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Distribution of genetic variability in mature and progeny populations of *Abies alba* Mill. from the Polish Western and Eastern Carpathians

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Abstract: This study aimed to compare patterns in the genetic structure of 27 mature stands and the natural regeneration of *A. alba* in the Eastern and Western Carpathians within the introgression zone of two refugial lineages from the Apennine and Balkan peninsulas. The distribution of the genetic diversity of fir stands was analysed using paternally inherited chloroplast DNA and five nuclear dominant inter simple sequence repeat markers (ISSRs). The study showed that the Balkan haplotype prevailed in both parental and progeny populations, and this haplotype was found in many mature Eastern Carpathian stands. Relatively high levels of genetic diversity were found in the mature stands (effective number of alleles *Ne* = 1.517, Shannon index *I* = 0.436, expected heterozygosity *He* = 0.295) and progeny (*Ne* = 1.515, *I* = 0.436 and *He* = 0.294) of silver fir. The analysis of molecular variance (AMOVA) revealed slight differences among the mature fir stands from the Western and Eastern Carpathians, with a value of 1.1%. According to principal coordinates analysis (PCoA) and STRUCTURE analyses, the populations, including stands of mature and progeny trees, were genetically separated into two groups. Slight genetic differences between the mature and progeny populations in the Polish Carpathians indicate sound gene pool transmission, which is essential for creating new selection and breeding programmes.

Keywords: chloroplast DNA; genetic diversity; genetic lineages; silver fir; simple sequence repeat markers (ISSR)

The current range of occurrence and the associated richness of the gene pool of tree species are the results of both demographic and evolutionary processes (Nosil, Feder 2013) and human influence (Hewitt 1999). In the future, the species composition of forests will depend on the scale, pace, and effects of the forecasted climate changes, including the response of individual species to those changes (Hamrick 2004; Kremer et al. 2012). To maintain the sustainability of future stands, there is a need to protect the genetic diversity of forest reproductive material (FRM) (Labra et al. 2006; Vinceti et al. 2020). To ensure the continuous development of forests, one of the essential tasks is to renew forests artificially or promote natural regeneration. In either case, the key element determining the value of new stands is the level of genetic variability in the parental population and the transfer of this

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variability to the progeny generations. The mating patterns are influenced by the management history, stand size or tree density. It is necessary to understand the genetic variability and diversity of forest species populations and the efficiency of the gene pool transmission between generations, especially for predicting the direction of spread and potential changes in the range of occurrence in the future.

At the European scale, a shift in the range of tree species occurrences towards the northeast is predicted (Bellard et al. 2012; Bréda, Peiffer 2014). Moreover, the mountain taxa are predicted to migrate to higher altitudes to compensate for climate change (Loarie et al. 2009). The resulting change scenarios are significant in the case of species such as silver fir (*Abies alba* Mill.), which forms the major component of mountain stands of central and southern Europe (Mauri et al. 2016). The presence of European silver fir will ensure the stability of mountain stands during future climate change (Tinner et al. 2013).

Some of the main factors shaping the population structure and distribution of genetic variation of different species in Europe are the recent glaciations and post-glacial migration to the northern areas (Bosela et al. 2016). The process of post-glacial colonisation of Europe by silver fir was found to involve migration from the Apennine and Balkan peninsulas (Liepelt et al. 2002, 2009; Gömöry et al. 2004, 2012). Willis and Van Andel (2004) indicated the Czech Republic and southern Poland as potential refuges of fir 42 000 and 20 000 years ago, respectively. Populations representing the western and eastern refugial lineages may have met in Croatia, Bosnia, and the Ukrainian Carpathians (Gömöry et al. 2004, 2012; Liepelt et al. 2009). The Polish gene pool of fir is the mixture of individuals derived from two distinct refugia located in the Apennine and Balkan Peninsulas (Litkowiec et al. 2016). The Western and Eastern Carpathians are an interesting region for assessing the genetic variation in *A. alba* populations, which remain under the influence of genetic pools originating from both refugial areas (Gömöry et al. 2004, 2012; Liepelt et al. 2009; Kempf et al. 2020).

A wide range of markers has been used to assess the variability in population structure, gene flow, and phylogenetic relationships of silver fir. The abundance of genetic resources of this species was estimated using isoenzymes (Konnert, Bergmann 1995), microsatellite analyses of chloroplast (Parducci et al. 2001; Awad et al. 2014), and mitochondrial (Liepelt et al. 2002; Gömöry et al. 2012, 2004) and nuclear (Cremer et al. 2006, 2012; Cvrčková et al. 2015) DNA loci. A useful but less frequently used method is the inter-simple sequence repeat (ISSR) analysis (Zietkiewicz et al. 1994), which can detect DNA variability at different levels. This method combines the simplicity of the RAPD (random amplification of polymorphic DNA) approach with the advantages of markers, such as SSRs (simple sequence repeats) or AFLPs (amplified fragment length polymorphisms) (Woo et al. 2008).

This study aimed to compare patterns in the genetic structure of 27 mature stands and the natural regeneration of *A. alba* in the Eastern and Western Carpathians, as well as the area located close to the introgression zone of two refugial lineages of this species in the Carpathians, as indicated in the literature (Gömöry et al. 2004, 2012; Liepelt et al. 2009). This study covered the area constituting an essential base for acquiring FRM of fir. The distribution of haplotype diversity was analysed using paternally inherited plastid DNA (chloroplast DNA), while the level of variability was estimated using ISSR markers. The objective of this research was to expand and supplement the current knowledge about the genetic variability in fir populations located in a region considered strategic in terms of the adaptation and evolutionary processes of this species in the Carpathians.

MATERIAL AND METHODS

The plant material used for the genetic analyses was collected from 27 fir stands in the Polish Carpathians (Table 1, Figure 1). At each location, the material was collected from 30 individuals from the parental generation and 30 naturally regenerating individuals. The collection process is described in detail in a paper by Paluch et al. (2019). A total of 1 620 samples were collected for the analysis. The needles were lyophilised for 120 h in a LabconcoFreeZone 2.5 lyophiliser (Labconco, USA) and ground into a powder using a Retsch MM400 mill (Retsch, Germany). Genomic DNA was extracted from the obtained powder using a CTAB (cetyltrimethylammonium bromide) protocol (Dumoulin et al. 1995). The extracted DNA pellets were dissolved in 50 ml TE buffer and diluted 10× with ddH_2O. DNA concentrations were detected with a Synergy™ 2 multi-detection microplate

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Table 1. Location of the sampled *Abies alba* populations with the samples divided into populations from the Western Carpathians (w1–w18) and Eastern Carpathians (e1–e9)

No.	Name	Code	Carpathians region	Longitude (E)	Latitude (N)	Altitude (m a.s.l.)
1	Ustron 61	w1	Western	18.7937	49.6927	661
2	Ustron 5	w2		18.8363	49.7276	504
3	Ustron 74	w3		18.9771	49.7111	661
4	W. Górka 22 Ficońka	w4		19.1869	49.5852	655
5	W. Górka Lipowa	w5		19.2185	49.5898	862
6	Korbielów	w6		19.3659	49.5461	797
7	Jeleśnia Dolna	w7		19.3679	49.6971	663
8	Jeleśnia Górna	w8		19.3775	49.6970	703
9	Limanowa 69	w9		20.1975	49.7142	880
10	Limanowa Łopień 58C	w10		20.2558	49.6979	764
11	Limanowa Skalne 112C	w11		20.2831	49.6229	637
12	Limanowa 219	w12		20.2894	49.6603	888
13	Łabowiec	w13		20.8242	49.4752	877
14	Leśny Zakład Doświadczalny 120	w14		20.9240	49.3510	650
15	Leśny Zakład Doświadczalny 52	w15		20.9251	49.3913	743
16	Leśny Zakład Doświadczalny 21	w16		20.9534	49.4447	739
17	Leśny Zakład Doświadczalny 107	w17		21.0016	49.4072	764
18	Leśny Zakład Doświadczalny 96	w18		21.0350	49.4298	711
19	Komańcza	e1	Eastern	22.0513	49.2713	616
20	Baligród	e2		22.1980	49.3220	644
21	Lutowiska 52	e3		22.6029	49.3130	582
22	Lutowiska 50	e4		22.6233	49.3050	671
23	Lutowiska 69	e5		22.6597	49.2581	662
24	Lutowiska 68	e6		22.6647	49.2461	698
25	Stuposiany 218	e7		22.7155	49.1335	781
26	Stuposiany 40	e8		22.7217	49.1625	630
27	Stuposiany 54	e9		22.7331	49.1634	687

reader (BioTek Instruments, USA). The prepared samples were used as matrix DNA in polymerase chain reactions (PCRs).

cpDNA analysis. This study used the parentally inherited chloroplast DNA marker (cpDNA) described by Liepelt et al. (2002). The cpDNA haplotype is a polymorphic form of a restriction site in the CP43 protein gene belonging to photosystem II (psbC).

Amplification was conducted in a total volume of 10 µL that contained 15–20 ng of DNA template, 10× concentrated reaction buffer (Thermo Scientific 10× Dream Taq™ Green Buffer), 20 mM $MgCl_2$, 0.2 mM dNTP, 0.1 µM of each primer (primer sequences: F-5'-GGTCGTGACCAAGAAACCAC-3' (Demesure et al. 1995); R-5'-GGACAGGTTCGAAATCACGA-3' (Wakasugi et al. 1994) and 0.5 U polymerase (Thermo Scientific DreamTaq DNA Polymerase). The PCR started with the predenaturation of the DNA at 95 °C, which lasted for 5 min. In the next step, the primers were attached at 57 °C for 45 s, followed by elongation at 72 °C for 150 s. Then, 36 cycles of fragmentation for 60 s at 95 °C, primer annealing for 45 s at 57 °C, and elongation for 150 s at 72 °C were carried out. The final chain elongation was performed at 72 °C for 8 min. The PCR products obtained were digested with Hae III (BsuRI) enzyme (Thermo Fisher Scientific Inc, Waltham, MA, USA) in the presence of R buffer at 37 °C for 16 h followed by enzyme inactivation for 20 min at 80 °C. The digestion

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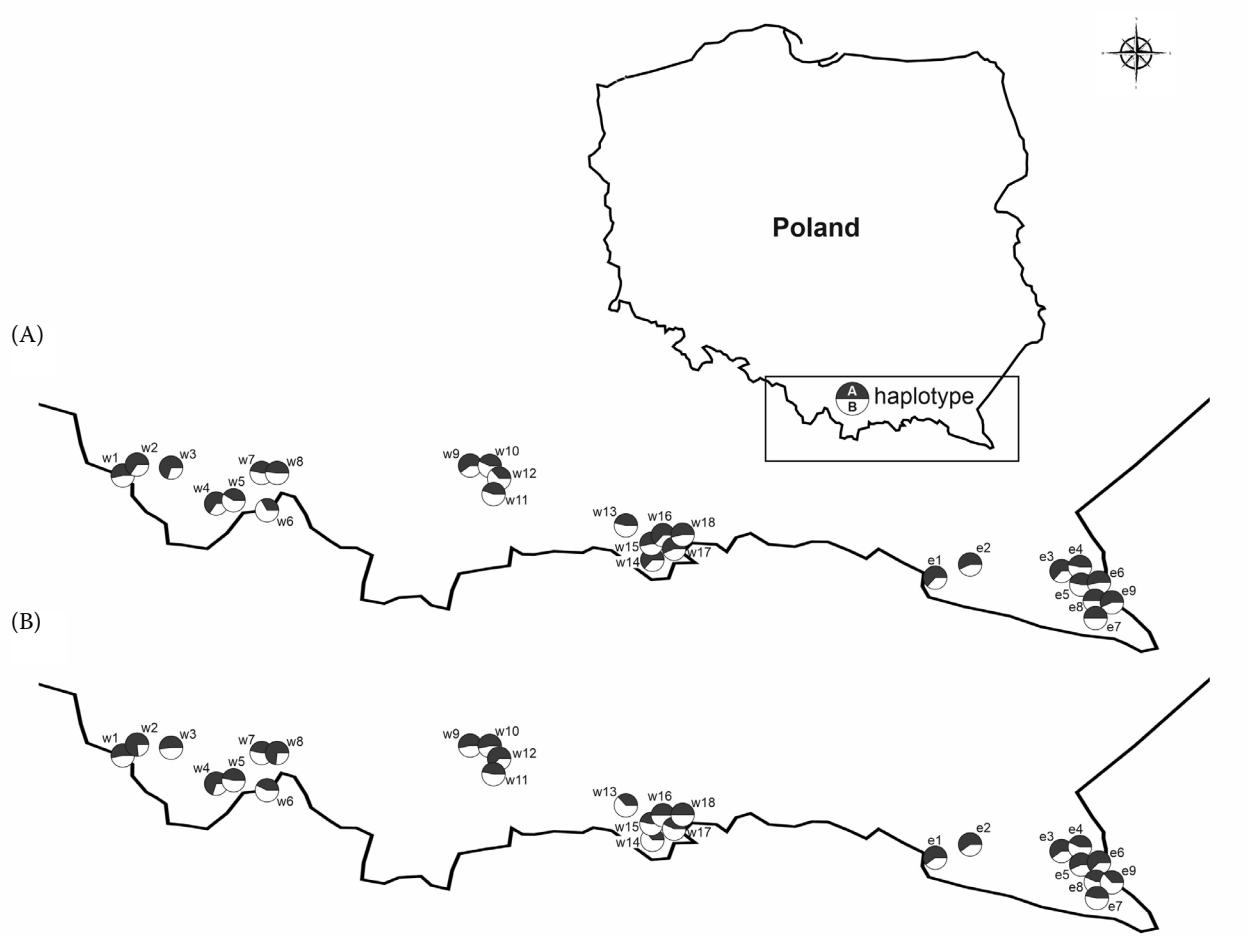

Figure 1. Locations of the 27 populations of *Abies alba* used in this study with the distribution of haplotypes in the chloroplast psbC marker in the (A) mature and (B) young stands; for population codes and the frequency distribution of chloroplast haplotypes A (Balkan refugium) and B (Apenine refugium) see in Table 1

products were stained with Midori Green Advance DNA Stain (Nippon Genetics, Europe GmbH, Duren, Germany) and separated by electrophoresis on 1.5% agarose gel (Agarose Basica LE, Prona, Burgos, Spain). The results were visualised under UV light, archived, and then read using Bio1D++ (Vilber Lourmat, France). A 100 bp Plus size marker (Thermo Fisher Scientific Inc, USA) was used as the length standard.

ISSR analysis. ISSR analysis was carried out using five primers (Table 2). DNA amplification was performed in a 10 µl mixture containing 5–20 ng

Table 2. ISSR primer information

No.	Primer name	Sequence (5'-3')	Fragment size range (bp)	Number of amplified loci	Polymorphism (%)
1	UBC815	CTC TCT CTC TCT CTC TG	500–3 000	17	100
2	UBC840	GAG AGA GAG AGA GAG AYT	370–1 870	15	100
3	UBC844	CTC TCT CTC TCT CTC TRC	400–3 000	21	100
4	UBC845	CTC TCT CTC TCT CTC TRG	290–3 000	18	100
5	UBC854	TCT CTC TCT CTC TCT CRG	580–3 000	13	100
Total				84	100

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DNA template, water, 10× concentrated reaction buffer (ThermoScientific 10× DreamTaq™ Green Buffer), 20 mM $MgCl_2$, 0.2 mM dNTP and 0.5 μM primer, 0.25 U polymerase (Thermo Scientific DreamTaq DNA Polymerase). The PCR was initiated by DNA predenaturation at 95 °C, lasting 5 min. Subsequently, 36 cycles of denaturation for 60 s at 95 °C, primer annealing for 45 s at 40 °C, and chain elongation for 150 s at 72 °C were performed. The final chain elongation was carried out at 72 °C for 480 s. The resulting PCR products were then stained with Midori Green Advance DNA Stain (Nippon Genetics, Europe GmbH, Germany) and separated by electrophoresis in 1.5% agarose gel (Agarose Basica LE, Prona, Spain). The results were visualised under UV light, archived, and then read using Bio1D++ (VilberLourmat, France). A 100-bp Plus size marker (Thermo Fisher Scientific Inc, USA) was used as the length standard. The amplified fragments were evaluated as present (1) or absent (0) at homologous bands and then transformed into a binary matrix.

Based on the binary ISSR data, the frequency of individual alleles, the percentage of polymorphic loci (*P*), the mean (*Na*) and effective (*Ne*) number of alleles in the locus, the expected heterozygosity (*He*) and the Shannon index (*I*) were calculated using GenAlEx 6.5 (Peakall, Smouse 2012). The total genetic diversity (*Ht*), intrapopulation genetic diversity (*Hs*), interpopulation genetic diversity ($Dst = Ht - Hs$), genetic differentiation coefficient ($Gst = Dst/Ht$), and gene flow $Nm = 0.5 \times [(1 - Gst)/Gst]$ were also calculated using PopGene32 v. 1.31. (Yeh et al. 1999).

To determine the difference in the observed variability between the populations of two different age groups and from two regions, an analysis of molecular variance (AMOVA) was performed. The significance of the genetic diversity was tested using 9 999 permutations. The genetic diversity between and within the populations was estimated using GenAlEx v. 6.5. The genetic distance (Nei 1972) between individuals was calculated using PopGene32v. 1.31. A dendrogram was constructed with the unweighted pair-group method of averages (UPGMA) using Statistica (Version 12, 2014). A principal coordinates analysis (PCoA) was carried out using GenAlEx v.6.5. Structure 2.3.4 (Pritchard et al. 2000), based on a Bayesian model, was used to analyse the population structure and assign individuals to populations according to ISSR genotypes. The number of reconstructed panmictic populations was estimated by testing *K* (logarithmic probability) = 1–10 for all the trees, assuming they are of unknown origin. Thirty independent runs were conducted for each *K*. The Markov Chain Monte Carlo (MCMC) sampling scheme was run for 200 000 iterations with a 150 000 burn-in period.

The admixture model was used in all the runs to estimate the number of clusters. Structure Harvester (Earl, von Holdt 2012), which implements the method of Evanno et al. (2005), was then used to estimate the most probable *K* value.

Pearson's correlation analysis used to infer relationships between geographic information about stands (i.e. latitude, longitude and altitude) and frequency of occurrence of haplotypes A and B of cpDNA in studied populations were conducted using Statistica.

RESULTS AND DISCUSSION

cpDNA. Two haplotypes were identified in the cpDNA analysis: A (eastern – Balkan type) and B (western – Apennine type) identified by Liepelt et al. (2002) and Litkowiec et al. (2016) (Figure 1). A slightly higher type A percentage was found in the parental and progeny generations (53.0% and 52.2%, respectively; Table 3). In the analysed populations, the frequency of haplotype A ranged from 33.3% (w6) to 70.0% (w3) in the mature populations and from 35.7% (w14) to 76.7% (w2) in the progeny populations. A slightly higher type A frequency was recorded in the Eastern Carpathians' parental stands than in the Western Carpathians' populations, with mean values of 54.1% and 52.5%, respectively. The study showed that the Balkan haplotype prevailed in parental and progeny populations. This haplotype was found in many mature Eastern Carpathians stands, possibly due to the proximity to the introgression zone. In a study by Litkowiec et al. (2016) on 81 fir populations throughout the range of firs in Poland, the frequency of this haplotype was observed to be very similar and amounted to 53%. The percentage of haplotypes changes along a cline from east to west, a characteristic identified by Liepelt et al. (2002) and Litkowiec et al. (2016). However, in the present study, no such correlation was observed, perhaps due to the smaller spatial scale of the analysed populations. It is worth noting that the frequency of haplotype A in some progeny populations from the Western Carpathians was as high as 70% (populations w2 and w8). This can

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Table 3. The distribution of the frequency (%) of chloroplast haplotypes A (Balkan refugium) and B (Apennine refugium) among *Abies alba* populations

No.	Code	Carpathians region	A (%)		B (%)	
			mature	young	mature	young
1	w1	Western	53.3	53.3	46.7	46.7
2	w2		65.5	76.7	34.5	23.3
3	w3		70.0	51.7	30.0	48.3
4	w4		65.5	70.0	34.5	30.0
5	w5		41.4	46.7	58.6	53.3
6	w6		33.3	42.9	66.7	57.1
7	w7		46.7	46.7	53.3	53.3
8	w8		48.3	73.3	51.7	26.7
9	w9		60.0	53.3	40.0	46.7
10	w10		43.3	53.3	56.7	46.7
11	w11		44.8	46.7	55.2	53.3
12	w12		36.7	63.3	63.3	36.7
13	w13		46.7	36.7	53.3	63.3
14	w14		63.3	35.7	36.7	64.3
15	w15		53.3	46.7	46.7	53.3
16	w16		63.3	50,0	36.7	50,0
17	w17		56.7	43.3	43.3	56.7
18	w18		53.3	50.0	46.7	50.0
19	e1	Eastern	63.3	60.0	36.7	40.0
20	e2		56.7	60.0	43.3	40.0
21	e3		63.3	60.0	36.7	40.0
22	e4		46.7	43.3	53.3	56.7
23	e5		46.7	56.7	53.3	43.3
24	e6		53.3	62.1	46.7	37.9
25	e7		50.0	46.7	50.0	53.3
26	e8		50.0	43.3	50.0	56.7
27	e9		56.7	36.7	43.3	63.3
Average		Western	52.5	52.2	47.5	47.8
		Eastern	54.1	52.1	45.9	47.9
		Total	53.0	52.2	47.0	47.8

be a result of the high volatility of pollen grains. Although the pollen grains of *A. alba* are quite large and heavy (a weight of ca. 251.1 × 10^{-6} g per 1 000 grains) and have a fast sedimentation velocity of ca. 0.12 m·s^{-1} (Pidek et al. 2013; Szczepanek et al. 2017), their range of influence could be much larger than seeds dispersal (Poska, Pidek 2010; Amm et al. 2012). Analysis of maternally inherited mitochondrial DNA completes the picture of fir genetic variation in the Carpathians. Investigations of Liepelt et al. (2002) and Gömöry et al. (2004, 2012) showed that in the modern range of *A. alba* in Europe, there are two highly conservative haplotypes, which were assigned to two glacial refugial areas, but only one western haplotype occurred in Poland (Pawlaczyk et al. 2013; Litkowiec et al. 2016; Kempf et al. 2020).

ISSR. The analysis of the selected ISSR markers showed a total of 84 alleles, and the polymorphism ratio was 100% (Table 2). The mean number of alleles per population in the mature and progeny trees was 72.18 and 72.11, respectively. The range of the number of alleles was similar in both groups (67–79 and 66–79; Table 4). The scope of extreme values was slightly lower in the parental population (64.29% for w17; 90.48% for w12) than in the progeny population (70.24% for w16; 91.67% for w10). The percentage of polymorphic loci was slightly higher in the Western Carpathians group of stands (82.67% and 82.47%) than in the Eastern Carpathians group of stands (80.42%). Similar percentages of polymorphic loci, exceeding 80% on average, were found in *Abies holophylla* using the same markers (*P* = 85.6%) (Kim et al. 2014) and *Abies nephrolepis* (*P* = 80.21%) (Woo et al. 2008). However, it should be remembered that due to their nature, ISSR markers generally give higher polymorphism values than other markers (Pradeep Reddy et al. 2002).

The mean (*Na*) and effective (*Ne*) number of alleles in the locus were estimated to be similar in the mature and progeny populations and the populations from the Eastern and Western Carpathians. The highest average number of alleles (*Na*) was observed in population w15 (1.821), which belongs to the mature stands, and population e9 (1.881), which belongs to the progeny populations. The highest average effective (*Ne*) number of alleles in the locus was observed in population w9 (1.603) (mature stands) and population w6 (1.605) (progeny generation). Similar results were reported in a study by Masternak et al. (2015). The same level of genetic diversity (indicated by the Shannon index) was observed in both the mature and progeny generations (0.436). Lower values were found in other species of the Pinaceae family. For *A. nephrolepis*, the Shannon index ranged from 0.336 to 0.396 (Woo et al. 2008); for *Pinus squamata* it was 0.03 (Zhang et al. 2005); and for *Pinus sylvestris* it ranged from 0.0857 to 0.2006 (Hui-yu et al. 2005). Higher values were recorded for *Taxus wallichiana* var. *mairei* (0.515) (Zhang et al. 2009).

The mean expected heterozygosity (*He*) in the mature stands was 0.295 (0.283 in the Eastern

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Table 4. Genetic diversity of 27 *Abies alba* populations in the Polish Carpathians based on the ISSR method

No.	Code	Carpathian region	No. of alleles		%*P*		*Na*		*Ne*		*I*		*He*	
			old	young	old	young	old	young	old	young	old	young	old	young
1	w1	W	76	76	85.71	85.71	1.762	1.762	1.561	1.543	0.466	0.463	0.317	0.312
2	w2		74	73	84.52	86.90	1.726	1.738	1.506	1.554	0.433	0.467	0.291	0.316
3	w3		76	76	89.29	85.71	1.798	1.762	1.524	1.585	0.455	0.478	0.304	0.327
4	w4		70	70	80.95	82.14	1.643	1.655	1.507	1.482	0.418	0.414	0.284	0.278
5	w5		74	70	88.10	83.33	1.762	1.667	1.535	1.515	0.456	0.431	0.306	0.291
6	w6		68	73	78.57	86.90	1.595	1.738	1.534	1.605	0.441	0.506	0.301	0.345
7	w7		68	69	80.95	82.14	1.619	1.643	1.516	1.546	0.427	0.454	0.290	0.309
8	w8		74	69	88.10	82.14	1.762	1.643	1.544	1.541	0.464	0.455	0.312	0.309
9	w9		75	71	89.29	82.14	1.786	1.667	1.603	1.551	0.484	0.444	0.332	0.304
10	w10		71	77	83.33	91.67	1.679	1.833	1.519	1.550	0.442	0.479	0.298	0.321
11	w11		70	69	83.33	82.14	1.667	1.643	1.600	1.586	0.478	0.474	0.330	0.326
12	w12		76	77	90.48	90.48	1.810	1.821	1.602	1.541	0.488	0.466	0.334	0.313
13	w13		71	72	82.14	79.76	1.667	1.655	1.534	1.475	0.444	0.407	0.301	0.273
14	w14		76	77	83.33	85.71	1.738	1.774	1.531	1.556	0.453	0.467	0.306	0.317
15	w15		79	70	88.10	71.43	1.821	1.548	1.532	1.428	0.461	0.368	0.309	0.248
16	w16		68	70	69.05	70.24	1.500	1.536	1.431	1.418	0.366	0.360	0.247	0.242
17	w17		67	76	64.29	78.57	1.440	1.690	1.444	1.467	0.362	0.406	0.248	0.272
18	w18		68	66	78.57	77.38	1.595	1.560	1.530	1.481	0.436	0.413	0.298	0.278
19	e1	E	73	72	71.43	77.38	1.583	1.631	1.459	1.486	0.384	0.418	0.261	0.281
20	e2		74	72	86.90	82.14	1.750	1.679	1.563	1.521	0.472	0.437	0.321	0.296
21	e3		73	72	77.38	77.38	1.643	1.631	1.495	1.516	0.412	0.421	0.280	0.287
22	e4		73	69	83.33	73.81	1.702	1.560	1.490	1.443	0.422	0.378	0.283	0.255
23	e5		73	70	79.76	80.95	1.667	1.643	1.458	1.532	0.403	0.448	0.268	0.304
24	e6		72	77	82.14	85.71	1.679	1.774	1.549	1.505	0.456	0.446	0.311	0.298
25	e7		75	69	85.71	73.81	1.750	1.560	1.461	1.404	0.414	0.364	0.273	0.240
26	e8		68	66	78.57	78.57	1.595	1.571	1.473	1.477	0.410	0.410	0.275	0.275
27	e9		67	79	78.57	94.05	1.583	1.881	1.481	1.604	0.413	0.503	0.278	0.341
Western			72.3	72.3	82.67	82.47	1.687	1.685	1.531	1.524	0.443	0.442	0.300	0.299
Eastern			72	71.8	80.42	80.42	1.661	1.658	1.492	1.498	0.420	0.424	0.283	0.286
Total			72.18	72.11	81.92	81.79	1.678	1.676	1.517	1.515	0.436	0.436	0.295	0.294

%*P* – percentage of polymorphic loci; *Na* – mean number of alleles per locus; *Ne* – effective number of alleles per locus; *I* – Shannon's diversity index; *He* – expected heterozygosity; W – Western Carpathians; E – Eastern Carpathians

Table 5. Results of the AMOVA (analysis of molecular variance) for 27 parental and progeny *Abies alba* populations in the Polish Western (W) and Eastern (E) Carpathians

Source	Group	df	*SS*	*MS*	Est. var.	%
Among regions W and E	mature	1	251.9	251.9	0.192	1.10***
Among populations		25	4 567.9	182.7	5.707	32.78***
Within populations		783	9 012.1	11.5	11.510	66.11***
Among regions W and E	progeny	1	191.4	191.4	0.028	0.16***
Among populations		25	4 536.8	181.5	5.667	33.01***
Within populations		783	8 981.5	11.5	11.471	66.83***

***$P < 0.001$; *SS* – sum of squares; *MS* – mean squares; est. var. – estimated variance

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Carpathians and 0.300 in the Western Carpathians), and the values of *He* ranged from 0.247 (w16) to 0.334 (w12). In the progeny populations, the mean *He* was 0.294, and the values varied from 0.240 (e7) to 0.345 (w6). A slightly higher value of expected heterozygosity (*He* = 0.396) was ob-

Table 6. Results of the AMOVA (analysis of molecular variance) for 27 paternal and progeny *Abies alba* populations

Source	df	*SS*	*MS*	Est. var.	%
Among mature and progeny	1	53.8	53.8	0.000	0
Among populations	52	9 548.0	183.6	5.737	33.3***
Within populations	1 566	17 993.7	11.5	11.490	66.7***

***$P < 0.001$; *SS* – sum of squares; *MS* – mean squares; est. var. – estimated variance

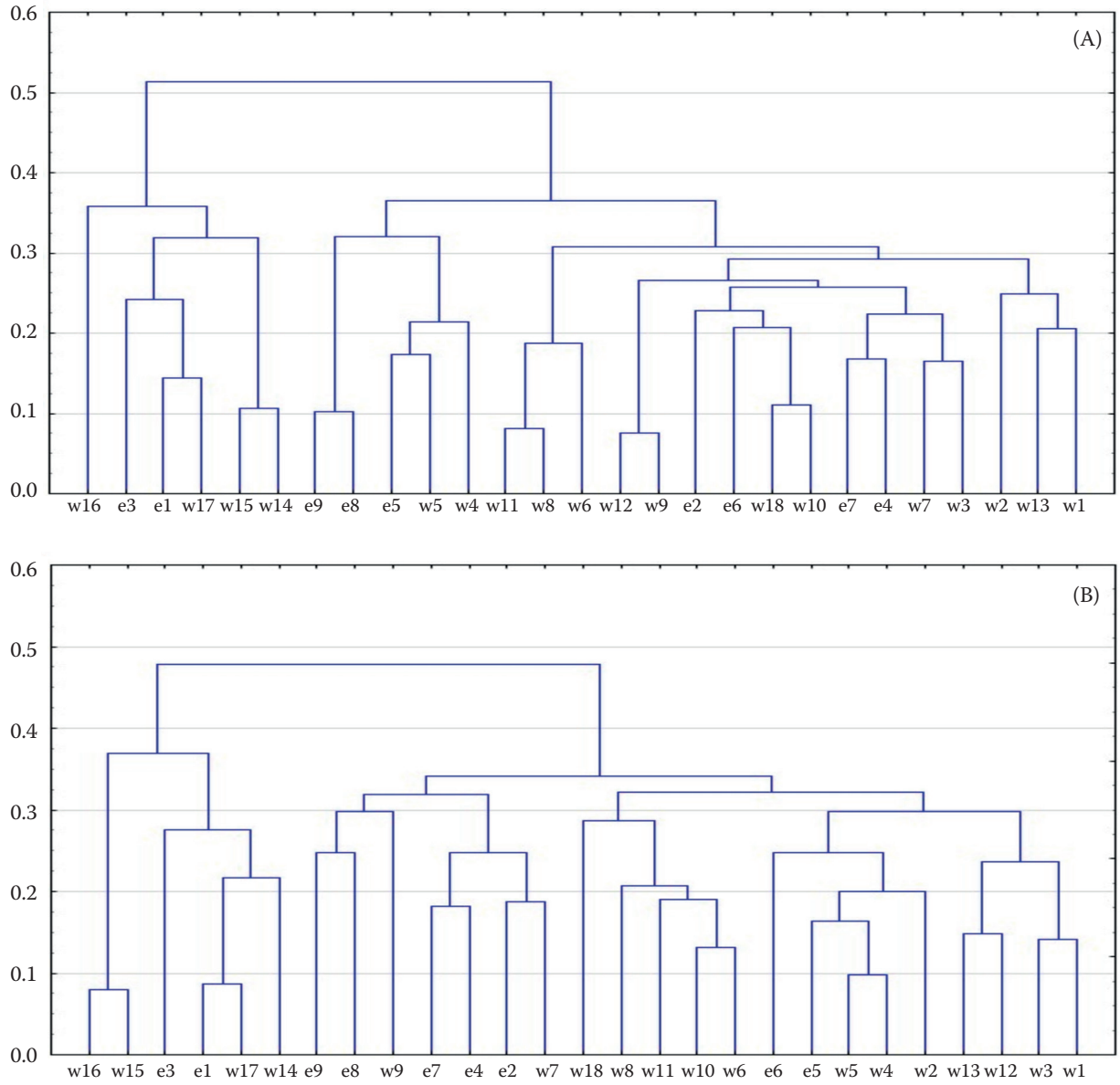

Figure 2. UPGMA dendrogram based on Nei's genetic distance of (A) mature and (B) progeny *Abies alba* populations in the Polish Carpathians; for population names see Table 1

UPGMA – unweighted pair-group method of averages

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served for *A. alba* by Masternak et al. (2015), and a similar value (*He* = 0.288) was observed for *A. holophylla* by Kim et al. (2014). Studies on other species using ISSR markers showed slightly lower values of this parameter (Meloni et al. 2006; Woo et al. 2008; Rubio-Moraga et al. 2012).

Ht, *Hs*, *Dst* and *Gst* were 0.395, 0.295, 0.101, and 0.255, respectively. The genetic differentiation among the populations demonstrated that the genetic diversity levels were low among populations (39.53%) and that variations mainly emerged within populations (60.47%). The average value of gene flow (*Nm*) among the populations was 1.464, indicating that a certain level of gene flow can lead to slight genetic differences among the populations.

In the parental stands, the AMOVA (Table 5) revealed the highest level of variability residues between the individuals within a population (66.1%), moderate variability between the populations (32.8%), and slight variability between the populations from the Eastern and Western Carpathians (1.1%). In the progeny trees, the variability between and within populations concerning age group and regions of occurrence was similar (66.8%, 33.0%, and 0.16%, respectively). All the comparisons were statistically significant ($P < 0.001$). No sig-

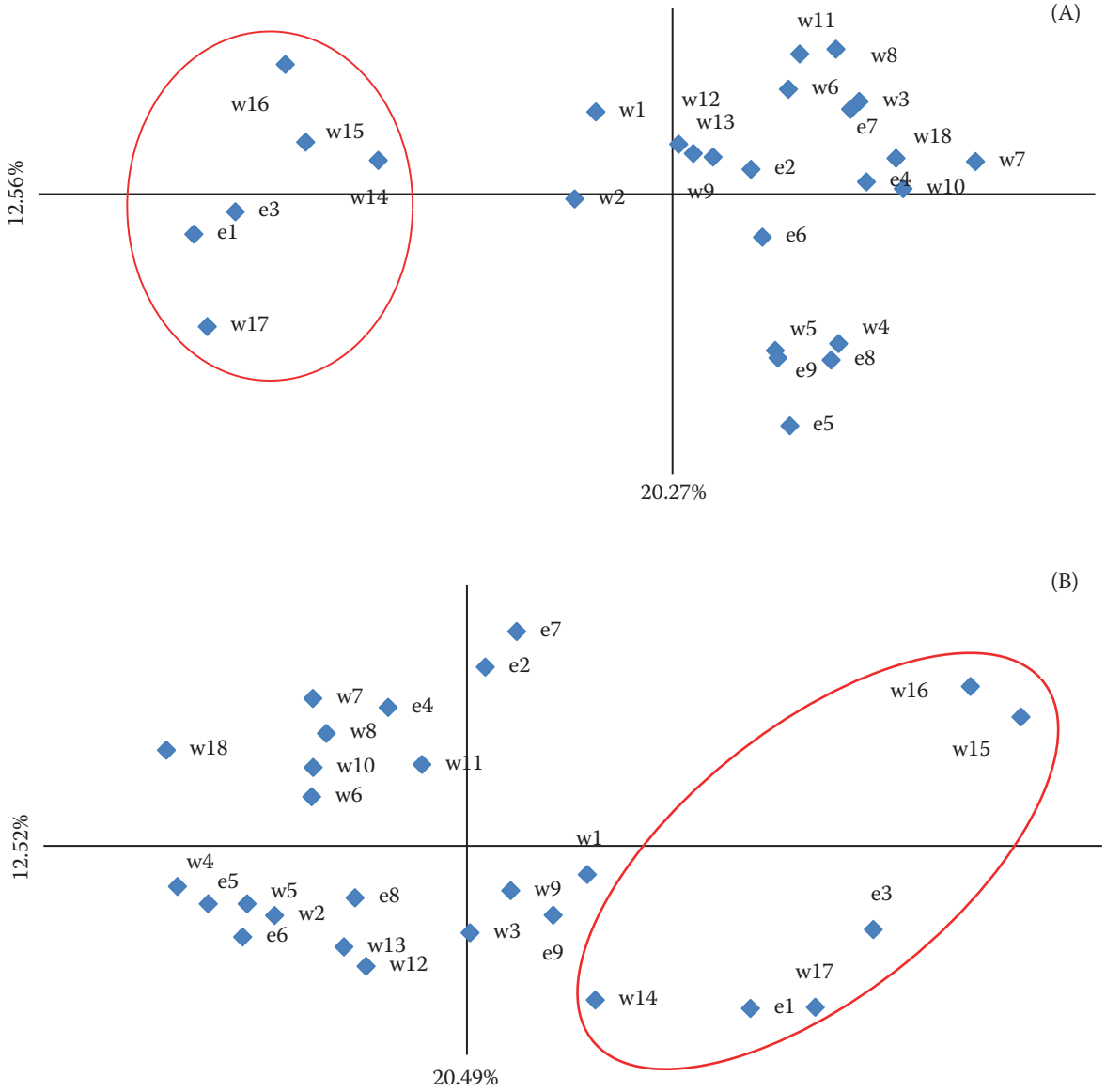

Figure 3. Biplot of a principal coordinates analysis (PCoA) of (A) mature and (B) progeny *Abies alba* populations in the Carpathian Mountains; for population names see Table 1

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nificant differences in the variability in individuals were found between the mature and progeny populations (Table 6). The interpopulation variability remained at 33.3%, and the intrapopulation variability remained at 66.7% (in both cases, $P < 0.001$). High genetic similarity was also found between the parental and progeny populations in *Picea asperata* by Wang et al. (2010), in *Picea abies* by Nowakowska et al. (2014) and *Pinus silvestris* by Kosinska et al. (2007).

The UPGMA tree revealed that populations w14–w17, e1 and e3 clustered in separate groups in the mature and progeny populations of *A. alba* from the Carpathian Mountains (Figure 2). Comparable results were obtained in the PCoA analysis, which clearly distinguished the same mature stands. The result was the same in the progeny generation, except that population w14 was separated from the other populations (Figure 3).

The Structure analysis showed the existence of two clusters ($K = 2$) in both the parental and progeny populations (Figure 4). The average membership of cluster 1 (blue) was similar in both groups and amounted to 0.654 and 0.688, while cluster 2 (orange) membership was 0.346 and 0.312, respectively. The average membership of cluster 1 was 0.614 and 0.707, and that of cluster 2 was 0.386 and 0.293, respectively, in the parental and progeny populations from the Western Carpathians, while for the Eastern Carpathians, the share of cluster 1 was 0.733 and 0.650, and that of cluster 2 was 0.267 and 0.350, respectively. A significant share of cluster 2, exceeding 90%, was observed in the parental generation in pop-

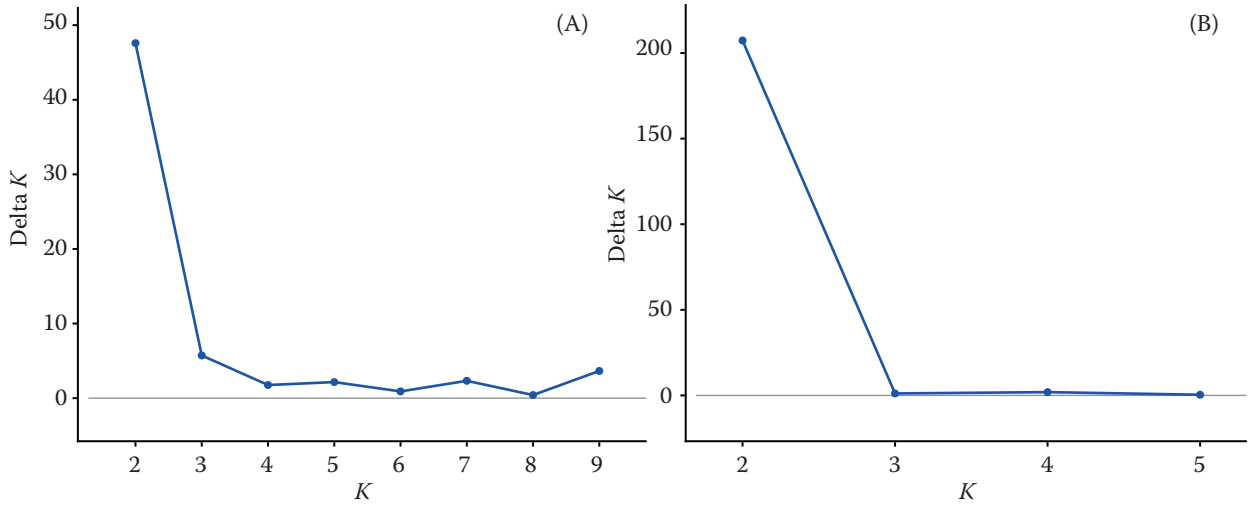

Figure 4. Delta *K* for the (A) mature and (B) young populations of *Abies alba* in the Carpathian Mountains

K – number of clusters

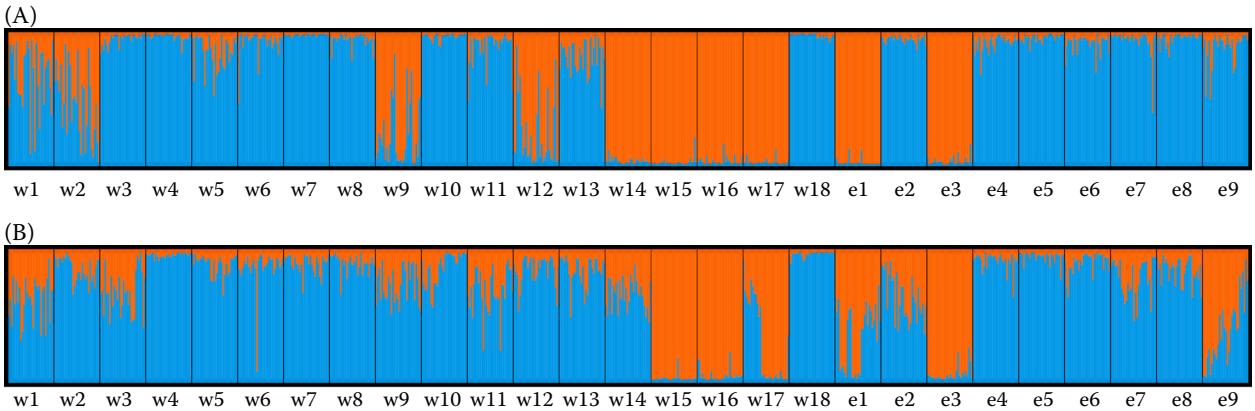

Figure 5. STRUCTURE bar plots for (A) mature and (B) young individuals ($K = 2$); for location names see Table 1.

Vertical bars – individuals; blue – cluster 1; orange – cluster 2; *K* – number of clusters

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ulations w14, w15, w16, w17, e1, and e3. In the progeny generation, the distribution of the two distinguished clusters was similar, while a high share of the second cluster, over 90%, was recorded in populations w15, w16, and e3 (Figure 5).

The correlation analysis showed a statistically significant relationship between the occurrence of haplotype B in the mature stands and altitude above sea level (r^2 = 0.2769, P = 0.0048). A similar relationship was observed in the progeny generation, but it was less clear and statistically insignificant (r^2 = 0.11, P = 0.09). No significant correlations were found between the haplotype frequency and the longitude or latitude in the mature or progeny populations. The analysis of the correlation between the ISSR clusters distinguished in the Structure analysis and cpDNA haplotypes showed a significant relationship between the share of cluster 1 and haplotype B cpDNA and the share of cluster 2 and haplotype A cpDNA in the mature stands (r^2 = 0.1583, P = 0.0399).

CONCLUSION

Molecular markers are considered important in plant breeding and protection programmes (Nybom, Bartish 2000; Grover, Sharma 2016). Studying the genetic diversity of tree populations can help create appropriate strategies for managing and conserving various species.

In this study, we indicated the influence of post-glacial expansion on the distribution of fir genetic variability. The analysis of cpDNA inherited through the paternal line showed that in the studied fir stands in the Carpathians, there was a mixing of two genealogical lineages, i.e. those from the Apennine and Balkan Peninsulas. In addition, the significant positive correlation between altitude above sea level and the percentage of haplotype B (Apennine) may result from differential selection regimes between low-elevated and high-elevated populations. Major et al. (2021) indicated that elevation and, to a lesser extent, post-glacial colonisation history were the best predictors of fine-scale spatial genetic structure across populations.

The low level of interpopulation differentiation observed in this study is consistent with other findings for woody species with large geographic ranges, outcrossing systems, and wind-dispersed seeds (Loveless, Hamrick 1984; Kosinska et al. 2007). These species have greater genetic diversity within species and populations but less variation among populations than other plant species. (Hamrick et al. 1992).

The study showed no differences between the groups of mature and progeny trees. The high genetic similarity between the parental and progeny populations may be proof of their durability and good adaptation to the environmental conditions in which they grow. The results of the present study indicate that the genetic structure of fir tree stands in the Carpathian Mountains has been preserved. This understanding is crucial for predicting responses to climate change and acquiring FRM. The analysis also showed that the region of origin had almost no impact on the variability in the stands; location contributed only 1.1% of the variability in the mature stands and 0.16% in the progeny stands.

The analysis distinguished six populations, namely, w14–w17, e1, and e3, which formed separate clusters in the analysis of both the mature and progeny generations. All these populations (except for the w14 population) were characterised by a very high share of cluster 1. Moreover, w16 and w17 populations were associated with deficient levels of genetic variability, as determined by the ISSR markers in both age groups. These populations are near the border between the Eastern and Western Carpathians.

The obtained results are an important expansion to the current knowledge about the variability of fir in the Carpathians. It is essential in creating new conservation and silvicultural strategies because decision-making under future climate warming scenarios should consider different phylogenetic origins (Vinceti et al. 2020).

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