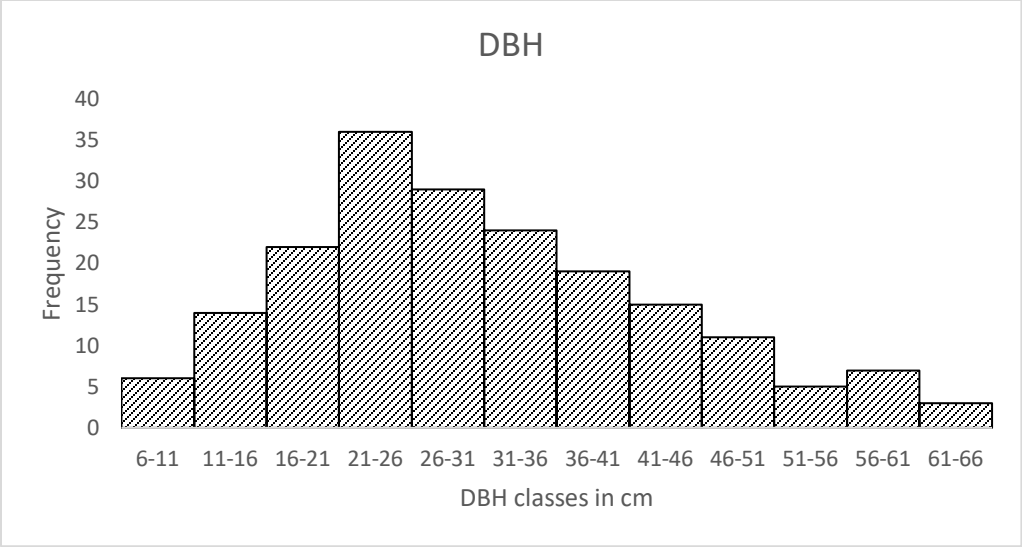


Supplementary file 1 Figure S1. DBH distribution histogram of 191 *Pinus gerardiana* trees in Paktia, Afghanistan.

Supplementary file 1 Table S1. List of EST-SSRs, genomic SSRs, and chloroplast SSRs that were tested for amplification and polymorphism in *Pinus gerardiana* samples. [a], allele size range; [b], quality class of microsatellites in *P. gerardiana*; [c], excluded from the further investigation because of missing data; [M], multiplex PCRs; 1, polymorphic; 2, monomorphic; N, no amplification; P, poor amplification; *, genomic SSRs and the expected size based on the Conifer Microsatellite Handbook (Auckland et al. 2002).

	Primer	Repeat motif	Allele size range		Sequence (5'–3')	Reference species	Quality [b]
			P.gerardiana [a]	Reference			
1	33255[M]	$(AAGGC)_5(GAG)_5$	200-220		F: TCAGCAACCAAACCATACCA R: TGCACTCGCTCCCTATCTTT	*P. bungeana*	1
2	34533[M]	$(CTCACC)_6$	265-277		F: ATCTCGGCCAATTTGTCATC R: TTGGTCCACCTTTCATCCTC	*P. bungeana*	1
3	66538[M]	$(GGGCGA)_4$	295-301		F: ATATTGATCAGGCGAGGCAG R: GGATTGTTGCAGGTTTTCGT	*P. bungeana*	1
4	24177	$(GGCTGC)_4$	262-292		F: CTGGGGAGTATGCACACCTT R: CAGTATCAACAGCAAGCCCA	*P. bungeana*	1
5	7028	$(TTC)_8$	230-251		F: AGCCATTTCTTCTGCTTCCA R: TTTTCACCCATTCTCCTTCG	*P. bungeana*	1
6	10962	$(TA)_{11}$	273-277		F: CGGCCTTTCACTTCTGGTAG R: TGCTGACAAACAAACCGAGA	*P. bungeana*	1
7	3534	$(AT)_{12}$	283-289		F: AAGCATCTGCACCTATTGGG R: GTGGAATTGAGATCGGCTGT	*P. bungeana*	1
8	72763	$(AAAACC)_4$	274-282		F: GGCAATTCTGCAGTAGCCTC R: ATGGTCTGTCCATTTCGGTG	*P. bungeana*	1
9	60339[c]	$(ACATAT)_4$	285		F: TCTCAATTAAGCGGACAAGTTACA R: AGCCCTCTGTGATTGGACTC	*P. bungeana*	1
10	5358	$(CAACCT)_4$	287		F: GCATTCGAATTCCTCTCAGC R: GCATTCGAATTCCTCTCAGC	*P. bungeana*	2
11	7309	$(CTG)_6$	245		F: TTGCTGCTGCTGTAACTGCT R: TGCAGCAGGTTCCCTCTAGT	*P. bungeana*	2
12	67970	$(GAC)_8$	220		F: AGCGAACGGATGAAAGATTG R: GTGAGGAAGCCAAGTTGGAG	*P. bungeana*	2
13	10335	$(TTGAT)_4$	288		F: CAACGTGGCCTCTGAGAAAT R: AGGGTTGCCTTCTTTCCAAT	*P. bungeana*	2
14	73317	$(TAATCC)_4$	174		F: AACGACATCGACAACGACAA R: CACGTGGTTTGTTGTGAAC	*P. bungeana*	2
15	1314	$(GAGGAA)_4$	184		F: GAGGAGGCAAATGAGCAAAG R: TTAGTCGCTTCTTTTGCGGT	*P. bungeana*	2
16	22642	$(ATT)_5(ATC)_6$	-		F: CCGTGTGCTTGATTGATCTG R: TCCTGTTTGTAATGGTTGCG	*P. bungeana*	N

17	10373	$(GCAGGG)_5$	165		F: GGCTTGCATCCATCAATTCT R: GAGGCTGGAGCTCTGTGAAC	*P. bungeana*	2
18	11371	$(AAATA)_5$	263		F: CAATTCGCGGTGTGATTATG R: TTCATCGAGCATGGAAACAG	*P. bungeana*	2
19	19808	$(CTGTG)_5$	261		F: CTCATTCTTCCGCCATTGTT R: AGAGGCACAGAACAGCACAA	*P. bungeana*	2
20	LOP1	$(TA)_{10}$	-	161-153	F: GGCTAATGGCCGGCCAGTGCT R: GCGATTACAGGGTTGCAGCCT	*P. taeda*	P
21	LOP3	$(TA)_9$	209	220-209	F: GTCTCCAGCCAGTTCACCTGC R: CAGTGGATCTGTCACCTCCTC	*P. taeda*	2
22	LOP5	$(TA)_{33}$	168	209-168	F: AGCCGTAAAAGCTATCTTGTG R: GGCATACTTACATTTTAATAA	*P. taeda*	2
23	LOP8	$(CCT)_6$	370	369-370	F: TATCCACCAGAAGGGCATC R: CGGGAGCTTTAATGATCTTGA	*P. taeda*	2
24	LOP9	$(GGC)_6$	131	142-131	F: GGATTCTCGTTGTGGCTGG R: TTGCCTTTGCACATAATATCT	*P. taeda*	2
25	LOP11	$(TA)_2T(TA)_{12}$	243	254-243	F: CCAGAAGGCTATAGTACAC R: CAACAATACAAGTAGCAATAC	*P. taeda*	2
26	ptTX3107*	$(CAT)_{14}$	-	182	F: AAACAAGCCCACATCGTCAATC R: TCCCCTGGATCTGAGGA	*P. taeda*	N
27	ptTX2123*	$(AGC)_8$	-	202	F: GAAGAACCCACAAACACAAG R: GGGCAAGAATTCAATGATAA	*P. taeda*	N
28	ptTX4001*	$(GT)_{15}$	-	224	F: CTATTTGAGTTAAGAAGGGAGTC R: CTGTGGGTAGCATCATC	*P. taeda*	N
29	ptTX2146*	$(TGC)_5CGC(TGC)_7C$ $GC(TGC)_7$	-	180	F: CCTGGGGATTTGGATTGGGTATTTG R: ACAGACCTTCCCCGTTCCTTTTATA	*P. taeda*	N
30	ptTX3025*	$(CAA)_{10}$	-	266	F: CACGCTGTATAATAACAATCTA R: TTCTATATTCGCTTTTAGTTTC	*P. taeda*	N
31	ptTX3030*	(TA)4…$(GGT)_{10}$	-	287	F: AATGAAAGGCAAGTGTCG R: GAGATGCAAGATAAAGGAAGTT	*P. taeda*	N
32	Pha6426	$(AT)_{10}$	367	349	F: TTACCTGTTGTACTGAATGGGT R: ATTGAACCTCTCTGGTTACTCC	*P. halepensis*	2
33	Pha1500	$(AT)_{16}$	-	369	F: TATGGGAGTTCTTTAACATTGC R: AATACACTTTCTCCACAGTTCC	*P. halepensis*	N
34	Pha4701	$(TA)_9$ $T(TA)_3$	391	375	F: GTACAGCAGCGACTTAATGATG R: GTATGTAATTGGAGTGCCTCTG	*P. halepensis*	2
35	Pha4497	$(CTG)_8$	342	440	F: TTGTATTGGAATTGGTAGGAAC R: AGACTCGAACCCTGTAGAAG	*P. halepensis*	2

36	Pha6062	$(TCC)_7$	343	371	F: CTCTCTTTCTCTTCGGTACTCA R: AAGTTTCAGAGGATTCACACTC	*P. halepensis*	2
37	Pt87268	$(T)_{14}$	151	165	F: GCCAGGGAAAATCGTAGG R: AGACGATTAGACATCCAACCC	*P. thunbergii*	2
38	Pt15169	$(C)_8(T)_8A(T)_8$	127	118	F: CTTGGATGGAATAGCAGCC R: GGAAGGGCATTAAGGTCATTA	*P. thunbergii*	2
39	Pt63718	$(T)_{10}$	94	93	F: CACAAAAGGATTTTTTTCAGTC R: CGACGTGAGTAAGAATGGTTG	*P. thunbergii*	2
40	Pt71936	$(T)_{16}$	150	148	F: TTCATTGGAAATACACTAGCCC R: AAAACCGTACATGAGATTCCC	*P. thunbergii*	2
41	Pt9383	$(T)_9AG(A)_{10}$	108	87	F: AGAATAAACTGACGTAGATGCCA R: AATTTTCAATTCCTTTCTTTCTCC	*P. thunbergii*	2
42	Pt26081	$(T)_{14}$	188	112	F: CCCGTATCCAGATATACTTCCA R: TGGTTTGATTCATTCGTTCAT	*P. thunbergii*	2
43	Pt30204	$(A)_{12}(G)_{10}$	181	145	F: TCATAGCGGAAGATCCTCTTT R: CGGATTGATCCTAACCATACC	*P. thunbergii*	2
44	Pt36480	$(T)_{11}$	175	147	F: TTTTGGCTTACAAAATAAAAGAGG R: AAATTCCTAAAGAAGGAAGAGCA	*P. thunbergii*	2

Supplementary file 1 Table S2. Transferability of genetic markers from related pine species to *Pinus gerardiana*.

Species	Number of markers	Amplified (%)	Polymorphic (%)
P. bungeana	19	18 (95%)	8 (42%)
P. taeda (EST-SSRs)	6	5 (83%)	-
P. taeda (genomic SSRs)	6	-	-
P. halepensis	5	4 (80%)	-
P. thunbergii	8	8 (100%)	-

Supplementary file 1 Table S3. Genetic variation over seven loci for each subplot and DBH class. N, number of samples; N_a, number of different alleles; N_{ae}, effective number of alleles; A_R (k = 28), allelic richness on a standardized sample of 28 gene copies; H_e, expected heterozygosity; H_o, observed heterozygosity; F_{is}, inbreeding coefficient; p-values of F_{is}.

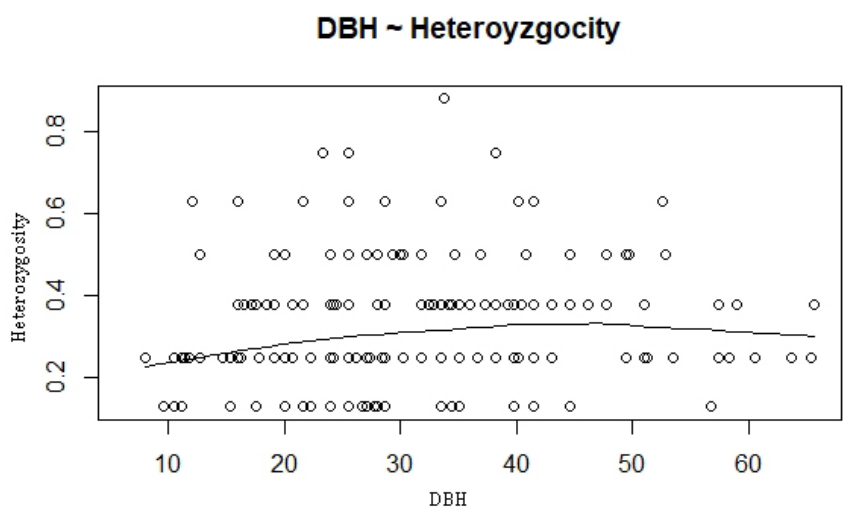
Subplot/population	**#N**	N_a	N_{ae}	A_R **(k = 28)**	H_e	H_o	F_{is}	**p value (F_{is})**
Gardiz1 (young)	29	3.86	1.67	3.28	0.350	0.304	0.135	0.350
Gardiz1 (old)	21	3.29	1.58	2.97	0.334	0.337	-0.009	0.834
Gardiz2 (young)	22	3.14	1.44	2.80	0.274	0.280	-0.022	0.738
Gardiz2 (old)	27	3.71	1.65	3.21	0.362	0.351	0.034	0.578
Gardiz3 (young)	23	3.43	1.64	3.11	0.318	0.351	-0.105	0.067
Gardiz3 (old)	27	3.57	1.60	3.14	0.340	0.361	-0.061	0.294
Gardiz4 (young)	27	3.57	1.66	3.14	0.354	0.339	0.044	0.466
Gardiz4 (old)	15	3.43	1.82	3.40	0.390	0.399	-0.023	0.747

Supplementary file 1 Table S4. Genetic variation over eight loci for each marker in the old cohort. N, number of samples; N_a, number of different alleles; N_{ae}, effective number of alleles; A_R (k = 168), allelic richness on a standardized sample of 168 gene copies; H_e, expected heterozygosity; H_o, observed heterozygosity; F_{is}, inbreeding coefficient; p values of F_{is}.

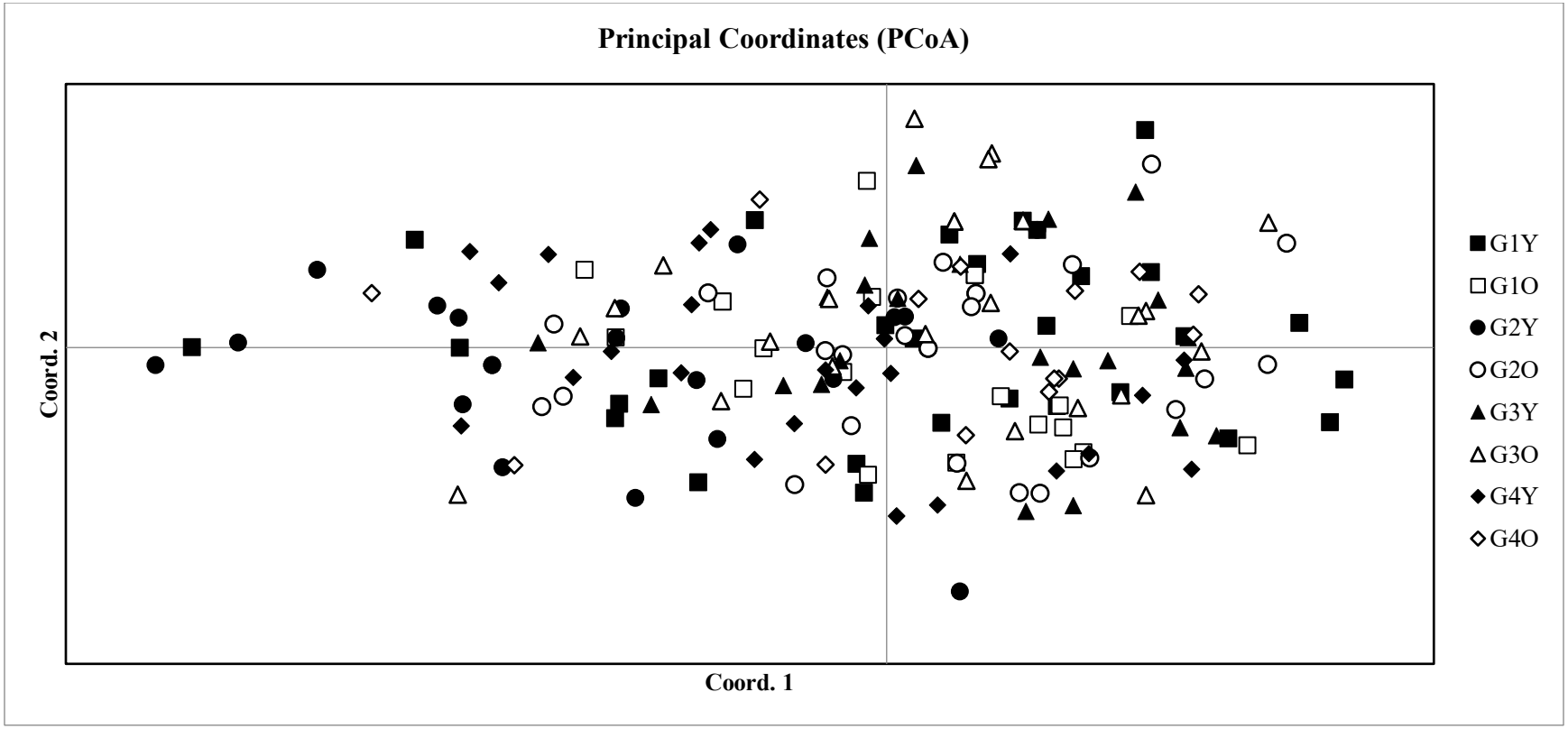
Marker	**#N**	N_a	N_{ae}	A_R **(k = 168)**	H_e	H_o	F_{is}	**p value (F_{is})**
33255	90	6	1.84	6.00	0.456	0.536	-0.175	0.008
34533	90	2	1.29	2.00	0.226	0.213	0.057	0.589
66568	90	2	1.37	2.00	0.271	0.278	-0.022	0.886
24177	90	8	2.12	7.95	0.527	0.489	0.075	0.281
7028	90	8	1.86	7.87	0.462	0.433	0.063	0.378
10962	90	3	1.68	3.00	0.405	0.420	-0.037	0.706
3534	90	4	1.44	4.00	0.305	0.306	-0.001	0.944
72763	90	2	1.15	2.00	0.130	0.140	-0.069	0.662
Mean	90	4.38	1.59	4.35	0.348	0.352	-0.010	0.748

Supplementary file 1 Table S5. Genetic variation over eight loci for each marker in the young cohort. N, number of samples; N_a, number of different alleles; N_{ae}, effective number of alleles; A_R (k = 186), allelic richness on a standardized sample of 186 gene copies; H_e, expected heterozygosity; H_o, observed heterozygosity; F_{is}, inbreeding coefficient; p-values of F_{is}.

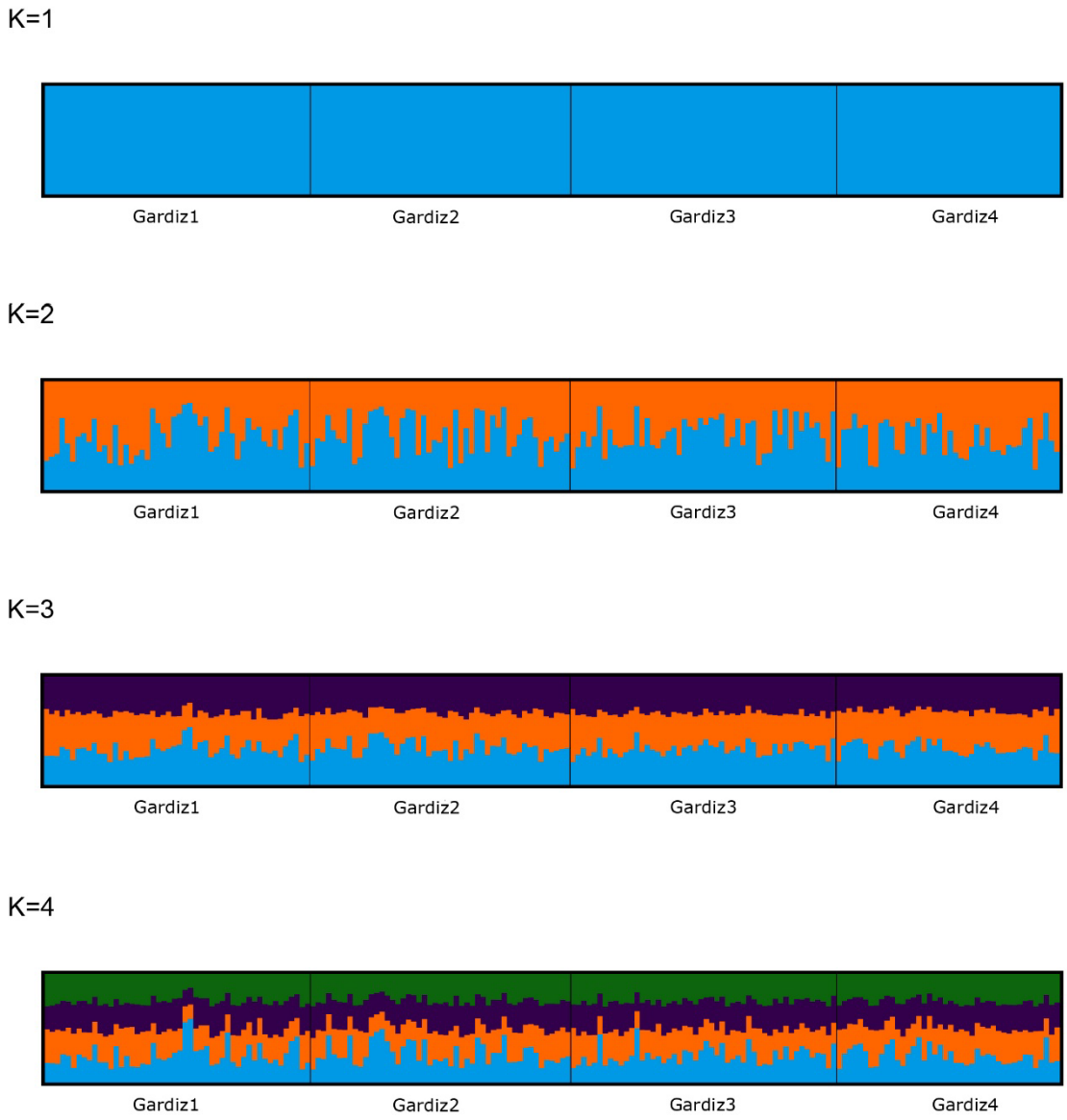
Marker	**#N**	N_a	N_{ae}	A_R **(k = 186)**	H_e	H_o	F_{is}	**p value (F_{is})**
33255	101	6	2.00	6.00	0.500	0.548	-0.095	0.150
34533	101	3	1.19	3.00	0.157	0.140	0.114	0.219
66568	101	2	1.23	2.00	0.187	0.188	-0.005	0.972
24177	101	7	2.02	7.00	0.503	0.450	0.107	0.079
7028	101	7	1.93	6.91	0.482	0.495	-0.027	0.723
10962	101	3	1.60	3.00	0.376	0.313	0.172	0.058
3534	101	4	1.44	4.00	0.304	0.221	0.274	0.002
72763	101	3	1.15	2.92	0.130	0.099	0.241	0.046
Mean	101	4.38	1.57	4.35	0.330	0.307	0.072	0.131



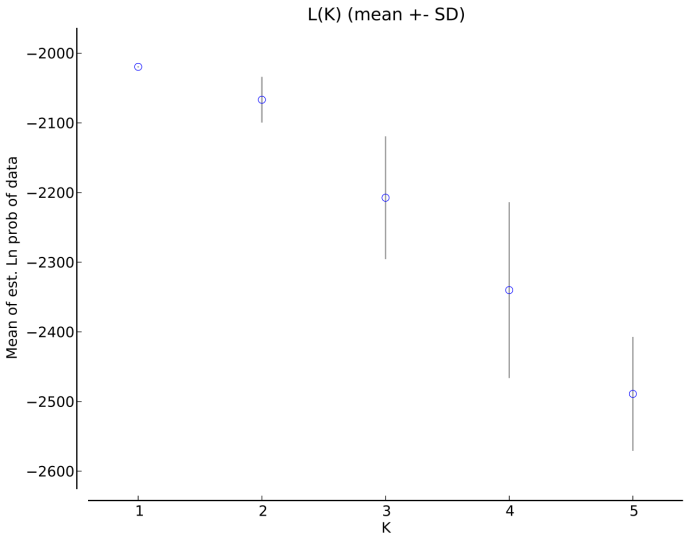
Supplementary file 1 Figure S2. Scatterplot showing the individual heterozygosity plotted against the diameter at breast height (DBH) with trend line.



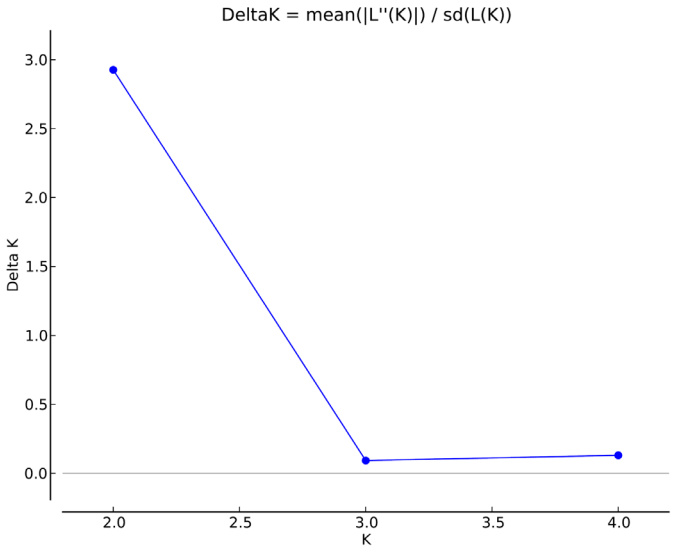
Supplementary file 1 Figure S3. PCoA based on genetic distances between all *Pinus gerardiana* samples from Paktia, Afghanistan (Peakall and Smouse 2012).



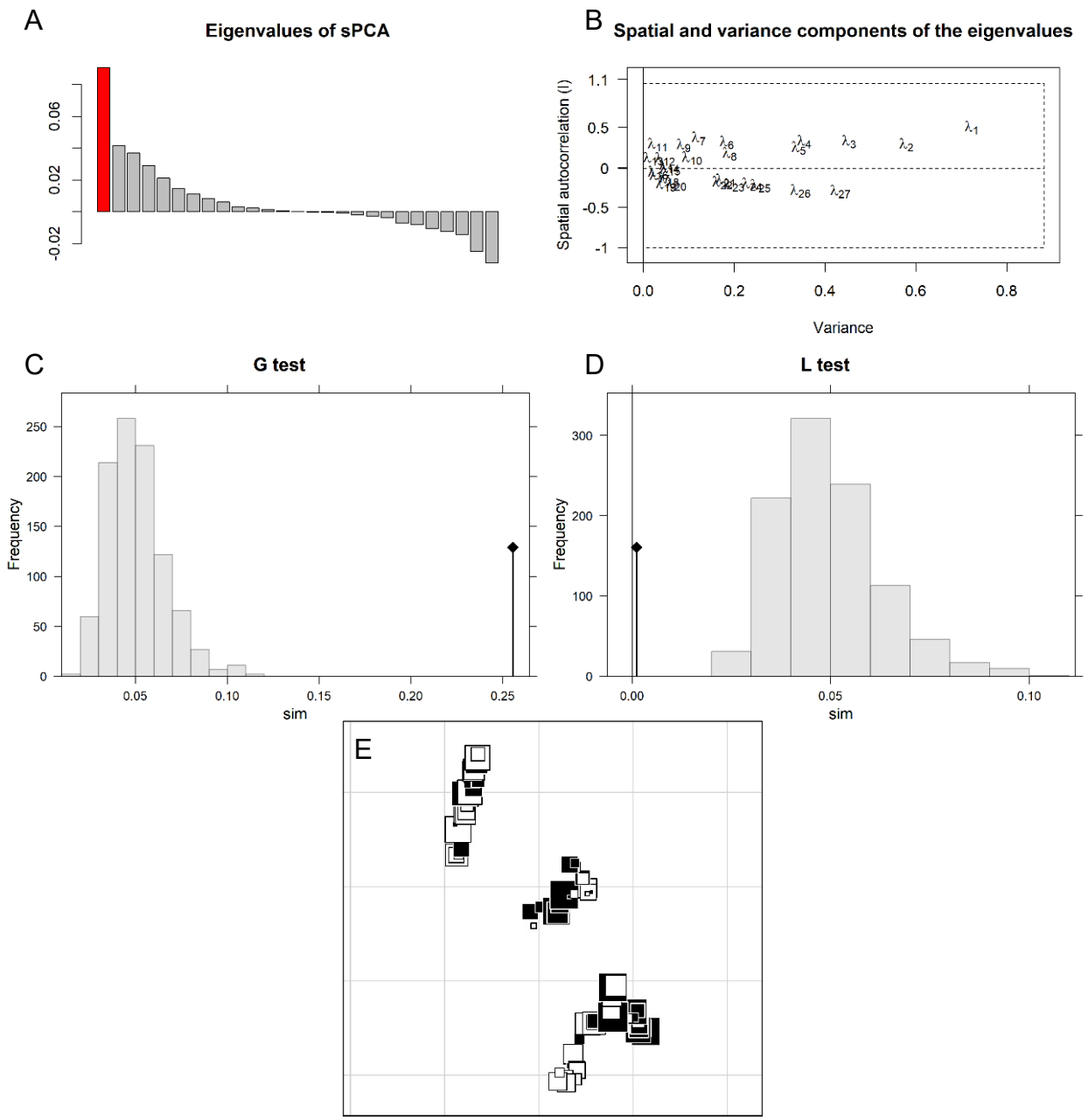
Supplementary file 1 Figure S4. Structure plots of 191 *Pinus gerardiana* samples for K = 2 and 3 sorted by sub-plot in Gardiz, Afghanistan. Structure (Burnin: 10000, MCMC: 100000, Number of Iterations: 10, K: 1–4.



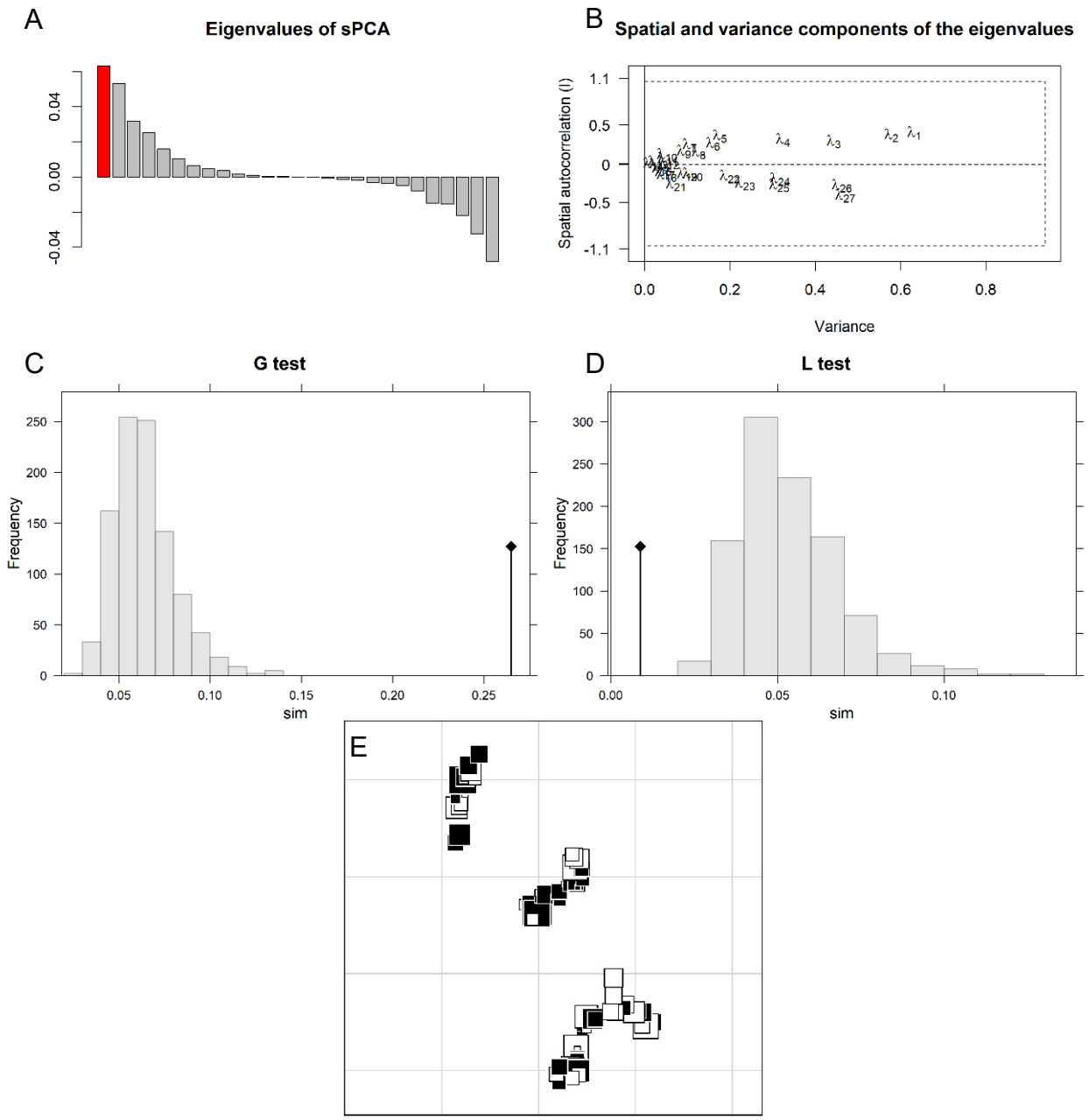
Supplementary file 1 Figure S5. Plot of mean likelihood L(K) and variance per K value from STRUCTURE software using STRUCTURE HARVESTER v.0.6.94 (Earl and vonHoldt 2012).



Supplementary file 1 Figure S6. Plot of delta K (Evanno et al. 2005) revealed by STRUCTURE HARVESTER v.0.6.94 (Earl and vonHoldt 2012).



Supplementary file 1 Figure S7. sPCA plots for the young cohort. **A**. The eigenvalues bar plot: positive eigenvalues (on the left) indicate global structure and negative eigenvalues (on the right) indicate local patterns. **B**. Decomposition of each eigenvalue into its spatial autocorrelation and variance components. **C**. G-test histogram. **D**. L-test histogram. **E**. PC1 scores plot.



Supplementary file 1 Figure S8. sPCA plots for the old cohort. **A**. The eigenvalues bar plot: positive eigenvalues (on the left) indicate global structure and negative eigenvalues (on the right) indicate local patterns. **B**. Decomposition of each eigenvalue into its spatial autocorrelation and variance components. **C**. G-test histogram. **D**. L-test histogram. **E**. PC1 scores plot.