

Evidence of subtle genetic structure in the sympatric species *Mullus barbatus* and *Mullus surmuletus* (Linnaeus, 1758) in the Mediterranean Sea

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Supplement Table S1. Origin of the studied samples of *Mullus barbatus* and *Mullus surmuletus*, and corresponding number of specimens amplified with the microsatellites markers and included in the analyses.

Species	Sea	Region, Country (GSA)	Geographic coordinates		Code	N
<i>M. barbatus</i> <i>M. surmuletus</i>	Mediterranean	North Adriatic, Croatia (GSA17)	44.943815°	13.605487°	CRO_NAS	48 50
<i>M. barbatus</i> <i>M. surmuletus</i>	Mediterranean	Middle Adriatic, Croatia (GSA17)	43.568418°	15.769985°	CRO_MAS	66 54
<i>M. barbatus</i> <i>M. surmuletus</i>	Mediterranean	South Adriatic, Croatia (GSA17)	42.615188°	17.779310°	CRO_SAS	50 49
<i>M. barbatus</i>	Mediterranean	North Adriatic, Italy (GSA17)	45.009044°	13.004869°	ITA_NAS	49
<i>M. barbatus</i> , <i>M. surmuletus</i>	Mediterranean	Middle Adriatic, Italy (GSA17)	43.750132°	13.757831°	ITA_MAS	50 47
<i>M. barbatus</i> , <i>M. surmuletus</i>	Mediterranean	South Adriatic, Italy (GSA17)	41.504365°	16.810572°	ITA_SAS	47 42
<i>M. barbatus</i> , <i>M. surmuletus</i>	Mediterranean	Tyrrhenian Sea, Italy (GSA9)	41.503912°	11.065175°	ITA_TS	49 45
<i>M. barbatus</i> , <i>M. surmuletus</i>	Mediterranean	South Adriatic, Montenegro (GSA18)	42.266861°	18.618152°	MN_AS	87 31
<i>M. barbatus</i>	Mediterranean	South Adriatic, Albania (GSA18)	41.162552°	19.259209°	AL_AS	67
<i>M. barbatus</i> , <i>M. surmuletus</i>	Mediterranean	Ionian Sea, Greece (GSA20)	38.171978°	22.651481°	GR_IS	59 59
<i>M. barbatus</i> , <i>M. surmuletus</i>	Mediterranean	Aegean Sea, Turkey (GSA22)	37.947463°	26.720771°	TR_AS	48 49
<i>M. barbatus</i> , <i>M. surmuletus</i>	Mediterranean	Levantine Sea, Israel (GSA27)	32.335961°	34.363010°	IS_LS	23 15
<i>M. barbatus</i> , <i>M. surmuletus</i>	Mediterranean	Levantine Sea, Cyprus (GSA25)	34.429656°	32.220743°	CP_LS	31 49
<i>M. barbatus</i> , <i>M. surmuletus</i>	Mediterranean	Balearic Sea, Spain (GSA6)	38.572230°	0.637080°	SP_BS	46 41
<i>M. surmuletus</i>	N-E Atlantic	Portugal	40.154904°	9.21806°	PT_AO	68

Supplement Table S2. Summery statistics of 13 microsatellite loci among samples of *Mullus barbatus* and *Mullus surmuletus*.

<i>M.barbatus</i>		Sampling code													
Locus		CRO NAS	CRO MAS	CRO SAS	ITA NAS	ITA MAS	ITA SAS	ITA TS	MN AS	AL AS	GR IS	TR LS	IS LS	CP LS	SP BS
Mbar 132	n	48	66	50	49	50	47	49	87	67	59	48	23	29	46
	nA	13	11	10	10	10	8	10	11	9	11	8	8	9	10
	HO	0.771	0.848	0.720	0.706	0.660	0.787	0.715	0.655	0.672	0.763	0.750	0.739	0.862	0.543
	HE	0.734	0.769	0.710	0.717	0.632	0.734	0.733	0.652	0.711	0.732	0.746	0.734	0.769	0.636
Mbar 14	n	47	66	50	49	50	45	48	87	65	59	48	22	20	46
	nA	31	33	31	27	30	32	31	36	35	31	31	20	20	28
	HO	0.914	0.924	0.860	0.857	0.820	0.867	0.833	0.851	0.923	0.864	0.896	0.772	0.850	0.804
	HE	0.963	0.939	0.948	0.949	0.960	0.968	0.955	0.957	0.957	0.952	0.949	0.948	0.958	0.938
Mbar 064	n	48	66	50	49	50	47	49	87	67	59	48	23	31	46
	nA	6	6	6	4	6	6	6	6	6	6	6	6	7	5
	HO	0.562	0.500	0.500	0.571	0.540	0.532	0.530	0.506	0.657	0.542	0.688	0.565	0.548	0.521
	HE	0.521	0.511	0.502	0.531	0.544	0.589	0.576	0.558	0.559	0.558	0.614	0.581	0.580	0.490
Mbar 051	n	48	66	50	49	50	47	49	87	67	59	48	23	31	46
	nA	10	8	8	9	7	9	7	9	11	10	9	9	7	10
	HO	0.625	0.606	0.640	0.673	0.620	0.787	0.694	0.724	0.657	0.661	0.625	0.609	0.580	0.696
	HE	0.656	0.602	0.611	0.703	0.599	0.787	0.665	0.688	0.666	0.631	0.675	0.726	0.652	0.688
Mbar 002	n	48	66	50	49	50	47	49	87	67	59	48	23	31	46
	nA	8	9	8	10	9	9	7	9	9	10	8	7	9	11
	HO	0.708	0.666	0.700	0.877	0.660	0.829	0.633	0.701	0.701	0.627	0.729	0.696	0.742	0.696
	HE	0.724	0.730	0.690	0.771	0.714	0.725	0.702	0.708	0.712	0.761	0.673	0.723	0.816	0.739
Mbar 63*	n	48	64	50	49	49	45	47	85	66	59	47	20	21	45
	nA	16	15	14	15	12	12	12	14	14	10	16	10	9	14
	HO	0.687	0.609	0.690	0.796	0.571	0.800	0.638	0.718	0.591	0.610	0.681	0.650	0.571	0.666
	HE	0.852	0.851	0.857	0.859	0.830	0.858	0.853	0.843	0.842	0.841	0.889	0.877	0.872	0.891
Mbar 101*	n	48	65	50	49	50	47	49	87	67	59	48	23	31	46
	nA	2	2	2	2	2	2	2	3	3	2	2	3	3	1
	HO	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.015	0.017	0.000	0.000	0.032	0.783
	HE	0.491	0.143	0.424	0.247	0.298	0.384	0.679	0.034	0.044	0.017	0.041	0.166	0.206	0.878
Mbar 55	n	48	65	50	49	50	45	47	87	67	58	48	23	27	46
	nA	16	17	17	17	17	17	18	19	19	14	13	13	14	13
	HO	0.708	0.800	0.850	0.755	0.840	0.867	0.851	0.839	0.851	0.741	0.792	0.696	0.778	0.782
	HE	0.889	0.876	0.889	0.877	0.911	0.916	0.921	0.888	0.900	0.904	0.879	0.922	0.899	0.877
Mbar 130*	n	40	55	44	41	43	37	46	73	61	50	44	18	21	36
	nA	11	13	11	13	12	17	10	11	8	11	12	7	9	10
	HO	0.250	0.345	0.254	0.293	0.302	0.479	0.174	0.164	0.229	0.100	0.227	0.056	0.142	0.305
	HE	0.836	0.829	0.782	0.788	0.796	0.868	0.737	0.699	0.789	0.819	0.837	0.678	0.829	0.825
Mbar 56	n	48	66	50	49	50	46	49	87	66	58	48	22	23	46
	nA	9	8	8	7	8	8	8	8	11	9	9	6	6	7
	HO	0.645	0.636	0.740	0.735	0.660	0.674	0.673	0.829	0.621	0.689	0.688	0.773	0.435	0.609
	HE	0.692	0.663	0.745	0.712	0.664	0.671	0.670	0.696	0.700	0.718	0.737	0.683	0.669	0.682
Mbar 3*	n	32	41	33	35	36	30	30	74	56	43	47	20	20	35
	nA	19	20	16	18	18	17	19	28	23	14	21	14	13	21
	HO	0.281	0.341	0.424	0.171	0.222	0.233	0.200	0.338	0.500	0.256	0.298	0.250	0.250	0.257
	HE	0.946	0.933	0.919	0.927	0.926	0.902	0.939	0.942	0.944	0.894	0.928	0.919	0.892	0.942
Mbar 11	n	48	65	50	49	50	46	49	87	67	59	48	22	26	46
	nA	13	13	13	12	16	12	9	13	12	12	11	9	11	12
	HO	0.666	0.575	0.600	0.653	0.640	0.652	0.551	0.609	0.955	0.695	0.583	0.682	0.692	0.608
	HE	0.670	0.652	0.668	0.626	0.688	0.716	0.618	0.622	0.553	0.702	0.660	0.682	0.674	0.650
Mbar 133	n	47	65	50	49	50	45	49	84	64	59	48	22	19	45
	nA	18	18	19	14	15	14	19	18	16	17	19	12	7	18
	HO	0.766	0.677	0.740	0.639	0.720	0.622	0.735	0.667	0.625	0.729	0.813	0.591	0.579	0.777
	HE	0.890	0.825	0.852	0.882	0.851	0.806	0.837	0.859	0.829	0.856	0.859	0.845	0.665	0.842

<i>M. surmuletus</i>		Sampling code													
Locus	Indices	CRO	CRO	CRO	ITA	ITA	ITA	MN	GR	TR	IS	CP	SP	PT	
		NAS	MAS	SAS	MAS	SAS	TS	AS	IS	LS	LS	LS	BS	AO	
Mbar 132	n	50	54	49	47	42	45	31	59	49	15	49	41	68	
	nA	14	12	14	11	12	13	13	13	15	7	14	13	16	
	HO	0.800	0.722	0.816	0.872	0.928	0.822	0.806	0.673	0.816	0.800	0.796	0.805	0.779	
	HE	0.841	0.818	0.973	0.833	0.835	0.856	0.852	0.825	0.814	0.752	0.875	0.853	0.855	
Mbar 14	n	50	54	48	47	42	45	31	59	49	15	49	41	68	
	nA	22	23	22	20	21	25	22	22	20	10	22	21	26	
	HO	0.880	0.888	0.937	0.936	0.871	0.977	1.000	0.763	0.939	0.933	0.877	0.951	0.897	
	HE	0.939	0.919	0.941	0.936	0.917	0.944	0.950	0.917	0.934	0.816	0.911	0.932	0.926	
Mbar 064	n	50	54	49	47	42	45	31	59	49	15	49	41	68	
	nA	5	4	3	2	2	4	5	6	2	2	5	2	4	
	HO	0.200	0.148	0.265	0.128	0.357	0.244	0.226	0.288	0.184	0.133	0.347	0.097	0.162	
	HE	0.302	0.173	0.282	0.121	0.297	0.223	0.343	0.312	0.201	0.129	0.371	0.178	0.255	
Mbar 051	n	50	53	49	47	42	45	31	59	47	15	49	41	68	
	nA	16	17	18	16	14	16	12	17	17	9	13	17	16	
	HO	0.660	0.830	0.755	0.745	0.715	0.777	0.645	0.831	0.875	0.667	0.633	0.732	0.618	
	HE	0.899	0.909	0.894	0.895	0.870	0.909	0.899	0.883	0.912	0.696	0.876	0.906	0.876	
Mbar 002	n	50	54	49	47	42	45	31	59	49	15	49	41	68	
	nA	10	9	9	8	7	9	8	9	7	6	10	8	9	
	HO	0.660	0.648	0.775	0.766	0.786	0.777	0.742	0.746	0.714	0.600	0.775	0.658	0.750	
	HE	0.712	0.772	0.787	0.772	0.691	0.757	0.827	0.767	0.779	0.825	0.750	0.776	0.756	
Mbar 63	n	50	54	49	47	42	45	31	59	49	15	49	41	68	
	nA	16	18	19	15	18	16	12	18	17	9	15	15	17	
	HO	0.700	0.833	0.837	0.808	0.833	0.689	0.677	0.798	0.796	0.533	0.551	0.805	0.823	
	HE	0.893	0.873	0.889	0.886	0.888	0.868	0.877	0.879	0.879	0.887	0.874	0.874	0.861	
Mbar 101	n	48	51	49	47	42	45	30	59	49	15	49	41	67	
	nA	7	7	6	6	6	8	7	8	6	4	6	9	7	
	HO	0.579	0.726	0.653	0.617	0.786	0.644	0.567	0.695	0.592	0.467	0.715	0.683	0.522	
	HE	0.684	0.645	0.704	0.686	0.656	0.692	0.708	0.680	0.661	0.494	0.627	0.714	0.572	
Mbar 55	n	50	54	49	46	42	45	31	59	49	15	49	41	68	
	nA	12	7	8	9	9	7	12	10	10	3	8	6	9	
	HO	0.780	0.685	0.633	0.739	0.857	0.644	0.742	0.644	0.755	0.667	0.775	0.658	0.521	
	HE	0.759	0.688	0.716	0.750	0.718	0.730	0.749	0.708	0.746	0.536	0.738	0.717	0.703	
Mbar 130*	n	45	37	41	37	42	37	30	52	45	15	40	36	51	
	nA	16	9	12	13	10	9	10	14	10	7	15	13	17	
	HO	0.244	0.063	0.171	0.270	0.214	0.270	0.300	0.288	0.133	0.267	0.125	0.139	0.216	
	HE	0.873	0.744	0.825	0.783	0.766	0.624	0.814	0.821	0.692	0.827	0.863	0.809	0.856	
Mbar 56	n	47	54	49	47	42	45	31	59	49	14	47	41	68	
	nA	7	7	7	7	7	7	8	7	7	6	7	6	7	
	HO	0.851	0.833	0.673	0.787	0.833	0.889	0.839	0.831	0.837	0.643	0.766	0.756	0.809	
	HE	0.806	0.814	0.777	0.761	0.763	0.798	0.800	0.779	0.806	0.754	0.817	0.757	0.766	
Mbar 3	n	33	48	46	47	42	44	30	58	48	10	37	33	64	
	nA	8	9	7	8	7	8	9	8	5	3	7	6	5	
	HO	0.333	0.500	0.500	0.638	0.595	0.341	0.367	0.431	0.431	0.500	0.297	0.424	0.312	
	HE	0.415	0.523	0.508	0.550	0.545	0.372	0.481	0.423	0.402	0.426	0.371	0.394	0.302	
Mbar 11	n	42	50	47	47	42	45	29	58	48	10	19	31	55	
	nA	9	7	9	8	7	8	12	11	9	7	14	7	7	
	HO	0.381	0.600	0.808	0.638	0.762	0.711	0.586	0.741	0.687	0.700	0.526	0.581	0.545	
	HE	0.759	0.730	0.785	0.777	0.684	0.744	0.821	0.768	0.783	0.863	0.824	0.743	0.699	
Mbar 133*	n	45	49	49	45	41	43	29	57	48	14	40	39	64	
	nA	11	14	13	10	13	13	13	16	12	7	12	12	12	
	HO	0.111	0.224	0.143	0.311	0.439	0.302	0.448	0.281	0.229	0.428	0.325	0.231	0.172	
	HE	0.866	0.907	0.874	0.874	0.884	0.907	0.892	0.900	0.884	0.873	0.877	0.868	0.882	

Supplement Table S3. Bottleneck results of one-tail Wilcoxon test for heterozygote excess and observed values of the M -ratio averaged over the number of polymorphic microsatellite loci for each *Mullus barbatus* (a) and *Mullus surmuletus* (b) contemporary sample considered in this study. For different ancestral theta (0.5, 1, 10), the p -value at which a bottleneck occurred is presented. NS = not significant.

a) <i>Mullus barbatus</i>	Bottleneck		M	M ratio (P -value)		
	IAM	TPM(90)		$\Theta = 0.5$	$\Theta = 1$	$\Theta = 10$
CRO_NAS	Ns	ns	0.754	ns	ns	ns
CRO_MAS	Ns	ns	0.763	ns	ns	ns
CRO_SAS	Ns	ns	0.796	ns	ns	ns
ITA_NAS	Ns	ns	0.792	ns	ns	ns
ITA_MAS	Ns	ns	0.73	< 0.05	ns	ns
ITA_SAS	Ns	ns	0.78	ns	ns	ns
ITA_TS	Ns	ns	0.806	ns	ns	ns
MN_AS	Ns	ns	0.806	ns	ns	ns
AL_AS	Ns	ns	0.77	ns	ns	ns
GR_IS	< 0.05	ns	0.741	< 0.001	< 0.001	< 0.001
TR_AS	< 0.05	ns	0.729	< 0.001	< 0.001	< 0.001
SP_BS	Ns	ns	0.773	ns	ns	ns

b) <i>Mullus surmuletus</i>	Bottleneck		M	M ratio (P -value)		
	IAM	TPM(90)		$\Theta = 0.5$	$\Theta = 1$	$\Theta = 10$
CRO_NAS	Ns	ns	0.723	< 0.03	ns	ns
CRO_MAS	Ns	ns	0.768	ns	ns	ns
CRO_SAS	Ns	ns	0.76	ns	ns	ns
ITA_MAS	Ns	ns	0.806	ns	ns	ns
ITA_SAS	Ns	ns	0.8	ns	ns	ns
ITA_TS	Ns	ns	0.765	ns	ns	ns
MN_AS	Ns	ns	0.695	< 0.02	ns	ns
GR_IS	Ns	ns	0.732	ns	ns	ns
TR_AS	< 0.01	ns	0.688	< 0.001	< 0.01	ns
CP_LS	Ns	ns	0.764	ns	ns	ns
SP_BS	Ns	ns	0.76	ns	ns	ns
PT_AO	Ns	ns	0.703	< 0.02	ns	ns

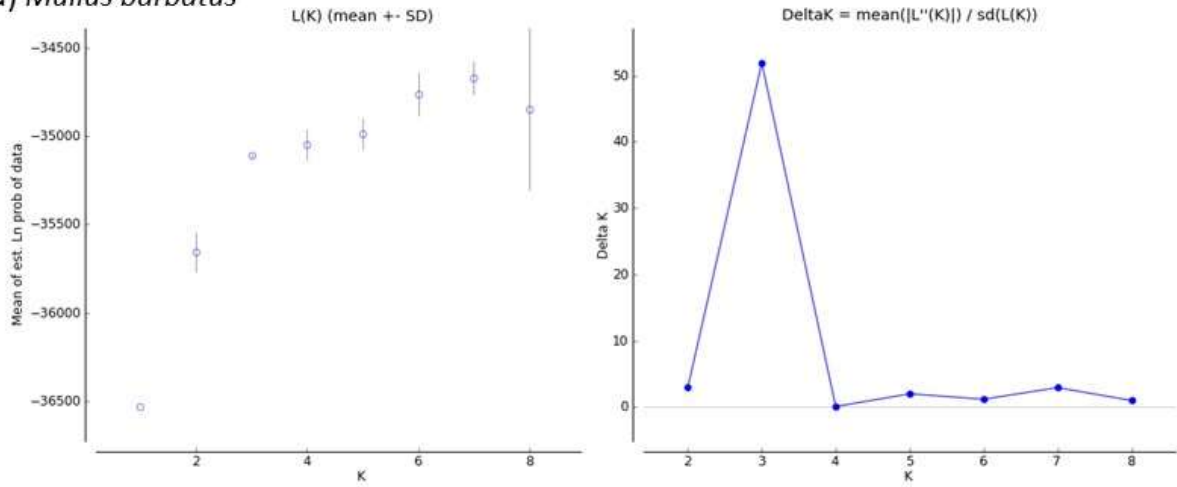
Supplement Table S4. GFCM stock assessment outcomes for *Mullus barbatus* in 2016⁴⁵ in relation with observed heterozygosity and M ratio values

GSA	<i>Mullus barbatus</i>			
	F_{cur}	State	Observed Heterozygosity	M-ratio
GSA 6 (Northern Spain)	$F_{cur} > F_{0.1}$	Overexploitation	0.67±0.1	0.773
GSA 9 (Ligurian Sea and Northern Tyrrhenian Sea)	$F_{cur} > F_{0.1}$	High overfishing	0.68±0.1	0.806
GSA 17 (Northern Adriatic Sea)	$F_{cur} > F_{0.1}$	High overfishing	0.68±0.1	0.754-0.763
			0.70±0.9(CRO)	(CRO)
			0.67±0.1	0.73-0.792
GSA 18 (Southern Adriatic Sea)	$F_{cur} < F_{0.1}$	Sustainable exploitation	0.73±0.1 (ITA)	(ITA)
			0.70±0.1(CRO)	0.796 (CRO)
GSA 20 (Eastern Ionian Sea)		n.a.	0.74±0.1 (ITA)	0.78 (ITA)
GSA 22 (Aegean Sea)		n.a.	0.69±0.1	0.741
GSA 25 (Cyprus)	$F_{cur} < F_{0.1}$	Sustainable exploitation	0.72±0.1	0.729
GSA 27 (Eastern Levant Sea)		n.a.	0.66±0.1	-
GSA 27 (Eastern Levant Sea)		n.a.	0.67±0.1	-

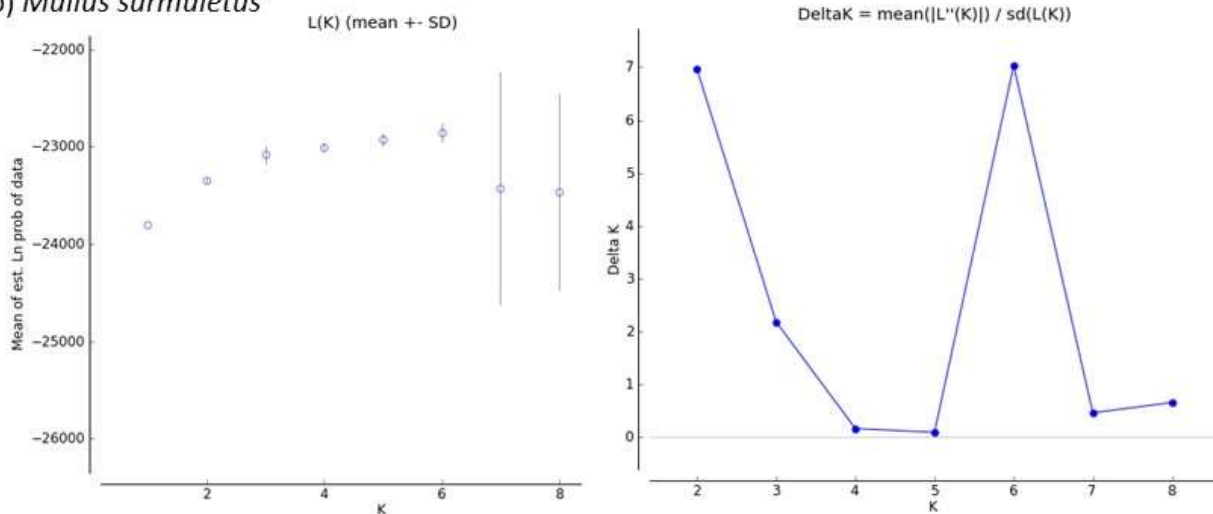
F_{cur} – current fishing mortality; $F_{0.1}$ – the fishing mortality rate at which the slope of the yield-per-recruit curve is only one-tenth the slope of the curve at its origin

Supplement Figures

a) *Mullus barbatus*

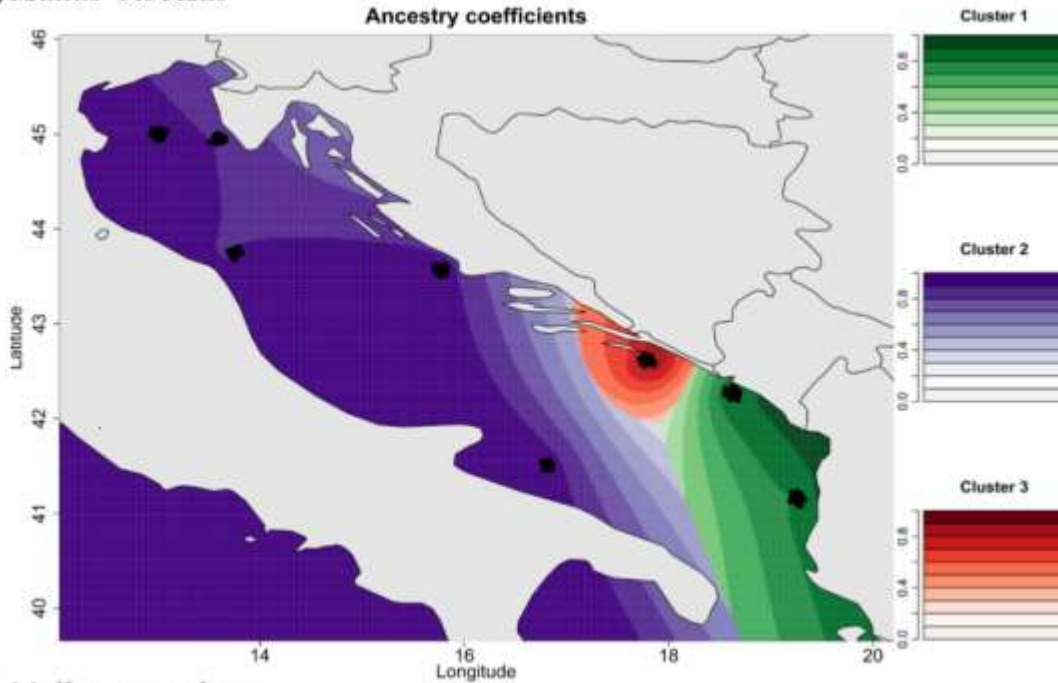


b) *Mullus surmuletus*

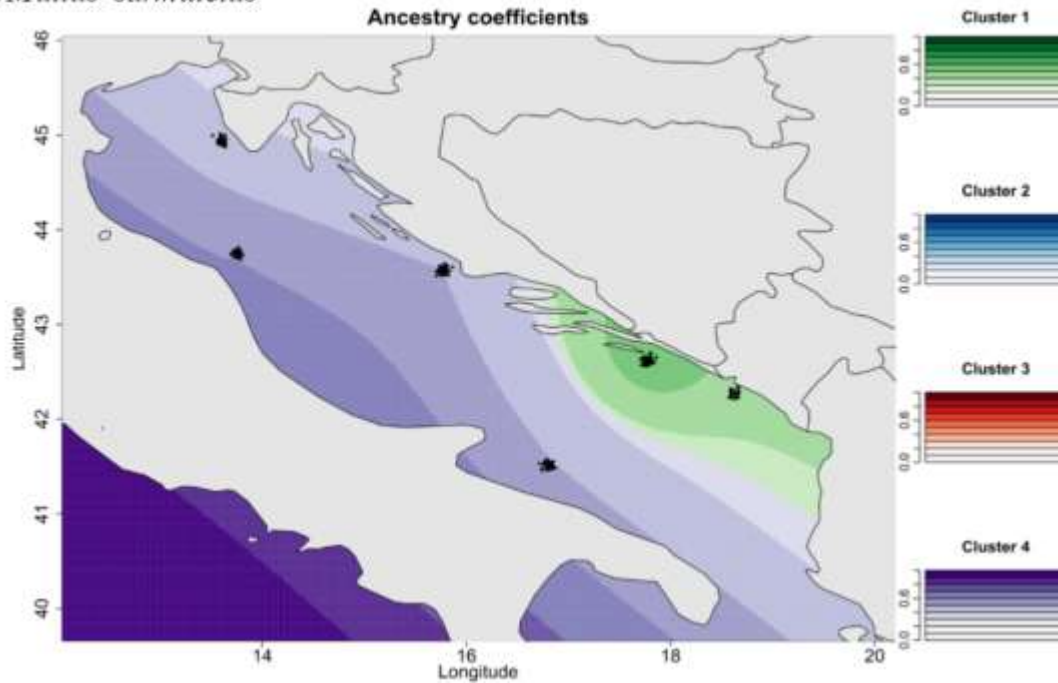


Suppl. Figure S1. Inference of the number of clusters in *Mullus barbatus* (a) and *Mullus surmuletus* (b) populations data. The first plot represents the values for the mean likelihood of each genetic cluster (K), where the error bar represents one standard deviation, while the second plot represents values of Delta K calculated as the mean of the second-order rate of change in the likelihood of K divided by the standard deviation of the likelihood of K.

a) *Mullus barbatus*



b) *Mullus surmuletus*



Suppl. Figure S2. STRUCTURE Q scores of *Mullus barbatus* (a) and *Mullus surmuletus* (b) individuals from Adriatic Sea displayed spatially by universal kriging. Q scores were represented on the ETOPO1 map (produced by NOAA and freely available as indicated here: https://www.ngdc.noaa.gov/mgg/global/dem_faq.html#sec-2.4⁸²) using the maps function from the POPSutilities.R⁷⁹ in R 3.3.3 software and by using the *max* option where only the cluster with the maximal local contribution to ancestry is represented at each geographic point of the map.