Supporting Information

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SI Text

Microsatellite Data Analysis. DNA extractions were performed from pectoral fin tissue following either HotSHOT protocol (1), or the Chelex 10% protocol (2). A total of 63 microsatellite loci (mean 9 loci per species) were successfully amplified by PCR (3–8). Amplified products were resolved on an ABI3100 or an ABI 3700 Genetic Analyser (Applied Biosystems). Allele scoring was carried out by using GeneMapper v 3.5 software (Applied Biosystems). The total number of alleles per locus and sample was obtained by using GENETIX v.4.02 (9). Observed and expected heterozygosities were determined by using the software package Arlequin v. 2.0 (10). Deviations from Hardy–Weinberg expectations were estimated according to the level of significance determined by a Markov chain method by using GENEPOP v. 3.4 (11). Bonferroni corrections were made for multiple tests.

The number of analyzed individuals and mean number of alleles for each species in each locality is given in Table S1. The majority of the assayed microsatellite loci showed high levels of polymorphism. The total number of alleles per locus, observed and expected heterozygosity as well as Hardy–Weinberg expectations, are listed in Table S2. No evidence of linkage disequilibrium was found between locus pairs. Significant deviations from Hardy–Weinberg expectations within sampling locations were observed for most species. Out of 252 tests across species, 30 remained significant after Bonferroni correction. One or two loci accounted for >90% of the deviations from Hardy–Weinberg expectations for a particular species (Table S2). These loci, *Dvul*2, *Dvul*63, *Msur*14, *Sc*05, *Td*09, *St*138, *Aimb*17, and *Aimb*22, were removed from further analysis. Exclusion of these loci made little difference to the overall estimates of population structure; although the magnitude of the F_{ST} values changed slightly, the statistical significance remained unaltered.

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Table S1. Number of analyzed individuals (*N*) and mean number of alleles (*Na*) for each species in each locality

Species	Herradura		Cabo de Gata		Mallorca		Blanes	
	N	*Na*	*N*	*Na*	*N*	*Na*	*N*	*Na*
Diplodus vulgaris	48	13.8	47	14	47	15.6	48	15.5
Mullus surmuletus	48	9.3	48	11.4	48	11.9	48	11.2
Serranus cabrilla	32	9	30	10.4	30	10.4	30	8.6
Oblada melanura	43	11.2	43	11.6	47	12.2	34	12
Tripterygion delaisi	35	18.4	33	16.6	42	16	47	18.7
Apogon imberbis	32	11.2	48	11.5	48	10.6	47	12.5
Symphodus tinca	40	12	57	13.6	47	12.3	51	14.1

Table S2. Summary statistics for microsatellite loci among sampling collections of *D. vulgaris* species

Species	Dvul1	Dvul2	Dvul33	Dvul4	Dvul61	Dvul63	Dvul84	Dvul38	Overall
					Locus				
D. vulgaris, HE									
N	48	48	47	48	43	44	46	47	
A	5	11	17	16	8	16	17	21	13.875
Rs	4.998	10.884	16.639	15.667	8	15.886	16.591	20.808	13.684
H_O	0.597	0.542	0.872	0.749	0.86	0.591	0.894	0.915	
H_E	0.635	0.824	0.9	0.824	0.796	0.827	0.918	0.932	
F_{IS}	0.092	*0.341**	0.032	0.123*	0.139	*0.285**	0.024	0.016	0.131
D. vulgaris, CG									
n	47	46	48	47	46	44	44	44	
a	2	10	15	16	26	15	15	13	14
Rs	2	9.927	14.769	15.731	25.652	15	14.909	12.789	13.847
H_O	0.298	0.5	0.875	0.875	0.913	0.758	0.752	0.897	
H_E	0.303	0.803	0.892	0.898	0.952	0.933	0.824	0.923	
F_{IS}	−0.044	*0.372**	0.015	0.027	0.042	*0.193**	0.113*	0.013	0.091
D. vulgaris, MA									
N	47	46	47	45	46	47	47	45	
A	4	12	16	14	26	17	19	17	15.625
Rs	3.993	11.92	15.488	13.908	25.576	16.81	18.299	18.197	15.523
H_O	0.277	0.435	0.861	0.911	0.896	0.809	0.809	0.927	
H_E	0.269	0.805	0.913	0.906	0.947	0.927	0.815	0.935	
F_{IS}	−0.101	*0.462**	0.101	−0.009	0.086	0.128*	0.008	0.034	0.088
D. vulgaris, BL									
N	48	43	47	48	48	44	48	48	
A	4	10	13	15	28	14	22	18	15.5
Rs	3.895	10	12.889	14.674	27.105	13.932	20.814	17.734	15.13
H_O	0.229	0.488	0.957	0.917	0.913	0.591	0.771	0.871	
H_E	0.246	0.841	0.875	0.882	0.957	0.919	0.756	0.917	
F_{IS}	0.066	*0.413**	−0.095	−0.041	0.081	*0.355**	−0.019	0.121	0.110

n, number of individuals analyzed; a, number of alleles; Rs, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. Asterisk represents significant F_{IS} at $\alpha = 0.05$. Significant values after Bonferroni corrections are shown in bold italics.

Table S3. Summary statistics for microsatellite loci among sampling collections of *M. surmuletus* species

Species	Mb3	Mb11	Mb14	Mb28	Mb46	Mb55	Mb63	Mb130	Mb132	Mb133	Overall
					Locus						
M. surmuletus, HE											
n	48	45	47	48	48	48	46	48	45	48	47.1
a	6	11	16	2	6	7	14	10	10	11	9.7
Rs	5.677	4.862	5.729	2	3.284	6.323	13.381	4.707	9.745	10.416	4.230
H_O	0.444	0.868	0.743	0.182	0.765	0.653	0.815	0.760	0.846	0.793	
H_E	0.419	0.880	0.877	0.189	0.873	0.704	0.893	0.785	0.833	0.848	
F_{IS}	−0.060	0.178	*0.243**	0.113	0.033	0.121	0.088	0.043	−0.015	0.122*	0.086
M. surmuletus, CG											
n	46	45	48	48	44	48	48	48	48	48	
a	7	18	17	2	5	7	16	15	10	17	11.4
Rs	2.963	6.029	5.923	1.251	2.424	3.434	5.481	5.219	4.712	5.749	4.318
H_O	0.520	0.862	0.651	0.068	0.528	0.658	0.850	0.814	0.790	0.829	
H_E	0.560	0.901	0.892	0.065	0.524	0.703	0.868	0.834	0.816	0.881	
F_{IS}	0.082	0.106	*0.246**	−0.024	0.097	0.119*	0.047	0.095	0.066	0.103	0.093
M. surmuletus, MA											
n	48	48	48	48	42	48	48	48	48	41	
a	8	11	17	3	5	10	18	14	12	21	11.9
Rs	2.733	4.517	6.114	1.756	2.373	3.731	6.423	4.727	5.416	6.502	4.429
H_O	0.326	0.720	0.722	0.178	0.492	0.660	0.930	0.768	0.857	0.896	
H_E	0.458	0.744	0.908	0.259	0.501	0.706	0.924	0.783	0.868	0.925	
F_{IS}	0.199*	0.097	*0.495**	0.119*	0.058	0.103	0.005	0.097	0.014	0.063	0.125
M. surmuletus, BL											
n	48	48	48	48	44	48	48	48	47	48	
a	8	21	17	3	4	9	17	13	9	11	11.2
Rs	2.711	6.371	6.087	1.57	2.611	3.903	5.583	4.928	4.179	5.338	4.328
H_O	0.467	0.863	0.891	0.174	0.565	0.761	0.851	0.778	0.826	0.849	
H_E	0.493	0.919	0.905	0.160	0.579	0.748	0.868	0.799	0.740	0.861	
F_{IS}	0.065	0.086	*0.226**	−0.073	0.091	−0.006	0.092	0.048	−0.105	0.071	0.049

n, number of individuals analyzed; a, number of alleles; Rs, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. Asterisk represents significant F_{IS} at $\alpha = 0.05$. Significant values after Bonferroni corrections are shown in bold italics.

Table S4. Summary statistics for microsatellite loci among sampling collections of *S. cabrilla* species

Species	Sc03	Sc04	Sc05	Sc06	Sc07	Sc08	Sc11	Sc12	Sc13	Sc14	Sc15	Overall
						Locus						
S. cabrilla, HE												
n	32	32	32	31	32	32	32	30	30	28	31	
a	8	7	17	10	6	7	4	4	12	16	8	9
Rs	7.5	6.861	16.579	9.613	5.986	6.875	3.986	4	11.73	16	7.797	8.811
H_O	0.688	0.750	0.656	0.871	0.779	0.688	0.719	0.6	0.9	0.821	0.613	
H_E	0.662	0.804	0.939	0.818	0.801	0.741	0.671	0.591	0.868	0.916	0.641	
F_{IS}	−0.039	0.068	*0.274**	−0.07	0.096	0.070	−0.086	−0.049	−0.037	0.1	0.045	0.033
S. cabrilla, CG												
n	30	30	30	29	30	30	30	29	29	29	29	
a	8	11	20	12	9	11	6	4	12	16	6	10.454
Rs	7.733	10.6	19.46	11.929	8.733	10.663	5.927	3.965	11.929	15.826	5.931	10.245
H_O	0.767	0.867	0.733	0.864	0.8	0.767	0.694	0.345	0.897	0.893	0.652	
H_E	0.7	0.832	0.946	0.883	0.814	0.797	0.728	0.559	0.872	0.908	0.691	
F_{IS}	−0.124	−0.041	*0.253**	0.069	0.018	0.062	0.063	0.163*	−0.03	0.029	0.09	0.050
S. cabrilla, MA												
n	30	30	30	30	30	30	30	30	30	30	30	
a	7	9	21	14	9	9	5	6	11	19	5	10.454
Rs	6.86	8.863	20.526	13.796	8.8	8.796	4.867	5.863	10.733	18.196	4.93	10.202
H_O	0.7	0.833	0.767	0.933	0.8	0.7	0.333	0.6	0.867	0.867	0.433	
H_E	0.688	0.846	0.954	0.888	0.816	0.728	0.38	0.574	0.816	0.892	0.484	
F_{IS}	−0.018	0.008	*0.198**	−0.051	0.01	0.039	0.051	−0.085	−0.062	0.03	0.06	0.016
S. cabrilla, BL												
n	30	30	30	30	30	29	30	30	30	30	30	
a	7	8	20	12	7	8	3	5	9	12	4	8.636
Rs	6.8	7.863	19.719	11.73	6.867	7.861	2.997	4.93	8.863	11.723	3.933	8.48
H_O	0.553	0.739	0.833	0.773	0.767	0.592	0.2	0.55	0.767	0.783	0.383	
H_E	0.579	0.771	0.952	0.829	0.763	0.64	0.215	0.621	0.799	0.822	0.401	
F_{IS}	0.038	0.094	*0.251**	0.112	−0.004	0.117	0.072	0.148*	0.033	0.087	0.051	0.09

n, number of individuals analyzed; a, number of alleles; Rs, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. Asterisk represents significant F_{IS} at $\alpha = 0.05$. Significant values after Bonferroni corrections are shown in bold italics.

Table S5. Summary statistics for microsatellite loci among sampling collections of *O. melanura* species

Species	Locus								
	Omel2	Omel3	Omel20	Omel27	Omel38	Omel54	Omel58	Omel61	Overall
O. melanura, HE									
n	43	40	41	43	40	43	43	41	
a	15	10	3	13	10	16	8	15	11.25
Rs	12.94	9.215	2.785	12.254	8.751	14.407	6.882	13.676	10.113
H_O	0.868	0.872	0.186	0.877	0.83	0.851	0.744	0.884	
H_E	0.925	0.885	0.23	0.925	0.86	0.906	0.785	0.904	
F_{IS}	*0.121**	0.086	0.088	0.051	0.106	*0.115**	0.035	0.145	0.093
O. melanura, CG									
n	43	43	41	41	43	41	43	42	
a	17	8	3	15	10	16	9	15	11.62
Rs	14.939	8.351	2.511	14.632	8.646	14.021	7.786	13.164	10.50
H_O	0.905	0.868	0.22	0.893	0.744	0.845	0.721	0.891	
H_E	0.908	0.887	0.223	0.906	0.833	0.928	0.746	0.927	
F_{IS}	0.003	0.019	0.088	0.137	0.166	*0.122**	0.053	0.045	0.079
O. melanura, MA									
n	46	45	47	47	45	47	46	46	
a	17	8	3	14	13	16	12	15	12.25
Rs	15.856	7.836	2.816	13.742	13.426	14.955	8.481	14.453	11.446
H_O	0.934	0.812	0.085	0.896	0.833	0.865	0.673	0.717	
H_E	0.917	0.827	0.103	0.913	0.852	0.889	0.732	0.919	
F_{IS}	−0.022	0.095	0.025	0.01	0.036	0.038	0.08	0.22	0.060
O. melanura, BL									
n	34	34	34	33	34	31	34	34	
a	13	9	3	14	15	16	9	17	12
Rs	11.047	8.793	3	13.917	14.028	14.587	8.404	15.152	11.116
H_O	0.776	0.872	0.378	0.859	0.861	0.848	0.788	0.911	
H_E	0.837	0.905	0.385	0.892	0.927	0.926	0.838	0.916	
F_{IS}	0.117	0.021	0.019	*0.113**	*0.137**	0.081	0.043	0.004	0.066

n, number of individuals analyzed; a, number of alleles; Rs, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. Asterisk represents significant F_{IS} at $\alpha = 0.05$. Significant values after Bonferroni corrections are shown in bold italics.

Table S6. Summary statistics for microsatellite loci among sampling collections of *T. delaisi* species

Species	Td01	Td02	Td04	Td05	Td06	Td07	Td08	Td09	Td10	Td11	Overall
T. delaisi, HE											
n	32	32	34	33	32	34	33	34	34	35	
a	14	12	19	27	31	8	23	9	31	10	18.4
Rs	13.905	11.875	18.437	26.561	30.559	7.735	22.624	8.558	29.722	9.507	17.948
H_O	0.938	0.969	0.884	0.974	0.968	0.794	0.843	0.618	0.971	0.486	
H_E	0.906	0.956	0.92	0.961	0.959	0.76	0.933	0.701	0.966	0.531	
F_{IS}	−0.034	−0.033	0.099	−0.03	−0.032	−0.061	0.127*	*0.131**	−0.036	0.087	0.021
T. delaisi, CG											
n	33	32	33	32	32	32	31	32	32	32	
a	14	13	17	25	23	7	26	6	27	8	16.6
Rs	13.815	12.843	16.801	24.684	22.778	6.969	26	5.968	26.652	7.938	16.444
H_O	0.879	0.906	0.909	0.906	0.875	0.684	0.933	0.531	0.875	0.73	
H_E	0.904	0.882	0.873	0.927	0.953	0.774	0.952	0.727	0.936	0.694	
F_{IS}	0.028	−0.092	−0.041	0.023	0.081	0.115*	0.052	*0.249**	0.083	−0.081	0.041
T. delaisi, MA											
n	42	41	42	42	42	42	42	42	42	42	
a	16	12	18	25	24	7	20	5	26	7	16
Rs	14.146	10.661	15.894	22.799	21.505	6.456	18.27	4.738	23.228	6.992	14.468
H_O	0.881	0.814	0.881	0.952	0.881	0.548	0.891	0.643	0.957	0.738	
H_E	0.9	0.734	0.891	0.95	0.938	0.615	0.922	0.564	0.946	0.71	
F_{IS}	0.022	−0.125	0.012	−0.002	0.06	0.09	0.094	*0.375**	−0.058	−0.039	0.042
T. delaisi, BL											
n	47	47	47	46	47	47	47	47	47	47	
a	12	16	20	25	31	8	29	9	27	10	18.7
Rs	11.28	15.416	17.867	22.505	26.374	6.865	24.589	8.181	23.897	9.383	16.635
H_O	0.872	0.887	0.915	0.978	0.915	0.702	0.845	0.362	0.894	0.681	
H_E	0.876	0.913	0.917	0.936	0.961	0.683	0.951	0.749	0.923	0.735	
F_{IS}	0.005	0.038	0.003	−0.045	0.046	−0.042	0.114*	*0.513**	0.063	0.062	0.075

n, number of individuals analyzed; a, number of alleles; Rs, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. Asterisk represents significant F_{IS} at $\alpha = 0.05$. Significant values after Bonferroni corrections are shown in bold italics.

Table S7. Summary statistics for microsatellite loci among sampling collections of *A. imberbis* species

| | Locus | | | | | | | | |
Species	Aimb14	Aimb17	Aimb2	Aimb22	Aimb28	Aimb29	Aimb41	Aimb74	Overall
A. imberbis, HE									
n	32	31	32	32	32	32	32	31	
a	13	20	8	5	7	13	15	9	11.25
Rs	12.874	20	7.968	5	7	12.874	14.842	9	11.194
H_O	0.843	0.806	0.75	0.312	0.741	0.812	0.812	0.709	
H_E	0.876	0.933	0.771	0.646	0.79	0.879	0.866	0.727	
F_{IS}	0.032	*0.138**	0.028	*0.505**	0.089	0.064	0.055	−0.005	0.113
A. imberbis, CG									
n	48	48	48	45	48	47	48	47	
a	13	20	8	5	6	18	14	8	11.5
Rs	11.729	18.599	7.926	5	5.863	15.91	11.955	7.609	10.573
H_O	0.854	0.812	0.77	0.5	0.685	0.914	0.784	0.744	
H_E	0.879	0.939	0.805	0.64	0.723	0.901	0.841	0.644	
F_{IS}	0.024	*0.137**	0.065	*0.174**	0.059	−0.016	0.097	−0.179	0.045
A. imberbis, MA									
n	46	42	48	47	48	48	48	48	
a	10	21	7	6	8	18	7	8	10.625
Rs	9.473	19.498	6.712	5.621	7.518	15.533	6.518	7.263	9.767
H_O	0.774	0.603	0.687	0.404	0.77	0.896	0.729	0.541	
H_E	0.833	0.934	0.736	0.622	0.822	0.895	0.679	0.624	
F_{IS}	0.048	*0.437**	0.087	*0.35**	0.057	−0.001	−0.109	0.133*	0.125
A. imberbis, BL									
n	47	47	46	42	47	45	46	47	
a	14	25	7	6	8	16	15	9	12.5
Rs	12.889	22.961	6.957	5.737	7.531	14.36	12.595	7.828	11.357
H_O	0.872	0.872	0.772	0.412	0.872	0.862	0.841	0.672	
H_E	0.836	0.938	0.843	0.64	0.749	0.873	0.872	0.728	
F_{IS}	0.084	0.089	0.112*	*0.583**	−0.052	0.037	0.067	0.011	0.116

n, number of individuals analyzed; a, number of alleles; Rs, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. Asterisk represents significant F_{IS} at $\alpha = 0.05$. Significant values after Bonferroni corrections are shown in bold italics.

Table S8. Summary statistics for 63 microsatellite loci among sampling collections of *S. tinca* species

Species	St138	St143	St155	St222	St287	St368	St245	St336	Overall
					Locus				
S. tinca, HE									
n	37	37	39	39	40	39	37	39	
a	15	9	11	10	9	17	12	13	12
Rs	15	9	10.897	9.895	8.915	16.791	11.987	12.879	11.92
H_O	0.568	0.749	0.897	0.821	0.825	0.886	0.872	0.845	
H_E	0.911	0.788	0.883	0.83	0.796	0.914	0.896	0.856	
F_{IS}	*0.378**	0.114	−0.023	0.005	−0.036	0.075	0.084	0.091	0.086
S. tinca, CG									
n	54	51	58	57	57	55	54	57	
a	17	11	13	12	11	17	15	13	13.625
Rs	15.625	10.916	12.174	10.847	9.471	15.448	14.724	12.677	12.735
H_O	0.759	0.843	0.897	0.895	0.789	0.836	0.847	0.798	
H_E	0.926	0.86	0.891	0.825	0.786	0.89	0.86	0.824	
F_{IS}	*0.179**	0.018	−0.005	−0.085	−0.008	0.061	0.008	0.027	0.024
S. tinca, MA									
n	45	47	47	47	47	46	45	47	
a	15	12	11	12	9	17	12	11	12.375
Rs	14.575	11.778	10.903	10.934	8.566	16.701	11.878	10.902	12.029
H_O	0.6	0.915	0.83	0.745	0.723	0.843	0.864	0.757	
H_E	0.902	0.886	0.878	0.796	0.793	0.904	0.878	0.769	
F_{IS}	*0.332**	−0.032	0.05	0.066	0.076	0.133*	0.06	0.078	0.095
S. tinca, BL									
n	51	49	51	51	45	50	49	51	
a	19	12	13	13	10	16	13	17	14.125
Rs	17.092	11.446	12.554	11.802	9.584	15.716	12.677	15.098	13.246
H_O	0.529	0.878	0.882	0.726	0.8	0.898	0.915	0.773	
H_E	0.907	0.874	0.894	0.8	0.792	0.926	0.927	0.784	
F_{IS}	*0.414**	−0.003	0.013	0.123*	−0.012	0.066	0.012	0.015	0.078

n, number of individuals analyzed; a, number of alleles; Rs, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. Asterisk represents significant F_{IS} at $\alpha = 0.05$. Significant values after Bonferroni corrections are shown in bold italics.