Supplementary materials

Differentiation and taxonomic identification of roburoid oaks in the Caucasian and Crimean regions using nuclear microsatellite markers

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Table S1. Characteristics of 18 nSSR loci from kit-1 and kit-2 [20] used to study the variability of *Quercus* species

Locus	Dye	Repeat motif	Amount of primers in PCR, pmol**
kit-1			
PIE020*	FAM	AG	1.25
PIE152	FAM	TA	4.4
PIE242	R6G	TA	3.6
PIE102	R6G	CT	2.2
PIE243	R6G	AG	2.2
PIE267	TAMRA	AG	2.2
PIE215	FAM	GAG	3.6
PIE227	ROX	TGG	2.2
PIE271	ROX	TC	4.4
PIE239	ROX	AT	3.6
kit-2			
QrZAG7	FAM	TC	1.25
MsQ13	FAM	GA	1.25
QrZAG112	R6G	GA	3
QrZAG20	R6G	TC	2.6
QrZAG110	ROX	AG	5
QrZAG96	TAMRA	TC	3.75
QrZAG11	TAMRA	TC	4.4
QpZAG15	ROX	AG	5

* primer sequences and other information, see in Guichoux et al., 2011 [20].

** Amplification conditions were based on Semerikova et al., 2023 [36]. PCR was performed in 10 µL reaction mixture containing 1X buffer (67 mM Tris-HCl (pH 8.8 at 25 C), 16.6 mM $(NH_4)_2SO_4$, 0.01% Tween-20), 2.5 mM $MgCl_2$, 200µM of each dNTP, primers, 10–100 ng of genomic DNA, and 0.32 U of Taq polymerase. The amount of each primer is indicated in Table S1. Thermal profile was as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles (94°C 30 s, annealing at 60°C/56°C (kit1/kit2) for 1 min, and extension at 72°C for 45 seconds), and final elongation at 60 °C for 20 minutes. After PCR, the products were diluted with water in a ratio of 1:5 (PCR product: water), then 1 µL was added to a mixture of 8.7 µL HiDi formamide and 0.3 µL SD-450 size standard (Syntol, Russia). After denaturation for 3 min at 95°C the samples were analyzed on a Nanofor 05 automatic sequencer (Institute of Analytical Instrumentation, Russian Academy of Sciences, Russia) at the Center for Collective Use “Modern Technologies for Ecological Research” of the IPAE, Ural Branch of RAS.

Table S2. Characteristics of 18 microsatellite loci and diversity measures over loci in seven roburoid oaks taxa

Locus	Range (bp)	*A*	N_a	N_e	H_o	H_e	F_{IS}	P value	F_{ST}
Q. petraea* ssp. *petraea									
PIE020	101–113	7	4.500	3.200	0.738	0.673	-0.096	0.6184	0.074
PIE152	235–281	20	10.000	5.476	0.871	0.798	-0.091	0.3619	0.063
PIE242	107–129	12	9.250	5.764	0.843	0.820	-0.028	0.8538	0.038
PIE102	137–171	14	10.000	5.014	0.819	0.790	-0.038	0.3662	0.037(ns)
PIE243	201–235	12	7.000	4.037	0.814	0.749	-0.087	0.2679	0.042
PIE267	94–111	10	7.000	4.923	0.788	0.792	0.006	0.4386	0.017(ns)
PIE215	192–219	7	6.250	3.735	0.778	0.727	-0.070	0.5990	0.059
PIE239	86–98	5	3.250	1.583	0.181	0.353	0.489*	<0.001	0.046
PIE227	160–178	7	4.750	2.795	0.567	0.635	0.107*	0.0351	0.051
PIE271	188–212	13	7.750	3.823	0.679	0.719	0.056	0.1663	0.078
QrZAG7	116–156	21	15.250	10.056	0.929	0.899	-0.034	0.9610	0.032(ns)
MsQ13	188–230	13	7.500	4.408	0.829	0.737	-0.125	0.3996	0.065
QrZAG112	85–105	8	3.750	1.640	0.457	0.378	-0.208	0.9992	0.035
QrZAG20	161–197	16	10.750	8.010	0.848	0.872	0.027	0.1298	0.042
QrZAG96	143–185	9	11.000	6.037	0.793	0.828	0.043*	0.1370	0.074
QrZAG11	232–277	20	8.750	3.401	0.612	0.697	0.122*	<0.001	0.059
QpZAG15	111–141	13	7.500	3.590	0.665	0.717	0.072	0.3243	0.072
QpZAG110	204–256	19	12.000	7.202	0.900	0.858	-0.049	0.7924	0.036
Mean		12.6	8.125	4.705	0.728	0.725	0.005	<0.001	0.051
SE			0.409	0.276	0.025	0.018	0.035		0.004
Q. petraea* ssp. *iberica									
PIE020	101–115	6	4.250	2.893	0.728	0.647	-0.125	0.9819	0.028
PIE152	235–265	18	9.750	5.653	0.801	0.807	0.007	0.8856	0.039(ns)
PIE242	107–127	11	8.000	5.978	0.937	0.829	-0.130	0.6444	0.023(ns)
PIE102	145–173	14	8.000	3.268	0.622	0.676	0.079*	0.2131	0.033(ns)
PIE243	205–227	12	7.250	3.505	0.738	0.686	-0.077	0.5388	0.031(ns)
PIE267	94–120	10	6.250	3.498	0.631	0.706	0.107	0.4516	0.028(ns)
PIE215	192–219	10	7.500	5.633	0.820	0.820	0.000	0.2083	0.032(ns)
PIE239	86–92	5	3.000	1.276	0.200	0.210	0.048	0.2874	0.022(ns)
PIE227	154–175	7	4.750	2.666	0.674	0.617	-0.092	0.8809	0.037
PIE271	188–206	10	8.000	5.953	0.844	0.824	-0.024	0.7423	0.021(ns)
QrZAG7	116–162	20	12.500	8.708	0.895	0.876	-0.022	0.8190	0.031(ns)
MsQ13	188–250	16	9.250	6.091	0.894	0.834	-0.071	0.9987	0.051
QrZAG112	89–103	7	3.500	1.488	0.276	0.275	-0.004	0.0233	0.074(ns)
QrZAG20	157–187	14	8.750	4.876	0.791	0.784	-0.009	0.7294	0.049
QrZAG96	143–189	19	10.500	6.455	0.730	0.796	0.083*	<0.001	0.076
QrZAG11	231–271	19	9.500	4.811	0.633	0.765	0.173*	0.0433	0.070
QpZAG15	113–147	13	6.750	2.680	0.581	0.571	-0.017	0.7757	0.053(ns)
QpZAG110	204–240	15	9.250	5.762	0.790	0.824	0.041*	0.1925	0.033
Mean		12.6	7.597	4.511	0.699	0.697	-0.002	0.0433	0.041
SE			0.405	0.268	0.025	0.024	0.019		0.004
Q. macranthera									
PIE020	101–111	6	4.000	2.662	0.638	0.607	-0.050	0.8753	0.018(ns)
PIE152	233–271	15	10.667	5.829	0.821	0.819	-0.003	0.9712	0.047
PIE242	109–127	10	7.667	5.365	0.856	0.812	-0.055	0.7205	0.028
PIE102	147–169	7	5.000	2.918	0.670	0.656	-0.020	0.8798	0.022(ns)

PIE243	201–227	10	6.000	2.700	0.622	0.616	-0.009	0.4288	0.025(ns)
PIE267	96–112	6	4.667	2.560	0.552	0.605	0.087	0.7532	0.021(ns)
PIE215	152–219	9	7.000	2.810	0.676	0.639	-0.058	0.0677	0.035
PIE239	87–88	2	1.667	1.059	0.057	0.054	-0.049	-	0.019(ns)
PIE227	160–175	6	4.667	2.565	0.652	0.604	-0.080	0.4210	0.036(ns)
PIE271	188–204	8	6.000	3.082	0.663	0.670	0.010	0.2980	0.015(ns)
QrZAG7	116–162	20	13.000	9.787	0.946	0.895	-0.057	0.8606	0.038
MsQ13	192–240	15	10.667	7.358	0.909	0.861	-0.056	0.9114	0.025(ns)
QrZAG112	91–103	5	3.667	2.393	0.703	0.577	-0.217	0.5553	0.041(ns)
QrZAG20	159–189	14	9.000	5.795	0.853	0.827	-0.032	0.0426	0.020(ns)
QrZAG96	145–171	7	3.667	1.455	0.356	0.306	-0.166	1	0.039
QrZAG11	232–285	15	14.667	9.156	0.756	0.890	0.151*	<0.001	0.033(ns)
QpZAG15	113–143	12	7.000	3.923	0.686	0.735	0.066	0.2182	0.040
QpZAG110	204–240	9	6.333	3.618	0.655	0.693	0.055	0.3278	0.049
Mean		9.7	6.963	4.169	0.671	0.659	-0.027	0.2273	0.031
SE			0.513	0.350	0.030	0.029	0.020		0.002
Q. petraea* ssp. *medwediewii									
PIE020	101–121	9	6.500	2.229	0.511	0.517	0.012	0.1778	0.031(ns)
PIE152	233–281	18	11.750	5.872	0.859	0.828	-0.038	0.9939	0.011(ns)
PIE242	111–135	12	8.500	5.128	0.690	0.799	0.136*	0.0341	0.051
PIE102	147–169	10	8.000	3.959	0.796	0.729	-0.092	0.9229	0.030(ns)
PIE243	203–223	9	6.250	2.311	0.668	0.566	-0.181*	0.7195	0.017(ns)
PIE267	94–110	7	5.750	3.539	0.817	0.699	-0.169*	0.5088	0.031(ns)
PIE215	195–213	7	5.500	3.828	0.747	0.736	-0.015	0.6249	0.036
PIE239	88–104	5	3.250	2.126	0.372	0.527	0.294*	0.0062	0.043(ns)
PIE227	160–169	3	3.000	1.234	0.183	0.187	0.023	0.8600	0.009(ns)
PIE271	188–206	8	5.750	2.521	0.527	0.596	0.115*	0.2516	0.030
QrZAG7	116–152	17	11.500	7.711	0.878	0.870	-0.010	0.2363	0.029
MsQ13	208–250	10	7.000	4.275	0.766	0.760	-0.008	0.5437	0.048
QrZAG112	85–117	9	4.000	1.191	0.166	0.154	-0.072	1	0.019(ns)
QrZAG20	161–185	10	6.250	3.463	0.708	0.707	-0.002	0.4728	0.025
QrZAG96	143–187	16	11.000	7.600	0.855	0.866	0.012	0.3954	0.050
QrZAG11	232–280	24	12.250	6.351	0.668	0.834	0.199*	<0.001	0.039
QpZAG15	107–139	12	7.000	2.795	0.633	0.623	-0.016	0.3115	0.046
QpZAG110	202–242	17	9.250	4.024	0.630	0.731	0.138*	0.0122	0.030
Mean		11.3	7.361	3.898	0.637	0.652	0.018	0.0025	0.032
SE			0.358	0.242	0.027	0.025	0.029		0.003
Q. pubescens									
PIE020	101–113	6	4.600	3.180	0.716	0.683	-0.047	0.9575	0.031
PIE152	235–262	17	12.000	7.716	0.892	0.869	-0.027	0.6973	0.026(ns)
PIE242	109–131	12	8.600	5.412	0.815	0.804	-0.013	0.2118	0.047
PIE102	145–181	16	11.400	5.964	0.782	0.822	0.049*	0.1058	0.056
PIE243	205–241	7	10.400	5.364	0.806	0.803	-0.004	0.7551	0.036
PIE267	94–114	9	7.000	4.653	0.829	0.783	-0.060	0.6818	0.040
PIE215	192–225	13	10.000	6.697	0.851	0.838	-0.016	0.7532	0.027(ns)
PIE239	86–100	12	6.800	2.758	0.533	0.632	0.156*	0.0026	0.029
PIE227	154–178	8	4.200	1.860	0.432	0.427	-0.013	0.5374	0.056
PIE271	188–210	11	8.800	4.409	0.787	0.758	-0.039	0.8651	0.095
QrZAG7	116–152	17	12.400	8.252	0.927	0.863	-0.074*	0.9972	0.038
MsQ13	188–232	19	11.800	6.665	0.804	0.843	0.046*	<0.001	0.037
QrZAG112	83–119	16	10.400	6.905	0.859	0.846	-0.015	0.6627	0.029(ns)
QrZAG20	157–197	18	12.400	8.114	0.867	0.875	0.009*	0.0710	0.021(ns)

QrZAG96	143–187	22	14.400	9.369	0.801	0.881	0.092*	0.0005	0.050
QrZAG11	231–279	28	12.800	6.758	0.700	0.844	0.171*	<0.001	0.062
QpZAG15	113–139	10	5.600	2.467	0.579	0.582	0.004	0.2959	0.049
QpZAG110	204–246	19	11.200	5.865	0.857	0.826	-0.037	0.6244	0.023(ns)
Mean		14.4	9.711	5.689	0.769	0.777	0.010	<0.001	0.042
SE			0.357	0.266	0.016	0.014	0.016		0.004
Q. robur									
PIE020	97–127	11	7.400	4.758	0.888	0.786	-0.131	0.0869	0.061
PIE152	236–269	17	9.800	5.638	0.855	0.811	-0.054	0.3728	0.077
PIE242	111–135	13	8.800	5.774	0.865	0.823	-0.052	0.7861	0.020(ns)
PIE102	139–173	12	5.600	1.963	0.436	0.421	-0.036	0.9530	0.065
PIE243	207–225	9	6.200	3.003	0.695	0.660	-0.054	0.5449	0.078
PIE267	90–119	11	6.200	3.523	0.643	0.681	0.055*	0.1072	0.147
PIE215	195–210	6	5.200	3.709	0.709	0.720	0.015	0.6465	0.065
PIE239	86–102	12	6.000	3.369	0.286	0.685	0.582*	<0.001	0.095
PIE227	160–175	5	3.400	2.068	0.504	0.495	-0.018	0.7375	0.065
PIE271	186–208	12	7.000	3.346	0.762	0.661	-0.153	0.6387	0.131
QrZAG7	116–158	18	10.600	6.899	0.867	0.845	-0.026	0.1291	0.051
MsQ13	188–228	10	5.400	2.952	0.690	0.650	-0.063	0.9534	0.113
QrZAG112	77–121	21	11.600	7.175	0.902	0.860	-0.049	0.3563	0.058
QrZAG20	157–197	19	9.800	5.611	0.838	0.812	-0.031	0.8552	0.064
QrZAG96	143–181	14	7.200	3.025	0.416	0.580	0.283*	<0.001	0.073
QrZAG11	236–271	27	10.400	6.461	0.668	0.839	0.204*	<0.001	0.093
QpZAG15	107–139	10	6.200	3.359	0.705	0.684	-0.031	0.8290	0.064
QpZAG110	202–256	21	10.200	5.217	0.741	0.773	0.042	0.1656	0.063
Mean		13.8	7.611	4.325	0.693	0.710	0.027	<0.001	0.077
SE			0.299	0.204	0.023	0.016	0.041		0.007
Q. hartwissiana									
PIE020	101–115	5	4.333	2.817	0.664	0.636	-0.045	0.7996	0.104
PIE152	235–257	13	9.667	7.088	0.789	0.858	0.081*	0.5504	0.031(ns)
PIE242	109–135	14	9.000	6.181	0.712	0.833	0.146*	0.0129	0.058
PIE102	145–159	5	3.667	2.595	0.567	0.613	0.074	0.4967	0.075
PIE243	205–227	7	4.000	1.849	0.360	0.414	0.129	0.6352	0.096
PIE267	96–114	7	4.667	3.487	0.766	0.701	-0.092	0.7490	0.047
PIE215	195–204	4	3.333	2.373	0.501	0.565	0.113	0.3406	0.034(ns)
PIE239	86–88	2	1.333	1.108	0.095	0.082	-0.167	-	0.100
PIE227	154–171	6	4.667	3.173	0.855	0.681	-0.255*	0.9978	0.074(ns)
PIE271	190–202	7	5.333	4.026	0.855	0.748	-0.143	0.9722	0.047(ns)
QrZAG7	116–142	9	6.333	3.454	0.795	0.697	-0.140	0.7260	0.030(ns)
MsQ13	192–230	8	5.333	3.142	0.712	0.665	-0.071	0.2502	0.046(ns)
QrZAG112	87–117	9	5.667	3.030	0.473	0.654	0.277*	<0.001	0.051
QrZAG20	163–185	10	6.000	3.577	0.718	0.706	-0.018	0.7068	0.048(ns)
QrZAG96	143–171	9	5.333	2.623	0.560	0.612	0.085*	0.1875	0.049
QrZAG11	237–285	20	9.333	6.345	0.843	0.831	-0.013	0.6158	0.073
QpZAG15	107–123	6	3.333	1.662	0.390	0.376	-0.036	0.9830	0.046(ns)
QpZAG110	206–232	10	6.000	3.541	0.776	0.717	-0.083	0.9972	0.044
Mean		8.4	5.407	3.448	0.635	0.633	-0.009	<0.541	0.059
SE			0.339	0.234	0.033	0.027	0.031		0.005

A – total number of alleles, N_a – mean number of alleles per population; N_e – effective number of alleles; H_o – observed heterozygosity; H_e – expected heterozygosity; F_{IS}, F_{ST} – fixation indexes; *P*-value – Hardy-Weinberg equilibrium Probability test; * – deviations from the Hardy-Weinberg equilibrium based on Global test (P<0.05) (ns) – F_{ST} non-significant

Table S3. Average pairwise interpopulation F_{ST} for seven *Quercus* taxa (range in parentheses)

	Q. petraea ssp. *petraea*	*Q. p.* ssp. *iberica*	*Q. macranthera*	*Q. p.* ssp. *medwediewii*	*Q. pubescens*	*Q. robur*	*Q. hartwissiana*
Q. petraea ssp. *petraea*	0.034 (0.018-0.047)						
Q. p. ssp. *iberica*	0.051 (0.034-0.063)	0.028 (0.021-0.034)					
Q. macranthera	0.077 (0.060-0.091)	0.067 (0.051-0.096)	0.023 (0.016-0.030)				
Q. p. ssp. *medwediewii*	0.086 (0.078-0.097)	0.089 (0.070-0.106)	0.106 (0.095-0.119)	0.022 (0.014-0.028)			
Q. pubescens	0.067 (0.054-0.076))	0.067 (0.050-0.086)	0.083 (0.071-0.099)	0.078 (0.057-0.097)	0.026 (0.015-0.035)		
Q. robur	0.089 (0.076-0.102)	0.105 (0.073-0.135)	0.118 (0.098-0.147)	0.124 (0.120-0.135)	0.085 (0.074-0.097)	0.050 (0.034-0.063)	
Q. hartwissiana	0.106 (0.091-0.127)	0.109 (0.090-0.139)	0.130 (0.120-0.145)	0.166 (0.153-0.174)	0.115 (0.101-0.122)	0.086 (0.071-0.110)	0.043 (0.040-0.049)

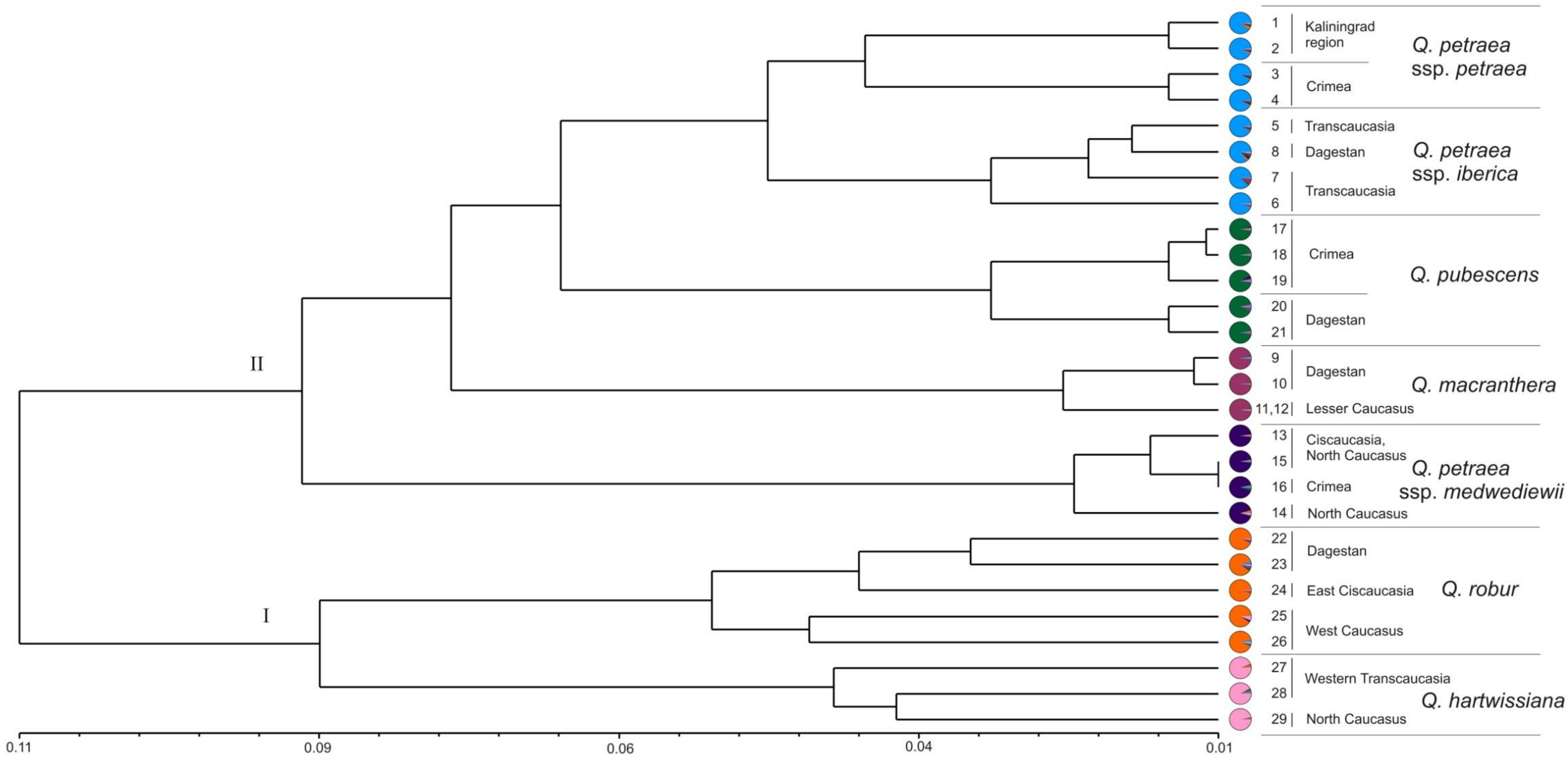


Figure S1. UPGMA - dendrogram of *Quercus* populations based on pairwise F_{ST} values and performed with the NTSYS-pc software [47]. The frequencies of STRUCTURE clusters (with K = 6, set 1) are presented in the form of pie charts, the colors of the clusters correspond to Figure 1b. Clade I (long pedunculate oaks) and clade II (short pedunculate oaks, sessile flower) – see text.

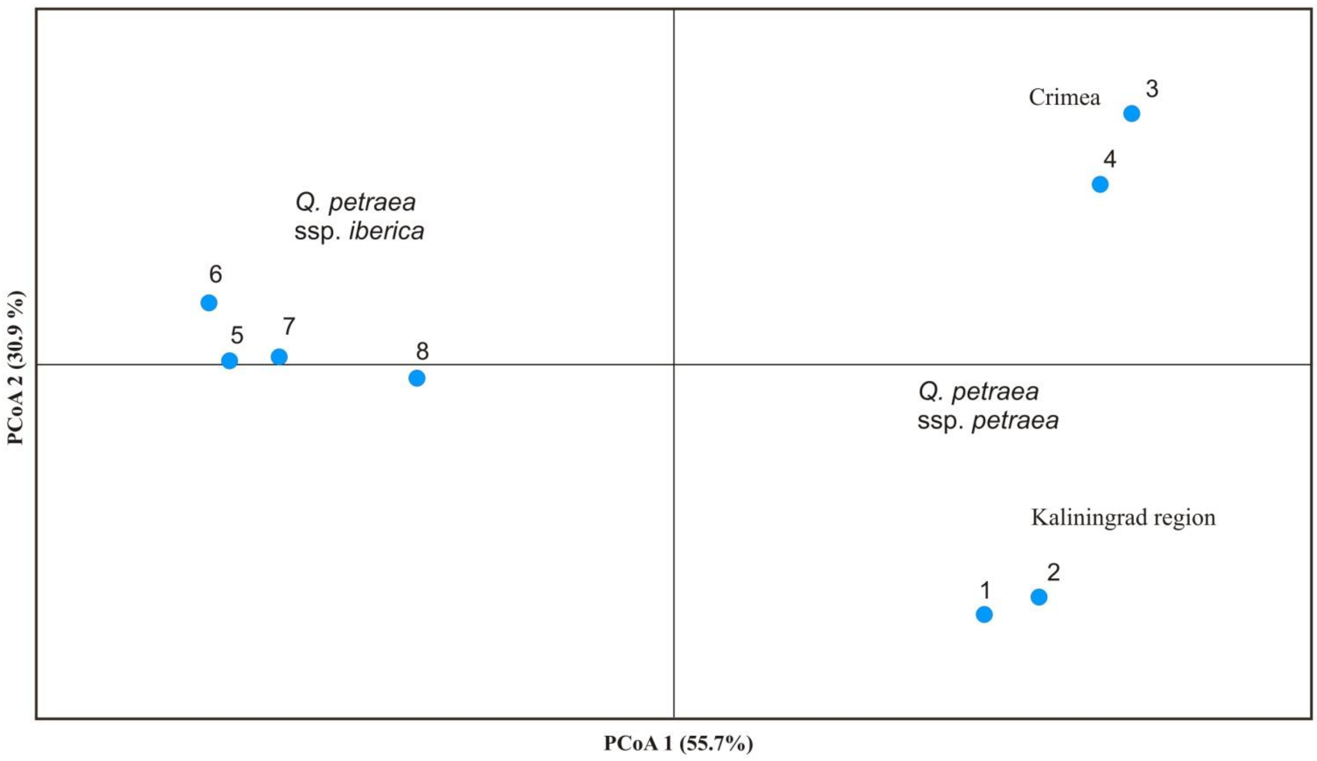
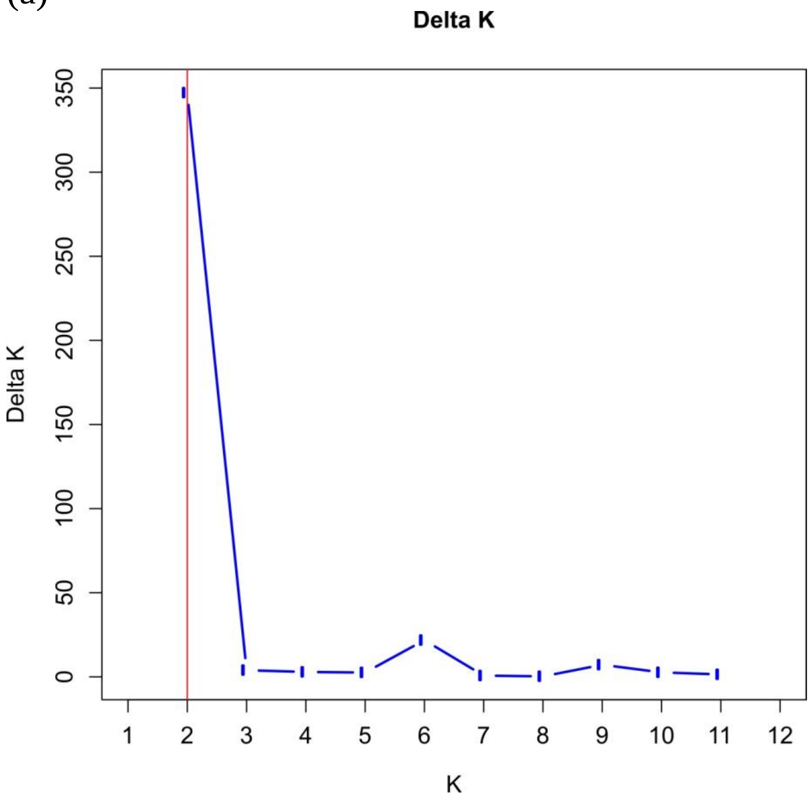


Figure S2. PCoA ordination of eight populations of *Q. petraea* ssp. *petraea* and *Q. petraea* ssp. *iberica* based on variability at 18 nSSR loci.

(a)



(b)

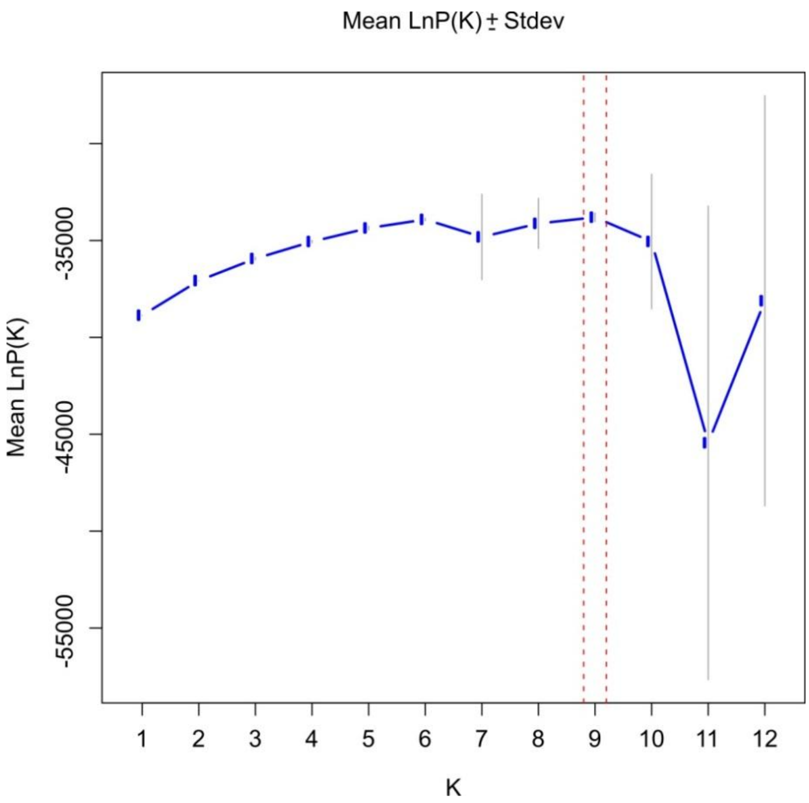


Figure S3. The tests of the most likely number of clusters (*K*) in the STRUCTURE analysis (set 1, all taxa) using 18 nSSR loci based on (a) *ΔK* [39] or (b) *LnP(D)* [38] approaches, performed with the online software StructureSelector [40].

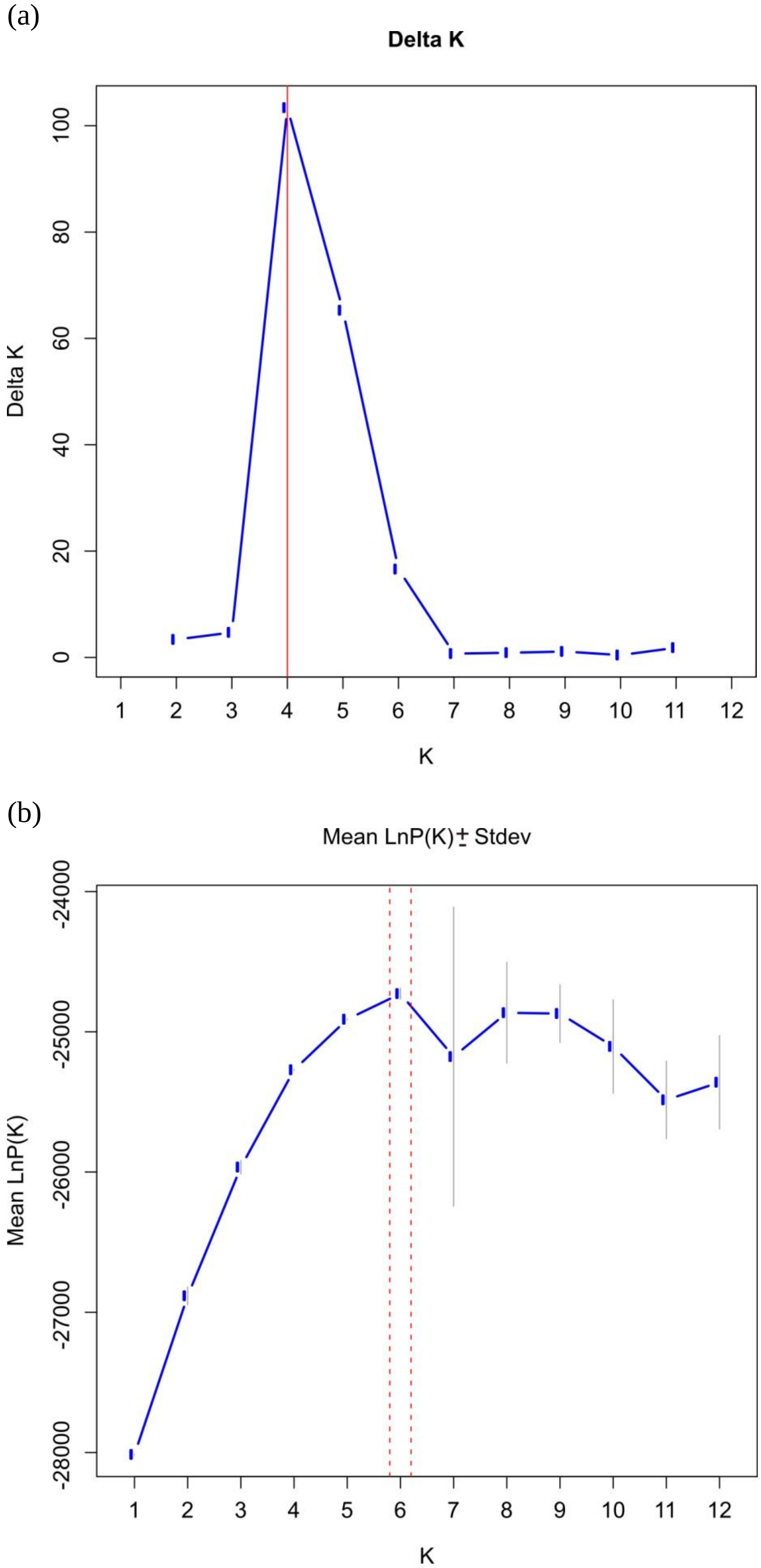
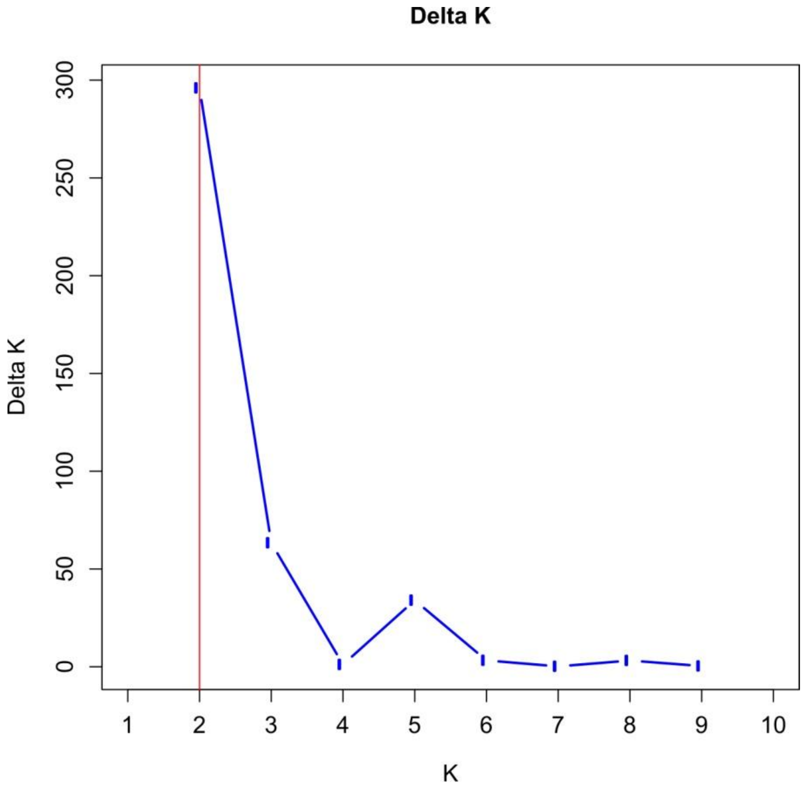


Figure S4. The tests of the most likely number of clusters (*K*) in the STRUCTURE analysis (set 2) using 18 nSSR loci based on (a) *ΔK* [39] or (b) *LnP(D)* [38] approaches, performed with StructureSelector [40].

(a)



(b)

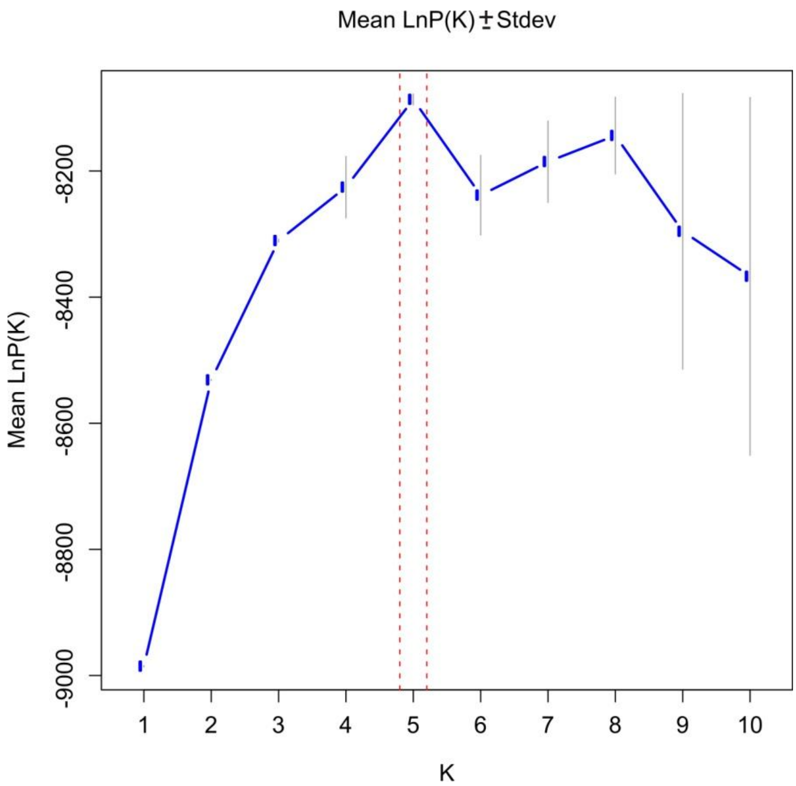


Figure S5. The tests of the most likely number of clusters (*K*) in the STRUCTURE analysis (set 3, *Q. robur* and *Q. hartwissiana*) using 18 nSSR loci based on (a) *ΔK* [39] or (b) *LnP(D)* [38] approaches, performed with StructureSelector [40].

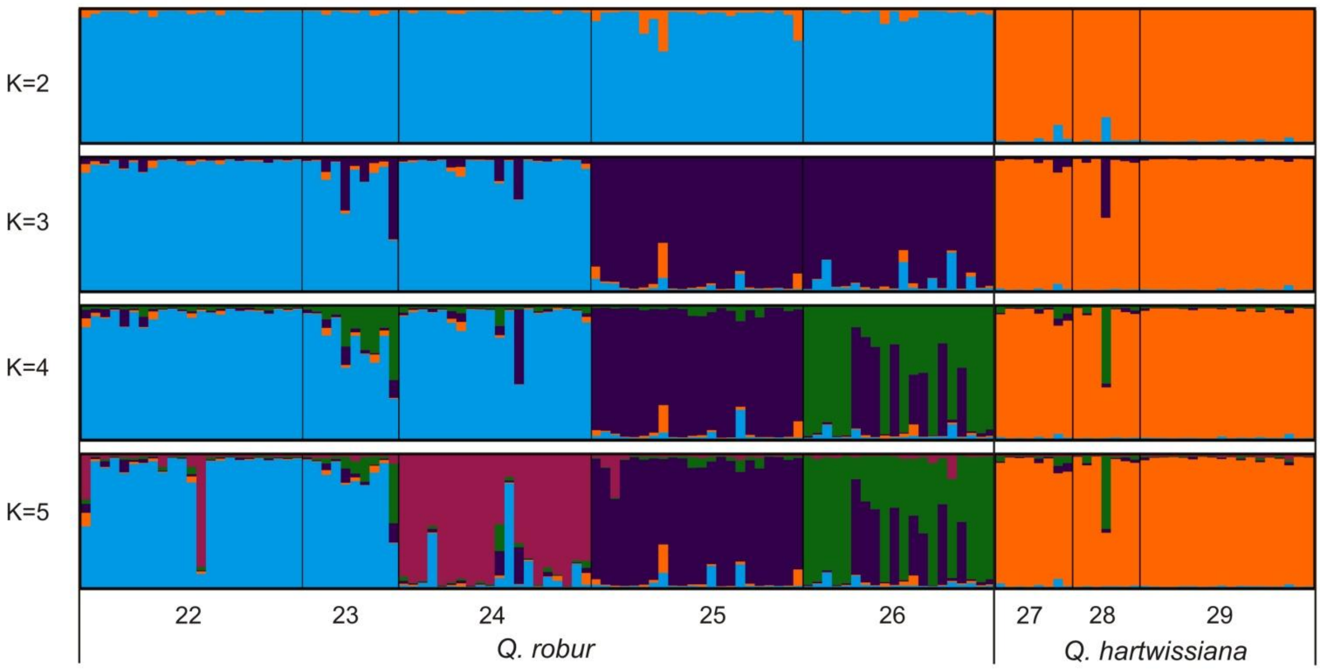


Figure S6. Genetic clusters computed by STRUCTURE based on 18 microsatellite loci for 128 individuals of *Q. robur* and *Q. hartwissiana* (set 3) with K = 2, 3, 4, 5. Each individual corresponds to a vertical bar divided into segments proportional to the participation of each genetic cluster.