

Table S1: Details of the 115 samples used in this study, including the accession number from INSDC (when available – the others were retrieved from DRYAD), locality, phylogeographic unit, latitude, longitude, sex, and year of collection. For the Occidental phylogeographic unit samples collected in this study, additional information is provided on watershed of origin.

Sample	Accession number	Locality	Phylogeographic Unit	Latitude	Longitude	Sex	Year of Collection	Watershed	Data Source Reference
SAMN18106615	SAMN18106615	Hija de Dios	Central System	40.5	-4.9	Male	2019	-	[1]
IBE-C1069	-	Range Rio Calamantio	Iberian Range	42.2	-2.9	Female	<1997?	-	[2]
IBE-C2736	-	Rio Caranyo-Covelo	Occidental	42.3	-8.4	Female	<1997?	-	[2]
IBE-C2737	-	Rio Riobo-A Estrada	Occidental	42.7	-8.4	Male	<1997?	-	[2]
IBE-C2739	-	Rio Cabras-Laza	Occidental	42.1	-7.4	Male	<1997?	-	[2]
IBE-C2740	-	Rio Meladas-Carballeda	Occidental	42.2	-6.8	Female	<1997?	-	[2]
IBE-C2749	-	Rio Termes-As Neves	Occidental	42.1	-8.4	Female	<1997?	-	[2]
IBE-C2755	-	Leitzaran-Berastegi	Pyrenees	43.1	-1.9	Male	2001	-	[3]
IBE-C2756	-	Rio Aiaiturrieta-Ataun	Pyrenees	43.0	-2.1	Female	<1997?	-	[2]
IBE-C2757	-	Amundarain-Zaldibia	Pyrenees	43.1	-2.0	Female	<1997?	-	[2]
IBE-C2758	-	Amundarain-Zaldibia	Pyrenees	43.0	-2.1	Female	2006	-	[3]
IBE-C2759	-	Amundarain-Zaldibia	Pyrenees	43.0	-2.1	Male	2001	-	[3]
IBE-C2760	-	Erasote-Leitza	Pyrenees	43.1	-1.9	Female	2010	-	[3]
IBE-C2761	-	Erasote-Leitza	Pyrenees	43.1	-1.9	Female	2010	-	[3]
IBE-C2762	-	Erasote-Leitza	Pyrenees	43.1	-1.9	Male	2010	-	[3]

Sample	Accession number	Locality	Phylogeographic Unit	Latitude	Longitude	Sex	Year of Collection	Watershed	Data Source Reference
IBE-C2763	-	Olazar-Eugi	Pyrenees	43.0	-1.5	Male	2008	-	[3]
IBE-C2765	-	Ezpelura-Urrotz	Pyrenees	43.1	-1.7	Male	2010	-	[3]
IBE-C2766	-	Ezpelura-Urrotz	Pyrenees	43.1	-1.7	Female	1999	-	[3]
SAMN18106612	SAMN18106612	Ezpelura-Urrotz	Pyrenees	43.1	-1.7	Male	1999	-	[1]
IBE-C2770	-	Ezpelura-Urrotz	Pyrenees	43.1	-1.7	Female	1999	-	[3]
IBE-C2771	-	Ezpelura-Urrotz	Pyrenees	43.1	-1.7	Male	1999	-	[3]
IBE-C2772	-	Ezpelura-Urrotz	Pyrenees	43.1	-1.7	Male	1999	-	[3]
IBE-C2773	-	Ezpelura-Urrotz	Pyrenees	43.1	-1.7	Male	1999	-	[3]
IBE-C2776	-	Ezpelura-Urrotz	Pyrenees	43.1	-1.7	Female	1999	-	[3]
IBE-C2778	-	Ezpelura-Urrotz	Pyrenees	43.1	-1.7	Male	1999	-	[3]
IBE-C2779	-	Ezpelura-Urrotz	Pyrenees	43.1	-1.7	Female	1999	-	[3]
IBE-C2780	-	Ezpelura-Urrotz	Pyrenees	43.1	-1.7	Male	1999	-	[3]
IBE-C2781	-	Amezti-Labaien	Pyrenees	43.1	-1.7	Female	1998	-	[3]
IBE-C2782	-	Amezti-Labaien	Pyrenees	43.1	-1.7	Female	1998	-	[3]
IBE-C2783	-	Amezti-Labaien	Pyrenees	43.1	-1.7	Female	1998	-	[3]
IBE-C2784	-	Amezti-Labaien	Pyrenees	43.1	-1.7	Male	1998	-	[3]
IBE-C2785	-	Amezti-Labaien	Pyrenees	43.1	-1.7	Female	1998	-	[3]
IBE-C2786	-	Amezti-Labaien	Pyrenees	43.1	-1.7	Male	1998	-	[3]

Sample	Accession number	Locality	Phylogeographic Unit	Latitude	Longitude	Sex	Year of Collection	Watershed	Data Source Reference
IBE-C2787	-	Amezti-Labaien	Pyrenees	43.1	-1.7	Male	1998	-	[3]
IBE-C2788	-	Amezti-Labaien	Pyrenees	43.1	-1.7	Female	1998	-	[3]
IBE-C2789	-	Amezti-Labaien	Pyrenees	43.1	-1.7	Female	1998	-	[3]
IBE-C2790	-	Amezti-Labaien	Pyrenees	43.1	-1.7	Male	1998	-	[3]
IBE-C2791	-	Urrobi-Auritz	Pyrenees	43.0	-1.3	Male	2001	-	[3]
IBE-C2792	-	Urrobi-Auritz	Pyrenees	43.0	-1.3	Female	2001	-	[3]
IBE-C2793	-	Urrobi-Auritz	Pyrenees	43.0	-1.3	Female	2001	-	[3]
IBE-C2794	-	Urrobi-Auritz	Pyrenees	43.0	-1.3	Male	2001	-	[3]
IBE-C2796	-	Sasoaran-Eugi	Pyrenees	43.0	-1.6	Male	1997	-	[3]
IBE-C2797	-	Sasoaran-Eugi	Pyrenees	43.0	-1.6	Female	1997	-	[3]
IBE-C2799	-	Elama-Artikutza	Pyrenees	43.2	-1.8	Male	2002	-	[3]
IBE-C2800	-	Elama-Artikutza	Pyrenees	43.2	-1.8	Male	2002	-	[3]
IBE-C2802	-	Elama-Artikutza	Pyrenees	43.2	-1.8	Female	2002	-	[3]
IBE-C2803	-	Elama-Artikutza	Pyrenees	43.2	-1.8	Male	2002	-	[3]
IBE-C2804	-	Elama-Artikutza	Pyrenees	43.2	-1.8	Male	2002	-	[3]
IBE-C2856	-	Mount. Rio Lamedo	Cantabria	43.1	-4.6	Female	<1997?	-	[2]
IBE-C2892	-	Mount. Rio Cares-Cain	Cantabria	43.2	-4.9	Male	<1997?	-	[2]
IBE-C2894	-	Mount. Rio Cares-Casiellos	Cantabria	43.2	-4.9	Female	<1997?	-	[2]

Sample	Accession number	Locality	Phylogeographic Unit	Latitude	Longitude	Sex	Year of Collection	Watershed	Data Source Reference
IBE-C3301	-	Paiva-Fraguas	Occidental	40.8	-7.8	Female	<1997?	-	[2]
IBE-C3303	-	Sabor-Macas-Quintanilla	Occidental	41.8	-6.6	Male	<1997?	-	[2]
IBE-C3305	-	Paiva-Fraguas	Occidental	40.8	-7.8	Male	<1997?	-	[2]
IBE-C3733	-	Oja Cabecera	Iberian Range	42.2	-3.1	Male	2011	-	[4]
SAMN18106613	SAMN18106613	Oja Cabecera	Iberian Range	42.2	-3.1	Female	2011	-	[1]
IBE-C3735	-	Oja Cabecera	Iberian Range	42.2	-3.1	Female	2011	-	[4]
IBE-C3736	-	Oja Cabecera	Iberian Range	42.2	-3.1	Female	2011	-	[4]
IBE-C3737	-	Oja Cabecera	Iberian Range	42.2	-3.1	Female	2011	-	[4]
IBE-C3738	-	Oja Azarrulla	Iberian Range	42.3	-3.0	Female	2011	-	[4]
IBE-C3739	-	Cíloria	Iberian Range	42.3	-3.1	Female	2011	-	[4]
IBE-C3740	-	Cíloria	Iberian Range	42.3	-3.1	Male	2011	-	[4]
IBE-C3741	-	Tobía	Iberian Range	42.3	-2.9	Female	2011	-	[4]
IBE-C3742	-	Tobía	Iberian Range	42.2	-2.9	Male	2011	-	[4]
IBE-C3743	-	Tobía	Iberian Range	42.3	-2.9	Male	2011	-	[4]
IBE-C3744	-	Tobía	Iberian Range	42.2	-2.9	Male	2011	-	[4]
IBE-C3745	-	Cárdenas	Iberian Range	42.3	-2.9	Female	2011	-	[4]
IBE-C3746	-	Roñas	Iberian Range	42.2	-2.8	Male	2011	-	[4]
IBE-C3747	-	Roñas	Iberian Range	42.2	-2.8	Female	2011	-	[4]

Sample	Accession number	Locality	Phylogeographic Unit	Latitude	Longitude	Sex	Year of Collection	Watershed	Data Source Reference
IBE-C3748	-	Roñas	Iberian Range	42.2	-2.8	Male	2011	-	[4]
IBE-C3749	-	Roñas	Iberian Range	42.2	-2.8	Female	2011	-	[4]
IBE-C3750	-	Range Iregua	Iberian Range	42.1	-2.7	Male	2011	-	[2]
IBE-C3751	-	Iregua-Cabecera	Iberian Range	42.1	-2.7	Male	2011	-	[4]
IBE-C3752	-	La Vieja	Iberian Range	42.1	-2.6	Male	2011	-	[4]
IBE-C3753	-	La Vieja	Iberian Range	42.0	-2.6	Male	2011	-	[4]
IBE-C3754	-	Range La Vieja	Iberian Range	42.0	-2.6	Female	2011	-	[2]
IBE-C3755	-	La Vieja	Iberian Range	42.1	-2.6	Female	2011	-	[4]
IBE-C3756	-	Range La Soledad	Iberian Range	42.2	-3.1	Male	<1997?	-	[2]
IBE-C3757	-	La Soledad	Iberian Range	42.2	-3.1	Female	2011	-	[4]
IBE-C3758	-	La Soledad	Iberian Range	42.2	-3.1	Female	2011	-	[4]
IBE-C3759	-	Urumea	Pyrenees	43.1	-1.8	Female	2011	-	[3]
IBE-C3760	-	Urumea	Pyrenees	43.1	-1.8	Male	2011	-	[3]
IBE-C3761	-	Urumea	Pyrenees	43.1	-1.8	Female	2011	-	[3]
IBE-C3763	-	Aritzakun	Pyrenees	43.3	-1.4	Male	2011	-	[3]
IBE-C3765	-	Urdanta	Iberian Range	42.3	-3.0	Female	2011	-	[4]
IBE-C3766	-	Urdanta	Iberian Range	42.3	-3.0	Female	2011	-	[4]
IBE-C3767	-	Urdanta	Iberian Range	42.3	-3.0	Male	2011	-	[4]

Sample	Accession number	Locality	Phylogeographic Unit	Latitude	Longitude	Sex	Year of Collection	Watershed	Data Source Reference
IBE-C3768	-	Urdanta	Iberian Range	42.3	-3.0	Female	2011	-	[4]
IBE-C3769	-	Urdanta	Iberian Range	42.3	-3.0	Male	2011	-	[4]
IBE-C3770	-	Urdanta	Iberian Range	42.3	-3.0	Female	2011	-	[4]
IBE-C3771	-	Ormázar	Iberian Range	42.1	-2.8	Male	2011	-	[4]
IBE-C3772	-	Mayor	Iberian Range	42.1	-2.7	Male	2011	-	[4]
SAMN18106614	SAMN18106614	Mayor	Iberian Range	42.1	-2.7	Male	2011	-	[1]
IBE-C3774	-	Iregua Achichuelo	Iberian Range	42.1	-2.7	Female	2011	-	[4]
IBE-C3775	-	Iregua Achichuelo	Iberian Range	42.1	-2.7	Female	2011	-	[4]
IBE-C422	-	Tor	Pyrenees	42.6	1.4	Female	<1997?	-	[2]
IBE-C485	-	System Rio Ambroz-Hervas	Central System	40.3	-5.8	Female	<1997?	-	[2]
SAMN18106611	SAMN18106611	Torán	Pyrenees	42.8	-0.7	Male	2017	-	[1]
SAMN18106616	SAMN18106616	Requejo	Occidental	42.03	-6.7	Male	2018	-	[1]
IBE-S1925	-	Riu Ritort-Camprodon	Pyrenees	42.3	2.4	Male	<1997?	-	[2]
1	SAMEA118002938	Espadanedo	Occidental	41.65158321	-6.933384732	Male	2015	Tua	This study
2	SAMEA118002939	Felgar	Occidental	41.22312188	-6.947056639	Female	2015	Sabor	This study
3	SAMEA118002940	Quintela de Lapaças	Occidental	41.63340474	-6.866797096	Female	2015	Sabor	This study
4	SAMEA118002941	Chacim	Occidental	41.46954975	-6.87620478	Female	2016	Sabor	This study
5	SAMEA118002942	Gebelim	Occidental	41.43624515	-6.91739072	Female	2016	Sabor	This study

Sample	Accession number	Locality	Phylogeographic Unit	Latitude	Longitude	Sex	Year of Collection	Watershed	Data Source Reference
6	SAMEA118002943	Coelhoso	Occidental	41.665592	-6.658344	Female	2016	Sabor	This study
7	SAMEA118002944	Rebordãos	Occidental	41.739392	-6.779879	Female	2016	Sabor	This study
8	SAMEA118002945	Serapicos	Occidental	41.647539	-6.724812	Female	2016	Sabor	This study
9	SAMEA118002946	Edrosa	Occidental	41.730551	-6.935536	Female	2016	Tua	This study
10	SAMEA118002947	Montouto	Occidental	41.92936672	-6.97219392	Female	2016	Tua	This study
11	SAMEA118002948	Soutelo	Occidental	41.90403	-6.796208333	Female	2016	Sabor	This study
12	SAMEA118002949	Outeiro	Occidental	42.531308	-7.117328	Male	2012	Minho	This study
13	SAMEA118002950	Ferramulín	Occidental	42.57475	-7.0592	Female	2012	Minho	This study
14	SAMEA118002951	Riobó	Occidental	42.735563	-8.401418	Male	2013	Ulla	This study
15	SAMEA118002952	Riobó	Occidental	42.735563	-8.401418	Male	2013	Ulla	This study

Table S2: Genetic distances between individuals (see text for further details): **a.** for the Occidental phylogenetic unit; **b.** for the Douro river system; **c.** for the Sabor watershed.

a														
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.0000													
2	0.2772	0.0000												
3	0.2569	0.2683	0.0000											
4	0.2792	0.2490	0.2561	0.0000										
5	0.2894	0.2484	0.2800	0.2726	0.0000									
6	0.2710	0.2597	0.2719	0.2720	0.2714	0.0000								
7	0.2677	0.2764	0.2783	0.2779	0.2885	0.2247	0.0000							
8	0.2803	0.2662	0.2786	0.2649	0.2836	0.2717	0.2766	0.0000						
9	0.2338	0.2760	0.2654	0.2786	0.2873	0.2745	0.2709	0.2778	0.0000					
10	0.2514	0.2783	0.2784	0.2914	0.2954	0.2793	0.2860	0.2956	0.2647	0.0000				
11	0.2571	0.2699	0.2697	0.2751	0.2853	0.2661	0.2685	0.2699	0.2595	0.2645	0.0000			
12	0.2863	0.2946	0.2955	0.3000	0.3074	0.2962	0.2992	0.3048	0.2856	0.2805	0.2750	0.0000		
13	0.2854	0.2978	0.3005	0.3049	0.3091	0.3017	0.2996	0.3063	0.2889	0.2813	0.2859	0.2583	0.0000	
14	0.2957	0.3017	0.3035	0.3134	0.3147	0.3013	0.3155	0.3196	0.3010	0.2906	0.2881	0.2850	0.2951	0.0000

b											
Sample	1	2	3	4	5	6	7	8	9	10	11
1	0.0000										
2	0.2559	0.0000									
3	0.2383	0.2470	0.0000								
4	0.2594	0.2337	0.2375	0.0000							
5	0.2673	0.2309	0.2577	0.2543	0.0000						
6	0.2504	0.2390	0.2496	0.2515	0.2498	0.0000					
7	0.2472	0.2532	0.2545	0.2577	0.2650	0.2122	0.0000				
8	0.2557	0.2429	0.2522	0.2433	0.2602	0.2464	0.2533	0.0000			
9	0.2209	0.2556	0.2451	0.2576	0.2663	0.2546	0.2505	0.2552	0.0000		
10	0.2373	0.2568	0.2562	0.2696	0.2744	0.2588	0.2647	0.2684	0.2471	0.0000	
11	0.2374	0.2464	0.2480	0.2515	0.2654	0.2449	0.2488	0.2465	0.2380	0.2414	0.0000

c								
Sample	1	2	3	4	5	6	7	8
1	0.0000							
2	0.2361	0.0000						
3	0.2180	0.2179	0.0000					
4	0.2166	0.2336	0.2235	0.0000				
5	0.2257	0.2262	0.2253	0.2219	0.0000			
6	0.2419	0.2379	0.2321	0.2374	0.1966	0.0000		
7	0.2320	0.2370	0.2217	0.2334	0.2219	0.2350	0.0000	
8	0.2590	0.2547	0.2523	0.2601	0.2466	0.2537	0.2521	0.0000

Table S3: Matrix of overland (dark green) and/or river (dark blue) distances between samples, in meters: **a.** for the Occidental phylogeographic unit; **b.** for the Douro river system; **c.** for the Douro river system using a mix of overland and river distances (in bold are the sample pairs from headwaters, using the overland distances instead of the river distances); **d.** for the Sabor watershed.

[illegible]

b Samples	Overland distances										
	1	2	3	4	5	6	7	8	9	10	11
1	0.00	47709.74	5897.46	20816.07	24008.33	22927.92	16073.40	17355.03	8792.47	31090.03	30321.85
2	165752.94	0.00	46161.13	28063.98	23854.03	54831.60	59137.87	50756.18	56494.92	78646.68	76832.59
3	235678.37	73962.62	0.00	18257.08	22348.95	17705.60	13835.28	11916.39	12231.44	34088.20	30690.65
4	212054.38	50338.64	28516.99	0.00	5055.04	28381.17	31090.39	23486.40	29471.33	51804.62	48821.18
5	200201.37	38485.62	94213.50	70589.52	0.00	33429.64	35635.48	28472.83	32796.80	55082.80	53039.58
6	239176.61	77460.86	84150.52	60526.54	97711.74	0.00	13020.11	5882.19	24147.74	39250.48	28904.56
7	260177.40	98461.65	105151.30	81527.32	118712.53	21000.78	0.00	11202.84	12967.85	26488.92	18377.41
8	233146.31	71430.57	78120.22	54496.24	91681.45	65302.24	86303.02	0.00	19805.35	37494.78	29161.16
9	40056.64	166913.14	236838.57	213214.59	201361.58	240336.82	261337.60	234306.52	0.00	22339.96	22506.68
10	83561.88	191051.96	260977.39	237353.41	225500.40	264475.64	285476.42	258445.34	84722.08	0.00	14848.06
11	283503.66	121787.91	128477.56	104853.58	142038.79	44327.05	45277.17	109629.29	284663.86	308802.69	0.00

River distances

c Samples	Mix distances										
	1	2	3	4	5	6	7	8	9	10	11
1	0.00										
2	165752.94	0.00									
3	5897.46	73962.62	0.00								
4	212054.38	50338.64	28516.99	0.00							
5	200201.37	38485.62	94213.50	70589.52	0.00						
6	239176.61	77460.86	84150.52	60526.54	97711.74	0.00					
7	260177.40	98461.65	105151.30	81527.32	118712.53	21000.78	0.00				
8	233146.31	71430.57	78120.22	54496.24	91681.45	65302.24	86303.02	0.00			
9	40056.64	166913.14	236838.57	213214.59	201361.58	240336.82	12967.85	234306.52	0.00		
10	83561.88	191051.96	260977.39	237353.41	225500.40	264475.64	285476.42	258445.34	84722.08	0.00	
11	283503.66	121787.91	128477.56	104853.58	142038.79	44327.05	45277.17	109629.29	284663.86	14848.06	0.00

d	Overland distances							
Samples	2	3	4	5	6	7	8	11
2	0.00	46161.13	28063.98	23854.03	54831.60	59137.87	50756.18	76832.59
3	73962.62	0.00	18257.08	22348.95	17705.60	13835.28	11916.39	30690.65
4	50338.64	28516.99	0.00	5055.04	28381.17	31090.39	23486.40	48821.18
5	38485.62	94213.50	70589.52	0.00	33429.64	35635.48	28472.83	53039.58
6	77460.86	84150.52	60526.54	97711.74	0.00	13020.11	5882.19	28904.56
7	98461.65	105151.30	81527.32	118712.53	21000.78	0.00	11202.84	18377.41
8	71430.57	78120.22	54496.24	91681.45	65302.24	86303.02	0.00	29161.16
11	121787.91	128477.56	104853.58	142038.79	44327.05	45277.17	109629.29	0.00

River distances

Table S4: Discriminant Analysis of Principal Components posterior probabilities for the Occidental phylogeographic unit, taking 6 principal components into account: **a.** for 2 groups; **b.** for 3 groups; **c.** for 4 groups. Highlighted cells indicate the samples assigned to each cluster (K) based on their highest posterior probability.

a Sample	Group (K)	
	1	2
1	1.00E+00	3.60E-31
2	1.00E+00	3.34E-26
3	1.00E+00	1.74E-29
4	1.00E+00	4.54E-25
5	1.00E+00	4.24E-30
6	1.00E+00	1.84E-27
7	1.00E+00	2.97E-29
8	1.00E+00	1.07E-29
9	1.00E+00	3.76E-34
10	1.00E+00	3.75E-18
11	1.00E+00	5.38E-18
12	6.40E-24	1.00E+00
13	3.17E-28	1.00E+00
14	6.06E-26	1.00E+00

b Sample	Group (K)		
	1	2	3
1	1.00E+00	7.91E-29	1.46E-29
2	4.04E-21	8.89E-48	1.00E+00
3	2.68E-27	2.35E-57	1.00E+00
4	4.11E-37	9.68E-64	1.00E+00
5	2.46E-34	2.13E-65	1.00E+00
6	1.00E+00	8.59E-26	8.38E-24
7	1.00E+00	1.36E-26	3.85E-37
8	1.00E+00	2.55E-27	4.31E-32
9	1.00E+00	1.48E-31	1.18E-29
10	1.00E+00	1.18E-16	2.28E-33
11	1.00E+00	7.04E-17	1.50E-27
12	6.26E-22	1.00E+00	6.01E-53
13	3.26E-26	1.00E+00	1.41E-62
14	4.65E-24	1.00E+00	1.99E-59

c Samples	Group (K)			
	1	2	3	4
1	1.00E+00	1.77E-64	9.40E-15	1.72E-34
2	3.80E-11	9.22E-91	1.00E+00	2.16E-44
3	1.00E+00	3.59E-67	1.80E-08	1.40E-34
4	2.30E-08	2.30E-87	1.00E+00	7.46E-39
5	2.17E-15	8.32E-104	1.00E+00	2.17E-51
6	9.42E-13	9.06E-91	1.00E+00	2.04E-49
7	4.59E-13	9.78E-102	1.00E+00	1.78E-46
8	7.88E-14	1.50E-99	1.00E+00	7.50E-50
9	1.00E+00	1.41E-68	2.29E-13	6.38E-37
10	1.00E+00	2.19E-52	5.44E-17	5.39E-23
11	1.00E+00	7.45E-55	9.46E-08	6.66E-27
12	1.39E-23	2.95E-61	7.51E-41	1.00E+00
13	3.53E-37	5.87E-94	1.06E-50	1.00E+00
14	4.27E-60	1.00E+00	4.15E-94	5.26E-77

Table S5: Permutational Multivariate Analysis of Variance results testing genetic differences between samples on a genetic distance matrix: **a.** for the Occidental phylogeographic unit (Douro vs other river systems); **b.** for the Douro river system (Tua vs Sabor watersheds). Df stands for degrees of freedom, SS for sum of squares, R² for proportion of variation explained and F for F-statistic. Values with p < 0.01 are highlighted in bold.

a	Df	SS	R²	F	p-value
Model	1	0.0688	0.1332	1.8447	0.005
Residual	12	0.4476	0.8668		
Total	13	0.5164	1.0000		

b	Df	SS	R²	F	p-value
Model	1	0.0432	0.1376	1.4354	0.005
Residual	9	0.2710	0.8625		
Total	10	0.3142	1.0000		

Table S6: Mantel test using genetic distances and overland, river and a mix of overland and river distances for the Occidental phylogeographic unit, Douro river system and the Sabor watershed showing the correlation coefficient (r) and p-value for each test. Values with p < 0.01 are highlighted in bold. Missing values (-) are due to differences in the spatial context of the analyses. For the Occidental unit, river distances were not calculated because the samples are distributed across different river systems, making direct river-based distances biologically meaningless. Similarly, for the Sabor watershed, mixed distances were not applicable, as all samples are within a single watershed and no adjacent headwaters between watersheds were considered.

	Overland distances		River distances		Mix distances	
	r	p-value	r	p-value	r	p-value
Occidental phylogeographic unit	0.7884	0.0001	-	-	-	-
Douro river system	0.3403	0.0174	0.4206	0.0026	0.5493	0.0001
Sabor watershed	0.4461	0.0553	0.7039	0.0001	-	-

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