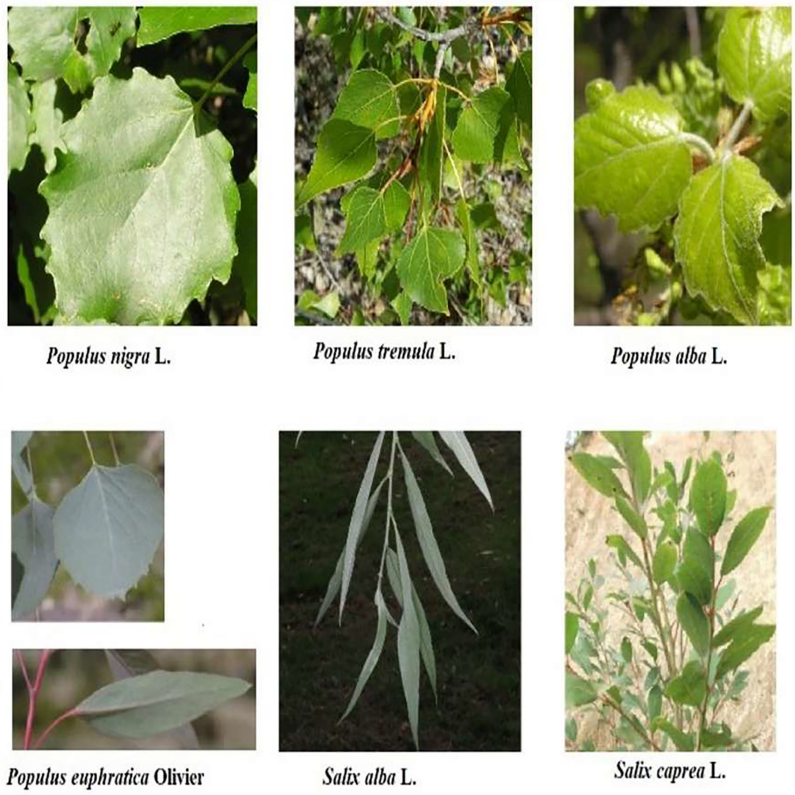

Figure 1 Leaf pattern of four *Populus* and two *Salix* species. POWO (2025).
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and tolerance to environmental stress, many *Populus* and *Salix* species are widely used in forestry, phytoremediation, erosion control, and ecological restoration.

Beyond their ecological and economic importance, *Populus* and *Salix* have emerged as model systems in plant genetics, evolutionary biology, and ecological physiology. Both genera are characterized by extensive geographic distributions, high morphological plasticity, frequent hybridization, and diverse reproductive strategies (*Tuskan et al., 2006*; *Smulders et al., 2008*; *Sanderson et al., 2023*; *Kaya et al., 2023*). For example, *Populus euphratica* exhibits exceptional tolerance to salinity and drought (*Rajput et al., 2016*), whereas many *Salix* species are highly effective in riparian restoration due to rapid vegetative propagation and strong root development.

Despite these shared traits, *Populus* and *Salix* differ markedly in leaf morphology, genome structure, and evolutionary history, making them valuable systems for comparative phylogenetic research (Fig. 1). Recent phylogenetic and phylogenomic studies have substantially advanced understanding of relationships within and between the two genera. Genome-wide analyses and nuclear single nucleotide polymorphism (SNP) datasets have clarified species boundaries, detected hybridization and introgression, and revealed patterns of reticulate evolution (*Wang et al., 2014*; *Liu et al., 2016*, *2022*; *Sanderson et al., 2023*; *Ogutcen et al., 2024*). These studies consistently support *Populus* and *Salix* as monophyletic sister genera (*Wang et al., 2014*; *Lauron-Moreau et al., 2015*; *Liu*

et al., 2016). Nevertheless, species delimitation within each genus remains challenging due to incomplete lineage sorting, interspecific hybridization, and overlapping morphological traits.

Türkiye hosts four native *Populus* species (*P. nigra*, *P. alba*, *P. euphratica*, *P. tremula*) and 27 native *Salix* species distributed across diverse ecological zones, including riparian corridors, wetlands, montane regions, and semi-arid landscapes (*Velioğlu, Bostancı & Akgül, 2020*). Despite this diversity and ecological significance, regional genetic relationships and evolutionary patterns within Turkish *Populus* and *Salix* remain poorly resolved. Previous studies in Türkiye have largely focused on morphology or single-marker plastid analyses, offering limited resolution of species boundaries, marker transferability, and multilocus genetic structure (*Çiftçi et al., 2017*; *Ciftci & Kaya, 2019*; *Acar et al., 2020*, *2022*; *Değirmenci et al., 2022*). To date, no study has applied an integrative multilocus framework combining nuclear simple sequence repeat (SSR) markers with plastid DNA barcodes across multiple native taxa and geographically distinct river systems.

This lack of regional multilocus data represents a significant knowledge gap, particularly for genera characterized by frequent hybridization and complex evolutionary histories, and is especially important given increasing habitat degradation and climate-driven stress across Türkiye. To address this gap, the present study employs an integrative approach using 12 nuclear SSR loci and three plastid barcode regions (*matK*, *rbcL*, *trnH–psbA*) to investigate phylogenetic relationships among six native *Populus* and *Salix* species. Specifically, the study aims to (i) assess genetic divergence within and between genera, (ii) evaluate SSR marker transferability across taxa, (iii) characterize plastid sequence variation among native species, and (iv) identify informative loci for molecular systematics and conservation genetics within the Salicaceae. By focusing on populations sampled from multiple river systems, this study provides the first multilocus, population-based molecular assessment of Turkish *Populus* and *Salix*, contributing regionally focused data that complement, rather than seek to redefine, existing global phylogenetic frameworks.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 149 individual trees representing six populations from four distinct river systems in Türkiye were sampled for this study. Specifically, leaf samples were collected from 26 individuals of *P. alba* (Tekirdağ River), 26 of *P. nigra* (Dicle River), 26 of *P. euphratica* and 26 of *S. alba* (Göksu River), 27 of *S. caprea* L. (Çoruh River), and 18 of *P. tremula* (Kızılırmak River). Healthy young leaves were collected in the field, immediately dried in silica gel, and transported to the laboratory for genomic DNA extraction.

The six river systems were selected to capture broad ecological and biogeographical variation across Türkiye. These rivers span contrasting environmental gradients—including coastal (Tekirdağ), semi-arid (Dicle), Mediterranean riparian (Göksu), montane (Çoruh), and Central Anatolian continental climates (Kızılırmak)—where *Populus* and *Salix* species experience distinct selective pressures. Several basins, particularly the Dicle and Göksu Rivers, also represent conservation priority areas due to hydrological alteration, habitat fragmentation, and increasing anthropogenic stress. Sampling across these diverse

regions allowed for a more comprehensive assessment of genetic differentiation associated with ecological heterogeneity and geographically structured populations.

Total genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method following the protocol of *Doyle & Doyle (1990)*, with minor modifications to optimize DNA yield and purity from woody plant tissues. The modification was carried out using chloroform instead of chloroform-isoamyl alcohol (24:1). Additionally, a mixture of 0.08 volumes of 7.5 M ammonium acetate and 0.54 volumes of isopropanol was used to remove the secondary metabolites that inhibit PCR amplification and to purify the DNA. DNA concentration and quality were assessed using a BioDrop µLITE spectrophotometer (BioDrop Ltd., Cambridge, UK) by measuring absorbance ratios at A260/280 nm. Only samples with optical density values between 1.7 and 2.0 were considered suitable for downstream PCR applications.

Microsatellite amplification

To investigate genetic diversity and structure, twelve highly polymorphic nuclear microsatellite loci were selected from the International Populus Genome Consortium (*IPGC, 2016*). Primer sequences and annealing temperatures were chosen based on established protocols optimized for interspecific transferability (*Van Der Schoot et al., 2000*; *Smulders et al., 2001*) (File S1, Table S1).

PCR reactions were performed in a 20 µL total volume containing 4 µL of 5× HOT FIREPol® Blend Master Mix (Solis BioDyne, Tartu, Estonia), 0.25 µM of each forward and reverse primer, 20 ng of template DNA, and nuclease-free water to reach the desired volume. Two thermal cycling protocols were used depending on primer requirements, both starting with an initial denaturation at 95 °C, followed by 30–35 cycles of denaturation, annealing, and extension, and ending with a final extension step at 72 °C (*Van Der Schoot et al., 2000*; *Smulders et al., 2001*). PCR products were initially visualized on 3% agarose gels to verify successful amplification prior to genotyping.

DNA barcoding amplification

To support species identification and phylogenetic reconstruction, three plastid barcode regions (*trn*H-*psb*A, *mat*K and *rbc*L) were targeted (File S1, Table S1). Although the nuclear ITS region is frequently used in plant barcoding, it is known to present challenges in *Populus* and *Salix* due to paralogous copies, incomplete concerted evolution, and hybridization, which may result in ambiguous or non-homologous sequences (*Hou et al., 2019*). Preliminary amplification tests conducted in this study produced inconsistent and low-quality ITS products, particularly in *Salix* species. To ensure data reliability and avoid complications arising from multi-copy nuclear loci, ITS was excluded, and only plastid markers (*matK*, *rbcL*, *trnH-psbA*)—which showed high amplification success and sequence quality—were used for phylogenetic reconstruction (*Hebert et al., 2003*). PCR reactions were carried out in a 20 µL reaction volume containing 5 µL HOT FIREPol® Blend Master Mix, 0.5 µL each of forward and reverse primers (10 µM), 5 µL template DNA, and 9 µL deionized water. Thermal cycling conditions included an initial denaturation at 94 °C for 4 min, followed by 30 cycles of 94 °C for 40 s, annealing at the appropriate primer-specific

temperature for 35 s (annealing temperature (Ta):57 for *rbcL* and *trn*H-*psb*A. Ta:58 for *mat*K), and 72 °C for 40 s. A final extension was conducted at 72 °C for 5 min. PCR products were visualized on 3% agarose gels stained with ethidium bromide and run at 90 V for 30 min.

Data analysis

Samples that failed to amplify were reattempted up to two additional PCR runs. Only loci producing clear, reproducible bands were retained for downstream SSR genotyping. PCR products from the barcode regions were sequenced bidirectionally by Sanger dideoxy sequencing at BM Labosis (Çankaya, Ankara). Sequencing reactions were carried out in a total volume of 25 µL using an ABI 310 Genetic Analyzer and an ABI 3730XL 96-capillary automated sequencer (Applied Biosystems, Foster City, CA, USA). All chromatograms were manually inspected in MEGA11. Low-quality reads, sequences containing multiple ambiguous base calls, or those with Phred quality scores <20 were removed. Forward and reverse reads were assembled only when both directions provided consistent, high-quality base calls. Sequences failing quality criteria were excluded from alignment and phylogenetic reconstruction. For each species, plastid barcode regions were successfully sequenced from three individuals.

SSR genotyping

Fluorescently labeled SSR amplicons were subjected to capillary electrophoresis using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems Foster City, CA, USA) at BM Laboratory Systems (Ankara, Türkiye). Allele sizes were scored using Peak Scanner v2.0 software (Applied Biosystems, Foster City, CA, USA), with GeneScan 400 ROX used as the internal size standard. Population-level genetic statistics (*e.g.*, He, Fst, Analysis of Molecular Variance (AMOVA)) were not computed because only three or four SSR loci amplified in several species, which does not provide sufficient marker coverage for reliable population genetic inference.

DNA sequence analysis and phylogenetics

Sequence chromatograms were manually inspected in MEGA v11 (*Tamura, Stecher & Kumar, 2021*) to ensure base-calling accuracy, and ambiguous sites were resolved by comparing forward and reverse reads. The resulting sequences for each locus were aligned using CLUSTALW (*Thompson, Higgins & Gibson, 1994*), and a consensus sequence was generated to represent each species for each barcode region. Sequence identity and coverage were verified using BLASTn searches against the NCBI GenBank database (*Altschul et al., 1990*), considering both query coverage and percentage identity. Consensus sequences obtained from Turkish populations were aligned and concatenated with publicly available reference sequences for the same species to enhance phylogenetic resolution from NCBI GenBank. Only sequences with verified species identification and complete locus coverage were included. Because the primary objective was to infer species-level relationships, population-specific variation within Türkiye was not analyzed separately. Phylogenetic tree was constructed using Maximum Likelihood (ML) method under the General Time

Reversible (GTR) model with gamma distribution and invariant sites (G+I), as implemented in MEGA11. The Neighbour-Joining (NJ) method (*Saitou & Nei, 1987*) was also employed to compare topologies. The robustness of branches was assessed by 1,000 bootstrap replicates. Phylogenetic trees were drawn to scale, with branch lengths proportional to genetic distance estimated using the maximum composite likelihood method.

RESULTS

Allelic diversity

A total of 12 SSR loci were analyzed across six species of *Populus* and *Salix* to examine allelic patterns. Although all primers were tested across species, amplification profiles revealed notable interspecific variation. The number of alleles per locus varied considerably among taxa. The loci WPMS14, WPMS15, and WPMS18 were successfully amplified in both *Populus* and *Salix* species. The PMGC2163 locus demonstrated broader amplification, being detected in *S. alba* and all *Populus* species. Amplification of WPMS03 and WPMS04 was restricted to *P. tremula* and *P. nigra*, whereas WPMS05, WPMS20, and PMGC93 consistently amplified across all *Populus* species, indicating their potential utility as robust cross-species markers. Additionally, WPMS09 and WPMS16 amplified in *P. tremula*, *P. alba*, and *P. nigra*, while WPMS07 was amplified in *P. tremula*, *P. euphratica*, and *P. nigra*. Among the amplified loci, *P. tremula*, *P. nigra*, and *P. alba* showed the highest allelic richness, particularly at WPMS05, PMGC2163, and PMGC93, respectively. In contrast, *P. euphratica* exhibited fewer alleles across all loci. A summary of allelic profiles per locus is presented in Table 1.

Conserved alleles among species

Shared alleles were identified at several loci. For instance, the 219 bp allele at the WPMS14 locus was found in *P. alba*, *P. euphratica*, *S. alba*, and *S. caprea*. Similarly, the 231 bp allele occurred in *P. alba*, *P. euphratica*, *P. nigra*, and *S. caprea*. Overall, WPMS14 showed allele sharing among at least two species, except *P. tremula*. At the WPMS15 locus, *P. euphratica* and *S. caprea* shared the 182 and 185 bp alleles. The 226 bp allele was common to *P. tremula* and *P. nigra*, while the 218 bp allele was shared by *P. alba* and *P. nigra*.

Among the loci, the WPMS18 exhibited the highest number of shared alleles. The 225, 228, and 231 bp alleles were detected in all *Populus* species, and the 225 and 231 bp alleles also appeared in *S. alba*. The 219 bp allele was shared by *S. caprea*, *P. tremula*, *P. euphratica*, and *S. alba*. At the PMGC93 locus, the 351, 354, and 357 bp alleles were conserved in *P. tremula*, *P. alba*, *P. euphratica*, and *P. nigra*. The 363 bp allele was present in all *Populus* species except *P. euphratica*, but absent in *Salix* species. WPMS20 alleles 251, 270, and 274 were shared between *P. tremula* and *P. nigra*, indicating a close phylogenetic relationship. The 145 and 150 bp alleles at WPMS16 were exclusive to *P. tremula* and *P. nigra*.

DNA barcoding and phylogenetic analysis

DNA sequences were successfully obtained for all three barcode regions (*matK*, *rbcL*, and *trnH-psbA*) from all sampled *Populus* and *Salix* species (File S2). Among these, the *matK*

Table 1 DNA fingerprinting based on 12 microsatellite markers (Bold, italics and underlined alleles common among species).

SRR loci	*P. tremula* (18 indv)	*P. alba* (26 indv)	*P. euphratica* (26 indv)	*P. nigra* (26 indv)	*S. alba* (26 indv)	*S. caprea* (27 indv)
WPMS03	210, ***224***, 230, ***234***, 236, 239, 242, 248, 251, 259	–	–	220, ***224***, 228, 232, ***234***	–	–
WPMS04	258, 266, ***274***	–	–	263, 270, 272, ***274***	–	–
WPMS05	***274, 278***, 280, ***282, 285, 288, 290***, 304	298, 306, 310, 312	286, ***288, 290***	232, ***274***, 276, ***278, 282, 285***, 301	–	–
WPMS07	***222, 228, 230, 236***, 238, 250, 262	–	215, 219	***222***, 224, ***228, 230***, 232, ***236***	–	–
WMPS09	***245, 250***, 256, 258, 260	224, 230, 236, ***245***, 248, ***250***	–	230, ***245, 250***, 252, 260, 274	–	–
WMPS14	251, 263, 266, 269, 272, 280	204, 207, ***219, 222, 228, 231***	213, ***219, 231, 237***	210, ***231, 237***, 242, 244	192, 198, 204, ***219***	***219, 222***, 225, ***228, 231***, 234
WPMS15	220, ***226***, 228, 233	195, 198, ***218***	***182, 185***	203, 210, 212, ***218, 22***6	158	***182, 185***
WPMS16	129, 134, ***145, 150***	166, 168, 172, 174, 178	–	***145, 150***	–	–
WMPS18	210, ***219, 225, 228, 231, 234***, 243	216, ***222, 225, 228, 231***, 237	***225, 228, 231***	***219, 225, 228, 231, 234***	213, ***219, 222, 225, 231***	***219***, 274, 298
WPMS20	***214***, 228, 232, ***251***, 256, 268, ***270, 27 4***	198, 200, 208, 210, 212	***214***	***251***, 263, ***270***, 272, ***274***, 280	–	–
PMGC2163	***224***, 230, 236, 242, ***248***, 252, 258, 264	192, ***194, 198, 200, 204, 206***	***194, 200, 204***	***194, 200***, 202, 208, ***212***, 222, ***224***, 240, ***248***	***194, 198, 200***, ***204, 206, 212***, 216	–
PMGC93	***351, 354, 357, 363***	***351, 354, 357***,360, ***363***, 366	348, ***351, 357***, 369	***351, 357, 363***	–	–

region exhibited the greatest variation in sequence length, ranging from 556 base pairs in *P. euphratica* to 788 base pairs in other *Populus* species. In contrast, the *trnH-psbA* region yielded the shortest sequences, ranging from 242 base pairs in *S. alba* to 305 base pairs in *P. tremula*. The *rbcL* region showed uniform sequence lengths across all species (586 base pairs) (File S2). In the BLAST search for the *rbcL* region, *P. tremula* (KX163000.1) and *P. alba* (KX162988.1) demonstrated 100% sequence identity with previously published sequences. *P. nigra*, *P. euphratica*, and *S. caprea* showed 99.83% sequence identity with reference sequences *P. nigra* (JF429910.1), *P. euphratica* (MK267314.1), and *S. caprea* (OZ037764.1), respectively. All examined species, except *P. nigra*, exhibited 100% identity with their respective aligned reference sequences. For the *trnH-psbA* region, *S. caprea* displayed the highest sequence identity with its reference sequence (OZ037764.1), whereas *P. tremula* showed the lowest identity with its respective reference (LC806940.1) (File 1, Table S2).

Phylogenetic analyses were performed on three plastid barcode regions (*matK*, *rbcL*, and *trnH–psbA*) using sequences retrieved from GenBank for four *Populus* and two *Salix* species (File S3). The coding *matK* region exhibited sequence lengths between 824 bp (*S. alba*) and 846 bp (*P. nigra*), with *P. nigra* and *P. alba* showing the most conserved sequences, while *P. tremula* and *P. euphratica* displayed higher variability. *P. tremula* had 35 variable sites (33 parsimony-informative), and *P. euphratica* contained the highest number of singletons ($n = 37$). *Salix* species showed very low variation (two variable sites,

Table 2 Phylogenetic analysis of four *Populus* and two *Salix* species based on *matK* barcode gene region.

Species/Filogenetic parameters	*Populus nigra*	*Populus tremula*	*Populus alba*	*Populus euphratica*	*Salix alba*	*Salix caprea*
Accession no.*	PX101359	PX101357	PX101360	PX101358	PX101361	PX101362
Number of aligned individuals	26	29	25	28	22	21
Sequence length	846	836	835	835	824	827
Conserved region	829	801	832	787	822	825
Variable region	17	35	3	37	2	2
Singleton region	14	2	0	37	1	1
Parsimony region	3	33	3	0	1	1
Indel	0	0	0	0	0	0
Nucleotide diversity	0.0	0.01	0.00	0.00	0.00	0.00
GC content %	33.5	33.8	33.8	34	33.9	34

Note:
* Accession numbers refer to sequences deposited in GenBank.

one singleton per species), and no indels were detected. Nucleotide diversity (π) was generally low (maximum π = 0.01 in *P. tremula*), with stable GC content across taxa (33.5–34.0%) (Table 2). The *rbcL* gene region showed uniform sequence lengths (513 bp for *Populus*, 549 bp for *Salix*) and high sequence conservation, particularly in *P. euphratica*. *P. tremula* had 69 variable sites (54 singletons, eight parsimony-informative), while *P. alba* exhibited 59 singletons without informative positions. Among *Salix* species, *S. alba* contained five singleton sites and one indel, and *S. caprea* had two variable sites (one parsimony-informative). Nucleotide diversity was highest in *P. alba* (π = 0.03), and GC content ranged from 43.0% to 45.3% (Table 3). In contrast, the non-coding *trnH–psbA* spacer displayed the greatest variability, with sequence lengths from 233 bp (*P. nigra*) to 367 bp (*P. alba* and *P. euphratica*). *P. tremula* showed the highest variation (52 variable and 43 singleton sites) followed by *P. nigra* (42 variable, 36 singleton sites), with 10 and six parsimony-informative sites, respectively. *S. caprea* also exhibited moderate variability (20 variable, 12 singleton, eight informative sites), whereas *S. alba* remained more conserved. Indels were observed in all taxa, most notably in *P. nigra* ($n = 8$) and *S. caprea* ($n = 7$), suggesting structural divergence in this intergenic region. Nucleotide diversity was highest in *P. tremula* (π = 0.05) and *P. nigra* (π = 0.04), while *Salix* species showed relatively low but consistent diversity (π = 0.01). GC content varied widely among taxa (20.5–32.7%) (Table 4).

To provide an integrated overview, the results from the three plastid DNA barcode regions (*matK*, *rbcL*, and *trnH–psbA*) were compared across *Populus*, *Salix*, and the combined dataset (Table 5). As detailed above, the *matK* region exhibited the highest number of parsimony-informative sites, the *trnH–psbA* spacer showed the greatest nucleotide diversity and indel frequency, and *rbcL* remained the most conserved marker with consistently high GC content. In *Populus* species, the average sequence lengths were 846 bp (*matK*), 513 bp (*rbcL*), and 235 bp (*trnH–psbA*), with conserved regions of 786, 444, and 170 bp, respectively. The highest number of singleton sites occurred in *rbcL* (55), followed by *trnH–psbA* (43) and *matK* (15). Parsimony-informative sites were most

Table 3 Phylogenetic analysis of four *Populus* and two *Salix* species based on *rbcL* barcode gene region.

Species/Filogenetic parameters	*Populus nigra*	*Populus tremula*	*Populus alba*	*Populus euphratica*	*Salix alba*	*Salix caprea*
Accession no.*	PX093670	PX093668	PX093671	PX093669	PX093673	PX093672
Number of aligned individuals	33	29	13	14	20	23
Sequence length	513	513	513	513	549	549
Conserved region	503	451	454	513	544	547
Variable region	10	69	59	0	5	2
Singleton region	3	54	59	0	5	1
Parsimony region	7	8	0	0	0	1
Indel	–	–	–	–	1	–
Nucleotide diversity	0.00	0.01	0.03	0.00	0.00	0.00
GC content %	45.3	43.5	43.5	43.4	43.7	43

Note:
* Accession numbers refer to sequences deposited in GenBank.

Table 4 Phylogenetic analysis of four *Populus* and two *Salix* species based on *trnH-psbA* barcode gene region.

Species/Filogenetic parameters	*Populus nigra*	*Populus tremula*	*Populus alba*	*Populus euphratica*	*Salix alba*	*Salix caprea*
Accession no.*	PX101364	PX101366	PX101365	PX101363	PX101368	PX101367
Number of aligned individuals	15	13	16	10	8	19
Sequence length	233	234	367	367	242	281
Conserved region	191	181	359	364	234	261
Variable region	42	52	8	3	8	20
Singleton region	36	43	2	0	6	12
Parsimony region	6	10	6	3	2	8
Indel	8	1	2	2	2	7
Nucleotide diversity	0.04	0.05	0.01	0.00	0.01	0.01
GC content %	20.5	26.4	32	32.7	29.7	30

Note:
* Accession numbers refer to sequences deposited in GenBank.

abundant in *matK* (45), with fewer in *trnH–psbA* (22) and *rbcL* (14). The *trnH–psbA* region exhibited 13 indels and the highest nucleotide diversity ($\pi = 0.03$), while *matK* and *rbcL* showed lower diversity ($\pi = 0.00$ and $\pi = 0.01$, respectively). GC content ranged from 26.1% in *trnH–psbA* to 34.5% in *rbcL*. In *Salix* species, average sequence lengths were 827 bp (*matK*), 513 bp (*rbcL*), and 265 bp (*trnH–psbA*), with conserved regions of 817, 507, and 248 bp, respectively. Variation across markers was limited: variable sites ranged from 6 in *rbcL* to 17 in *trnH–psbA*, and singleton counts were low (2–6 per region). Parsimony-informative sites were few (4–11 per marker). Indels were found in *rbcL* ($n = 1$) and *trnH–psbA* ($n = 9$). All markers displayed minimal nucleotide diversity ($\pi \leq 0.01$), with *rbcL* showing the highest GC content (43.6%). *trnH–psbA* contributed the greatest sequence variation (variable sites = 93; $\pi = 0.04$) when all species were analyzed together, whereas *matK* and *rbcL* provided complementary phylogenetic signals at higher taxonomic levels (Table 5). These results highlight distinct marker-specific patterns in

Table 5 Phylogenetic analysis of members of Salicaceae family based on *mat*K, *rbcL* and *trn*H- *psb*A barcode gene regions.

	Populus species			*Salix* species			*All species*		
Species/Filogenetic parameters	**matK**	**rbcL**	**trnH-psbA**	**matK**	**rbcL**	**trnH-psbA**	**matK**	**rbcL**	**trnH-psbA**
Number of aligned species	112	89	54	43	43	27	151	132	81
Sequence length	846	513	235	827	513	265	788	513	233
Conserved region	786	444	170	817	507	248	712	436	140
Variable region	60	69	65	10	6	17	76	77	93
Singleton region	15	55	43	2	2	6	15	55	45
Parsimony region	45	14	22	8	4	11	61	22	48
Indel	0	0	13	0	1	9	0.00	1	22
Nucleotide diversity	0.0	0.01	0.03	0.00	0.00	0.01	0.01	0.01	0.04
GC content %	33.8	34.5	26.1	33.9	43.6	30.5	32.3	43.5	26.1

sequence variability and demonstrate the advantage of combining plastid loci for robust phylogenetic inference within Salicaceae.

When all species were analyzed together, 151 *matK*, 132 *rbcL*, and 81 *trnH-psbA* sequences were aligned. The average sequence lengths were 788 bp (*matK*), 513 bp (*rbcL*), and 233 bp (*trnH-psbA*). Conserved regions spanned 712, 436, and 140 bp, respectively. *trnH-psbA* exhibited the highest number of variable sites (93), parsimony-informative sites (48), the highest indel count (22). This region also showed the highest nucleotide diversity ($\pi = 0.04$) and the lowest GC content (26.1%), highlighting its strong resolution potential for closely related taxa. In contrast, *matK* and *rbcL* showed lower variability ($\pi = 0.01$) but provided complementary phylogenetic signals, particularly at higher taxonomic levels (Table 5).

A phylogenetic tree was reconstructed using a concatenated alignment of the three plastid barcode regions (*matK*, *rbcL*, and *trnH–psbA*). The combined plastid dataset clearly resolved *Populus* and *Salix* as two distinct and well-supported clades. Within *Salix*, *S. caprea* and *S. alba* formed a strongly supported clade (bootstrap support >99). Within *Populus*, *P. nigra*, *P. alba*, and *P. tremula* clustered together, while *P. euphratica* was resolved as a distinct lineage sister to this group with high bootstrap support. Overall, the concatenated plastid phylogeny provided stronger support for intergeneric relationships and major intrageneric groupings than individual barcode trees, while maintaining patterns consistent with marker-specific analyses (Fig. 2).

DISCUSSION

This study integrates nuclear SSR markers and plastid DNA barcodes to evaluate genetic relationships among selected *Populus* and *Salix* species sampled from Türkiye. The regional setting is central to interpreting these results: Türkiye lies at the intersection of the Euro-Siberian, Irano-Turanian, and Mediterranean floristic zones, and its river corridors span strong climatic and ecological gradients. This biogeographic complexity is directly relevant to patterns of divergence, potential secondary contact, and local adaptation in riparian trees, yet Turkish populations remain underrepresented in many broader

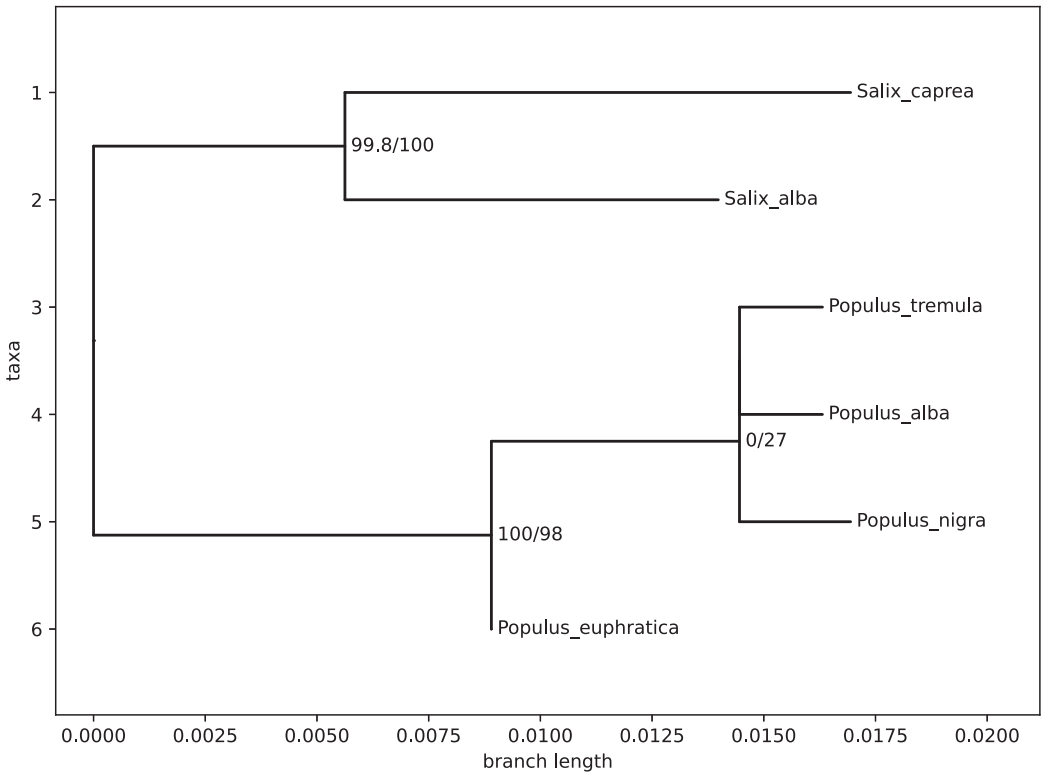

Figure 2 Phylogenetic tree reconstructed from a concatenated alignment of matK, rbcL, and trnH–psbA plastid sequences for *Populus* and *Salix* species. Analyses were performed using the Maximum Likelihood method under the GTR + G + I model. Numbers on nodes indicate bootstrap support values (%) based on 1,000 replicates; values <50% are not shown. Branch lengths represent substitutions per site. Full-size DOI: 10.7717/peerj.20936/fig-2

Salicaceae phylogenetic and phylogeographic syntheses. The present dataset therefore contributes regionally focused molecular evidence that can help improve the geographic representation of *Populus* and *Salix* lineages and inform conservation priorities in threatened riparian systems.

Nuclear SSR patterns and marker performance

SSR analysis revealed substantial interspecific polymorphism and clear differentiation between *Populus* and *Salix*, supporting the utility of nuclear fingerprinting for species-level comparisons in these genera. Among the loci tested, WPMS05, PMGC2163, and PMGC93 amplified consistently across all *Populus* species, suggesting their potential value as broadly transferable markers within the genus. By contrast, amplification success was lower and more variable in *Salix*, which likely reflects genomic divergence affecting primer binding sites or structural differences (*Smulders et al., 2001*; *Tuskan et al., 2006*; *Perdereau et al., 2014*; *Tokdemir et al., 2023*; *Ogutcen et al., 2024*), and may be further influenced by polyploidy and complex genome architecture that can reduce cross-species amplification efficiency (*Wagner, Gramlich & Hörandl, 2018*; *Sanderson et al., 2023*).

Across species, allelic richness was highest in *P. tremula*, *P. nigra*, and *P. alba*, consistent with their broader ecological ranges and adaptive flexibility (*Fussi, Lexer & Heinze, 2010*; *Jansson & Douglas, 2007*). In contrast, *P. euphratica* exhibited reduced allelic variation, which is plausibly associated with ecological specialization and demographic constraints in arid and saline habitats (*Hampe & Petit, 2005*; *Zhang et al., 2019*; *Kansu & Kaya, 2020*). These contrasts are compatible with a regional interpretation in which differing habitat breadth and landscape fragmentation contribute to divergent levels of nuclear diversity across Turkish lineages.

Locus-specific allele distributions also provide insight into evolutionary dynamics. Conserved alleles at WPMS14, WPMS15, and WPMS18 observed across *Populus* and *S. alba* may represent evolutionarily stable regions maintained by purifying or stabilizing selection (*Hou et al., 2019*), or ancestral polymorphisms retained through lineage sorting. Shared alleles between closely related taxa within *Populus* are consistent with recent divergence and/or gene flow, such as allele sharing at WPMS20 between *P. tremula* and *P. nigra*. By contrast, lineage-specific patterns at WPMS16—conserved in *P. nigra* and *P. tremula* but distinct in *P. alba*—suggest differential evolutionary trajectories among *Populus* species (*Cicatelli et al., 2012*; *Bonthala & Stich, 2022*). The overlap between alleles reported for *P. deltoides* (*Uluğ, 2022*) and alleles observed here further supports shared genetic patterns among closely related *Populus* taxa (*Çiftçi et al., 2017*). Allele sharing between *P. euphratica* and *S. caprea* at WPMS15 is notable, but it should be interpreted cautiously. SSR loci are prone to homoplasy (*Selkoe & Toonen, 2006*), and technical factors such as null alleles, size-calling inconsistencies, or differential amplification can generate apparent similarity unrelated to shared ancestry (*Dakin & Avise, 2004*). Thus, while such patterns may be compatible with retained ancestral polymorphism or convergence (*Chen et al., 2010*; *Liu et al., 2018*), robust inference would require sequence-based validation or genome-wide nuclear markers. Overall, the SSR results highlight pronounced divergence between *Populus* and *Salix* and meaningful differentiation among *Populus* species, potentially shaped by selective pressures and drift following lineage divergence (*Slavov & Zhelev, 2010*; *Değirmenci, Acar & Kaya, 2019*; *Hou et al., 2019*; *Ciftci & Kaya, 2019*; *Uluğ, 2022*; *Porth, Goessen & Heinze, 2024*; *Uzan Eken et al., 2024*).

Plastid barcoding, marker-specific resolution, and phylogenetic discordance

The marker-specific patterns observed among the three plastid barcode regions are consistent with their known evolutionary dynamics and reported performance in Salicaceae and other angiosperms. The coding regions *matK* and *rbcL* exhibited relatively low nucleotide diversity and high sequence conservation, particularly in *Salix*, reflecting their slower evolutionary rates and suitability for resolving higher-level relationships rather than closely related taxa (*Newmaster, Fazekas & Ragupathy, 2006*; *Fazekas et al., 2008*; *CBOL Plant Working Group, 2009*; *Hollingsworth, 2011*). In contrast, the non-coding *trnH–psbA* spacer showed substantially higher levels of sequence variation, indel frequency, and nucleotide diversity—especially within *Populus*—highlighting its greater resolution potential at lower taxonomic levels (*Kress et al., 2005*; *Shaw et al., 2007*). The

elevated variability detected in *P. tremula* and *P. nigra* across plastid markers is consistent with their broader ecological amplitudes and complex demographic histories, whereas the lower plastid diversity observed in *Salix* species aligns with previous reports of plastid uniformity and limited discriminatory power in willows (*Chen et al., 2010*; *Percy et al., 2014*). Collectively, these results emphasize the complementary nature of plastid barcode regions and support the use of multiple loci to balance phylogenetic resolution across taxonomic scales within the Salicaceae.

The concatenated plastid phylogeny recovered *Populus* and *Salix* as two well-supported and clearly separated clades, reinforcing the genus-level distinction consistently observed in the individual marker trees. Within *Salix*, *S. caprea* and *S. alba* formed a strongly supported clade (bootstrap support >99), consistent with results from all single-marker analyses and reflecting their placement in different subgenera. This concordance indicates that plastid signal across loci is sufficient for resolving deeper splits within Salicaceae.

Within *Populus*, the concatenated tree recovered *P. nigra*, *P. alba*, and *P. tremula* as a closely related group, while *P. euphratica* was resolved as a distinct lineage sister to this cluster with high bootstrap support. This topology is broadly consistent with the dominant signal observed across the individual plastid markers and provides a more stable representation of plastid relationships than any single locus alone. Importantly, the concatenated analysis reduces some of the marker-specific topological instability observed in the individual trees, particularly those associated with the lower phylogenetic resolution of *rbcL*, while still reflecting the underlying plastid history.

Nevertheless, the concatenated plastid phylogeny should be interpreted cautiously. As a maternally inherited genome, plastid DNA represents a single evolutionary history and may be influenced by processes such as chloroplast capture, incomplete lineage sorting, and historical hybridization, all of which are well documented in *Populus* and *Salix* (*Tsitrone, Kirkpatrick & Levin, 2003*; *Hamzeh & Dayanandan, 2004*; *Wang et al., 2014*; *Jelic et al., 2015*). The placement of *P. euphratica* as sister to the remaining *Populus* species in the concatenated tree is consistent with plastid-based ambiguities reported in previous studies (*Chen et al., 2010*; *Wang et al., 2014*; *Lauron-Moreau et al., 2015*) and may reflect retained ancestral polymorphism or historical introgression rather than true species relationships.

When considered together, the individual and concatenated plastid phylogenies demonstrate that combining barcode regions improves support for deeper nodes and genus-level relationships but does not fully resolve species-level relationships within *Populus*. This outcome underscores the limitations of plastid data alone for closely related and hybridizing lineages and highlights the importance of integrating plastid barcodes with nuclear markers. In this study, congruence between the concatenated plastid topology and nuclear SSR patterns—particularly the distinctiveness of *P. euphratica* and the close relationship between *P. nigra*, *P. alba*, and *P. tremula*—supports the value of a multilocus framework while reinforcing the need for genome-wide nuclear data to achieve robust species-level phylogenetic inference in Salicaceae (*Sanderson et al., 2023*).

Consistency with broader phylogenomic evidence and regional contribution

The broader literature consistently supports *Populus* and *Salix* as monophyletic sister genera (*Wang et al., 2014*; *Lauron-Moreau et al., 2015*; *Sanderson et al., 2023*; *Ogutcen et al., 2024*), despite earlier morphology-based interpretations suggesting overlap or convergence (*Eckenwalder, 1996*). The present study does not aim to revise these well-established relationships; rather, it contributes regionally focused molecular data from Türkiye. The plastid and SSR results are consistent with these multilocus and phylogenomic studies in recovering clear separation of *Populus* and *Salix*. Patterns of SSR allele sharing across some loci, alongside the absence of shared alleles at others, further support close relatedness as sister lineages while reflecting post-divergence differentiation.

Implications, limitations, and future directions in a Turkish context

From a conservation perspective, the results are relevant to Türkiye's riparian ecosystems, which are increasingly affected by hydrological alteration and climate-driven stress. *Populus* and *Salix* function as foundation taxa in riverbank stabilization, hydrological regulation, and habitat formation, making the maintenance of genetic diversity a practical management priority. The pronounced distinctiveness of *P. euphratica* highlights the importance of conserving populations that may harbor adaptive traits associated with drought and salinity tolerance under intensifying aridity. More broadly, the lineage-specific alleles and genetically cohesive clusters observed here can inform seed sourcing, restoration planning, and germplasm conservation by emphasizing locally adapted gene pools.

At the same time, the scope of inference remains limited. Only six of the more than thirty Salicaceae species native to Türkiye were included, and uneven SSR amplification across taxa reduced marker comparability. The sampling strategy targeted species-level representation rather than population-level structure, precluding robust population genetic analyses and making population-level phylogenies methodologically inappropriate for the current dataset. Broader taxonomic and geographic sampling within Türkiye—combined with genome-wide markers and ecological correlates—will be essential for evaluating the generality of these patterns and for resolving the roles of hybridization, historical demography, and environmental gradients in shaping Salicaceae diversity. In this context, Anatolia's recognized role as a major Pleistocene refugium and secondary contact zone for temperate trees (*Albayrak et al., 2024*) underscores the value of incorporating Turkish populations into future Eurasian-scale evolutionary analyses.

CONCLUSIONS

This study presents a regionally focused assessment of species-level phylogenetic relationships among native *Populus* and *Salix* taxa occurring in major river systems of Türkiye. By integrating nuclear SSR markers with plastid DNA barcodes, the analysis evaluates marker transferability, identifies conserved and lineage-specific alleles, and reveals patterns of genetic similarity among locally adapted species. Although the findings are consistent with established phylogenetic frameworks for the Salicaceae, they contribute

novel molecular data from a biogeographically underrepresented region and emphasize the importance of incorporating local populations into broader systematic and conservation-oriented research. The study does not aim to address global phylogenetic relationships within the family but instead helps fill geographic and molecular gaps in existing datasets. By highlighting regional genetic differentiation and marker-specific performance, the results provide a foundation for future investigations. Expanded taxonomic sampling and the application of genome-wide markers across Türkiye will be essential to further elucidate patterns of diversification, adaptive variation, and evolutionary dynamics in these ecologically and economically important riparian tree lineages.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Funda Özdemir Değirmenci conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (*i.e.*, approving body and any reference numbers):

The plant specimens used in this study were collected under official field collection permits issued by the Republic of Türkiye Ministry of Agriculture and Forestry. These permits were obtained during earlier nationally funded research projects supported by TÜBİTAK (The Scientific and Technological Research Council of Türkiye), a national funding agency for scientific research.

All fieldwork was conducted in accordance with national regulations. Although these projects included large-scale sampling efforts, only a subset of the collected specimens relevant to this study were selected and used in the current manuscript.

The samples used here were collected within the scope of the following TÜBİTAK projects, all of which included official field permit approval:

- TÜBİTAK TOVAG 110O570 (2011–2014)
- TÜBİTAK TOVAG 213O154 (2014–2017)
- TÜBİTAK KBAG 117Z018 (2017–2020)

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DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

The matK (PX101357–PX101362), trnH-psbA (PX101363–PX101368), and rbcL (PX093668–PX093673) sequences described in this study are available at GenBank.

Data Availability

The following information was supplied regarding data availability:

The clean sequence data for the matK, rbcL, and trnH-psbA regions used in phylogenetic analyses are available in the Supplemental Files.

The matK (PX101357–PX101362), trnH-psbA (PX101363–PX101368), and rbcL (PX093668–PX093673) sequences described in this study are available at GenBank.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.20936#supplemental-information.

REFERENCES

Acar P, Değirmenci FO, Duman H, Kaya Z. 2022. Molecular phylogenetic analysis resolving the taxonomic discrepancies among *Salix* L. species naturally found in Turkey. *Dendrobiology* **87**:13–26 DOI 10.12657/denbio.087.002.

Acar P, Taşkıran B, Özdemir Değirmenci F, Kaya Z. 2020. Turkish Salix species: molecular phylogeny and morphology. *Forestist* **70(2)**:141–150 DOI 10.5152/forestist.2020.19029.

Albayrak T, Tunçel T, Öğe P, Tietze DT, Forcina G. 2024. Anatolia: a hotspot of avian genetic diversity in the western Palaearctic. *Diversity* **16(6)**:339 DOI 10.3390/d16060339.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215(3)**:403–410 DOI 10.1016/s0022-2836(05)80360-2.

Bonthala VS, Stich B. 2022. Genetic divergence of lineage-specific tandemly duplicated gene clusters in four diploid potato genotypes. *Frontiers in Plant Science* **13**:875202 DOI 10.3389/fpls.2022.875202.

CBOL Plant Working Group. 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America* **106(31)**:12794–12797 DOI 10.1073/pnas.0905845106.

Chen J, Sun H, Wen J, Yang Y. 2010. Molecular phylogeny of Salix (Salicaceae) inferred from three chloroplast datasets and its systematic implications. *Taxon* **59(1)**:29–37 DOI 10.1002/tax.591004.

Cicatelli A, Sala F, Yfan H, Lupi R. 2012. Genetic biodiversity and phylogenetic studies in poplar by means of the metallothionein multigene family. *The Molecular Basis of Plant Genetic Diversity* 113–134.

Çiftçi A, Karatay H, Küçükosmanoğlu F, Karahan A, Kaya Z. 2017. Genetic differentiation between clone collections and natural populations of European black poplar (*Populus nigra* L.) in Turkey. *Tree Genetics and Genomes* **13**:69 DOI 10.1007/s11295-017-1154-8.

Ciftci A, Kaya Z. 2019. Genetic diversity and structure of *Populus nigra* populations in two highly fragmented river ecosystems from Türkiye. *Tree Genetics and Genomes* **15(4)**:66 DOI 10.1007/s11295-019-1370-5.

Dakin EE, Avise JC. 2004. Microsatellite null alleles in parentage analysis. *Heredity* **93(5)**:504–509 DOI 10.1038/sj.hdy.6800545.

Değirmenci FO, Acar P, Kaya Z. 2019. Consequences of habitat fragmentation on genetic diversity and structure of *Salix alba* L. populations in two major river systems of Türkiye. *Tree Genetics and Genomes* **15(4)**:59 DOI 10.1007/s11295-019-1365-2.

Değirmenci FÖ, Çiftçi A, Acar P, Kaya Z. 2022. Genetic diversity and population structure of Salix alba across river systems in Turkey and their importance in conservation management. *Plant Ecology & Diversity* **14(5–6)**:293–304 DOI 10.1080/17550874.2022.2053601.

Dickmann DI, Kuzovkina YA. 2014. Poplars and willows of the world, with emphasis on silviculturally important species. In: Isebrands JG, Richardson J, eds. *Poplars and Willows of the World, with Emphasis on Silviculturally Important Species, Poplars and Willows: Trees for Society and the Environment*. Wallingford, UK: CABI, 8–91.

Doyle JJ, Doyle JL. 1990. A rapid total DNA preparation procedure for fresh plant tissue. *Focus* **12**:13–15.

Eckenwalder JE. 1996. Systematics and evolution of *Populus*. In: Stettler RF, Bradshaw HDK Jr, Heilman PE, Hinckley TM, eds. *Biology of Populus and its Implications for Management and Conservation*. Ottawa, Canada: NRC Research Press, National Research Council of Canada, 7–32.

Fazekas AJ, Burgess KS, Kesanakurti PR, Graham SW, Newmaster SG, Husband BC, Percy DM, Hajibabaei M, Barrett SC. 2008. Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *PLOS ONE* **3(7)**:e2802 DOI 10.1371/journal.pone.0002802.

Fussi B, Lexer C, Heinze B. 2010. Phylogeography of *Populus alba* (L.) and *Populus tremula* (L.) in Central Europe: secondary contact and hybridisation during recolonisation from disconnected refugia. *Tree Genetics & Genomes* **6(3)**:439–450 DOI 10.1007/s11295-009-0262-5.

Hampe A, Petit RJ. 2005. Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters* **8(5)**:461–467 DOI 10.1111/j.1461-0248.2005.00739.x.

Hamzeh M, Dayanandan S. 2004. Phylogeny of Populus using chloroplast and nuclear markers. *Canadian Journal of Botany* **82(9)**:263–272 DOI 10.3732/ajb.91.9.1398.

Hebert PD, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* **270(1512)**:313–321 DOI 10.1098/rspb.2002.2218.

Hollingsworth PM. 2011. Refining the DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America* **108(49)**:19451–19452 DOI 10.1073/pnas.1116812108.

Hou L, Zhang G, Lu D, Hu J, Jia H. 2019. Analysis of the genetic diversity and population structure of *Salix psammophila* based on phenotypic traits and simple sequence repeat markers. *PeerJ* **7(1)**:e6419 DOI 10.7717/peerj.6419.

IPGC. 2016. International Populus genome consortium [online]. *Available at https://phytozome-next.jgi.doe.gov/info/Ptrichocarpa_v3_0* (accessed 2020).

Jansson S, Douglas CJ. 2007. *Populus*: a model system for plant biology. *Annual Review of Plant Biology* **58(1)**:435–458 DOI 10.1146/annurev.arplant.58.032806.103956.

Jelic M, Patenkovic A, Skoric M, Misic D, Kurbalija Novicic Z, Bordács S, Várhidi F, Vasić I, Benke A, Frank G, Šiler B. 2015. Indigenous forest of European black poplar along the Danube River: genetic structure and reliable detection of introgression. *Tree Genetics and Genomes* **11**:89.

Kansu C, Kaya Z. 2020. Genetic diversity of marginal populations of *Populus euphratica* Oliv. from highly fragmented river ecosystems. *Silvae Genetica* **69(1)**:139–151 DOI 10.2478/sg-2020-0019.

Karrenberg S, Edwards PJ, Kollmann J. 2002. The life history of Salicaceae living in the active zone of floodplains. *Freshwater Biology* **47(4)**:733–748 DOI 10.1046/j.1365-2427.2002.00894.x.

Kaya Z, Taşkıran B, Uluğ A, Güvendiren A, Wegrzyn J, Neale D. 2023. Association genetics of wood traits in European black poplar (*Populus nigra* L.). *Tree Genetics & Genomes* **19**:16 DOI 10.1007/s11295-023-01612-y.

Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH. 2005. Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America* **102(23)**:8369–8374 DOI 10.1073/pnas.0503123102.

Kuzovkina YA, Volk TA. 2009. The characterization of willow (*Salix* L.) varieties for use in ecological engineering applications: coarse screening and field performance. *Ecological Engineering* **35(8)**:1178–1189 DOI 10.1016/j.ecoleng.2009.03.010.

Lauron-Moreau A, Pitre FE, Argus GW, Labrecque M, Brouillet L. 2015. Phylogenetic relationships of American willows (*Salix* L., Salicaceae). *PLOS ONE* **10(4)**:e0121965 DOI 10.1371/journal.pone.0121965.

Liu G, Xie Y, Zhang D, Chen H. 2018. Analysis of SSR loci and development of SSR primers in Eucalyptus. *Journal of Forestry Research* **29(2)**:273–282 DOI 10.1007/s11676-017-0434-3.

Liu X, Wang Z, Wang W, Huang Q, Zeng Y, Jin Y, Li H, Du S, Zhang J. 2022. Origin and evolutionary history of Populus (Salicaceae): further insights based on time divergence and biogeographic analysis. *Frontiers in Plant Science* **13**:1031087 DOI 10.3389/fpls.2022.1031087.

Liu X, Wang Z, Wang D, Zhang J. 2016. Phylogeny of *Populus-Salix* (Salicaceae) and their relative genera using molecular datasets. *Biochemical Systematics and Ecology* **68**:210–215 DOI 10.1016/j.bse.2016.07.016.

Newmaster SG, Fazekas AJ, Ragupathy S. 2006. DNA barcoding in land plants: evaluation of *rbcL* in a multigene tiered approach. *Canadian Journal of Botany* **84(3)**:335–341 DOI 10.1139/b06-020.

Ogutcen E, de Lima Ferreira P, Wagner ND, Marinček P, Vir Leong J, Aubona G, Cavender-Bares J, Michálek J, Schroeder L, Sedio BE, Vašut RJ, Volf M. 2024. Phylogenetic insights into the Salicaceae: the evolution of willows and beyond. *Molecular Phylogenetics and Evolution* **199(4)**:108161 DOI 10.1016/j.ympev.2024.108161.

Percy DM, Argus GW, Cronk QC, Fazekas AJ, Kesanakurti PR, Burgess KS, Husband BC, Newmaster SG, Barrett SC, Graham SW. 2014. Understanding the spectacular failure of DNA barcoding in willows (Salix): does this result from a trans-specific selective sweep? *Molecular Ecology* **23(19)**:4737–4756 DOI 10.1111/mec.12837.

Perdereau AC, Kelleher CT, Douglas GC, Hodkinson TR. 2014. High levels of gene flow and genetic diversity in Irish populations of *Salix caprea* L. inferred from chloroplast and nuclear SSR markers. *BMC Plant Biology* **14(1)**:202 DOI 10.1186/s12870-014-0202-x.

Porth I, Goessen R, Heinze B. 2024. Poplar genomics: an introduction. In: *The Poplar Genome*. Cham: Springer International Publishing, 1–31.

Rajput V, Minkina T, Yaning C, Sushkova S, Chapligin V, Mandzhieva S. 2016. A review on salinity adaptation mechanism and characteristics of *Populus euphratica*, a boon for arid ecosystems. *Acta Ecologica Sinica* **36(6)**:497–503 DOI 10.1016/j.chnaes.2016.08.001.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**:406–425 DOI 10.1093/oxfordjournals.molbev.a040454.

Sanderson BJ, Gambhir D, Feng G, Hu N, Cronk QC, Percy DM, Freaner FM, Johnson MG, Smart LB, Keefover-Ring K, Yin T, Ma T, DiFazio SP, Liu J, Olson MS. 2023. Phylogenomics reveals patterns of ancient hybridization and differential diversification that contribute to phylogenetic conflict in willows, poplars, and close relatives. *Systematic Biology* **72(6)**:1220–1232 DOI 10.1093/sysbio/syad042.

Selkoe KA, Toonen RJ. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters* **9(5)**:615–629 DOI 10.1111/j.1461-0248.2006.00889.x.

Shaw J, Lickey EB, Schilling EE, Small RL. 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(3)**:275–288 DOI 10.3732/ajb.94.3.275.

Slavov G, Zhelev P. 2010. Salient biological features, systematics, and genetic variation of *Populus*. In: Jansson S, Bhalerao R, Groover A, eds. *Genetics and Genomics of Populus*. New York, NY, USA: Springer, 15–38 DOI 10.1007/978-1-4419-1541-2_2.

Smulders MJM, Beringen R, Volosyanchuk R, Vanden Broeck A, van der Schoot J, Arens P, Vosman B. 2008. Natural hybridisation between *Populus nigra* L. and *P.* × *canadensis* Moench. Hybrid offspring competes for niches along the Rhine River in the Netherlands. *Tree Genetics and Genomes* **4(4)**:663–675 DOI 10.1007/s11295-008-0141-5.

Smulders MJM, Van Der Schoot J, Arens P, Vosman B. 2001. Trinucleotide repeat microsatellite markers for black poplar (*Populus nigra* L.). *Molecular Ecology Notes* **1(3)**:188–190 DOI 10.1046/j.1471-8278.2001.00071.x.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* **38(7)**:3022–3027 DOI 10.1093/molbev/msab120.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22(22)**:4673–4680 DOI 10.1093/nar/22.22.4673.

Tokdemir Y, Özdemir Değirmenci F, Uluğ A, Acar P, Kaya Z. 2023. Genetic diversity structure of *Salix caprea* L. populations from fragmented riparian habitats. *Research Square* **7(4)**:15 DOI 10.21203/rs.3.rs-2816148/v1.

Tsitrone A, Kirkpatrick M, Levin DA. 2003. A model for chloroplast capture. *Evolution Education and Outreach* **57(8)**:1776–1782 DOI 10.1111/j.0014-3820.2003.tb00585.x.

Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A, Schein J. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **313(5793)**:1596–1604 DOI 10.1126/science.1128691.

Uluğ A. 2022. Detection and confirmation of diagnostic microsatellite loci in *Populus nigra* and *Populus deltoides* to identify their hybrids (*P.* × *canadensis*). *Turkish Journal of Botany* **46(6)**:6–613 DOI 10.55730/1300-008X.2735.

Uzan Eken B, Kırdök E, Velioğlu E, Özden Çiftçi Y. 2024. Genetic variation in white poplar (*Populus alba* L.) populations as characterized by SSR markers. *Turkish Journal of Botany* **48(1)**:2 DOI 10.55730/1300-008X.2790.

Van Der Schoot J, Pospiskova M, Vosman B, Smulders MJM. 2000. Development and characterization of microsatellite markers in black poplar (*Populus nigra* L.). *Theoretical and Applied Genetics* **101(1–2)**:317–322 DOI 10.1007/s001220051485.

Velioğlu E, Bostancı YS, Akgül S. 2020. Poplars, willows, and other fast-growing species in Türkiye. In: *Country Progress Report for the International Poplar Commission, Time Period: 2016–2019*.

Wagner ND, Gramlich S, Hörandl E. 2018. RAD sequencing resolved phylogenetic relationships in European shrub willows (*Salix* L. subg. *Chamaetia* and subg. *Vetrix*) and revealed multiple evolution of dwarf shrubs. *Ecology and Evolution* **8(16)**:8243–8255 DOI 10.1002/ece3.4360.

Wang ZS, Du SH, Dayanandan S, Wang DS, Zeng YF, Zhang JG. 2014. Phylogeny reconstruction and hybrid analysis of *Populus* (Salicaceae) based on nucleotide sequences of multiple single-copy nuclear genes and plastid fragments. *PLOS ONE* **9(8)**:e103645 DOI 10.1371/journal.pone.0103645.

Zhang X, Liu L, Chen B, Qin Z, Xiao Y, Zhang Y, Yao R, Liu H, Yang H. 2019. Progress in understanding the physiological and molecular responses of *Populus* to salt stress. *International Journal of Molecular Sciences* **20(6)**:1312 DOI 10.3390/ijms20061312.