Amphibia-Reptilia

Well-known species, unexpected results: High genetic diversity in declining *Vipera ursinii* in Central, Eastern and Southeastern Europe

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Supplementary material

Table S1. detail of the samples analysed, with their location, the number of specimens analysed with microsatellite and mitochondrial markers. For each locality, the number of different haplotypes found is indicated; the total number of haplotypes per subspecies is not directly the sum of haplotypes per locality as the same haplotype could be shared between localities. We also included the mtDNA sequences available on GenBank, and gathered them with the other samples when they come from the same locality.

Subspecies	Locality	Region	map code	Country	Lat	Long	Different haplotypes	µsat	mtDNA	hapl
V. u. rakosiensis	Hanság, Fűzfa-szigetek	Hanság	1	Hungary	47.76	17.33	HAN1;HAN2	31	4	2
V. u. rakosiensis	Dabas-Gyón, Göböly-járás	Kisksunság 2	2	Hungary	47.15	19.31	KIS5; KIS2	9	7	2
V. u. rakosiensis	Peszéradacs, Bányatavi-rét	Kisksunság 3	3	Hungary	47.08	19.27	KIS1; KIS2; VT48;VT40	25	10	4
V. u. rakosiensis	Bugac, Nagypuszta	Kisksunság 1	4	Hungary	46.68	19.60	KIS1; KIS2; KIS3; KIS4	32	8	4
V. u. rakosiensis	Cluj-Napoca, Transylvania	Transylvania 3	5	Romania	46.83	23.69	TRA1	7	6	1
V. u. rakosiensis	Bogata, Transylvania	Transylvania 1	6	Romania	46.47	23.81	TRA1;TRA2	12	2	2
V. u. rakosiensis	Cicârd, Transylvania	Transylvania 2	7	Romania	46.27	23.91	TRA3;TRA4;TRA5	21	4	3
V. u. rakosiensis	near Alba Lulia	Transylvania	8	Romania			ROU159; ROU148; ROU152; ROU155	-	4	4
Total								**137**	**45**	**18**
V. u. moldavica	Dealul lui Dumnezeu, Iasi	Iasi 1	9	Romania	47.28	27.41	IAS1	11	5	1
V. u. moldavica	Valea lui David, Iasi	Iasi 2	10	Romania	47.19	27.47	IAS2; IAS3; IAS4	6	3	3
V. u. moldavica	Periteasca, Danube Delta	Danube Delta 1	11	Romania	44.77	29.14	DAN1	28	2	1
V. u. moldavica	Sfantu Gheorghe, Danube Delta	Danube Delta 2	12	Romania	44.94	29.63	DAN2;DAN3; DAN4; DAN5; Mol1; Mol2; Mol3	16	7	7
Total								**61**	**17**	**12**
V. u. macrops	Velebit Mt. Veliki Štirovac,	Western Dinarides	13	Croatia	44.32	15.57	DIW1	62	3	1
V. u. macrops	Lisac Mt.	Western Dinarides	14	Croatia	44.33	15.99	DIW2	-	1	1
V. u. macrops	Dinara Mt., Poštak	Western Dinarides	15	Croatia	44.25	16.11	DIW2	-	2	1
V. u. macrops	Dinara Mt., Poštak	Western Dinarides	17	Croatia	44.07	16.38	DIW3; DIW2	-	2	1
V. u. macrops	Ilica Mt.	Western Dinarides	16	Bosnia and Herzegovina	44.23	16.27	DIW2	-	1	1
V. u. macrops	Troglav Mt.	Western Dinarides	18	Bosnia and Herzegovina	43.94	16.60	DIW4; DIW5	-	4	2
V. u. macrops	Cincar Mt.	Western Dinarides	19	Bosnia and Herzegovina	43.90	17.05	DIW6	-	1	1
V. u. macrops	Velež Mt.	Eastern Dinarides	20	Bosnia and Herzegovina	43.36	17.93	DIE1	-	2	1
V. u. macrops	Zelengora Mt.	Eastern Dinarides	21	Bosnia and Herzegovina	43.36	18.50	DIE2; DIE3; DIE1	-	3	3
V. u. macrops	Korita	Eastern Dinarides	22	Bosnia and Herzegovina	43.04	18.49	MacJ	-	1	1
V. u. macrops	Durmitor Mt.	Eastern Dinarides	23	Montenegro	43.13	18.99	DIE4	-	1	1
V. u. macrops	Bjelasica Mt.	Eastern Dinarides	24	Montenegro	42.90	19.58	DIE5	43	4	1
V. u. macrops	Paštrik Mt.	Eastern Dinarides	25	Kosovo province	42.22	20.52	DIE6; DIE7	-	2	2
V. u. macrops	Šar planina Mt., Brezovica	Hellenides	26	Kosovo province	42.19	20.90	DIE7	-	2	1
V. u. macrops	Šar planina Mt., Lukovo, Pole	Hellenides	27	North Macedonia	41.83	20.63	DIE8	-	1	1
V. u. macrops	Šar planina Mt., Vakaf	Hellenides	28	North Macedonia	42.00	20.86	DIE9	-	1	1
V. u. macrops	Korab Mt., Kobilino Pole	Hellenides	29	North Macedonia	41.78	20.55	DIE10;DIE8	-	2	2
Total								**105**	**34**	**17**
V. u. macrops	Bistra Mt., Medenica and Tonivoda	Hellenides	30	North Macedonia	41.63	20.67	MED2;MED3; MED1;TON1	6	6	4

Total								**6**	**6**	**4**
V. renardi	Orlov Island	Black Sea	31	Ukraine	46.27	31.73		4	-	
V. renardi	Yagorlytskyi Kut, Black Sea Biosphere Reserve	Black Sea	32	Ukraine	46.30	31.83	BLA1	4	2	1
V. renardi	Kherson region, Jarylgach isl.	Black Sea	33	Ukraine	46.02	32.98		1	-	
V. renardi	Crimea, near Armyansk	Black Sea	34	Ukraine	46.09	33.65	BLA5	1	1	1
V. renardi	Khersonskaja province, Island Churjuk	Black Sea	35	Ukraine	46.10	34.24		1	-	
V. renardi	Crimea, Prisivashje, s. Jasnopoljanskoe, Dzhankoiskij district	Black Sea	36	Ukraine	45.95	34.43	BLA5	2	1	1
V. renardi	Khersonskaja province, Island Kujuk-Tuk	Black Sea	37	Ukraine	46.11	34.49		10	-	
V. renardi	Obytichna Kosa, Zaporizhzha Prov.	Black Sea	38	Ukraine	46.52	36.19	BLA2	2	1	1
V. renardi	Crimea, Kerch Peninsula,cape Chauda, Leninskij distdict	Black Sea	39	Ukraine	45.00	35.84	BLA3; BLA4	4	2	2
V. renardi	Crimea, West Coast, Sakskij distr., lake Sasyk, st. Pribrezhnaja	Black Sea	40	Ukraine	45.15	33.50	CRI1	1	1	1
V. renardi	Crimea, Foothills (Low-Mountain), Sympheropol distr., v. Krasnolesje	Crimean Ridge	41	Ukraine	44.82	34.24	CRI1	1	1	1
V. renardi	Crimea, Main Ridge, Sympheropol distr.; north slope of Jaila Chatyrdagh near v. Pereval'noje	Crimean Ridge	42	Ukraine	44.80	34.29	CRI2	6	1	1
V. renardi	Poltava region, Poltava district, vicinities of Burty	East European Plains	43	Ukraine	49.66	34.89		1	-	-
V. renardi	Belgorod region, Rovenki district, near Nagolnoe villiage, the Sarma river valley slopes	East European Plains	44	Russia	49.98	38.98	BLA5	10	1	1
V. renardi	Borki, Valuyki District, Belgorod Province	East European Plains	45	Russia	50.13	37.88	BLA5	1	1	1
V. renardi	Lugansk region, Novopskov district, near Novo-Belaya village	East European Plains	46	Ukraine	49.77	39.15	BLA5	1	1	1
V. renardi	Tatarstan, Rayon Spassky		47	Russia	55.01	49.07	REN28	-	1	1
Total								**50**	**14**	**8**
outgroups										
V. u. ursinii	unknown			Italy			UR001		1	1
V. u. ursinii	Pop10			France			THIE7		1	1
V. graeca	Stavros			Greece			Gre3		1	1
V. berus	Swiss Jura Mountains			Switzerland			Vber416		1	1

Lat: latitude; Long: Longitude; µsat: number of individuals amplified for the different microsatellite markers; ntDNA: number of individuals amplified for the different mtDNA markers; hapl: number of different haplotypes found in the locality.

Table S2. NCBI GeneBank accession numbers of the different haplotypes for the mitochondrial cytb and ND4 fragments mentioned in supplementary table S1, with their locality and country information.

Subspecies	haplotype	Locality	Country	GenBank cytb	GenBank ND4
V. u. rakosiensis	HAN1	Hanság, Fűzfa-szigetek	Hungary	OP777956	OP778004
V. u. rakosiensis	HAN2	Hanság, Fűzfa-szigetek	Hungary	OP777957	OP778005
V. u. rakosiensis	KIS1	Peszéradacs, Bányatavi / Bugac, Nagypuszta	Hungary	OP777949	OP777997
V. u. rakosiensis	KIS2	Peszéradacs, Bányatavi / Bugac, Nagypuszta	Hungary	OP777950	OP777998
V. u. rakosiensis	KIS3	Bugac, Nagypuszta	Hungary	OP777951	OP777999
V. u. rakosiensis	KIS4	Bugac, Nagypuszta	Hungary	OP777952	OP778000
V. u. rakosiensis	KIS5	Dabas-Gyón, Göböly-járás	Hungary	OP777953	OP778001
V. u. rakosiensis	VT48	Peszéradacs, Bányatavi-rét	Hungary	FR745963	FR745912
V. u. rakosiensis	VT40	Peszéradacs, Bányatavi-rét	Hungary	FR745960	FR745909
V. u. rakosiensis	TRA1	Cluj-Napoca, Transylvania / Bogata, Transylvania	Romania	OP777944	OP777992
V. u. rakosiensis	TRA2	Bogata, Transylvania	Romania	OP777945	OP777993
V. u. rakosiensis	TRA3	Cicârd, Transylvania	Romania	OP777946	OP777994
V. u. rakosiensis	TRA4	Cicârd, Transylvania	Romania	OP777947	OP777995
V. u. rakosiensis	TRA5	Cicârd, Transylvania	Romania	OP777948	OP777996
V. u. rakosiensis	ROU159	near Alba Lulia	Romania	FR727086	FR727016
V. u. rakosiensis	ROU148	near Alba Lulia	Romania	FR727085	FR727010
V. u. rakosiensis	ROU152	near Alba Lulia	Romania	FR727081	FR727015
V. u. rakosiensis	ROU155	near Alba Lulia	Romania	FR727084	FR727012
V. u. moldavica	IAS1	Dealul lui Dumnezeu, Iasi	Romania	OP777931	OP777979
V. u. moldavica	IAS2	Valea lui David, Iasi	Romania	OP777932	OP777980
V. u. moldavica	IAS3	Valea lui David, Iasi	Romania	OP777933	OP777981
V. u. moldavica	IAS4	Valea lui David, Iasi	Romania	OP777934	OP777982
V. u. moldavica	DAN1	Periteasca, Danube Delta	Romania	OP777935	OP777983
V. u. moldavica	DAN2	Sfantu Gheorghe, Danube Delta	Romania	OP777936	OP777984
V. u. moldavica	DAN3	Sfantu Gheorghe, Danube Delta	Romania	OP777937	OP777985
V. u. moldavica	DAN4	Sfantu Gheorghe, Danube Delta	Romania	OP777938	OP777986
V. u. moldavica	DAN5	Sfantu Gheorghe, Danube Delta	Romania	OP777939	OP777987
V. u. moldavica	Mol1	Sfantu Gheorghe, Danube Delta	Romania	FR745979	FR745896
V. u. moldavica	Mol2	Sfantu Gheorghe, Danube Delta	Romania	FR745980	FR745897
V. u. moldavica	Mol3	Sfantu Gheorghe, Danube Delta	Romania	FR745981	FR745898

V. u. macrops	DIW1	Velebit Mt. Veliki Štirovac,	Croatia	OP777965	OP778013
V. u. macrops	DIW2	Lisac Mt. / Dinara Mt., Poštak / Ilica Mt.	Croatia / Bosnia and Herzegovina	OP777966	OP778014
V. u. macrops	DIW3	Dinara Mt., Poštak	Croatia	OP777967	OP778015
V. u. macrops	DIW4	Troglav Mt.	Bosnia and Herzegovina	OP777968	OP778016
V. u. macrops	DIW5	Troglav Mt.	Bosnia and Herzegovina	OP785697	OP785699
V. u. macrops	DIW6	Cincar Mt.	Bosnia and Herzegovina	OP785698	OP785700
V. u. macrops	DIE1	Velež Mt. / Zelengora Mt.	Bosnia and Herzegovina	OP777969	OP778017
V. u. macrops	DIE2	Zelengora Mt.	Bosnia and Herzegovina	OP777970	OP778018
V. u. macrops	DIE3	Zelengora Mt.	Bosnia and Herzegovina	OP777971	OP778019
V. u. macrops	DIE4	Durmitor Mt.	Montenegro	OP777972	OP778020
V. u. macrops	DIE5	Bjelasica Mt.	Montenegro	OP777973	OP778021
V. u. macrops	DIE6	Paštrik Mt.	Kosovo province	OP777974	OP778022
V. u. macrops	DIE7	Paštrik Mt. / Šar planina Mt., Brezovica	Kosovo province	OP777975	OP778023
V. u. macrops	DIE8	Šar planina Mt., Lukovo, Pole / Korab Mt., Kobilino Pole	North Macedonia	OP777976	OP778024
V. u. macrops	DIE9	Šar planina Mt., Vakaf	North Macedonia	OP777977	OP778025
V. u. macrops	DIE10	Korab Mt., Kobilino Pole	North Macedonia	OP777978	OP778026
V. u. macrops	MacJ	Korita	Bosnia and Herzegovina	FR727051	FR726982
V. u. macrops	MED1	Bistra Mt., Medenica and Tonivoda	North Macedonia	OP777940	OP777988
V. u. macrops	MED2	Bistra Mt., Medenica and Tonivoda	North Macedonia	OP777941	OP777989
V. u. macrops	MED3	Bistra Mt., Medenica and Tonivoda	North Macedonia	OP777942	OP777990
V. u. macrops	TON1	Bistra Mt., Medenica and Tonivoda	North Macedonia	OP777943	OP777991
V. renardi	BLA1	Yagorlytskyi Kut, Black Sea Biosphere Reserve	Ukraine	OP777958	OP778006
V. renardi	BLA2	Obytichna Kosa, Zaporizhzha Prov.	Ukraine	OP777959	OP778007
V. renardi	BLA3	Crimea, Kerch Peninsula,cape Chauda, Leninskij distdict	Ukraine	OP777960	OP778008
V. renardi	BLA4	Crimea, Kerch Peninsula,cape Chauda, Leninskij distdict	Ukraine	OP777961	OP778009
V. renardi	BLA5	Crimea, near Armyansk / Crimea, Prisivashje, s. Jasnopoljanskoe, Dzhankoiskij district / Belgorod region, Rovenki district, near Nagolnoe villiage, the Sarma river valley slopes / Borki, Valuyki District, Belgorod Province / Lugansk region, Novopskov district, near Novo-Belaya village	Ukraine and Russia	OP777962	OP778010
V. renardi	CRI1	Crimea, West Coast, Sakskij distr., lake Sasyk, st. Pribrezhnaja / Crimea, Foothills (Low-Mountain), Sympheropol distr., v. Krasnolesje	Ukraine	OP777964	OP778012
V. renardi	CRI2	Crimea, Main Ridge, Sympheropol distr.; north slope of Jaila Chatyrdagh near v. Pereval'noje	Ukraine	OP777963	OP778011
V. renardi	REN28	Tatarstan, Rayon Spassky	Russia	FR727101	FR727033
outgroups					
V. u. ursinii	UR001	unknown	Italy	FM955139	FR726973
V. u. ursinii	THIE7	Pop10	France	FM955137	FR726965
V. graeca	Gre3	Stavros	Greece	FR727089	FR727020
V. berus	Vber416	Swiss Jura Mountains	Switzerland	FR727105	FR727036

Table S3. Genetic diversity based on 9 microsatellite markers calculated with GenAlEx 6.51b2 (Smouse et al., 2017) and FSTAT (Goudet, 1995) for the allelic richness (4 diploid individuals).

Population	Nb samples	Ho	He	F_{IS}	Allelic richness
Romania 1	12	0.687	0.533	-0.315	2.262
Romania 2	21	0.479	0.585	0.152	3.919
Romania 3	7	0.485	0.467	-0.045	1.676
Hanság	31	0.526	0.617	0.130	5.04
Kiskunság 1	32	0.580	0.690	0.159	4.995
Kiskunság 2	9	0.548	0.562	-0.007	1.988
Kiskunság 3	25	0.617	0.678	0.118	4.681

Figure S1. TCS network of the mtDNA (concatenated samples) created with PopART v1.8 (Leigh & Bryant, 2015), using the information of the species and subspecies for colouring the different haplotypes.

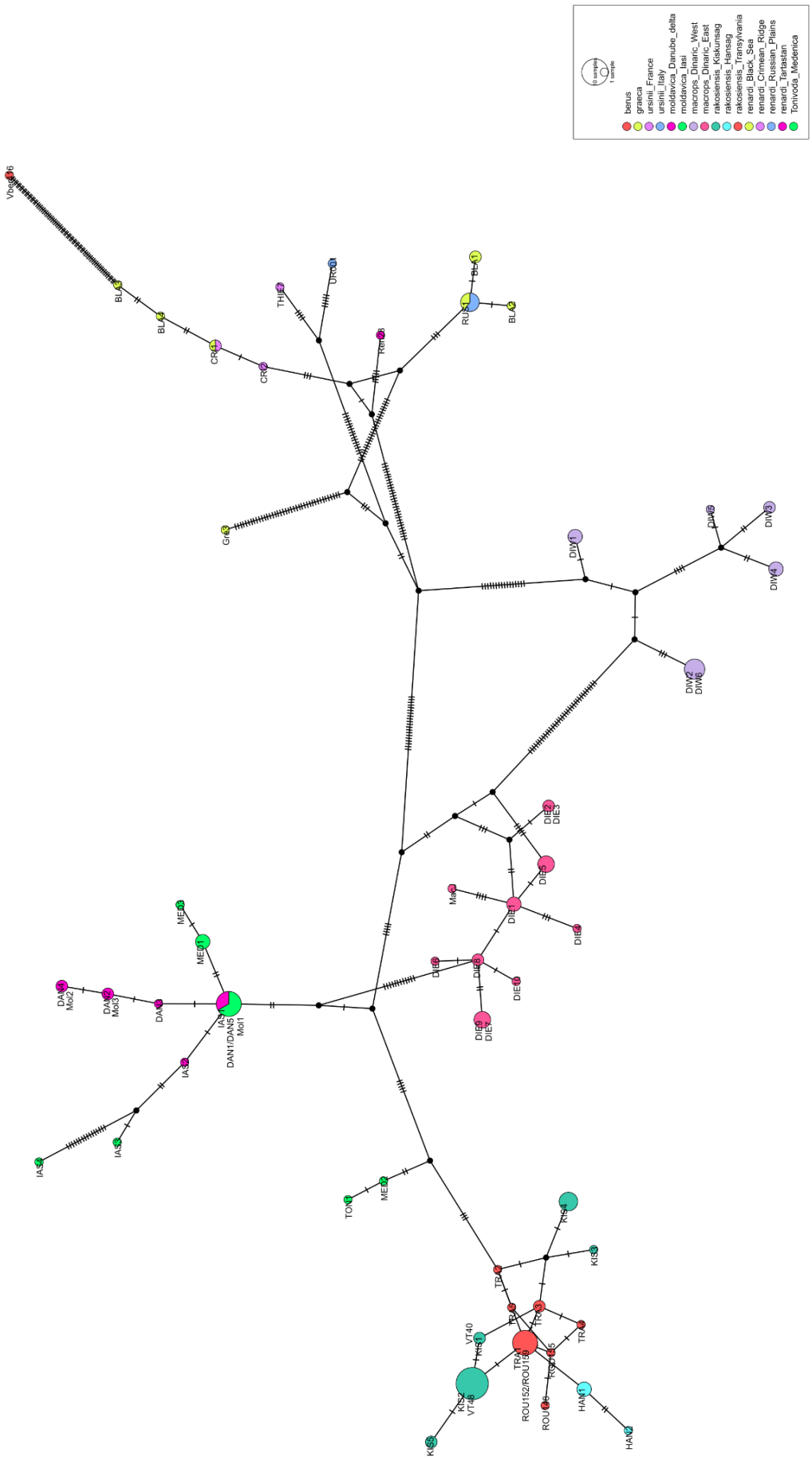
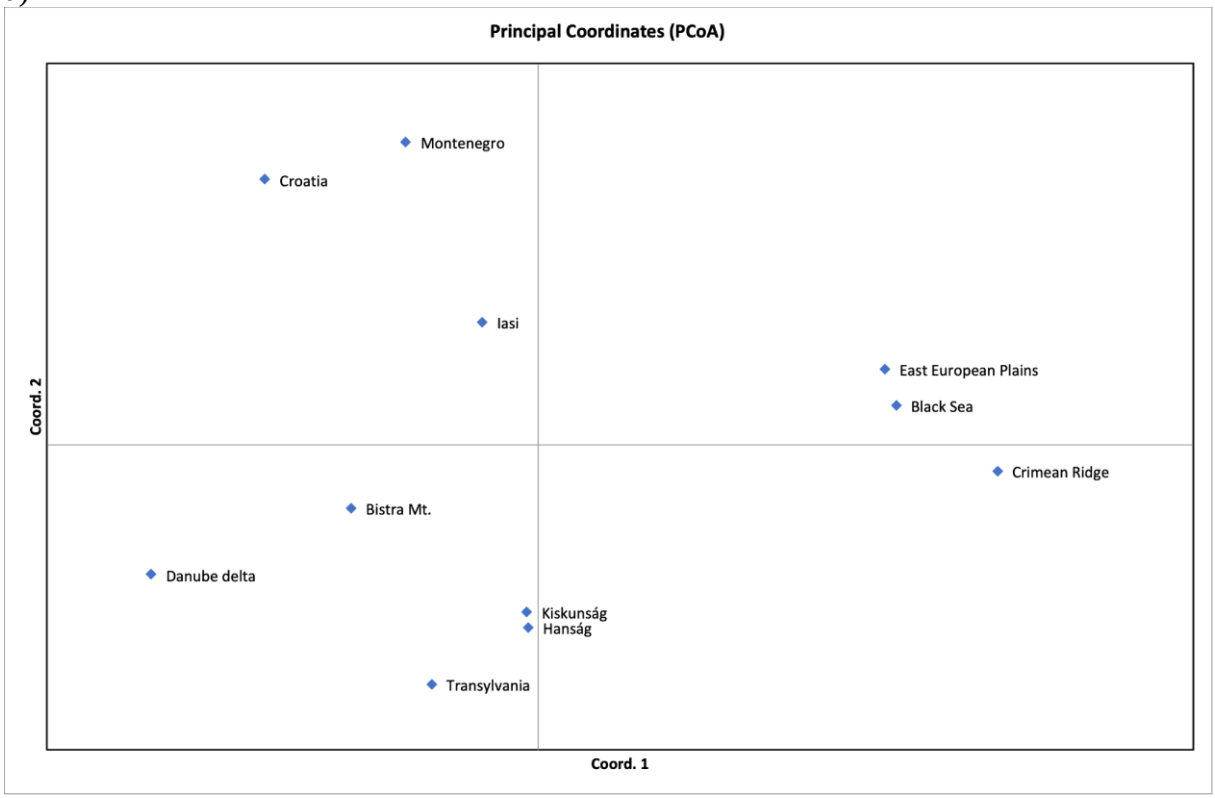

10 samples
1 sample
berus
graeca
ursinii_France
ursinii_Italy
moldavica_Danube_delta
moldavica_Iasi
macrops_Dinaric_West
macrops_Dinaric_East
rakosiensis_Kiskunsag
rakosiensis_Hansag
rakosiensis_Transylvania
renardi_Black_Sea
renardi_Crimean_Ridge
renardi_Russian_Plains
renardi_Tartastan
Tonivoda_Medenica

Figure S2. Principal Coordinates Analysis (PCoA) at the locality scale (a) and at the individual level (b) conducted with GenAlEx 6.51b2 (Smose et al., 2017) using 5 microsatellite markers.

a)



b)

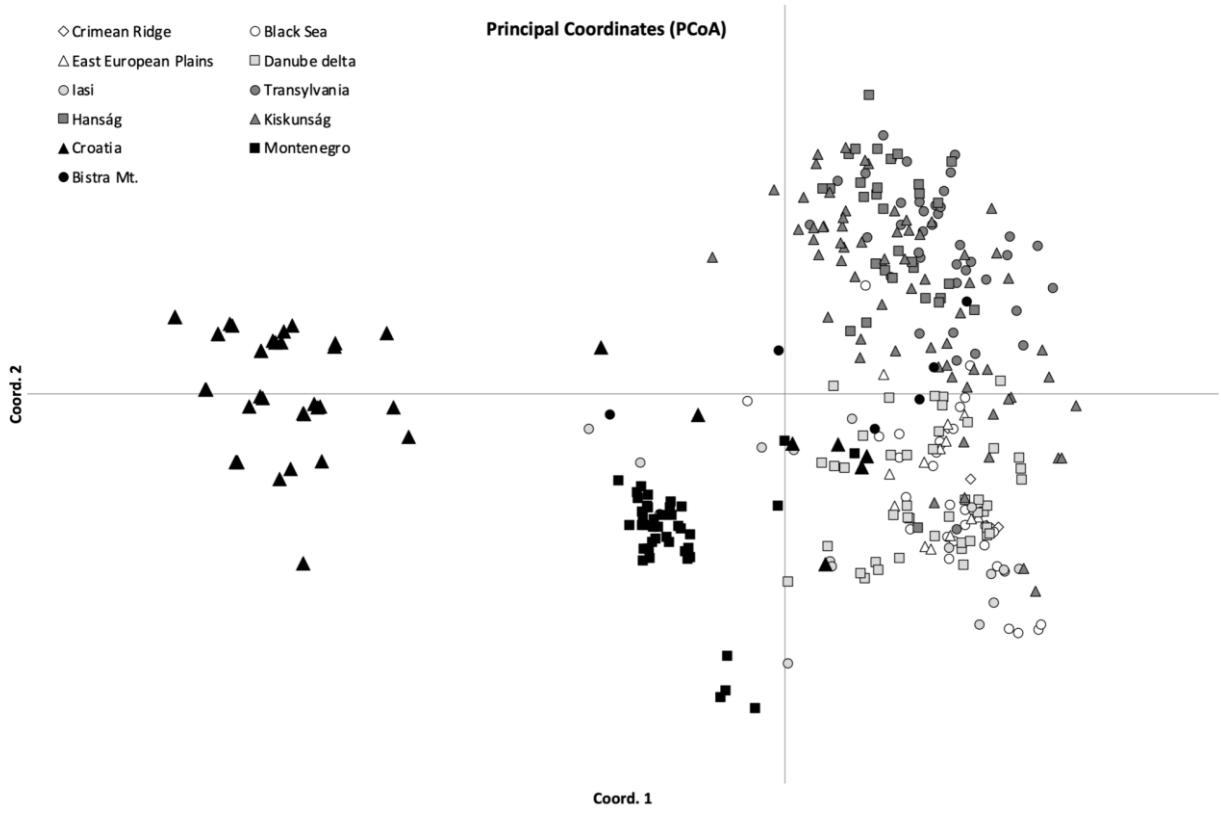
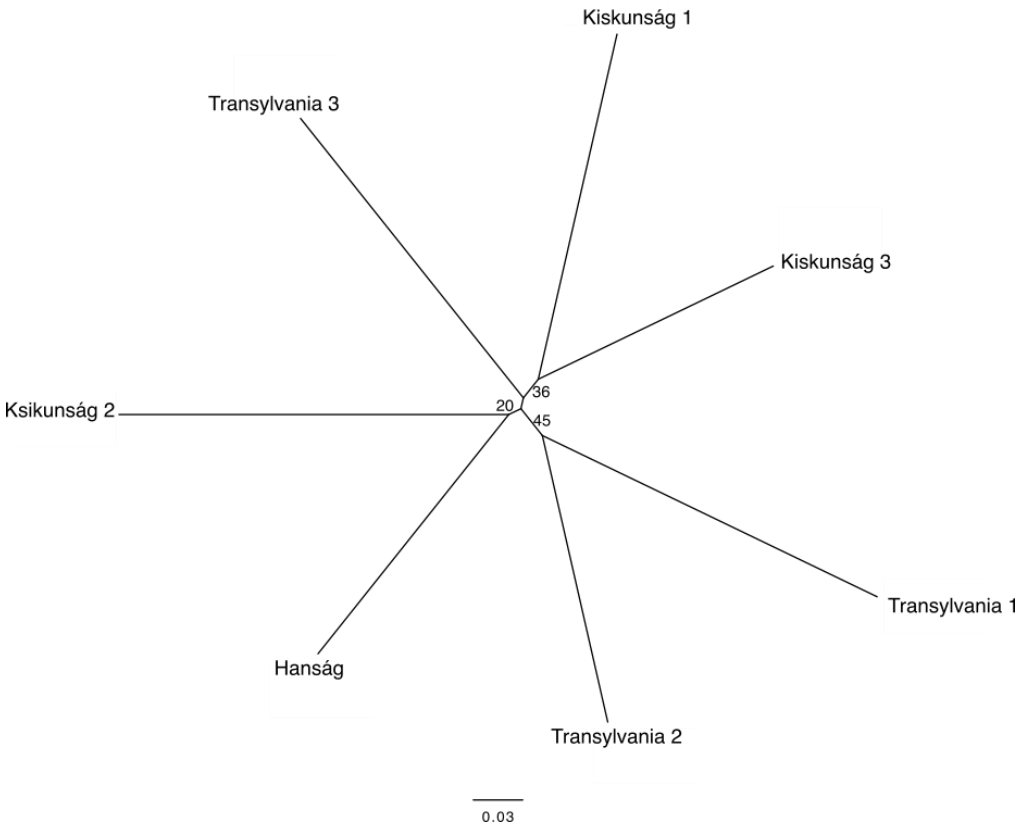


Figure S3. Phylogenetic reconstruction of the genetic relationship between locations calculated using Cavalli-Sforza and Edwards *Dc* distance (Cavalli-Sforza & Edward, 1967) using the programme POPULATIONS 1.2.28 (Langella, 1999) within a) *V. u. rakosiensis* and b) *V. u moldavica*

a)



b)

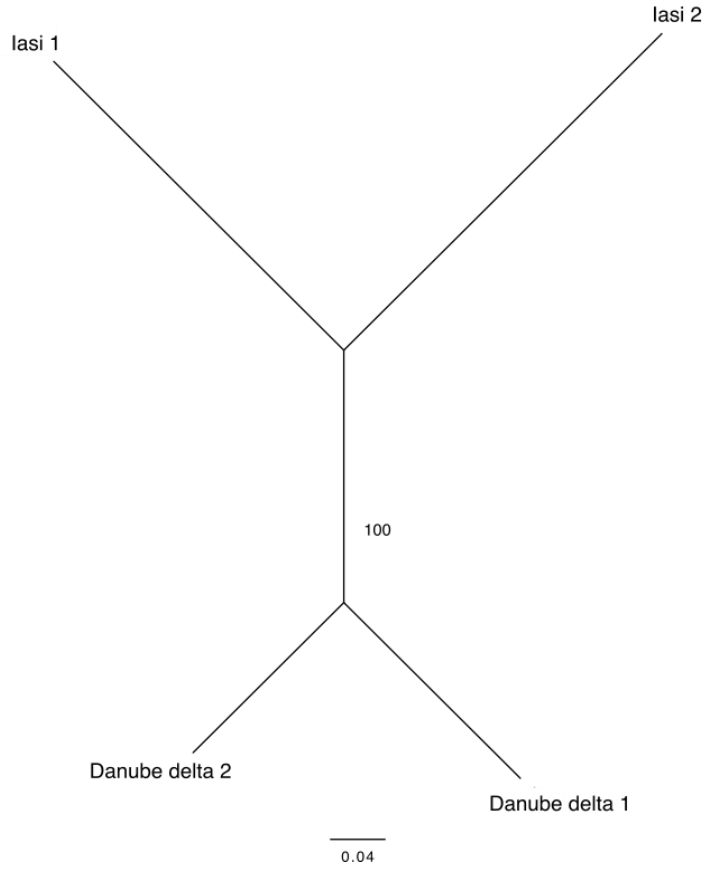
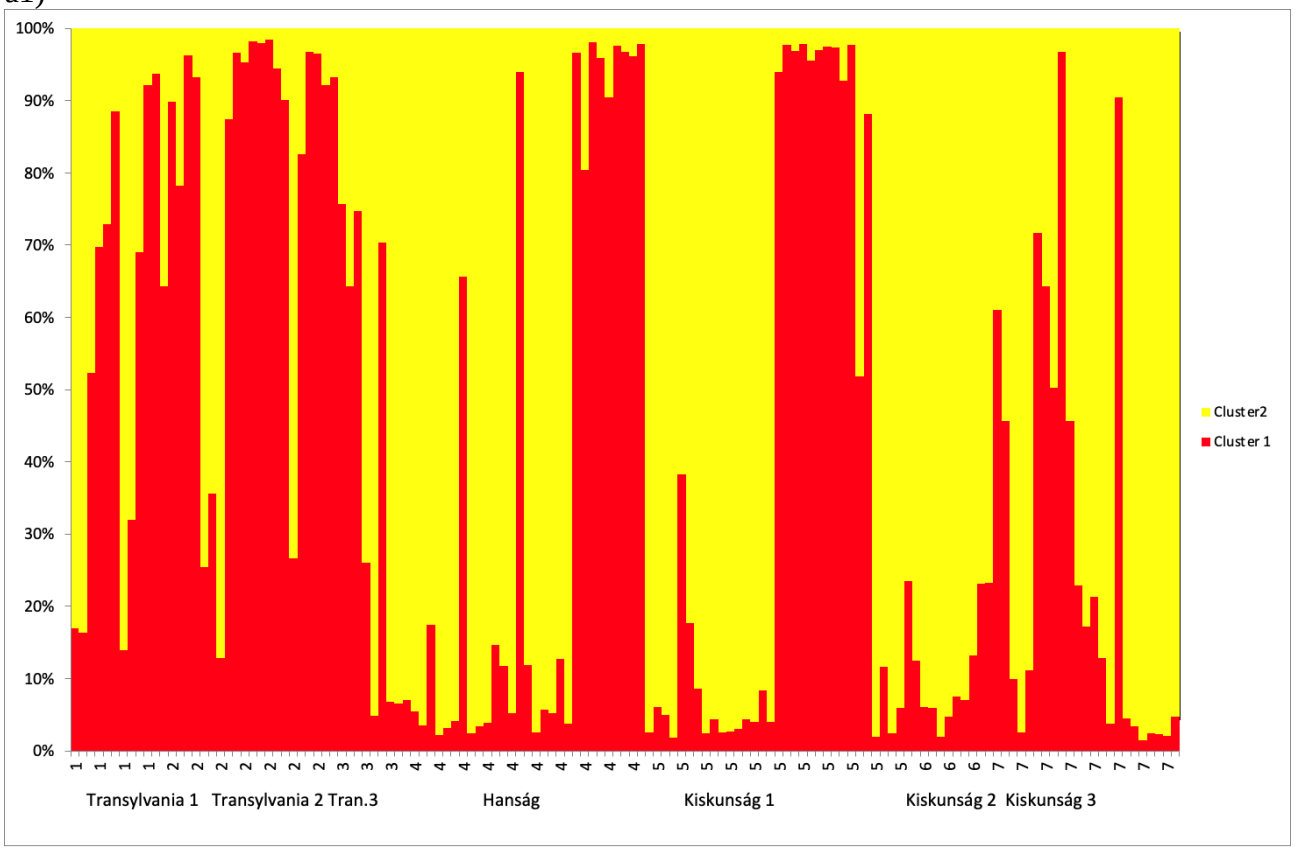
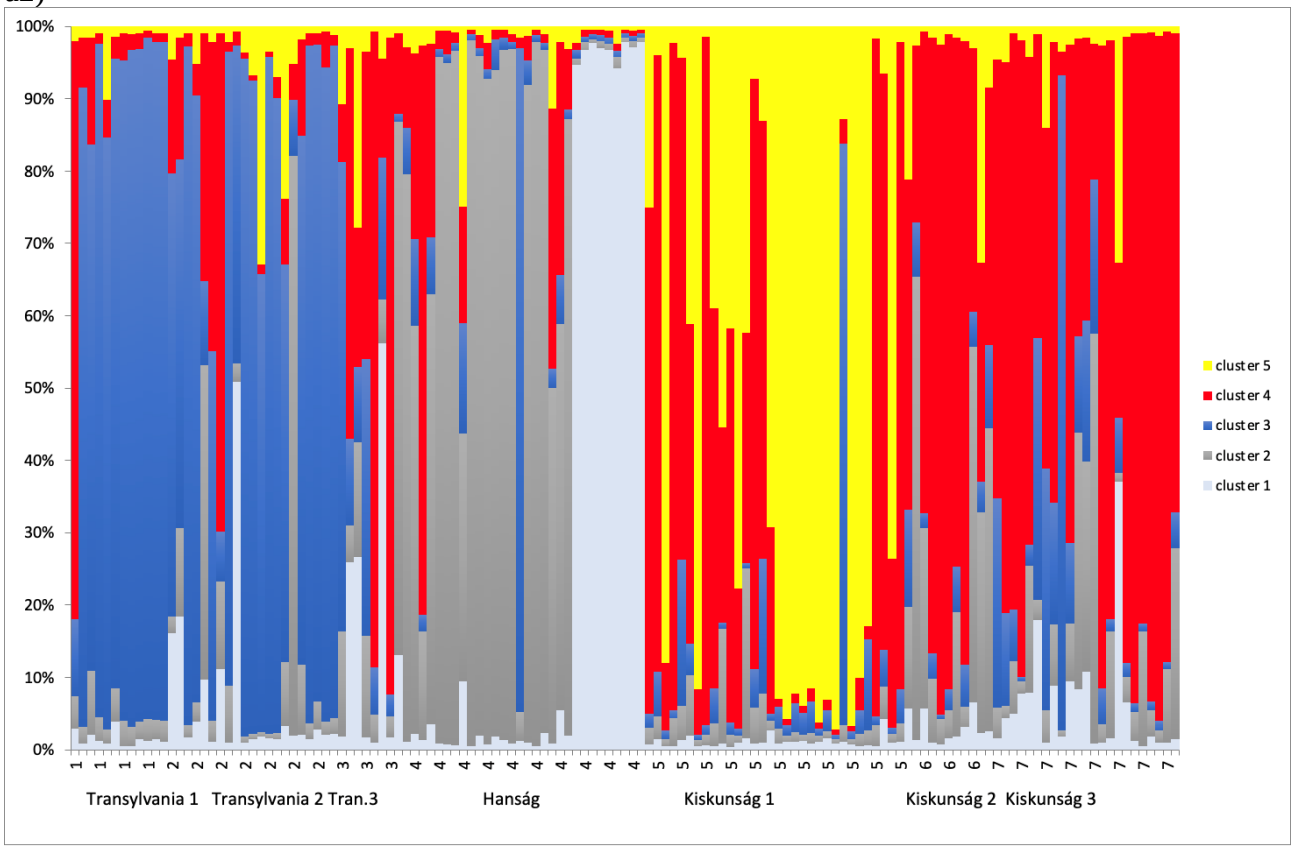


Figure S4. Best Structure runs for *V. u. rakosiensis* (a1: following Evanno et al. (2005): K = 2; a2: best L(K) value: K = 5); *V. u moldavica* (b1: K = 2; b2: K = 4), and *V. macrops* (c1: K = 2; c2: K = 7).

a1)



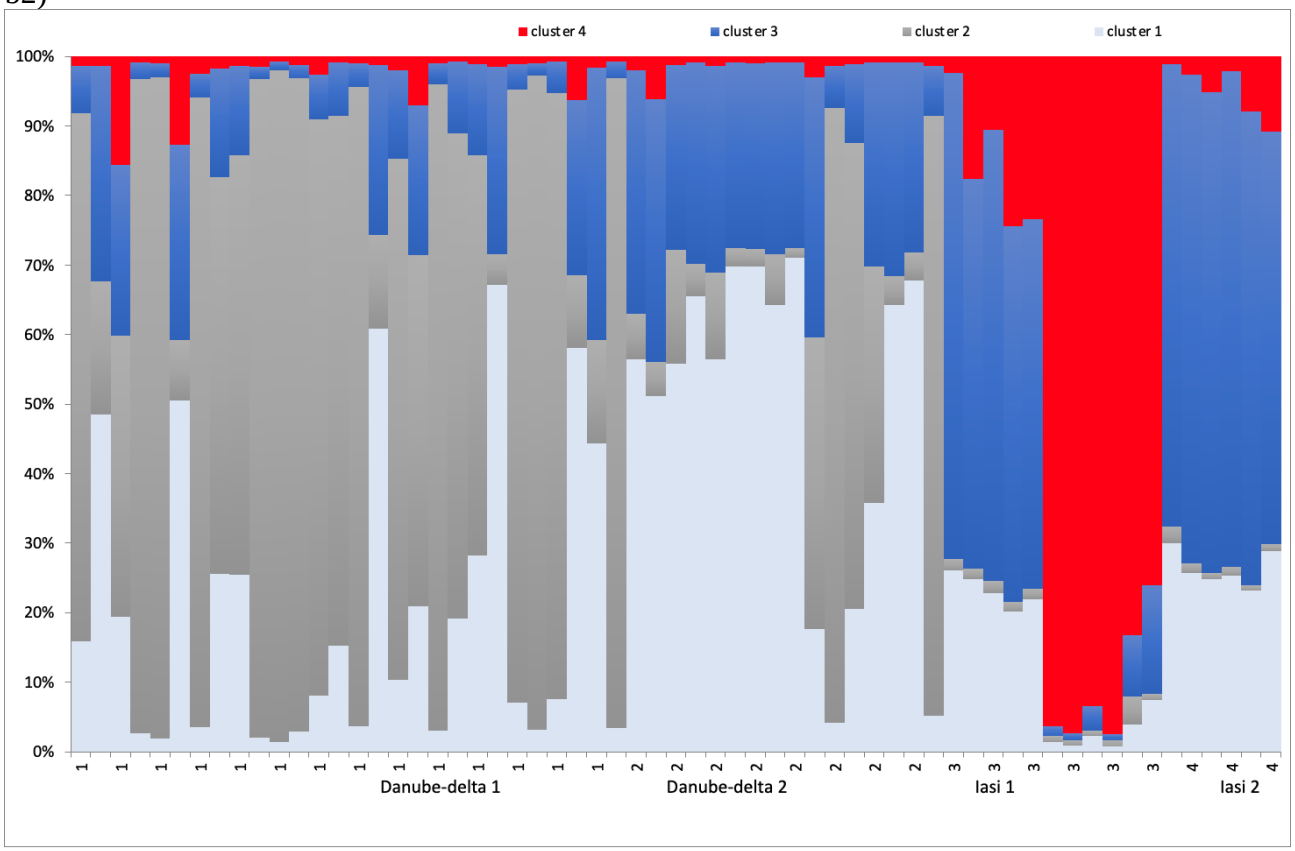
a2)



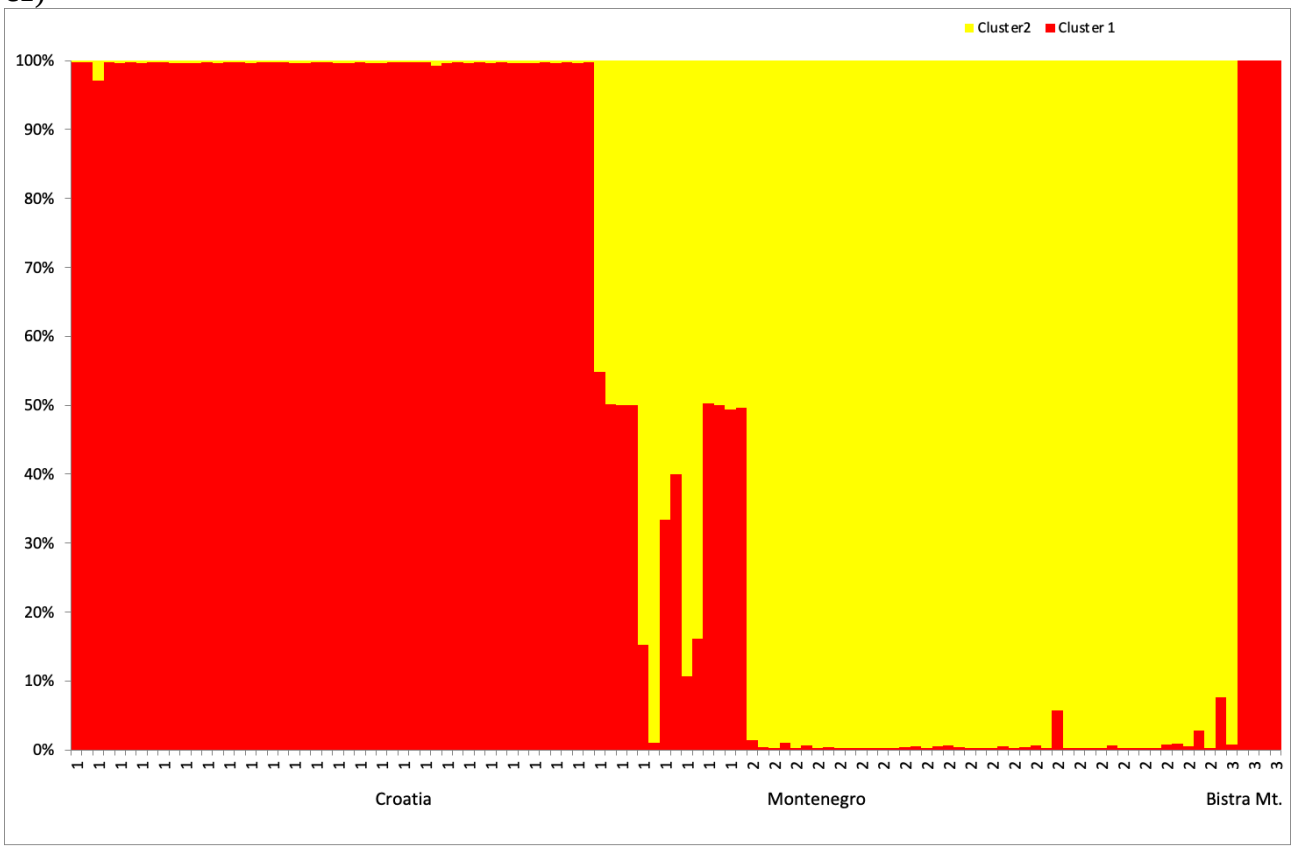
b1)


Cluster2
Cluster 1
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Danube-delta 1
Danube-delta 2
Iasi 1
Iasi 2

b2)


cluster 4
cluster 3
cluster 2
cluster 1
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Danube-delta 1
Danube-delta 2
Iasi 1
Iasi 2

c1)


Cluster2
Cluster 1
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Croatia
Montenegro
Bistra Mt.

c2)

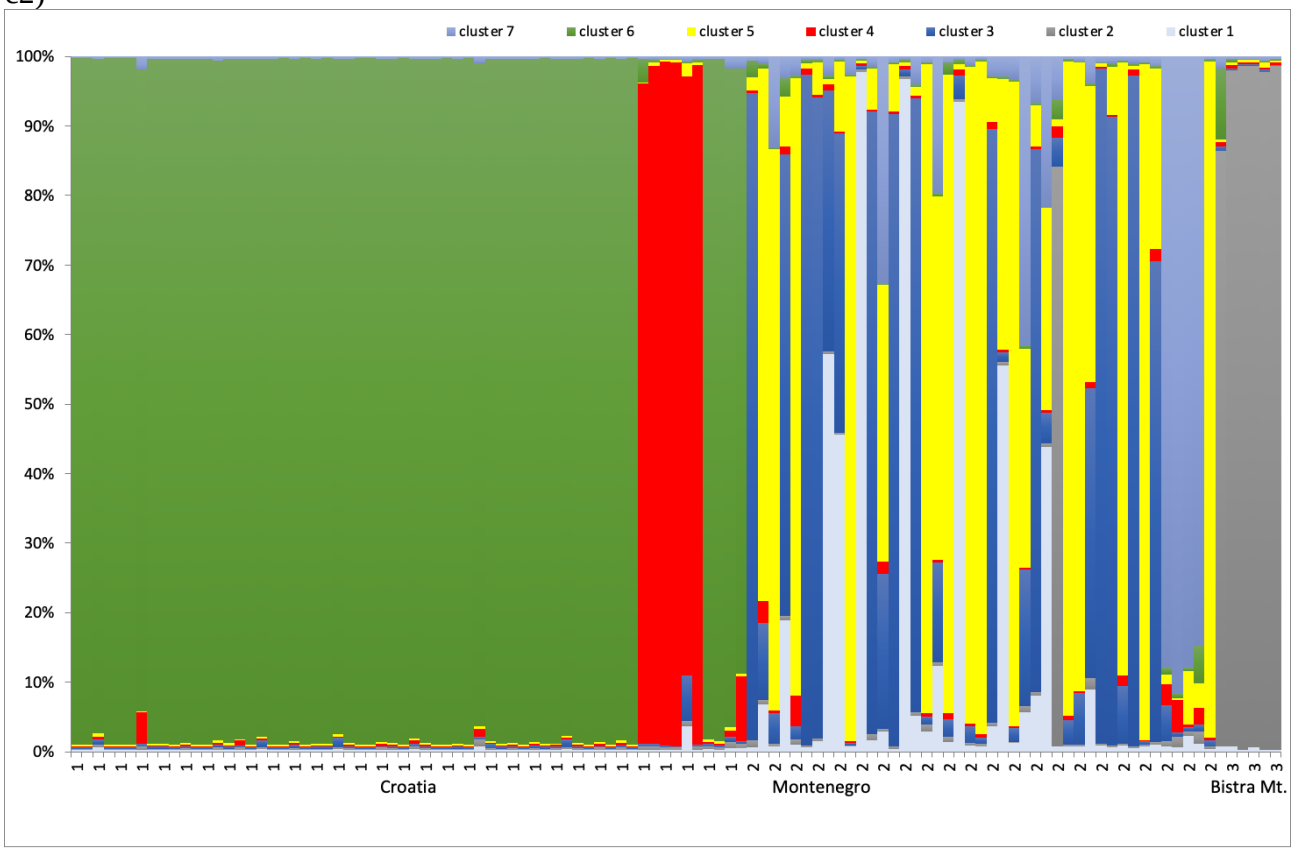
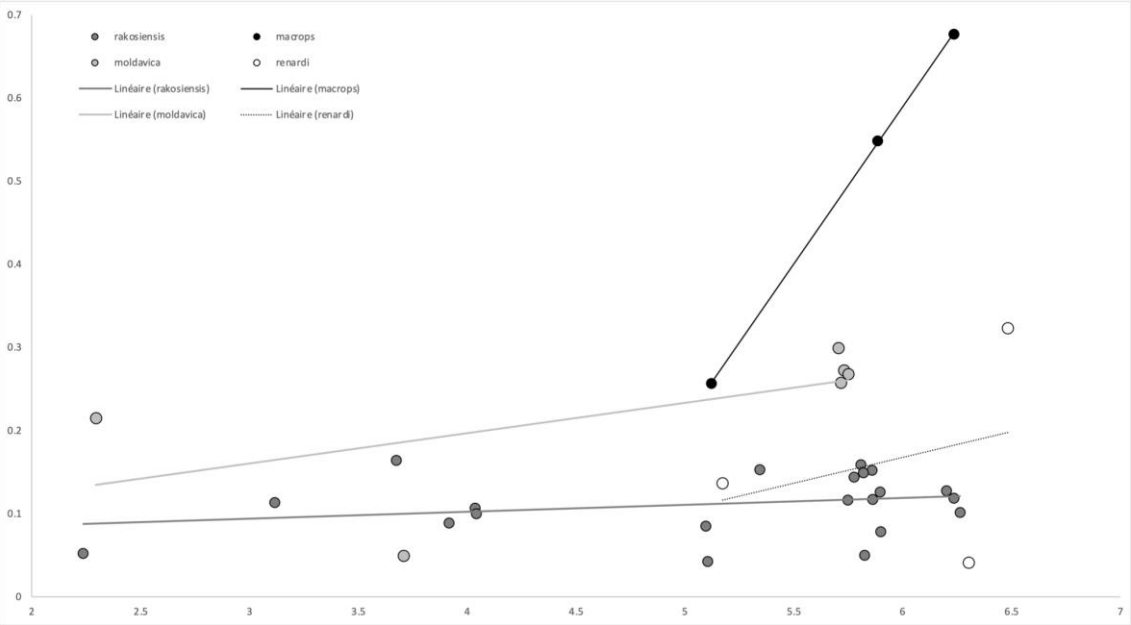

cluster 7
cluster 6
cluster 5
cluster 4
cluster 3
cluster 2
cluster 1
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Croatia
Montenegro
Bistra Mt.

Figure S5. Relation between geographical distance and genetic distance. The distance is ln corrected (kilometers), whereas the genetic distance was corrected following Rousset (1997) with (F_{ST}/[1-F_{ST}]). The test was not significant for *V. u. rakosiensis* (r^2 = 0.070; p = 0.247), *V. u. moldavica* (r^2 = 0.350; p = 0.216) and for *V. renardi* (r^2 = 0.093; p = 0.802), it was significant for *V. u. macrops* (r^2 = 1.000; p = 0.007)



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