

Mitochondrial ghost lineages blur phylogeography and taxonomy of *Natrix helvetica* and *N. natrix* in Italy and Corsica

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Supporting Information, *Zoologica Scripta*

TABLE S1 Grass snake samples used in the present study. Haplotypes in red font were newly identified in this study. STRUCTURE cluster memberships are colour-coded as in Figure 4; grey = admixed. Haplotypes with asterisks could not be identified more precisely because obtained DNA sequences were too short. Clade 4 of *Natrix natrix* (Kindler et al., 2013) corresponds to the ‘red lineage’ of Kindler et al. (2017). Lab codes of those samples used in the three HYBRIDLAB calculations bear symbols ([#] first calculation, [§] second calculation, [§] third calculation)

Sample/ Voucher	Locality	Latitude (N)	Longitude (E)	Clade	Haplotypes		First STRUCTURE run (K=2)		Second STRUCTURE run (K=3)			Third STRUCTURE run (K=2)		Reference
					ND4+ tRNAs	cyt <i>b</i>	<i>helv.</i>	<i>nat.</i>	<i>cetti</i>	<i>helv.</i>	<i>sic.</i>	<i>helv.</i>	<i>sic.</i>	
ZFMK 54705 [#]	Croatia: 25 km N Rijeka: Gomance	45.589056	14.529190	4	r18	—	0.003	0.997	—	—	—	—	—	Kindler et al. (2013)
MTD D 32031 [#]	Croatia: Istria: Rovinj	45.081763	13.640156	4	r4	r34	0.004	0.996	—	—	—	—	—	Kindler et al. (2013)
MSNPV CR1076	Italy: Emilia Romagna: Ferrara: Argenta, Argine Agosta	44.616756	11.833736	4	r16	—	—	—	—	—	—	—	—	This study
MFSNUD 01426 [#]	Italy: Friuli-Venezia Giulia: Gorizia: Dolegna del Collio	46.003624	13.466196	4	r4	r34	0.003	0.997	—	—	—	—	—	This study
MFSNUD 01447	Italy: Friuli-Venezia Giulia: Udine: Buja	46.215164	13.113595	4	r4	r34	0.042	0.958	—	—	—	—	—	This study
MFSNUD 01253	Italy: Friuli-Venezia Giulia: Udine: Fagagna	46.111702	13.087960	4	r16	—	—	—	—	—	—	—	—	This study
MFSNUD 01322	Italy: Friuli-Venezia Giulia: Udine: Majano	46.178210	13.090022	4	r16	r*	0.04	0.96	—	—	—	—	—	This study
MFSNUD 01419 [#]	Italy: Friuli-Venezia Giulia: Udine: San Daniele del Friuli	46.129921	12.970615	4	r4	—	0.003	0.997	—	—	—	—	—	This study

TABLE S1 – Continued

Sample/ Voucher	Locality	Latitude (N)	Longitude (E)	Clade	Haplotypes		First STRUCTURE run (K=2)		Second STRUCTURE run (K=3)			Third STRUCTURE run (K=2)		Reference
					ND4+ tRNAs	cyt <i>b</i>	<i>helv.</i>	<i>nat.</i>	<i>cetti</i>	<i>helv.</i>	<i>sic.</i>	<i>helv.</i>	<i>sic.</i>	
MFSNUD 01328 [#]	Italy: Friuli-Venezia Giulia: Udine: Faedis	46.149555	13.345848	4	r*	—	0.004	0.996	—	—	—	—	—	This study
MSNVE 24722	Italy: Veneto: Rovigo, Po Delta	44.965223	12.373656	4	r4	—	—	—	—	—	—	—	—	This study
MSNVE 19373	Italy: Veneto: Treviso: Bagaggiolo, Roncade	45.566591	12.404380	4	r4	r34	0.066	0.934	—	—	—	—	—	This study
MSNVE 718	Italy: Veneto: Treviso: Giavera del Montello, Santi Angeli	45.823510	12.149826	4	r17	—	0.445	0.555	—	—	—	—	—	This study
MSNVE 21655	Italy: Veneto: Treviso: Vedelago, sources of the Sile River	45.651506	12.021525	4	r4	r34	0.031	0.969	—	—	—	—	—	This study
MTD T 19771	Italy: Veneto: Venezia: Stigliano	45.526771	12.042860	4	r*	—	0.034	0.966	—	—	—	—	—	This study
MSNVE 19375	Italy: Veneto: Venezia: Ca'Noghera	45.523337	12.359767	4	r4	r*	0.039	0.961	—	—	—	—	—	This study
MSNVE 19822	Italy: Veneto: Venezia: Cavallino-Treporti, Punta Sabbioni	45.446682	12.432142	4	r4	r34	0.065	0.935	—	—	—	—	—	This study
MSNVE 21656	Italy: Veneto: Venezia: Dese, near Forte Cosenz	45.521305	12.281556	4	r4	—	—	—	—	—	—	—	—	This study
MSNVE 24735	Italy: Veneto: Venezia: Malcontenta, Mira	45.432885	12.198514	4	r35	r34	0.03	0.97	—	—	—	—	—	This study
MSNVE 6384	Italy: Veneto: Venezia: Marcon, Gaggio, Cave di Gaggio Nord	45.561357	12.331562	4	—	r*	—	—	—	—	—	—	—	This study
MSNVE 25066	Italy: Veneto: Venezia: Marcon, Gaggio, Cave di Gaggio Nord	45.561357	12.331562	4	r34	r34	0.005	0.995	—	—	—	—	—	This study
MSNVE 19374	Italy: Veneto: Venezia: Marcon, Gaggio, Cave di Gaggio Nord	45.562085	12.323511	4	r16	r34	0.004	0.996	—	—	—	—	—	This study
MSNVE 19815	Italy: Veneto: Venezia: Marcon, Praello	45.538254	12.308798	4	r21	r34	0.003	0.997	—	—	—	—	—	This study
MSNVE 6383	Italy: Veneto: Venezia: Porto Marghera	45.448694	12.235927	4	r16	—	0.005	0.995	—	—	—	—	—	This study
MSNVE 23107	Italy: Veneto: Venezia: Porto Marghera	45.448694	12.235927	4	r4	r37	0.028	0.972	—	—	—	—	—	This study
MSNVE 24730	Italy: Veneto: Venezia: Porto Marghera	45.448694	12.235927	4	r*	r*	0.008	0.992	—	—	—	—	—	This study
MSNVE 619	Italy: Veneto: Venezia: Porto Marghera	45.443282	12.221668	4	r16	—	0.008	0.992	—	—	—	—	—	This study
MSNVE 23106	Italy: Veneto: Venezia: Porto Marghera	45.443282	12.221668	4	r4	r34	0.363	0.637	—	—	—	—	—	This study
MTD T 12729 [#]	Slovenia: Bilje, Vipava River	45.889456	13.636068	4	r4	r42	0.007	0.993	—	—	—	—	—	Kindler et al. (2017)
MTD T 13540 [#]	Slovenia: Borjana	46.255920	13.479990	4	r4	r34	0.004	0.996	—	—	—	—	—	Kindler et al. (2017)

TABLE S1 – Continued

Sample/ Voucher	Locality	Latitude (N)	Longitude (E)	Clade	Haplotypes		First STRUCTURE run (K=2)		Second STRUCTURE run (K=3)			Third STRUCTURE run (K=2)		Reference
					ND4+ tRNAs	cyt <i>b</i>	<i>helv.</i>	<i>nat.</i>	<i>cetti</i>	<i>helv.</i>	<i>sic.</i>	<i>helv.</i>	<i>sic.</i>	
MTD T 11209 [#]	Slovenia: Borovnica	45.919154	14.363565	4	r4	r34	0.004	0.996	—	—	—	—	—	Kindler et al. (2017)
MTD T 11116 [#]	Slovenia: Borovnica	45.919393	14.364252	4	r4	r47	0.005	0.995	—	—	—	—	—	Kindler et al. (2017)
MTD T 11535	Slovenia: Cerknica Lake	45.772120	14.357790	4	r4	r38	0.004	0.996	—	—	—	—	—	Kindler et al. (2017)
MTD T 11536	Slovenia: Cerknica Lake	45.772120	14.357790	4	r4	r38	0.006	0.994	—	—	—	—	—	Kindler et al. (2017)
MTD T 11537	Slovenia: Cerknica Lake	45.772120	14.357790	4	r4	r38	0.003	0.997	—	—	—	—	—	Kindler et al. (2017)
MTD T 11538 [#]	Slovenia: Cerknica Lake	45.752283	14.357321	4	r4	r34	0.003	0.997	—	—	—	—	—	Kindler et al. (2017)
MTD T 11528 [#]	Slovenia: Divača (Kal Globočaj pond)	45.712652	13.956882	4	r4	r49	0.003	0.997	—	—	—	—	—	Kindler et al. (2017)
MTD T 13541	Slovenia: Dragonje	45.448150	13.676490	4	r4	r34	0.003	0.997	—	—	—	—	—	Kindler et al. (2017)
MTD T 11213 [#]	Slovenia: Ljubljansko barje: Bistra	45.944600	14.335600	4	r4	r34	0.002	0.998	—	—	—	—	—	Kindler et al. (2017)
MTD T 10900	Slovenia: Ljubljansko barje: Pako	45.940705	14.369674	4	r4	r34	0.004	0.996	—	—	—	—	—	Kindler et al. (2017)
MTD T 10901	Slovenia: Ljubljansko barje: Pako	45.940705	14.369674	4	r4	r34	0.003	0.997	—	—	—	—	—	Kindler et al. (2017)
MTD T 10902 [#]	Slovenia: Ljubljansko barje: Pako	45.940705	14.369674	4	r4	r34	0.002	0.998	—	—	—	—	—	Kindler et al. (2017)
MTD T 11526 [#]	Slovenia: near Bled (Kreda pond)	46.368253	14.112182	4	r4	r34	0.002	0.998	—	—	—	—	—	Kindler et al. (2017)
MTD T 11208 [#]	Slovenia: Podlipa	46.005786	14.223075	4	r4	r34	0.009	0.991	—	—	—	—	—	Kindler et al. (2017)
MTD T 19727 ^s	Italy: Calabria	39.241381	16.605621	A	a3	—	0.99	0.01	0.005	0.008	0.987	0.045	0.955	This study
MTD T 19728	Italy: Calabria	39.241381	16.605621	A	a3	a5	0.994	0.006	0.007	0.037	0.956	0.074	0.926	This study
MTD T 19729 ^s	Italy: Calabria	39.241381	16.605621	A	a3	a6	0.991	0.009	0.007	0.007	0.986	0.032	0.968	This study
MTD T 19718	Italy: Calabria: Cosenza, Thurio	39.694444	16.475000	A	a*	—	—	—	—	—	—	—	—	This study
MTD T 19708	Italy: Calabria: Lago di Rumia	38.181389	15.847500	A	a3	—	—	—	—	—	—	—	—	This study
MTD T 20366 ^{ss}	Italy: Calabria: San Giovanni in Fiora	39.260895	16.695693	A	a3	a3	0.994	0.006	0.012	0.011	0.977	0.049	0.951	This study
—	Italy: Calabria: Serra San Bruno	38.578567	16.331935	A	a3	a3	—	—	—	—	—	—	—	Kindler et al. (2013)

TABLE S1 – Continued

Sample/ Voucher	Locality	Latitude (N)	Longitude (E)	Clade	Haplotypes		First STRUCTURE run (K=2)		Second STRUCTURE run (K=3)			Third STRUCTURE run (K=2)		Reference	
					ND4+ tRNAs	cyt <i>b</i>	<i>helv.</i>	<i>nat.</i>	<i>cetti</i>	<i>helv.</i>	<i>sic.</i>	<i>helv.</i>	<i>sic.</i>		
MTD T 19715	Italy: Calabria: Rende, Università di Calabria, Botanical Garden	39.361111	16.228611	A	a*	—	—	—	—	—	—	—	—	—	This study
MTD T 2116 ^{ss}	Italy: Sicily: Agrigento: Siculiana	37.335566	13.421466	A	a1	—	0.989	0.011	0.013	0.007	0.98	0.043	0.957	Kindler et al. (2013)	
MZUF 24175 ^s	Italy: Sicily: Enna: Lago di Pergusa	37.519291	14.304621	A	—	a3	0.94	0.06	—	—	—	—	—	Kindler et al. (2013)	
—	Italy: Sicily: Messina: Caronia, Castello di Marescotto	37.973432	14.477005	A	a1	a3	—	—	—	—	—	—	—	Kindler et al. (2013)	
NME R 0331/01 ^{ss}	Italy: Sicily: Messina: Mojo Alcantara	37.900797	15.049710	A	a2	a4	0.995	0.005	0.008	0.007	0.985	0.032	0.968	Kindler et al. (2018a)	
MTD T 11605 ^s	Italy: Sicily: Siracusa: 3 km NE Cassaro	37.127450	14.991020	A	a1	a2	0.996	0.004	0.008	0.015	0.977	0.08	0.92	Kindler et al. (2018a)	
MTD T 8464 ^s	Italy: Sicily: Siracusa: Vendicari	36.814782	15.092411	A	a1	a1	0.994	0.006	0.004	0.007	0.989	0.033	0.967	Kindler et al. (2013)	
MTD T 9902 ^s	France: Corsica: Etang de Loto	42.718878	9.229499	B	b1	—	0.998	0.002	0.977	0.014	0.009	—	—	Kindler et al. (2013)	
MTD D 39091 ^s	France: Corsica: Gulf of Sagone	42.110417	8.700607	B	b2	b5	0.997	0.003	0.978	0.012	0.01	—	—	Kindler et al. (2013)	
BEV. 10935 ^s	France: Corsica: near Solenzara	41.857056	9.368389	B	b1	b1	0.997	0.003	0.993	0.003	0.004	—	—	Kindler et al. (2018a)	
MTD D 35388	France: Corsica: Porto-Vecchio	41.590797	9.281960	B	b1	b4	—	—	—	—	—	—	—	Fritz et al. (2012)	
MTD D 42489	France: Corsica: Santa Giulia	41.533447	9.276481	B	b2	b5	—	—	—	—	—	—	—	Fritz et al. (2012)	
MTD T 19155 ^s	Italy: Sardinia: Cagliari: Sinnai	39.293505	9.392273	B	b1	b4	0.997	0.003	0.874	0.046	0.081	—	—	This study	
ZFMK 60736 ^s	Italy: Sardinia: Limbara Mountains	40.850436	9.174557	B	b1	b2	0.997	0.003	0.945	0.013	0.042	—	—	Kindler et al. (2013)	
ZFMK 60737 ^s	Italy: Sardinia: Limbara Mountains	40.850436	9.174557	B	b1	b2	0.997	0.003	0.983	0.008	0.009	—	—	Kindler et al. (2013)	
MTD T 12017	Italy: Sardinia: Limbara Mountains	40.848878	9.177647	B	b1	—	—	—	—	—	—	—	—	Kindler and Fritz (2018)	
MTD T 18915 ^s	Italy: Sardinia: Monte Albo	40.534375	9.584363	B	b1	b6	0.998	0.002	0.992	0.004	0.004	—	—	This study	
MTD T 20220 ^s	Italy: Sardinia: near Grotta de su Ventu	40.243836	9.490072	B	b1	b*	0.995	0.005	0.966	0.004	0.03	—	—	This study	
MTD T 20221 ^s	Italy: Sardinia: near Grotta de su Ventu	40.243836	9.490072	B	b1	b*	0.996	0.004	0.925	0.023	0.052	—	—	This study	
MZUF 40268	Italy: Sardinia: Nuoro: Belvi	39.965543	9.185071	B	b1	b3	—	—	—	—	—	—	—	Fritz et al. (2012)	
MTD T 18916 ^s	Italy: Sardinia: Sette Fratelli	39.259560	9.408487	B	b1	b6	0.995	0.005	0.979	0.004	0.017	—	—	This study	

TABLE S1 – Continued

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					ND4+ tRNAs	cyt b	helv.	nat.	cetti	helv.	sic.	helv.	sic.	
MTD T 18918 ^s	Italy: Sardinia: Sette Fratelli	39.264079	9.409667	B	b3	b7	0.998	0.002	0.993	0.003	0.004	—	—	This study
MTD T 18917 ^s	Italy: Sardinia: Sette Fratelli	39.259784	9.411856	B	b1	b6	0.994	0.006	0.99	0.003	0.007	—	—	This study
MSNVE 24723	Italy: Emilia Romagna: Bologna: Quarto Inferiore	44.528973	11.419917	C	c*	—	—	—	—	—	—	—	—	This study
MTD T 19154 [#]	Italy: Emilia Romagna: Piacenza: Caorso	45.051052	9.875917	C	c2	c1	0.996	0.004	0.004	0.943	0.053	0.567	0.433	This study
MFSNUD 01335	Italy: Friuli-Venezia Giulia: Udine: Pavia di Udine, Cortello	45.991657	13.260642	C	c*	—	—	—	—	—	—	—	—	This study
MSNVE 684 [#]	Italy: Liguria: Imperia: Taggia	43.861661	7.854473	C	c*	—	0.996	0.004	0.004	0.104	0.892	0.158	0.842	This study
MTD T 12004 [#]	Italy: Lombardy: Milano: 30 km W Milano	45.467354	8.801308	C	c1	—	0.998	0.002	0.009	0.323	0.668	0.351	0.649	Kindler and Fritz (2018)
MSNPV CR1079 [#]	Italy: Lombardy: Milano: Noviglio, Mairano	45.357297	9.069940	C	c*	—	0.997	0.003	0.013	0.016	0.971	0.087	0.913	This study
MSNPV CR0891	Italy: Lombardy: Pavia: Bereguardo, Zelata di Bereguardo	45.263800	9.001272	C	c1	c1	0.988	0.012	0.006	0.045	0.949	0.143	0.857	This study
MSNPV CR0776 [#]	Italy: Lombardy: Pavia: Borgo Ticino	45.179625	9.152816	C	c1	c1	0.997	0.003	0.006	0.736	0.258	0.399	0.601	This study
MSNPV CR0780 [#]	Italy: Lombardy: Pavia: Bosco G. Negri	45.172504	9.140546	C	c1	c*	0.996	0.004	0.004	0.078	0.918	0.181	0.819	This study
MSNPV CR1230 [#]	Italy: Lombardy: Pavia: Marcignago	45.252598	9.075981	C	c3	c12	0.997	0.003	0.009	0.67	0.321	0.5	0.5	This study
MTD T 19153 ^{#s}	Italy: Lombardy: Sondrio: Samolaco	46.233863	9.413885	C	c1	c1	0.997	0.003	0.007	0.017	0.976	0.054	0.946	This study
MTD T 14521 [#]	Italy: Piedmont: Torino, Venaria Reale	45.145547	7.600656	C	—	c1	0.993	0.007	0.009	0.164	0.827	0.248	0.752	Kindler and Fritz (2018)
MTD T 14522	Italy: Piedmont: Torino, Venaria Reale	45.145547	7.600656	C	c1	c1	—	—	—	—	—	—	—	Kindler et al. (2018a)
ZMH R09039 [#]	Italy: Trentino-Alto Adige/Südtirol	46.621147	11.782379	C	c1	—	0.996	0.004	0.024	0.023	0.953	0.107	0.893	Kindler and Fritz (2018)
MSNVE 19385 ^{#s}	Italy: Trentino-Alto Adige/Südtirol: South Tirol: Fortezza/Franzensfeste, Le Cave/Grasstein	46.822018	11.538727	C	c1	c1	0.995	0.005	0.016	0.031	0.953	0.067	0.933	This study
MSNVE 25067	Italy: Trentino-Alto Adige/Südtirol: South Tirol: Mules/Mauls, Campo di Trens/Freienfeld	46.841681	11.527847	C	c1	—	—	—	—	—	—	—	—	This study
MSNVE 24725	Italy: Trentino-Alto Adige/Südtirol: Naz-Sciaves/Natz- Schabs, Sciaves/Schabs	46.770736	11.669115	C	c1	c1	0.94	0.06	0.006	0.021	0.973	0.078	0.922	This study
MTD T 11593 ^s	Italy: Trentino-Alto Adige/Südtirol: between Ballino and Fiave	45.988733	10.834551	C	c1	c1	0.982	0.018	0.011	0.011	0.978	0.043	0.957	Kindler and Fritz (2018)
MTD T 9656 [#]	Italy: Trentino-Alto Adige/Südtirol: Vela	46.090000	11.100000	C	c1	c1	0.993	0.007	0.008	0.035	0.957	0.114	0.886	Kindler et al. (2013)

TABLE S1 – Continued

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					ND4+ tRNAs	cyt <i>b</i>	<i>helv.</i>	<i>nat.</i>	<i>cetti</i>	<i>helv.</i>	<i>sic.</i>	<i>helv.</i>	<i>sic.</i>	
ZSM DNA 199	Italy: Veneto: Belluno, Piave River 3 km N of Ospitale di Cardore	46.349947	12.337599	C	c1	c1	0.921	0.079	0.011	0.028	0.961	0.114	0.886	Kindler and Fritz (2018)
MSNVE 22129 ^s	Italy: Veneto: Belluno: Cesiomaggiore, Val Canzoi	46.132328	11.945827	C	c1	c7	0.984	0.016	0.015	0.008	0.977	0.026	0.974	This study
MSNVE 24211 [#]	Italy: Veneto: Belluno: Frassenè, Fonzaso	46.010201	11.790969	C	c1	c8	0.997	0.003	0.014	0.024	0.962	0.085	0.915	This study
MFSNUD 01188	Italy: Veneto: Belluno: Masarè di Rocca Pietore	46.433749	11.976826	C	c1	c6	0.894	0.106	—	—	—	—	—	This study
MTD T 14523	Italy: Veneto: Belluno: Solagnot: Longarone	46.271985	12.301006	C	c1	c5	—	—	—	—	—	—	—	Kindler et al. (2018a)
MSNVE 24746 ^{#s}	Italy: Veneto: Treviso: Arfanta, Tarzo, Mondragon	45.957126	12.208244	C	c1	c7	0.991	0.009	0.005	0.019	0.976	0.053	0.947	This study
MSNVE 23998	Italy: Veneto: Treviso: Castelcucco	45.834619	11.871820	C	c1	c1	0.591	0.409	—	—	—	—	—	This study
MSNVE 24222	Italy: Veneto: Treviso: Cornuda, Bosco del Fagarè	45.840863	11.972676	C	c1	c9	—	—	—	—	—	—	—	This study
MSNVE 23424	Italy: Veneto: Treviso: Crespano del Grappa, Rosset	45.813722	11.842167	C	c1	c1	0.892	0.108	—	—	—	—	—	This study
MSNVE 24221	Italy: Veneto: Treviso: Miane	45.921895	12.091197	C	c1	c11	0.755	0.245	—	—	—	—	—	This study
MSNVE 547[A]	Italy: Veneto: Treviso: Mogliano, Marocco	45.539983	12.242959	C	c1	c7	0.963	0.037	0.004	0.009	0.987	0.027	0.973	This study
MTD T 19780	Italy: Veneto: Treviso: Moriago, Sernaglia della Battaglia, Palù	45.874222	12.097889	C	c1	c1	—	—	—	—	—	—	—	This study
MSNVE 24716	Italy: Veneto: Venezia: Porto Marghera	45.443282	12.221668	C	c*	—	0.059	0.941	—	—	—	—	—	This study
MSNVE 24717	Italy: Veneto: Venezia: Porto Marghera, Rana	45.450287	12.214032	C	c*	—	0.005	0.995	—	—	—	—	—	This study
MTD T 19777 ^s	Italy: Veneto: Vicenza, near shooting polygon	45.572056	11.541457	C	c1	c10	0.99	0.01	0.037	0.009	0.954	0.034	0.966	This study
—	Italy: Veneto: Vicenza: NW Asiago	45.874951	11.510868	C	c1	c1	—	—	—	—	—	—	—	Kindler et al. (2013)
MUE05	Switzerland: Ticino: Lago Ritóm	46.535675	8.674061	C	c1	c1	0.989	0.011	0.009	0.109	0.882	0.169	0.831	Kindler and Fritz (2018)
MTD T 11146 [#]	Switzerland: Valais (Wallis): Niedergesteln	46.312316	7.782440	C	c1	c1	0.998	0.002	0.005	0.321	0.674	0.381	0.619	Kindler et al. (2018a)
MTD T 18962 ^s	Italy: Apulia: Bari: Conversano, Castiglione	40.926582	17.128559	D	d2	d3	0.997	0.003	0.01	0.012	0.978	0.058	0.942	This study
—	Italy: Apulia: Brindisi: Torre San Gennaro	40.537156	18.069670	D	d1	d1	—	—	—	—	—	—	—	Guicking et al. (2006)
MSNVE 23929	Italy: Apulia: Lecce: Otranto, Alimini Lakes	40.190367	18.446439	D	d2	d2	—	—	—	—	—	—	—	This study

TABLE S1 – Continued

Sample/ Voucher	Locality	Latitude (N)	Longitude (E)	Clade	Haplotypes		First STRUCTURE run (K=2)		Second STRUCTURE run (K=3)			Third STRUCTURE run (K=2)		Reference
					ND4+ tRNAs	cyt <i>b</i>	<i>helv.</i>	<i>nat.</i>	<i>cetti</i>	<i>helv.</i>	<i>sic.</i>	<i>helv.</i>	<i>sic.</i>	
MSNVE 23920[A]	Italy: Apulia: Lecce: San Cataldo	40.396372	18.294305	D	d2	d5	0.992	0.008	0.11	0.011	0.879	—	—	This study
MSNVE 23920[B] [§]	Italy: Apulia: Lecce: San Cataldo	40.396372	18.294305	D	d2	d5	0.997	0.003	0.022	0.007	0.971	0.03	0.97	This study
MTD T 18963	Italy: Apulia: Lecce: Vernole, Cesine	40.343492	18.350628	D	d2	d2	0.998	0.002	0.289	0.015	0.696	—	—	This study
MTD T 18964 [§]	Italy: Apulia: Lecce: Vernole, Cesine	40.353583	18.334452	D	d2	d4	0.997	0.003	0.009	0.008	0.983	0.035	0.965	This study
MTD T 18965 [§]	Italy: Apulia: Lecce: Vernole, Cesine	40.362190	18.327296	D	d2	d5	0.998	0.002	0.013	0.008	0.979	0.035	0.965	This study
MTD T 18966	Italy: Apulia: Lecce: Vernole, Cesine	40.350430	18.321180	D	d2	d5	0.997	0.003	0.261	0.015	0.724	—	—	This study
MTD T 18967 [§]	Italy: Apulia: Lecce: Vernole, Cesine	40.338950	18.349210	D	d3	d5	0.997	0.003	0.056	0.012	0.932	0.057	0.943	This study
MTD T 18968	Italy: Apulia: Lecce: Vernole, Cesine	40.349820	18.322060	D	d1	d6	0.997	0.003	0.227	0.009	0.764	—	—	This study
BEV.1690	France: Alpes-de-Haute-Provence: Clumanc	44.020000	6.380000	E	h7	—	0.997	0.003	0.004	0.762	0.234	0.445	0.555	Pokrant et al. (2016)
ZFMK 54711 [§]	France: Ardèche: 10 km N Tournon-sur-Rhône	45.068550	4.831724	E	h1	h1	0.998	0.002	0.005	0.858	0.137	0.666	0.334	Kindler et al. (2013)
BEV.9397	France: Bouches-du-Rhône: between Fos-sur-Mer and Port-Saint-Louis-du-Rhône	43.424692	4.882669	E	h1	—	0.998	0.002	0.005	0.855	0.14	0.584	0.416	Pokrant et al. (2016)
ZMH R07572	France: Bouches-du-Rhône: Camargue: Salin de Badon	43.480359	4.646308	E	h1	h1	0.998	0.002	0.101	0.774	0.125	—	—	Pokrant et al. (2016)
ZMH R07573	France: Bouches-du-Rhône: Camargue: Salin de Badon	43.480359	4.646308	E	h1	h1	0.995	0.005	0.005	0.947	0.048	0.747	0.253	Pokrant et al. (2016)
ZMH R06950 [§]	France: Bouches-du-Rhône: Camargue: Tour du Valat	43.501001	4.672594	E	h1	h1	0.996	0.004	0.008	0.929	0.063	0.78	0.22	Pokrant et al. (2016)
ZMH R06951	France: Bouches-du-Rhône: Camargue: Tour du Valat	43.501001	4.672594	E	h1	h1	0.997	0.003	0.005	0.976	0.019	0.762	0.238	Pokrant et al. (2016)
ZMH R06952	France: Bouches-du-Rhône: Camargue: Tour du Valat	43.501001	4.672594	E	h1	h1	0.998	0.002	0.008	0.977	0.015	0.897	0.103	Pokrant et al. (2016)
ZMH R07062	France: Bouches-du-Rhône: Camargue: Tour du Valat	43.501001	4.672594	E	h1	h1	0.998	0.002	0.006	0.941	0.053	0.774	0.226	Pokrant et al. (2016)
ZMH R06997	France: Bouches-du-Rhône: Camargue: Tour du Valat	43.501001	4.672594	E	h1	—	0.996	0.004	0.009	0.891	0.1	0.7	0.3	Pokrant et al. (2016)
ZFMK 54710	France: Bouches-du-Rhône: Saint-Martin-de-Crau	43.639057	4.810925	E	h1	h1	0.997	0.003	0.005	0.961	0.034	0.845	0.155	Kindler et al. (2013)
BEV.9401	France: Bouches-du-Rhône: Saint-Martin-de-Crau: Mas de Pernes	43.756713	4.666586	E	h1	—	0.997	0.003	0.006	0.809	0.185	0.61	0.39	Pokrant et al. (2016)
BEV. 9293	France: Isère: Clelles	44.827509	5.622582	E	h1	—	0.998	0.002	0.183	0.805	0.012	—	—	Pokrant et al. (2016)

TABLE S1 – Continued

Sample/ Voucher	Locality	Latitude (N)	Longitude (E)	Clade	Haplotypes		First STRUCTURE run (K=2)		Second STRUCTURE run (K=3)			Third STRUCTURE run (K=2)		Reference
					ND4+ tRNAs	cyt <i>b</i>	<i>helv.</i>	<i>nat.</i>	<i>cetti</i>	<i>helv.</i>	<i>sic.</i>	<i>helv.</i>	<i>sic.</i>	
BEV. 11530	France: Vaucluse: Saint-Saturnin-lès-Apt	43.946361	5.382500	E	h1	—	0.997	0.003	0.006	0.892	0.102	0.658	0.342	Pokrant et al. (2016)
MTD T 19710	Italy: Calabria: Potame	39.188611	16.202500	E	h*	—	—	—	—	—	—	—	—	This study
MTD T 11572	Italy: Piedmont: between Viozene and Ponte di Nava	44.122962	7.819319	E	h1	h1	0.996	0.004	0.006	0.839	0.155	0.533	0.467	Kindler et al. (2018b)
MTD T 13084	Italy: Tuscany: Carrara	44.079200	10.098095	E	h1	—	0.991	0.009	0.018	0.961	0.021	0.843	0.157	This study
BBE01 ^{ss}	Switzerland: Bern: Bern Airport	46.918189	7.499929	E	h1	h1	0.998	0.002	0.004	0.991	0.005	0.972	0.028	Kindler et al. (2017)
BBE02 ^s	Switzerland: Bern: Bern Airport	46.918189	7.499929	E	h1	h18	0.998	0.002	0.007	0.986	0.007	0.961	0.039	Kindler et al. (2017)
BBE03 ^s	Switzerland: Bern: Bern Airport	46.918189	7.499929	E	h1	h1	0.998	0.002	0.004	0.991	0.005	0.977	0.023	Kindler et al. (2017)
BBE04 ^s	Switzerland: Bern: Bern Airport	46.918189	7.499929	E	h1	h23	0.997	0.003	0.008	0.987	0.005	0.977	0.023	Kindler et al. (2017)
BBE05 ^s	Switzerland: Bern: Bern Airport	46.913130	7.507865	E	h1	h23	0.998	0.002	0.004	0.991	0.005	0.978	0.022	Kindler et al. (2017)
BBE06 ^s	Switzerland: Bern: Bern Airport	46.913103	7.507957	E	h1	h1	0.998	0.002	0.004	0.991	0.005	0.975	0.025	Kindler et al. (2017)
MBE10 ^s	Switzerland: Bern: Bern Airport	46.910032	7.513558	E	h1	h1	0.998	0.002	0.004	0.99	0.006	0.961	0.039	Kindler et al. (2017)
MTD T 10084	Switzerland: Bern: Grosses Moos	46.983333	7.133333	E	h1	—	0.995	0.005	0.049	0.702	0.249	0.596	0.404	Kindler et al. (2013)
MBE11	Switzerland: Bern: Mühleberg	46.965053	7.251749	E	h1	h2	0.997	0.003	0.039	0.934	0.027	0.78	0.22	Kindler et al. (2017)
MTD T 10085 ^s	Switzerland: Bern: Gadmental near Meiringen	46.716667	8.316667	E	—	h1	0.997	0.003	0.004	0.986	0.01	0.923	0.077	Kindler et al. (2013)
MTD T 10083 ^s	Switzerland: Bern: near Bern	46.913340	7.505280	E	h1	h1	0.998	0.002	0.008	0.986	0.006	0.941	0.059	Kindler et al. (2013)
MBE09	Switzerland: Bern: Sutz-Lattrigen	47.101150	7.211438	E	h1	h23	0.998	0.002	0.045	0.944	0.011	0.832	0.168	Kindler et al. (2017)
MTD T 10079	Switzerland: Vaud/Waadt: Vinzel	46.449444	6.273333	E	h1	—	0.998	0.002	0.023	0.928	0.049	0.728	0.272	Kindler et al. (2013)
ZFMK 90574	Italy: Calabria	39.010648	16.532593	F	f1	f4	0.994	0.006	0.039	0.023	0.938	0.083	0.917	Kindler et al. (2013)
MTD T 20369	Italy: Campania: Caserta, Valle Agricola	41.424899	14.255653	F	f1	f4	—	—	—	—	—	—	—	This study
MTD T 20367 ^s	Italy: Campania: Caserta, San Gregorio Matese	41.383824	14.418093	F	f1	f2	0.996	0.004	0.01	0.019	0.971	0.056	0.944	This study
MTD T 20368	Italy: Campania: Caserta, San Gregorio Matese	41.383824	14.418093	F	f2	—	0.996	0.004	0.005	0.025	0.97	0.06	0.94	This study

TABLE S1 – Continued

Sample/ Voucher	Locality	Latitude (N)	Longitude (E)	Clade	Haplotypes		First STRUCTURE run (K=2)		Second STRUCTURE run (K=3)			Third STRUCTURE run (K=2)		Reference
					ND4+ tRNAs	cyt <i>b</i>	<i>helv.</i>	<i>nat.</i>	<i>cetti</i>	<i>helv.</i>	<i>sic.</i>	<i>helv.</i>	<i>sic.</i>	
MTD T 20370 ^s	Italy: Campania: Caserta, San Gregorio Matese	41.383824	14.418093	F	f1	f2	0.992	0.008	0.007	0.023	0.97	0.065	0.935	This study
BEV. 14432	Italy: Liguria: La Spezia: Carrodano	44.242070	9.677360	F	f1	f2	0.993	0.007	0.007	0.036	0.957	0.081	0.919	This study
MZUF 31620 ^s	Italy: Lazio: Monti della Tolfa	42.133431	11.900496	F	f1	f*	0.994	0.006	0.007	0.009	0.984	0.043	0.957	Kindler et al. (2013)
MTD T 20245	Italy: Lazio: Roma, Canale Monterano	42.139340	12.103292	F	f1	f2	0.996	0.004	0.007	0.026	0.967	0.101	0.899	This study
MTD T 20248	Italy: Lazio: Roma, Insugherata	41.953959	12.429020	F	f1	—	0.997	0.003	0.005	0.027	0.968	0.067	0.933	This study
MTD T 20244 ^s	Italy: Lazio: Roma, Monterotondo Scalo	42.064017	12.584303	F	f1	f7	0.993	0.007	0.006	0.007	0.987	0.032	0.968	This study
MTD T 20246	Italy: Lazio: Roma, Ostia Antica	41.757742	12.300021	F	f*	—	—	—	—	—	—	—	—	This study
MTD T 20247 ^s	Italy: Lazio: Viterbo: Capranica	42.259543	12.174631	F	f*	—	0.994	0.006	0.017	0.02	0.963	0.07	0.93	This study
MSNVE 24726	Italy: Trentino-Alto Adige/Südtirol: Mules/Mauls, Campo di Trens/Freienfeld	46.841681	11.527944	F	f1	f2	—	—	—	—	—	—	—	This study
ZFMK 64931 ^s	Italy: Tuscany: Arezzo: Chiaveretto	43.548890	11.928399	F	f1	f2	0.997	0.003	0.011	0.019	0.97	0.058	0.942	Kindler et al. (2013)
MZUF 7388	Italy: Tuscany: Grosseto: near Monti dell'Uccellina	42.671392	11.030774	F	f1	f*	0.997	0.003	0.007	0.066	0.927	0.14	0.86	Kindler et al. (2013)
—	Italy: Tuscany: Lucca: E Campagrina	44.067854	10.259056	F	f1	f*	—	—	—	—	—	—	—	Kindler et al. (2013)
ZMH R09025 ^s	Italy: Tuscany: Lucca: Pugliano	44.207312	10.198646	F	f1	—	0.998	0.002	0.007	0.115	0.878	0.235	0.765	Kindler and Fritz (2018)
MZUF 39870 ^s	Italy: Tuscany: Firenze	43.776300	11.249943	F	f1	f3	0.997	0.003	0.038	0.313	0.649	0.361	0.639	Kindler et al. (2013)
MZUF 38124	Italy: Tuscany: Firenze: Scandicci	43.757457	11.190548	F	—	f*	0.994	0.006	0.013	0.022	0.965	0.08	0.92	Kindler et al. (2013)
MZUF 40267	Italy: Tuscany: Firenze: Vaglia	43.910570	11.279390	F	f1	f2	—	—	—	—	—	—	—	Fritz et al. (2012)
MTD T 9981 ^s	Italy: Tuscany: Livorno	43.550539	10.311642	F	f1	f1	0.991	0.009	0.01	0.021	0.969	0.089	0.911	Kindler et al. (2013)
MTD D 33721 ^s	Italy: Tuscany: Pisa	43.722482	10.401936	F	f1	f5	0.997	0.003	0.004	0.024	0.972	0.081	0.919	Kindler et al. (2013)
MTD T 20241 ^s	Italy: Tuscany: Pistoia: mountains near Pistoia, Sambuca	44.054827	10.990569	F	f1	f6	0.997	0.003	0.011	0.038	0.951	0.116	0.884	This study
MZUF 40189	Italy: Tuscany: Siena: Gaiole in Chianti, Piazzole	43.468868	11.436653	F	f1	f2	0.996	0.004	0.009	0.182	0.809	0.258	0.742	Kindler et al. (2013)
MTD T 18970 ^s	Italy: Basilicata: Potenza: Montemilone, Strada San Giuseppe	41.051240	16.018140	G	g1	g1	0.998	0.002	0.019	0.056	0.925	0.211	0.789	This study

TABLE S1 – Continued

Sample/ Voucher	Locality	Latitude (N)	Longitude (E)	Clade	Haplotypes		First STRUCTURE run (K=2)		Second STRUCTURE run (K=3)			Third STRUCTURE run (K=2)		Reference
					ND4+ tRNAs	cyt <i>b</i>	<i>helv.</i>	<i>nat.</i>	<i>cetti</i>	<i>helv.</i>	<i>sic.</i>	<i>helv.</i>	<i>sic.</i>	
MTD T 20371 ^{ss}	Italy: Campania: Salerno: Amalfi	40.632910	14.591539	G	g2	—	0.993	0.007	0.009	0.028	0.963	0.08	0.92	This study
MTD T 11977 ^s	Italy: Tuscany: Castiglione della Pescaia	42.766449	10.876129	G	g1	—	0.997	0.003	0.005	0.011	0.984	0.045	0.955	Kindler and Fritz (2018)
BEV. 7054 ^s	France: Corsica: between Bonifacio and Porto-Vecchio	41.478747	9.238014	?	—	—	0.998	0.002	0.987	0.005	0.008	—	—	This study
BEV. 7053 ^s	France: Corsica: Fango valley	42.420196	8.665662	?	—	—	0.997	0.003	0.992	0.004	0.004	—	—	This study
MTD T 18961	Italy: Apulia: Bari: Conversano: Sassano	40.968759	17.097258	?	—	—	0.997	0.003	0.007	0.034	0.959	0.137	0.863	This study
MTD T 18960	Italy: Apulia: Bari: Ruvo di Puglia, Taverna nuova	40.995271	16.300986	?	—	—	0.997	0.003	0.007	0.067	0.926	0.185	0.815	This study
MTD T 18973	Italy: Apulia: Tarent: Martina Franca	40.746396	17.259578	?	—	—	0.996	0.004	0.051	0.029	0.92	0.086	0.914	This study
MSNPV CR0823	Italy: Emilia Romagna: Piacenza: Vallerenzo, Pecorara	44.891161	9.372501	?	—	—	0.996	0.004	0.02	0.024	0.956	0.071	0.929	This study
MSNPV CR1187	Italy: Lombardy: Pavia	45.181849	9.187354	?	—	—	0.958	0.042	0.007	0.37	0.623	0.375	0.625	This study
MSNVE 24724	Italy: Tuscany: Firenze: Fucecchio	43.722598	10.807614	?	—	—	0.987	0.013	0.005	0.048	0.947	0.108	0.892	This study
MSNVE 766[A]	Italy: Veneto: Venezia, Martellago	45.542107	12.167987	?	—	—	0.342	0.658	—	—	—	—	—	This study
MSNVE 24718	Italy: Veneto: Venezia: Porto Marghera	45.448694	12.235927	?	—	—	0.005	0.995	—	—	—	—	—	This study
MSNVE 24744	Italy: Veneto: Venezia: Quarto d'Altino, Trepalade	45.568646	12.397719	?	—	—	0.06	0.94	—	—	—	—	—	This study
MSNVE 6387	Italy: Veneto: Venezia: Salzano	45.530139	12.133016	?	—	—	0.037	0.963	—	—	—	—	—	This study

Additional samples included for third STRUCTURE run														
MTD T 19287 ^s	France: Aveyron: Laguiole	44.682045	2.926011	E	—	—	—	—	—	—	—	0.926	0.074	This study
BEV. 6430 ^s	France: Aveyron: Peyrusse-le-Roc	44.494914	2.140861	E	—	—	—	—	—	—	—	0.974	0.026	Pokrant et al. (2016)
MTD T 19078 ^s	France: Aveyron: St-Chély-d'Aubrac	44.591311	2.921214	E	—	—	—	—	—	—	—	0.923	0.077	This study
ZFMK 54712 ^s	France: Bouches-du-Rhône: Camargue	43.514697	4.541244	E	—	—	—	—	—	—	—	0.956	0.044	Kindler et al. (2013)
BEV.284	France: Bouches-du-Rhône: Camargue: Arles	43.519920	4.656654	E	—	—	—	—	—	—	—	0.493	0.507	Pokrant et al. (2016)

TABLE S1 – Continued

Sample/ Voucher	Locality	Latitude (N)	Longitude (E)	Clade	Haplotypes		First STRUCTURE run (K=2)		Second STRUCTURE run (K=3)			Third STRUCTURE run (K=2)		Reference
					ND4+ tRNAs	cyt <i>b</i>	<i>helv.</i>	<i>nat.</i>	<i>cetti</i>	<i>helv.</i>	<i>sic.</i>	<i>helv.</i>	<i>sic.</i>	
BEV.6425	France: Bouches-du-Rhône: Camargue: Clos de Lange	43.573124	4.562843	E	—	—	—	—	—	—	—	0.686	0.314	Pokrant et al. (2016)
BEV.9400	France: Gard: Saint-Hippolyte-du-Fort	43.966257	3.857374	E	—	—	—	—	—	—	—	0.856	0.144	Pokrant et al. (2016)
BEV.T 9264	France: Hérault: Avène dam	43.767270	3.094450	E	—	—	—	—	—	—	—	0.905	0.095	Pokrant et al. (2016)
BEV.T 9258	France: Hérault: La Tour-sur-Orb, Le Mas Blanc	43.650330	3.171910	E	—	—	—	—	—	—	—	0.885	0.115	Pokrant et al. (2016)
BEV.T 9266	France: Hérault: Lattes	43.552279	3.905801	E	—	—	—	—	—	—	—	0.589	0.411	Pokrant et al. (2016)
BEV.9049	France: Hérault: Le Bosc, Loiras	43.705916	3.395204	E	—	—	—	—	—	—	—	0.628	0.372	Pokrant et al. (2016)
BEV.T 9565 ^s	France: Hérault: Les Corrèges	43.670781	3.691793	E	—	—	—	—	—	—	—	0.931	0.069	Kindler et al. (2017)
BEV. 8499 ^s	France: Hérault: Rosis, Massif du Caroux	43.614073	2.985004	E	—	—	—	—	—	—	—	0.958	0.042	Pokrant et al. (2016)
BEV.847	France: Hérault: Saint-Pierre-de-la-Fage	43.794022	3.422570	E	—	—	—	—	—	—	—	0.729	0.271	Pokrant et al. (2016)
BEV. 12694 ^s	France: Loire: Cleppé, étang des Varennes	45.775100	4.173700	E	—	—	—	—	—	—	—	0.91	0.09	This study
BEV. 14516	France: Loire: Cleppé, 700 m au sud de «La Roche »	45.783900	4.174200	E	—	—	—	—	—	—	—	0.88	0.12	This study
BEV. 14107 ^s	France: Loire: Cleppé, le Bourg	45.768500	4.178300	E	—	—	—	—	—	—	—	0.961	0.039	This study
BEV. 8324 ^s	France: Loire: Sainte-Foy-Saint-Sulpice	45.768361	4.129114	E	—	—	—	—	—	—	—	0.94	0.06	Pokrant et al. (2016)
BEV. 14105 ^s	France: Loire: Valeille, route au coin NE de l'étang Vieux Ouest	45.716800	4.252200	E	—	—	—	—	—	—	—	0.961	0.039	This study
BEV. 14106 ^s	France: Loire: Valeille, route au coin NE de l'étang Vieux Ouest	45.716800	4.252200	E	—	—	—	—	—	—	—	0.915	0.085	This study
MTD T 10092	France: Puy-de-Dôme: Chambon-sur-Lac: Monneaux	45.558900	2.869270	E	—	—	—	—	—	—	—	0.812	0.188	Kindler et al. (2013)
MTD T 19008 ^s	France: Saône-et-Loire: Etang neuf	46.555268	4.425845	E	—	—	—	—	—	—	—	0.98	0.02	This study
MTD T 19009 ^s	France: Saône-et-Loire: Etang neuf	46.555268	4.425845	E	—	—	—	—	—	—	—	0.919	0.081	This study
MTD T 19010 ^s	France: Saône-et-Loire: Etang neuf	46.555268	4.425845	E	—	—	—	—	—	—	—	0.97	0.03	This study
MTD T 19007 ^s	France: Saône-et-Loire: Le Rousset	46.581401	4.452109	E	—	—	—	—	—	—	—	0.958	0.042	This study
BEV. 11533	France: Gard: Alzon	43.967121	3.440666	?	—	—	—	—	—	—	—	0.879	0.121	Pokrant et al. (2016)

TABLE S1 – Continued

Sample/ Voucher	Locality	Latitude (N)	Longitude (E)	Clade	Haplotypes		First STRUCTURE run (K=2)		Second STRUCTURE run (K=3)			Third STRUCTURE run (K=2)		Reference
					ND4+ tRNAs	cyt <i>b</i>	<i>helv.</i>	<i>nat.</i>	<i>cetti</i>	<i>helv.</i>	<i>sic.</i>	<i>helv.</i>	<i>sic.</i>	
BEV.9041	France: Hérault: Avène, Truscas	43.731662	3.120883	?	—	—	—	—	—	—	—	0.764	0.236	Pokrant et al. (2016)
BEV. 6421 ^s	France: Hérault: Rosis, Massif du Caroux	43.614073	2.985004	?	—	—	—	—	—	—	—	0.965	0.035	Pokrant et al. (2016)

Collection acronyms for Tables S1 and S2:

BEV – Biogéographie et Ecologie des Vertébrés, Centre d’Ecologie Fonctionnelle & Evolutive, Montpellier

CIBIO – Research Centre in Biodiversity and Genetic Resources, Vila do Conde

MNCN – Museo Nacional de Ciencias Naturales, Madrid

MTD D – Museum of Zoology, Senckenberg Dresden (Herpetological Collection)

MTD T – Museum of Zoology, Senckenberg Dresden (Tissue Collection)

MFSNUD – Sezione Zoologica del Museo Friulano di Storia Naturale, Udine

MSNPV – Museo di Storia Naturale, Università degli Studi di Pavia

MSNVE – Museo di Storia Naturale di Venezia

MZUF – Museo di Storia Naturale dell’Università di Firenze, Sede “La Specola”

NME – Naturkundemuseum Erfurt

ROM – Royal Ontario Museum, Department of Natural History, Toronto, Ontario

ZFMK – Zoologisches Forschungsmuseum Alexander Koenig, Bonn

ZMH – Zoologisches Museum Hamburg

ZSM – Zoologische Staatssammlung München

Remaining acronyms refer to samples processed for previous studies from the Department of Environmental Sciences, Section of Conservation Biology, University of Basel, Switzerland, and the Institute of Evolutionary Biology (CSIC-Universitat Pompeu Fabra), Barcelona, Spain.

TABLE S2 Additional mtDNA sequences used for phylogenetic tree calculations. Clade names follow those of the cited references. ME = Maghreb East; MW = Maghreb West

Sample/ Voucher	Taxon	Clade	Locality	Accession Numbers		Reference
				ND4 + tRNAs	cyt <i>b</i>	
MTD T 14359	<i>Natrix helvetica</i>	E	Great Britain: Yorkshire: Skipwith Common	LT839097	LT839248	Kindler et al. (2017)
MTD T 13943	<i>Natrix helvetica</i>	E	Netherlands: Houten	LT839092	LT839257	Kindler et al. (2017)
MTD T 13939	<i>Natrix helvetica</i>	E	Netherlands: Leersum	LT839092	LT839229	Kindler et al. (2017)
BEV.T5722	<i>Natrix natrix</i>	1	Azerbaijan: Hajikabul: near Chadzygabal Lake	LT900417	LT900468	Kindler et al. (2018a)
MTD D 48550	<i>Natrix natrix</i>	1	Azerbaijan: S Lerik	LT900417	LT900469	Kindler et al. (2018a)
MTD T 8954	<i>Natrix natrix</i>	1	Iran: Nowshahr	HF679880	HF680120	Kindler et al. (2013)
MTD T 8959	<i>Natrix natrix</i>	2	Georgia: Telavi	HF679858	HF680158	Kindler et al. (2013)
BEV.T5723	<i>Natrix natrix</i>	2	Azerbaijan: Hajikabul: near Chadzygabal Lake	LT900419	LT900474	Kindler et al. (2018a)
MTD T 8958	<i>Natrix natrix</i>	2	Georgia: Telavi	HF679857	HF680157	Kindler et al. (2013)
MTD T 14551	<i>Natrix natrix</i>	3	Denmark: Zealand: S Hillerød: Børstingerød Mose	LT839122	LT839258	Kindler et al. (2017)
MTD T 14508	<i>Natrix natrix</i>	3	Germany: Mecklenburg-Western Pomerania: Rostock	LT839113	LT839274	Kindler et al. (2017)
MTD T 14380	<i>Natrix natrix</i>	3	Germany: Brandenburg: Golm	LT839104	LT839262	Kindler et al. (2017)
MTD T 14351	<i>Natrix natrix</i>	4	Germany: Saxony: Klingenberg	LT839150	LT839333	Kindler et al. (2017)
MTD T 14362	<i>Natrix natrix</i>	4	Germany: Brandenburg: Golm	LT839149	LT839309	Kindler et al. (2017)
MTD T 14448	<i>Natrix natrix</i>	4	Slovakia: Istragov	LT839176	LT839302	Kindler et al. (2017)
MTD T 11602	<i>Natrix natrix</i>	5	Albania: Fitore	LT839184	LT839373	Kindler et al. (2017)
MTD T 11598	<i>Natrix natrix</i>	5	Albania: near Sukë: Trebeshinë Mountains	LT839199	LT839385	Kindler et al. (2017)
MTD T 11599	<i>Natrix natrix</i>	5	Albania: Shalës	LT839185	LT839375	Kindler et al. (2017)
ZFMK 71176	<i>Natrix natrix</i>	6	Turkey: Mediterranean Region: Burmaz (near Botaş)	HF679903	HF680201	Kindler et al. (2013)
e6116x10K	<i>Natrix natrix</i>	6	Turkey: Mediterranean Region: SW Osmaniye	KC570263	KC570308	Kindler et al. (2013)
MTD T 12965	<i>Natrix natrix</i>	7	Bulgaria: Pomorie	LT839203	LT839398	Kindler et al. (2017)
MTD T 14446	<i>Natrix natrix</i>	7	Bulgaria: Lozenets	LT839200	LT839391	Kindler et al. (2017)
MTD T 11816	<i>Natrix natrix</i>	7	Greece: Samothrace	LT839205	LT839396	Kindler et al. (2017)
MTD T 14757	<i>Natrix natrix</i>	8	Armenia: Nshkhark	LT839220	LT839417	Kindler et al. (2017)

TABLE S2 – Continued

Sample/ Voucher	Taxon	Clade	Locality	Accession Numbers		Reference
				ND4 + tRNAs	cyt <i>b</i>	
MTD T 13545	<i>Natrix natrix</i>	8	Russia: Cherusti	LT839211	LT839401	Kindler et al. (2017)
MTD T 14770	<i>Natrix natrix</i>	8	Georgia: Kobuleti	LT839225	LT839413	Kindler et al. (2017)
CIBIO 868	<i>Natrix astreptophora</i>	ME	Algeria: Béjaïa: Kabylia: Akfadou	LT900406	LT900451	Kindler et al. (2018a)
ZFMK 67196	<i>Natrix astreptophora</i>	ME	Tunisia: Bizerta: Cap Serrat	LT900409	LT900447	Kindler et al. (2013)
CIBIO 1536	<i>Natrix astreptophora</i>	ME	Tunisia: Jendouba: 10 km E of Tabarka	LT900408	LT900449	Kindler et al. (2018a)
CIBIO 10510	<i>Natrix astreptophora</i>	MW	Morocco: Fès-Meknès: Taza: Jbel Tazekka	LT900404	LT900444	Kindler et al. (2018a)
E21023x8M	<i>Natrix astreptophora</i>	MW	Morocco: Tanger-Tétouan-Al Hoceïma: Rif: 1 km SW of Dardara	LT960371	LT900445	Kindler et al. (2018a)
ENAT1M	<i>Natrix astreptophora</i>	MW	Morocco: Tanger-Tétouan-Al Hoceïma: 30 km NW of Chefchaouen	LT900405	LT900446	Kindler et al. (2018a)
MTD T 14353	<i>Natrix astreptophora</i>	Eu	Spain: Galicia: Fonsagrada: Penamaior	LT900391	LT900442	Pokrant et al. (2016)
ZFMK 87516	<i>Natrix astreptophora</i>	Eu	Portugal: Setúbal Peninsula: S Setúbal: Torre	LT900399	LT900426	Kindler et al. (2013)
MTD T 10631	<i>Natrix astreptophora</i>	Eu	Spain: Castilla y León: Soria: Garray	LT900401	LT900435	Kindler et al. (2018a)
Outgroups:						
MNCN 12016	<i>Natrix maura</i>	—	Spain: Southern Spain	AY873708	AY866530	Guicking et al. (2006)
ROM 23418	<i>Natrix tessellata</i>	—	Armenia: Geolazar	AY873734	AY866531	Guicking et al. (2006)
—	<i>Nerodia sipedon</i>	—	USA: Tennessee	JF964960	JF964960	GenBank

TABLE S3 Microsatellite loci and multiplex sets used in this study

Locus	Primer sequences (5' - 3')	Repeat motif	Allele size range [bp]	Number of alleles	Multiplex set	Annealing temp. [°C]	Fluorescent label	Original reference
Natnat09	for – TGTAATAAACAACACTGTACCATTTTGG rev – TGACTGGGCAACAGAAAAGC	(AC) ₂₂	80–144	25	1	55	FAM	Meister et al. (2009)
Natnat05	for – TCTGCACTGGGGATAGGAAG rev – GTCCCTTTTTTCAGTGCTGTTG	(GT) ₁₆	136–194	25	1	55	HEX	Meister et al. (2009)
μNt8new	for – GTATCGTCCTTCCAGACAAG rev – GCAAATCAAATAAATCTCACTGG	(AC) ₁₅	75–125	22	1	55	Atto565	Meister et al. (2009)
Nsμ3	for – CTGACTCACTTCTGACCCTAAT rev – AATATTTGCTTGGCTCAAAC	(ATCT) ₁₄ ATC(CA) ₂₀	139–457	39	1	55	Atto550	Prosser et al. (1999)
μNt3	for – GGCAGGCTATTGGAGAAATG rev – GGCAAAACTCCAGGTGCTAC	(AC) ₁₆	111–173	24	2	60	FAM	Gautschi et al. (2000)
μNt7	for – TTTGAAAGGAGAATGAATCGTG rev – CGCGAGGAATCAGAATGAAC	(AC) ₁₇	162–212	26	2	60	HEX	Gautschi et al. (2000)
Hb30	for – CCCACTGGCTCATTTC AAGT rev – CCACATTTGCATCGGAGTG	(CA) ₁₄	225–279	26	2	60	Atto565	Burns and Houlden (1999)
Natnat11	for – GGCTGTTTTCCAGTGAAGC rev – GGTCTGGGGAAAAAGAAAGG	(GA) ₁₃	102–228	32	3	55	FAM	Meister et al. (2009)
Natnat06	for – AATGGCATTCTCTCCAGCTC rev – ACCCATATCCGTATCCATATCC	(GT) ₂₁	145–187	16	3	55	HEX	Meister et al. (2009)
3TS	for – GGTCACTTAAATACAACGAAATTGGTTAGCT rev – CGGACAGCTCTGGCTCCCTTG	(GATA) ₁₉	186–282	22	3	55	Atto550	Garner et al. (2002)
Tbu_A09	for – CATCTCAACCAAAGTCGCTTC rev – GGATGTTGTGGGGTGTTC	(AC) ₇	110–160	20	3	55	Atto565	Sloss et al. (2012)
Eobμ1	for – ATCAGTAGGAGTGAGAGCAACT rev – CTGCATACTCTTCCAGAACC	(TG) ₂₁	120–142	12	—	51	FAM	Blouin-Demers and Gibbs (2003)
Eobμ13	for – TGATCTGAGTCTCTTTCTGG rev – CAATTCAAATCCATTGGTTT	(AC) ₂₀	118–164	20	—	51	FAM	Blouin-Demers and Gibbs (2003)

TABLE S4 Primers used for amplification and sequencing of mitochondrial genes (ND4+tRNAs, *cyt b*)

mtDNA fragment	Primer name	Direction	Primer sequence (5' - 3')	Reference
ND4+tRNAs	ND4ab	Forward	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Guicking et al. (2006)
ND4+tRNAs	tRNA-leu	Reverse	CATTACTTTTACTTGGATTTGCACCA	Guicking et al. (2006)
ND4+tRNAs	Natrix_ND4_For3	Forward	CCCAGCATTAAATTTYACDGG	Kindler et al. (2013)
ND4+tRNAs	Natrix_ND4_Rev2	Reverse	AATTGTTGTTGGGCATCA	Kindler et al. (2013)
ND4+tRNAs	Natrix_ND4_For4	Forward	TCATCAGCACTCTTYTGCCTAGC	This study
ND4+tRNAs	Natrix_ND4_Rev4	Reverse	AAATTAATGCTGGGRGGRGT	This study
<i>cyt b</i>	L14724NAT	Forward	GACCTGCGGTCCGAAAAACCA	Guicking et al. (2006)
<i>cyt b</i>	Thrsnr2	Reverse	CTTTGGTTTACAAGAACAATGCTTTA	Guicking et al. (2006)
<i>cyt b</i>	Natrix_Cytb_Rev2	Reverse	AGGGCAAAGAATCGGGTT	Kindler et al. (2013)
<i>cyt b</i>	Natrix_Cytb_For3	Forward	CTAGGAAAYACCCTCACAACC	Kindler et al. (2013)
<i>cyt b</i>	Natrix_Cytb_Rev3	Reverse	TTAATGTGTTGYGGGGTTACTA	Kindler et al. (2013)

TABLE S5 Partitions and best-fit evolutionary models for phylogenetic tree analyses as calculated with PARTITIONFINDER 2

Gene	Position	Partition	Evolutionary model
ND4	1-696	Codon position 1	GTR + G
		Codon position 2	HKY + I
		Codon position 3	GTR + G
tRNAs	697-867	tRNAs	K80 + I
<i>cyt b</i>	868-1984	Codon position 1	HKY + G
		Codon position 2	HKY + I
		Codon position 3	GTR + G

TABLE S6 HYBRIDLAB simulations for the data set containing all samples and covering the contact zone between *Natrix natrix* and *N. helvetica*. Sample size for each parental species and each hybrid class (F₁, F₂, backcrosses) was 15. The optimal threshold for *N. natrix* was 93% and for *N. helvetica*, 91%

STRUCTURE	Parent <i>N. natrix</i>	F ₁	F ₂	Backcross	Parent <i>N. helvetica</i>	F ₁	F ₂	Backcross
	('red lineage')	Referred to <i>Natrix natrix</i>			(lineage C)	Referred to <i>N. helvetica</i>		
Average <i>Q</i> score	0.957	0.52	0.541	0.781	0.952	0.48	0.459	0.81
SD	0.014	0.053	0.144	0.093	0.018	0.053	0.144	0.101
min	0.925	0.413	0.361	0.612	0.91	0.402	0.155	0.61
max	0.974	0.598	0.845	0.947	0.972	0.587	0.639	0.923
Misassignment (if $Q \geq 0.93/0.91$)	0	0	0	0.067	0	0	0	0.2

TABLE S7 HYBRIDLAB simulations for the data set containing only samples of *Natrix helvetica*, including Corsica and Sardinia (lineage B). Sample size for lineage B was 14; for all remaining lineages from the Tyrrhenian coast together (A: $n = 5$, E: $n = 3$, F: $n = 5$, G: $n = 2$) and each hybrid class (F₁, F₂, backcrosses), 15. The optimal threshold for lineage B was 73%; for the remaining lineages, 90%

STRUCTURE	Parent	F ₁	F ₂	Backcross	Parent	F ₁	F ₂	Backcross
	Lineage B	Referred to lineage B			Remaining lineages	Referred to remaining lineages		
Average <i>Q</i> score	0.885	0.398	0.333	0.701	0.937	0.602	0.667	0.84
SD	0.075	0.067	0.145	0.093	0.017	0.067	0.145	0.048
min	0.734	0.288	0.155	0.472	0.905	0.489	0.382	0.769
max	0.961	0.511	0.618	0.864	0.958	0.712	0.845	0.92
Misassignment (if $Q \geq 0.73/0.9$)	0	0	0	0.333	0	0	0	0.2

TABLE S8 HYBRIDLAB simulations for the data set containing only samples of *Natrix helvetica* exclusive of snakes from Corsica and Sardinia and their putative hybrids (see text). In the group from mainland Italy and Sicily ($n = 25$), lineages A, C, and F were represented by 6 samples each, lineage D by 5 samples, and lineage G by 2 samples. Sample size for lineage E and each hybrid class (F₁, F₂, backcrosses) was 25. The optimal threshold for lineage E was 80%; for the remaining lineages from mainland Italy plus Sicily, 86%

STRUCTURE	Parent	F ₁	F ₂	Backcross	Parent	F ₁	F ₂	Backcross
	Lineage E	Referred to lineage E			Mainland Italy + Sicily	Referred to mainland Italy + Sicily		
Average Q score	0.899	0.498	0.464	0.723	0.914	0.502	0.536	0.737
SD	0.056	0.097	0.134	0.099	0.028	0.097	0.134	0.096
min	0.741	0.227	0.242	0.515	0.86	0.328	0.263	0.516
max	0.959	0.672	0.737	0.93	0.955	0.773	0.758	0.881
Misassignment (if $Q \geq 0.8/0.86$)	0.08	0	0	0.12	0	0	0	0.16

TABLE S9 Akaike Information Criterion (AIC) scores for clines under different models as calculated by the R package HZAR (Derryberry et al. 2014). Bold values indicate best-fit model for each calculation. Q is the proportion of ancestry estimated by STRUCTURE using microsatellite loci. The lowest score indicates the best cline model

	<i>natrrix vs. helvetica cline</i>		<i>helvetica cline</i>	
	Q	mtDNA	Q	mtDNA
Null Model	45.972	47.079	28.088	68.790
Model 1	7.814	7.376	6.725	12.354
Model 2	6.790	n/a	6.756	n/a
Model 3	11.188	12.124	11.261	14.530
Model 4	14.793	14.381	15.932	19.292
Model 5	14.921	n/a	16.025	n/a
Model 6	20.990	21.961	21.315	24.916
Model 7	11.968	9.410	11.049	14.286
Model 8	9.510	n/a	11.168	n/a
Model 9	14.934	17.163	15.986	19.486
Model 10	11.680	11.957	11.252	16.947
Model 11	11.217	n/a	11.282	n/a
Model 12	16.062	17.177	16.118	19.466
Model 13	9.987	11.302	11.252	15.611
Model 14	10.002	n/a	11.283	n/a
Model 15	15.052	17.177	16.127	19.442

TABLE S10 Currently known haplotypes of ND4+tRNAs and *cyt b* and their ENA accession numbers for *Natrix helvetica* and the ‘red lineage’ of *N. natrix*

ND4+tRNAs			<i>cyt b</i>		
Haplotype	Accession number	Reference	Haplotype	Accession number	Reference
<i>Natrix helvetica</i> haplotypes					
Lineage A of Kindler et al. (2013)					
a1	LT900410	Kindler et al. (2018a)	a1	LT900452	Kindler et al. (2018a)
a2	LT900411	Kindler et al. (2018a)	a2	LT900453	Kindler et al. (2018a)
a3	LT900412	Kindler et al. (2018a)	a3	LT900454	Kindler et al. (2018a)
			a4	LT900455	Kindler et al. (2018a)
			a5	LR722580	This study
			a6	LR722581	This study
Lineage B of Kindler et al. (2013)					
b1	LT900413	Kindler et al. (2018a)	b1	LT900456	Kindler et al. (2018a)
b2	LT900414	Kindler et al. (2018a)	b2	LT900457	Kindler et al. (2018a)
b3	LR721654	This study	b3	LT900458	Kindler et al. (2018a)
			b4	LT900459	Kindler et al. (2018a)
			b5	LT900460	Kindler et al. (2018a)
			b6	LR722582	This study
			b7	LR722583	This study
Lineage C of Kindler et al. (2013)					
c1	LT839227	Kindler et al. (2017)	c1	LT839422	Kindler et al. (2017)
c2	LR721655	This study	c2	LT839423	Kindler et al. (2017)
c3	LR721656	This study	c3	LT900461	Kindler et al. (2018a)
			c4	LT900462	Kindler et al. (2018a)
			c5	LT900463	Kindler et al. (2018a)
			c6	LR722584	This study
			c7	LR722585	This study
			c8	LR722586	This study
			c9	LR722587	This study
			c10	LR722588	This study
			c11	LR722589	This study

TABLE S10 – Continued ND4+tRNAs			cyt b		
Haplotype	Accession number	Reference	Haplotype	Accession number	Reference
			c12	LR722590	This study
Lineage D of Kindler et al. (2013)					
d1	AY873715	Guicking et al. (2006)	d1	AY487733	Guicking et al. (2006)
d2	LR721657	This study	d2	LR722591	This study
d3	LR721658	This study	d3	LR722592	This study
			d4	LR722593	This study
			d5	LR722594	This study
			d6	LR722595	This study
Lineage E of Kindler et al. (2013)					
h1	LT839092	Kindler et al. (2017)	h1	LT839229	Kindler et al. (2017)
h2	LT839093	Kindler et al. (2017)	h2	LT839230	Kindler et al. (2017)
h3	LT839094	Kindler et al. (2017)	h3	LT839231	Kindler et al. (2017)
h4	LT839095	Kindler et al. (2017)	h4	LT839232	Kindler et al. (2017)
h5	LT839096	Kindler et al. (2017)	h5	LT839233	Kindler et al. (2017)
h6	LT839097	Kindler et al. (2017)	h6	LT839234	Kindler et al. (2017)
h7	LT839098	Kindler et al. (2017)	h7	LT839235	Kindler et al. (2017)
h8	LT839099	Kindler et al. (2017)	h8	LT839236	Kindler et al. (2017)
h9	LT839100	Kindler et al. (2017)	h9	LT839237	Kindler et al. (2017)
h10	LT839101	Kindler et al. (2017)	h10	LT839238	Kindler et al. (2017)
h11	LT839102	Kindler et al. (2017)	h11	LT839239	Kindler et al. (2017)
h12	LT839103	Kindler et al. (2017)	h12	LT839240	Kindler et al. (2017)
			h13	LT839241	Kindler et al. (2017)
			h14	LT839242	Kindler et al. (2017)
			h15	LT839243	Kindler et al. (2017)
			h16	LT839244	Kindler et al. (2017)
			h17	LT839245	Kindler et al. (2017)
			h18	LT839246	Kindler et al. (2017)
			h19	LT839247	Kindler et al. (2017)
			h20	LT839248	Kindler et al. (2017)
			h21	LT839249	Kindler et al. (2017)
			h22	LT839250	Kindler et al. (2017)

TABLE S10 – Continued ND4+tRNAs			cyt b		
Haplotype	Accession number	Reference	Haplotype	Accession number	Reference
			h23	LT839251	Kindler et al. (2017)
			h24	LT839252	Kindler et al. (2017)
			h25	LT839253	Kindler et al. (2017)
			h26	LT839254	Kindler et al. (2017)
			h27	LT839255	Kindler et al. (2017)
			h28	LT839256	Kindler et al. (2017)
			h29	LT839257	Kindler et al. (2017)
Lineage F of Kindler et al. (2013)					
f1	LT839228	Kindler et al. (2017)	f1	LT839424	Kindler et al. (2017)
f2	LR722601	This study	f2	LT900464	Kindler et al. (2018a)
			f3	LT900465	Kindler et al. (2018a)
			f4	LT900466	Kindler et al. (2018a)
			f5	LT900467	Kindler et al. (2018a)
			f6	LR722596	This study
			f7	LR722597	This study
Lineage G (this study)					
g1	LT900415	This study	g1	LR722598	This study
g2	LR721659	This study			
<i>Matrix matrix haplotypes</i>					
‘Red lineage’ = lineage 4 of Kindler et al. (2013)					
r1	LT839147	Kindler et al. (2017)	r1	LT839300	Kindler et al. (2017)
r2	LT839148	Kindler et al. (2017)	r2	LT839301	Kindler et al. (2017)
r3	LT839149	Kindler et al. (2017)	r3	LT839302	Kindler et al. (2017)
r4	LT839150	Kindler et al. (2017)	r4	LT839303	Kindler et al. (2017)
r5	LT839151	Kindler et al. (2017)	r5	LT839304	Kindler et al. (2017)
r6	LT839152	Kindler et al. (2017)	r6	LT839305	Kindler et al. (2017)
r7	LT839153	Kindler et al. (2017)	r7	LT839306	Kindler et al. (2017)
r8	LT839154	Kindler et al. (2017)	r8	LT839307	Kindler et al. (2017)
r9	LT839155	Kindler et al. (2017)	r9	LT839308	Kindler et al. (2017)
r10	LT839156	Kindler et al. (2017)	r10	LT839309	Kindler et al. (2017)
r11	LT839157	Kindler et al. (2017)	r11	LT839310	Kindler et al. (2017)

TABLE S10 – Continued ND4+tRNAs			cyt b		
Haplotype	Accession number	Reference	Haplotype	Accession number	Reference
r12	LT839158	Kindler et al. (2017)	r12	LT839311	Kindler et al. (2017)
r13	LT839159	Kindler et al. (2017)	r13	LT839312	Kindler et al. (2017)
r14	LT839160	Kindler et al. (2017)	r14	LT839313	Kindler et al. (2017)
r15	LT839161	Kindler et al. (2017)	r15	LT839314	Kindler et al. (2017)
r16	LT839162	Kindler et al. (2017)	r16	LT839315	Kindler et al. (2017)
r17	LT839163	Kindler et al. (2017)	r17	LT839316	Kindler et al. (2017)
r18	LT839164	Kindler et al. (2017)	r18	LT839317	Kindler et al. (2017)
r19	LT839165	Kindler et al. (2017)	r19	LT839318	Kindler et al. (2017)
r20	LT839166	Kindler et al. (2017)	r20	LT839319	Kindler et al. (2017)
r21	LT839167	Kindler et al. (2017)	r21	LT839320	Kindler et al. (2017)
r22	LT839168	Kindler et al. (2017)	r22	LT839321	Kindler et al. (2017)
r23	LT839169	Kindler et al. (2017)	r23	LT839322	Kindler et al. (2017)
r24	LT839170	Kindler et al. (2017)	r24	LT839323	Kindler et al. (2017)
r25	LT839171	Kindler et al. (2017)	r25	LT839324	Kindler et al. (2017)
r26	LT839172	Kindler et al. (2017)	r26	LT839325	Kindler et al. (2017)
r27	LT839173	Kindler et al. (2017)	r27	LT839326	Kindler et al. (2017)
r28	LT839174	Kindler et al. (2017)	r28	LT839327	Kindler et al. (2017)
r29	LT839175	Kindler et al. (2017)	r29	LT839328	Kindler et al. (2017)
r30	LT839176	Kindler et al. (2017)	r30	LT839329	Kindler et al. (2017)
r31	LT839177	Kindler et al. (2017)	r31	LT839330	Kindler et al. (2017)
r32	LT839178	Kindler et al. (2017)	r32	LT839331	Kindler et al. (2017)
r33	LT839179	Kindler et al. (2017)	r33	LT839332	Kindler et al. (2017)
r34	LR721660	This study	r34	LT839333	Kindler et al. (2017)
r35	LR721661	This study	r35	LT839334	Kindler et al. (2017)
			r36	LT839335	Kindler et al. (2017)
			r37	LT839336	Kindler et al. (2017)
			r38	LT839337	Kindler et al. (2017)
			r39	LT839338	Kindler et al. (2017)
			r40	LT839339	Kindler et al. (2017)
			r41	LT839340	Kindler et al. (2017)
			r42	LT839341	Kindler et al. (2017)
			r43	LT839342	Kindler et al. (2017)

TABLE S10 – <i>Continued</i> ND4+tRNAs			cyt <i>b</i>		
Haplotype	Accession number	Reference	Haplotype	Accession number	Reference
			r44	LT839343	Kindler et al. (2017)
			r45	LT839344	Kindler et al. (2017)
			r46	LT839345	Kindler et al. (2017)
			r47	LT839346	Kindler et al. (2017)
			r48	LT839347	Kindler et al. (2017)
			r49	LT839348	Kindler et al. (2017)
			r50	LT839349	Kindler et al. (2017)
			r51	LT839350	Kindler et al. (2017)
			r52	LT839351	Kindler et al. (2017)
			r53	LT839352	Kindler et al. (2017)
			r54	LT839353	Kindler et al. (2017)
			r55	LT839354	Kindler et al. (2017)
			r56	LT839355	Kindler et al. (2017)
			r57	LT839356	Kindler et al. (2017)
			r58	LT839357	Kindler et al. (2017)
			r59	LT839358	Kindler et al. (2017)
			r60	LT839359	Kindler et al. (2017)
			r61	LT839360	Kindler et al. (2017)
			r62	LT839361	Kindler et al. (2017)
			r63	LT839362	Kindler et al. (2017)
			r64	LT839363	Kindler et al. (2017)
			r65	LT839364	Kindler et al. (2017)
			r66	LT839365	Kindler et al. (2017)

TABLE S11 Uncorrected p distances for the mitochondrial clades in the study region, based on distinct haplotypes. Between group values for ND4+tRNAs above the diagonal on the left; for *cyt b*, below the diagonal. For information on haplotype clusters outside of our study region, see Kindler et al. (2018a). The mtDNA fragment containing the partial ND4 gene plus tRNA genes (867 bp) had 122 variable sites and 94 parsimony-informative sites across all haplotypes; for the *cyt b* gene (1117 bp) the respective values were 194 and 125

Lineage	Between-group values								Within-group values			
	'Red'	A	B	C	D	E	F	G	n_h	ND4+tRNAs	n_h	<i>cyt b</i>
'Red lin.'	—	0.066	0.053	0.055	0.064	0.059	0.061	0.063	35	0.004	66	0.006
A	0.059	—	0.042	0.047	0.051	0.051	0.050	0.048	3	0.002	6	0.002
B	0.065	0.050	—	0.025	0.029	0.026	0.025	0.028	3	0.002	7	0.004
C	0.059	0.047	0.032	—	0.026	0.023	0.023	0.027	3	0.002	11	0.002
D	0.059	0.054	0.036	0.022	—	0.008	0.008	0.010	3	0.002	6	0.002
E	0.063	0.052	0.036	0.026	0.012	—	0.004	0.009	12	0.002	29	0.002
F	0.063	0.051	0.033	0.024	0.009	0.004	—	0.008	2	0.001	7	0.001
G	0.060	0.057	0.039	0.027	0.007	0.016	0.014	—	2	0.001	1	n/a

n_h – number of haplotypes

TABLE S12 Allele size ranges and number of alleles per microsatellite locus for the STRUCTURE clusters, obtained in CONVERT 1.31. Individuals with mixed ancestries were excluded

Locus	First STRUCTURE run ($K=2$)				Second STRUCTURE run ($K=3$)						Third STRUCTURE run ($K=2$)			
	<i>natrix</i>		<i>helvetica</i>		<i>helvetica</i> NW		<i>helvetica</i> S		Corsica/Sardinia		<i>helvetica</i> NW		<i>helvetica</i> S	
	ASR	n_A	ASR	n_A	ASR	n_A	ASR	n_A	ASR	n_A	ASR	n_A	ASR	n_A
Natnat09	94–124	10	92–134	20	102–134	9	92–130	17	94–116	9	102–114	5	92–130	16
Natnat05	136–180	8	156–184	15	158–180	7	156–184	15	170–180	5	158–180	6	156–184	15
μ Nt8new	81–95	7	75–123	14	75–123	7	75–99	11	93–101	3	81–93	4	75–99	11
N μ 3	147–191	16	149–183	12	—	—	149–183	12	151–159	2	151–159	2	149–183	11
μ Nt3	123–159	12	121–131	5	123–131	3	123–127	3	121–127	4	123–131	3	123–127	3
μ Nt7	166–208	19	166–198	13	176–184	4	168–198	12	168–178	3	176–184	4	168–198	12
Hb30	241–261	11	239–275	14	257–263	4	239–275	13	251–259	4	253–261	5	239–275	13
Natnat11	106–124	4	106–156	15	106–118	3	106–156	13	114–122	5	106–118	4	106–156	12
Natnat06	147–183	11	159–187	14	159–183	6	159–187	12	161–171	4	159–185	10	159–187	12
3TS	198–254	9	154–246	9	194–246	5	154–242	7	194–234	3	194–246	6	154–242	6
Eob μ 1	122–138	5	120–134	5	128–134	4	120–132	4	128–130	2	128–134	4	120–132	4
Eob μ 13	130–156	10	128–164	17	134–156	5	130–164	15	128–134	2	134–156	7	130–162	14
TBuA09	112–138	8	106–144	17	114–142	8	106–144	15	118–130	5	114–142	10	106–144	14

ASR – Allele size range; n_A – number of alleles.

TABLE S13 Genetic diversity of STRUCTURE clusters based on 13 microsatellite loci. Individuals with mixed ancestries were excluded

Cluster	Microsatellites								ND4+tRNAs				cyt <i>b</i>					
	<i>n</i>	<i>n_A</i>	<i>n_Ā</i>	<i>n_P</i>	<i>AR</i>	<i>H_O</i>	<i>H_E</i>	<i>F_{IS}</i>	<i>n</i>	<i>n_{HT}</i>	<i>n_{pHT}</i>	<i>h</i>	π	<i>n</i>	<i>n_{HT}</i>	<i>n_{pHT}</i>	<i>h</i>	π
First STRUCTURE analysis (<i>K</i>=2)																		
<i>natrix</i>	41	130	10.00	42	8.749	0.571	0.708	0.096*	33	6	6	1.000	0.0026	27	6	6	1.000	0.0023
<i>helvetica</i>	112	170	13.08	82	9.182	0.593	0.785	0.279*	97	18	18	1.000	0.0264	76	34	34	0.995	0.0287
Second STRUCTURE analysis (<i>K</i>=3)																		
<i>helvetica</i> NW	20	65	5.00	9	4.474 [§]	0.493	0.569	0.083	19	2	2	1.000	0.0219	18	5	4	1.000	0.0107
<i>helvetica</i> S	52	149	11.46	72	7.320 [§]	0.634	0.783	0.196*	44	10	10	1.000	0.0297	34	20	19	0.995	0.0295
Corsica/Sardinia	14	51	3.92	6	3.852 [§]	0.502	0.498	0.089	12	3	3	1.000	0.0015	9	6	6	1.000	0.0036
Third STRUCTURE analysis (<i>K</i>=2)																		
<i>helvetica</i> NW	35	70	5.38	12	2.830	0.518	0.548	0.064	12	1	1	—	—	12	3	3	1.000	0.0012
<i>helvetica</i> S	48	143	11.00	85	4.637	0.629	0.781	0.191*	41	10	10	1.000	0.0284	32	19	19	0.994	0.0295

* $p < 0.05$; [§] without locus Nsm3 (missing data for *helvetica*); *n*, number of individuals; *n_A*, number of alleles; *n_Ā*, average number of alleles per locus; *n_P*, number of private alleles; *AR*, allelic richness; *H_O*, average observed heterozygosity; *H_E*, average expected heterozygosity; *F_{IS}*, inbreeding coefficient; *n_{HT}*, number of haplotypes; *n_{pHT}*, number of private haplotypes; *h*, haplotype diversity; π , nucleotide diversity.

AR was calculated in FSTAT 2.9.3.2 (Goudet, 1995); *h* and π , in DNASP 6 (Rozas et al., 2017), *n_P* was derived from an allele frequency table obtained in CONVERT 1.31 (Glaubitz, 2004); and the remaining values were calculated locus-by-locus using ARLEQUIN 3.5.1.3 (Excoffier & Lischer, 2010) with 1,000,000 Markov chain and 100,000 dememorization steps.

TABLE S14 AMOVA results for STRUCTURE clusters, calculated for microsatellites locus-by-locus in ARLEQUIN 3.5.1.3 using 10,000 permutations. For mtDNA, a standard AMOVA in haplotypic format was run using 10,000 permutations. Individuals with mixed ancestries, and for mtDNA with incomplete haplotype sequences (asterisks in Table S1), were excluded

	First STRUCTURE analysis (K=2)			Second STRUCTURE analysis (K=3)			Third STRUCTURE analysis (K=2)		
	Microsatellites	ND4+tRNAs	cyt <i>b</i>	Microsatellites	ND4+tRNAs	cyt <i>b</i>	Microsatellites	ND4+tRNAs	cyt <i>b</i>
Between populations	19.79	77.07	74.33	21.28	43.30	49.91	19.08	22.52	31.65
Within populations	80.21	22.93	25.67	78.72	56.70	50.09	80.92	77.48	68.35

TABLE S15 Fixation indices (F_{ST}) for the STRUCTURE clusters based on 13 microsatellite loci, calculated in ARLEQUIN 3.5.1.3 using 1,000 permutations and a significance level of 0.05. Individuals with mixed ancestries were excluded. All F_{ST} values were statistically significant ($p < 0.05$)

First STRUCTURE analysis (K=2)			Second STRUCTURE analysis (K=3)				Third STRUCTURE analysis (K=2)		
<i>natrix</i>	<i>helvetica</i>		<i>helv</i> NW	<i>helv</i> S	Cors/Sard		<i>helv</i> NW	<i>helv</i> S	
<i>natrix</i>	—		<i>helvetica</i> NW	—		<i>helvetica</i> NW	—		
<i>helvetica</i>	0.198	—	<i>helvetica</i> S	0.162	—	<i>helvetica</i> S	0.191	—	
			Corsica/Sardinia	0.348	0.227				

TABLE S16 Fixation indices (F_{ST}) for the STRUCTURE clusters based on mitochondrial haplotypes, calculated in ARLEQUIN 3.5.1.3 using 1,000 permutations and a significance level of 0.05. Above the diagonal are F_{ST} values for cyt *b*; below the diagonal, for ND4+tRNAs. All F_{ST} values were statistically significant ($p < 0.05$). Individuals with mixed ancestries or incomplete haplotype sequences (asterisks in Table S1) were excluded

First STRUCTURE analysis (K=2)			Second STRUCTURE analysis (K=3)				Third STRUCTURE analysis (K=2)		
<i>natrix</i>	<i>helvetica</i>		<i>helv</i> NW	<i>helv</i> S	Cors/Sard		<i>helv</i> NW	<i>helv</i> S	
<i>natrix</i>	—	0.743	<i>helvetica</i> NW	—	0.299	0.902	<i>helvetica</i> NW	—	0.317
<i>helvetica</i>	0.771	—	<i>helvetica</i> S	0.223	—	0.519	<i>helvetica</i> S	0.225	—
			Corsica/Sardinia	0.936	0.460	—			

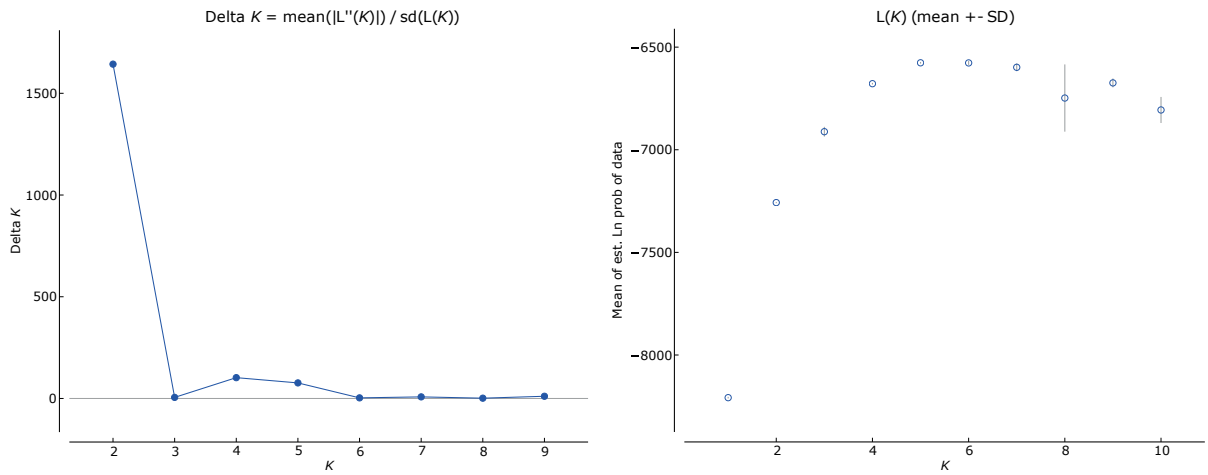


FIGURE S1 ΔK values (left) and posterior probabilities for K s (right) for the first STRUCTURE analysis visualized by STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Data set of all 160 samples of *Matrix natrix* and *N. helvetica* for which microsatellite data were available

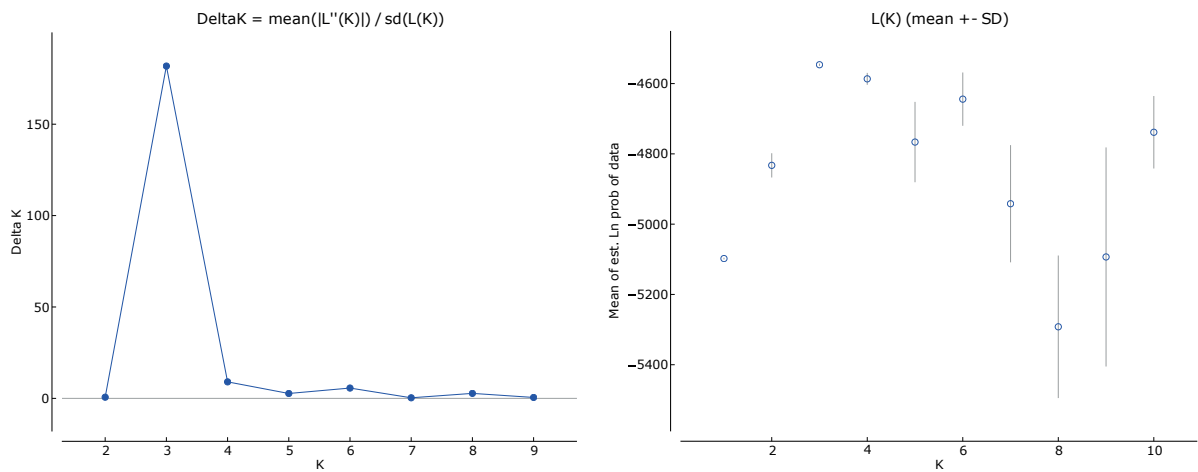


FIGURE S2 ΔK values (left) and posterior probabilities for K s (right) for the second STRUCTURE analysis visualized by STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Data set of 112 *Matrix helvetica* without genetic impact from *N. natrix*

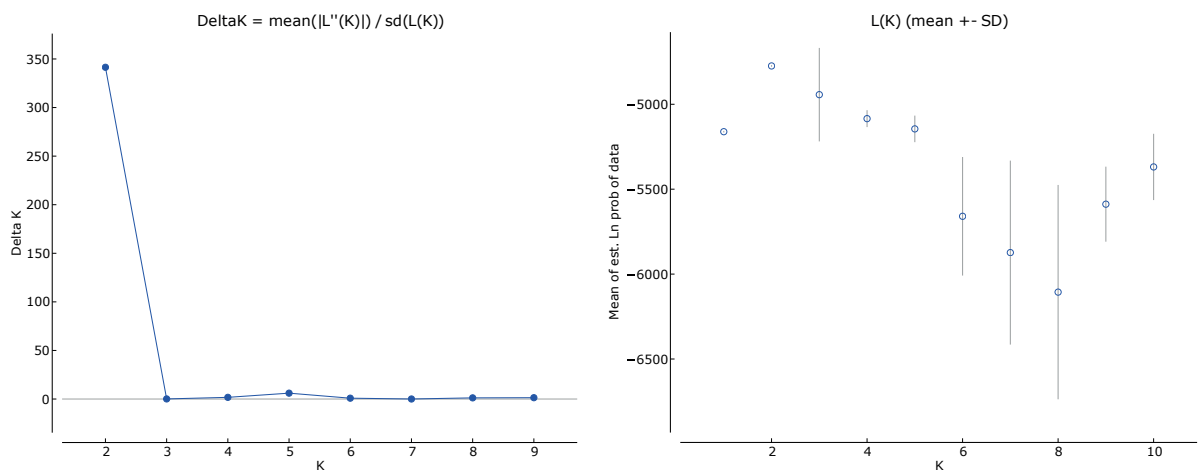


FIGURE S3 ΔK values (left) and posterior probabilities for K s (right) for the third STRUCTURE analysis visualized by STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Data set of 120 *Matrix helvetica* without samples corresponding to or with genetic impact from the Corso-Sardinian cluster

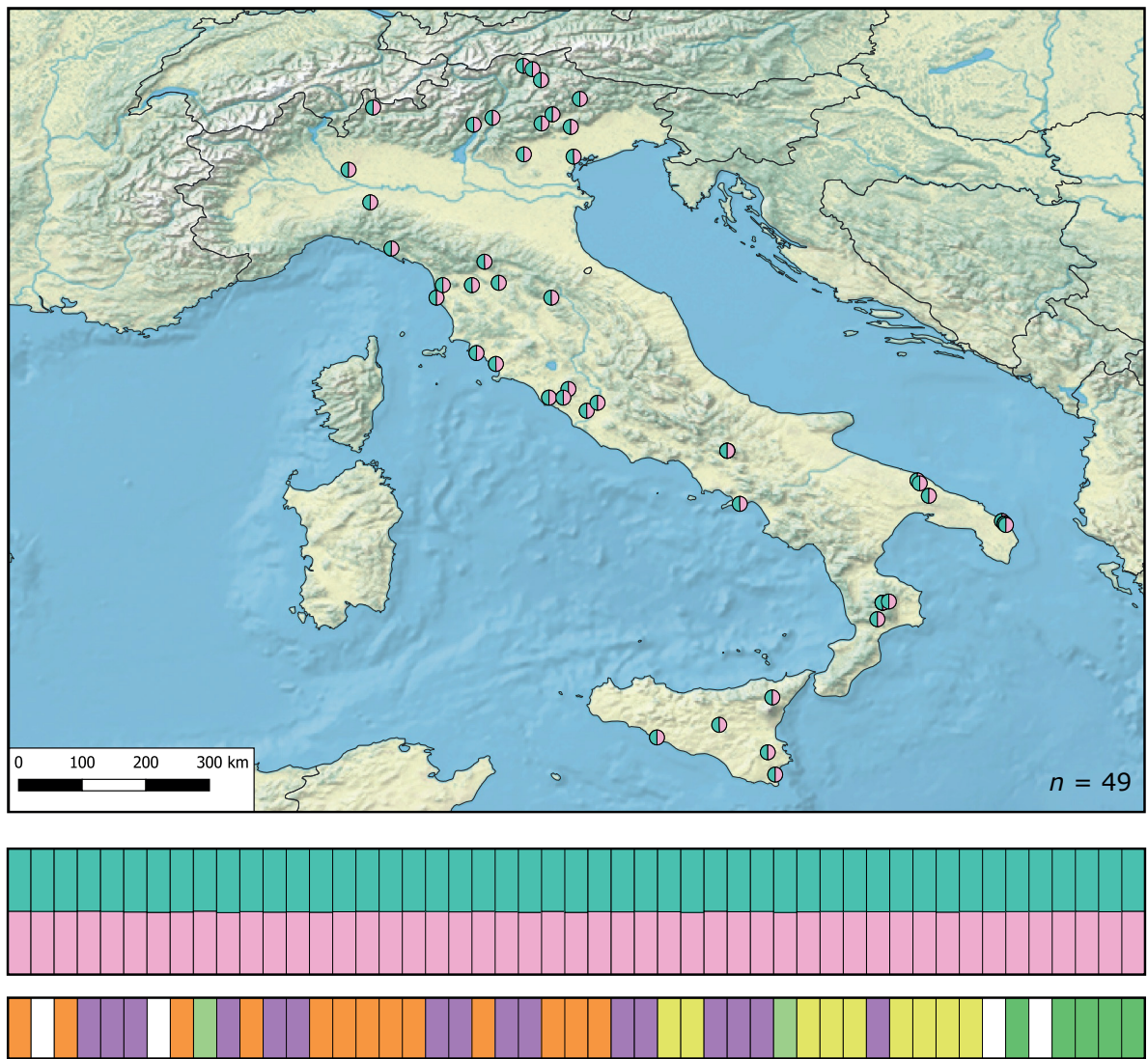


FIGURE S4 Fourth STRUCTURE analysis for *Natrix helvetica* samples from mainland Italy and Sicily excluding *N. h. helvetica* and samples showing genetic impact from the *N. h. helvetica* cluster. Symbol colours indicate cluster membership; admixed ancestries are depicted as pie charts reflecting Q values. In the bar plots below the map, each sample is represented by two bars, indicating its inferred cluster membership (upper bar) and its mitochondrial lineage (lower bar). Mitochondrial lineages colour-coded as in Figures 1-3; white bars indicate missing mtDNA data. Samples are arranged from northwest to southeast

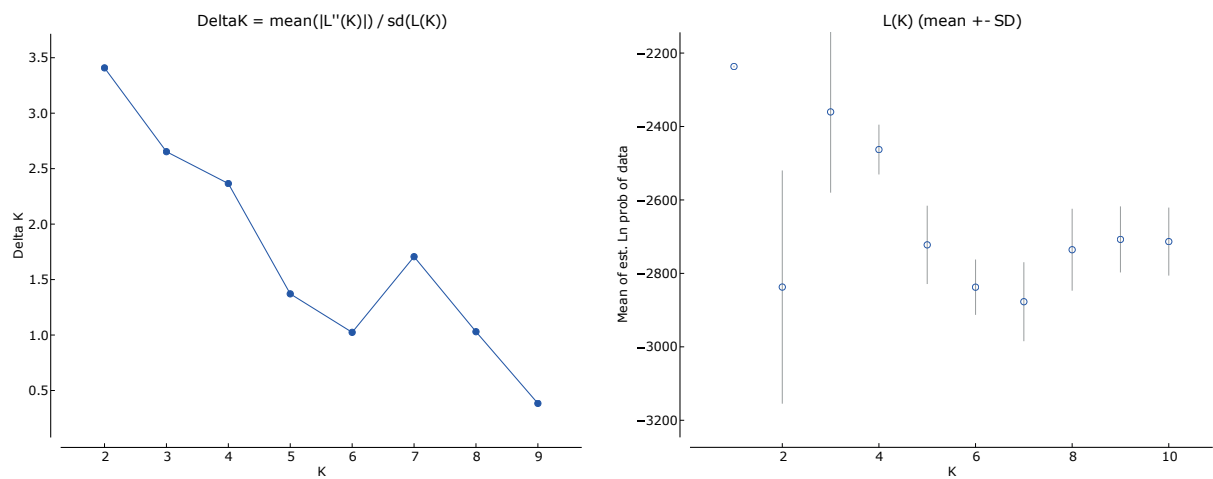


FIGURE S5 ΔK values (left) and posterior probabilities for K s (right) for the fourth STRUCTURE analysis visualized by STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Data set of 49 *Natrix helvetica* from mainland Italy and Sicily without *N. h. helvetica* and samples showing genetic impact from the *N. h. helvetica* cluster

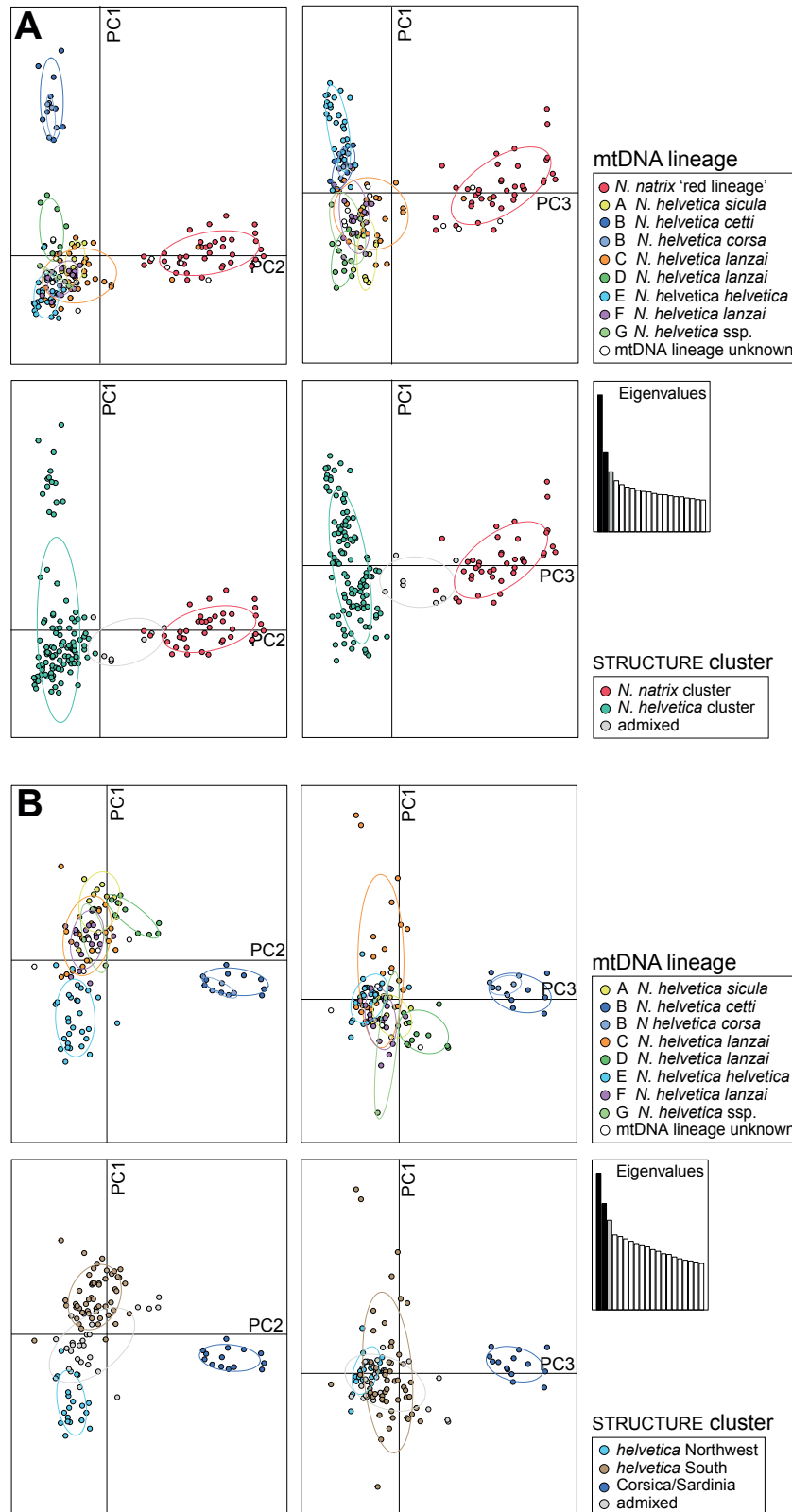


FIGURE S6 (A, B) Principal Component Analyses for microsatellite data of (A) all 160 samples (*Natrix helvetica*, *N. natrix*) and (B) only pure *N. helvetica* inclusive of Corso-Sardinian grass snakes ($n = 112$; same data sets as used for STRUCTURE analyses). Samples are coloured according to their mitochondrial lineage or STRUCTURE cluster membership. The thresholds for admixed samples were the same as used for STRUCTURE and derived from HYBRIDLAB simulations. Oval outlines depict 95% confidence intervals. For (A), PC1 explains 5.89% of the variance, PC2: 3.44%, and PC3: 2.58%; for (B) the values are PC1: 4.95%, PC2: 3.86%, and PC3: 3.26%

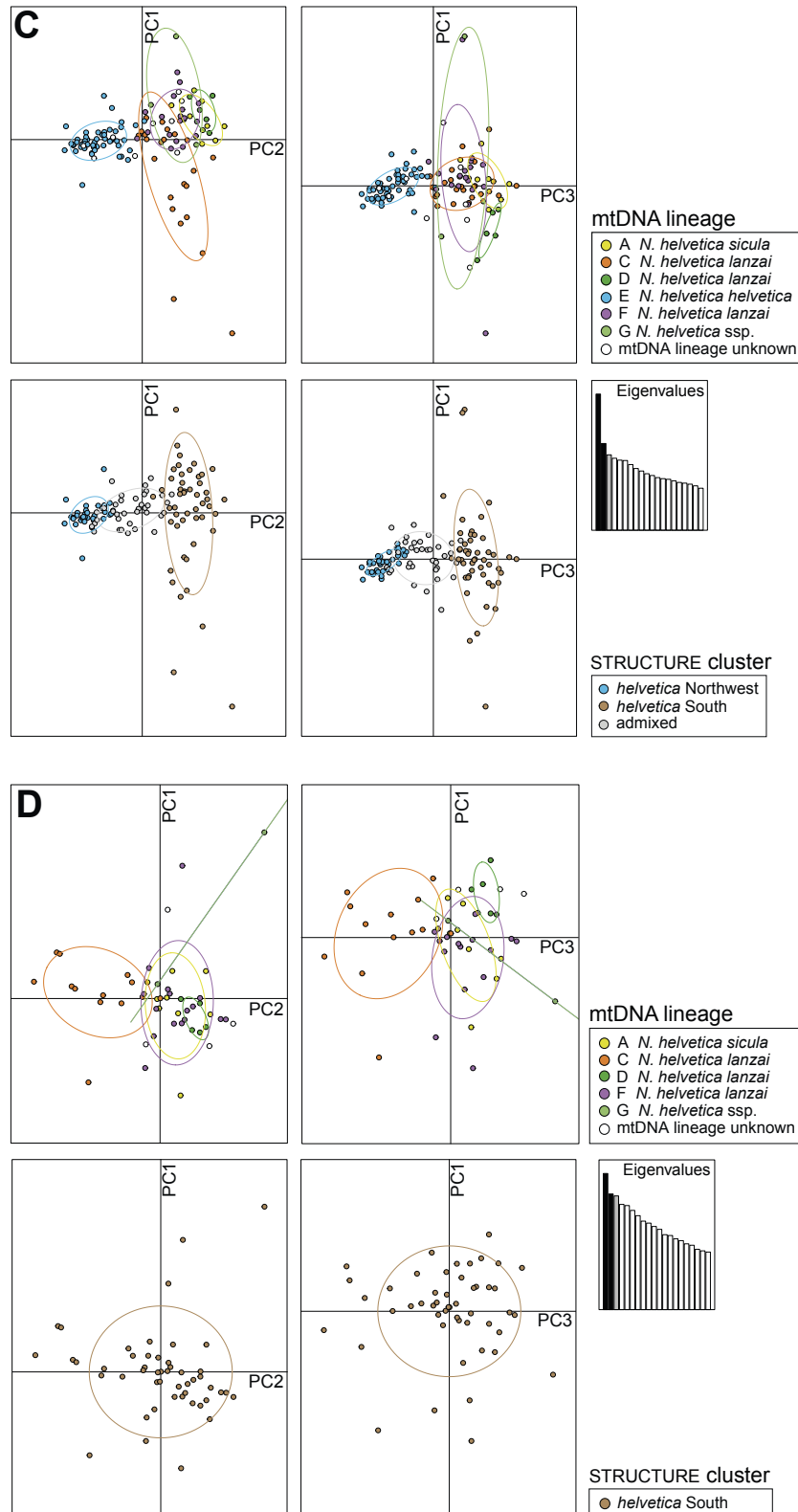


FIGURE S6 (C, D) Principal Component Analyses for microsatellite data of (C) pure *Natrix helvetica* exclusive of Corso-Sardinian grass snakes ($n = 120$) and (D) pure *N. helvetica* exclusive of the nominotypical subspecies ($n = 49$; same data sets as used for STRUCTURE analyses). Samples are coloured according to their mitochondrial lineage or STRUCTURE cluster membership. The thresholds for admixed samples were the same as used for STRUCTURE and derived from HYBRIDLAB simulations. Oval outlines depict 95% confidence intervals. For (C), PC1 explains 5.16% of the variance, PC2: 3.29%, and PC3: 2.86%; for (D) the values are PC1: 5.41%, PC2: 4.59%, and PC3: 4.52%

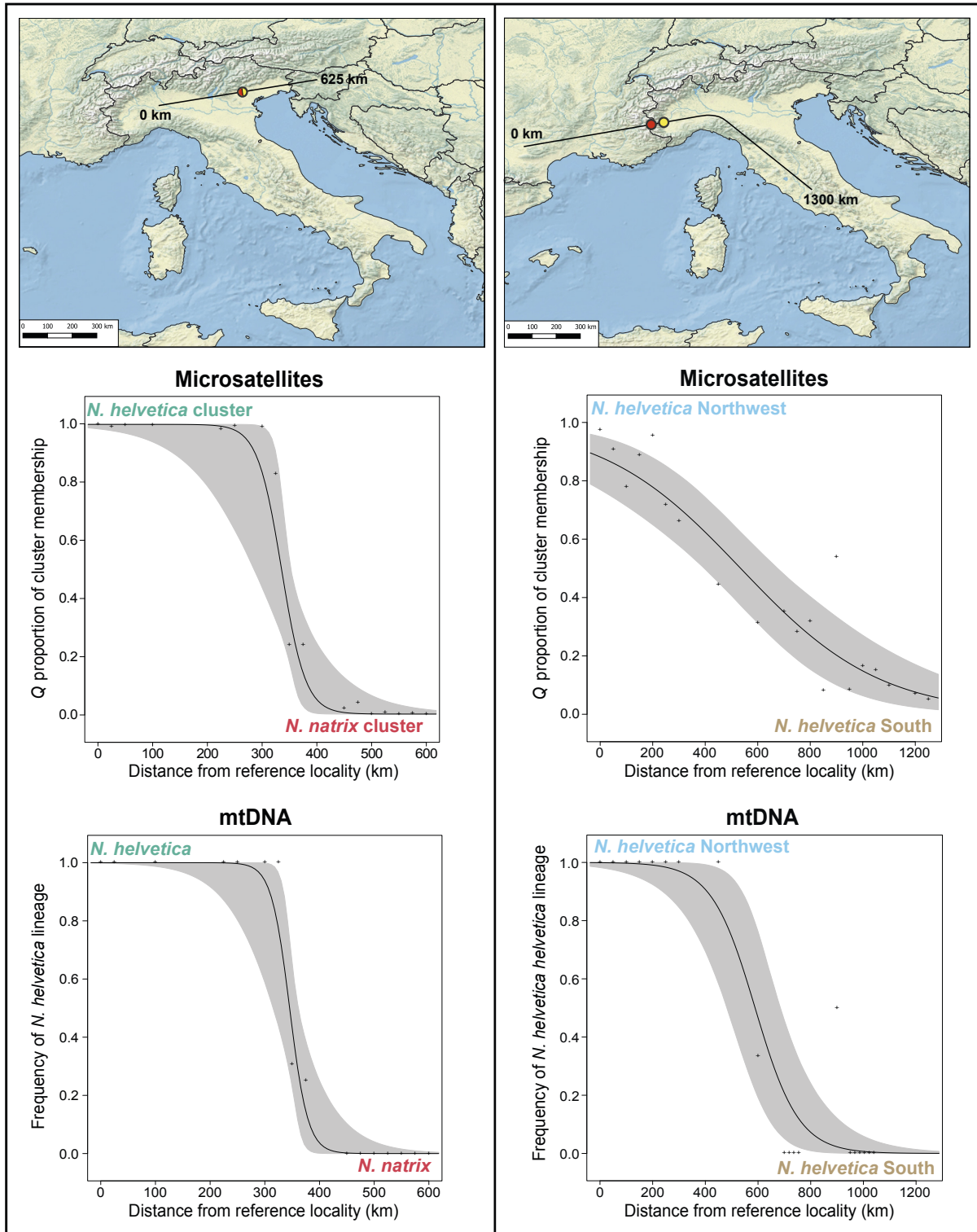


FIGURE S7 Cline analyses. Transects across the two contact zones (top), Maximum Likelihood clines for microsatellite data (centre), and mtDNA (bottom); fuzzy cline region (95% credibility) in grey. Cline centres depicted as points: red = microsatellites, yellow = mtDNA

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