It takes two to tango – Phylogeography, taxonomy and hybridization in grass snakes and dice snakes (Serpentes: Natricidae: *Natrix natrix*, *N. tessellata*)

Marika Asztalos, Dinçer Ayaz, Yusuf Bayrakcı, Murat Afsar, Cemal Varol Tok, Carolin Kindler, Daniel Jablonski, Uwe Fritz

Vertebrate Zoology

Supplementary Information

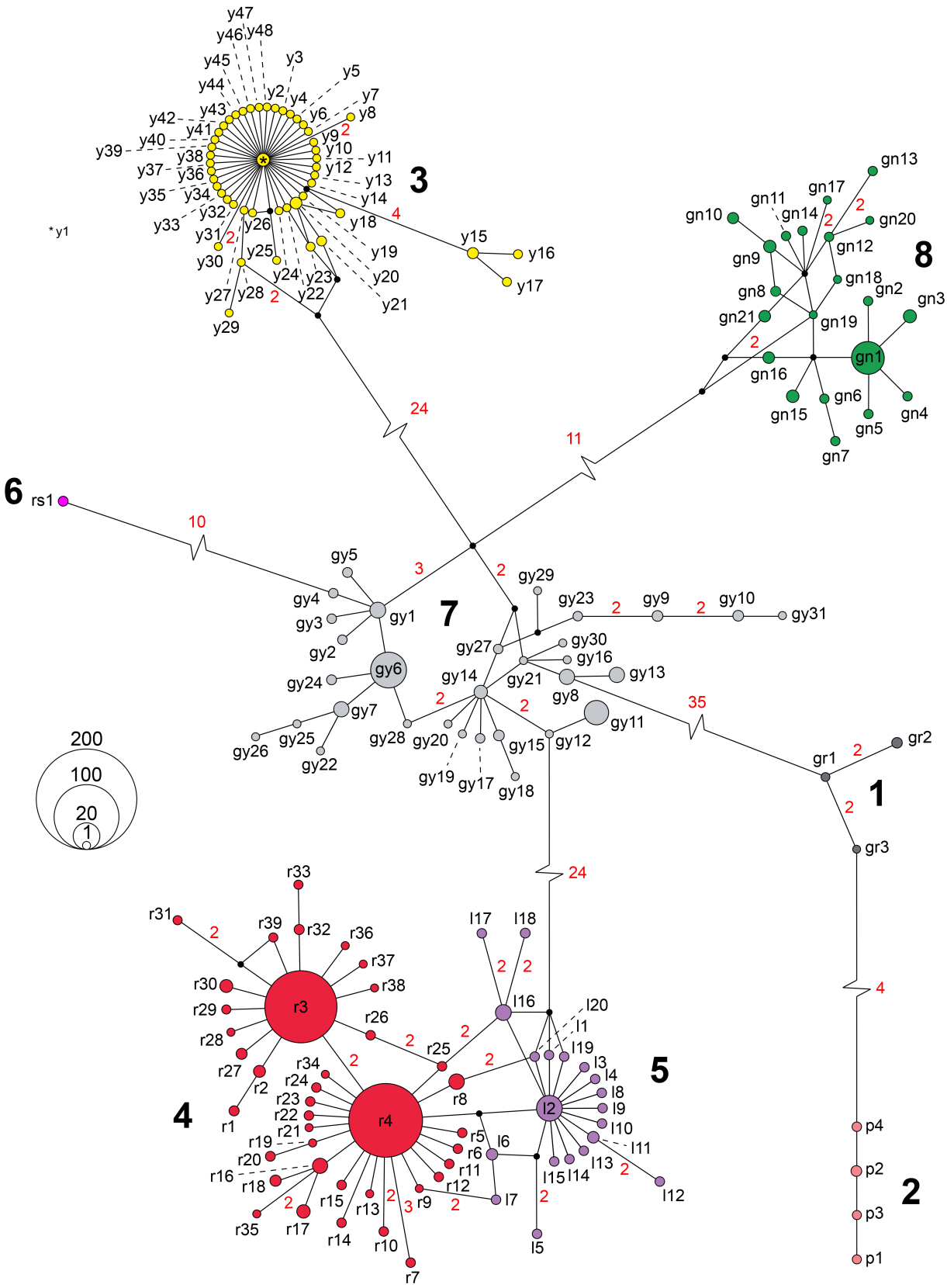

Figure S1. Parsimony network for 722 ND4+tRNAs sequences of *Natrix natrix*. Symbol sizes reflect haplotype frequencies. Small black circles are missing node haplotypes; each line connecting two haplotypes corresponds to one mutation step, if not otherwise indicated by red numbers along lines. Haplotype colors correspond to lineages.

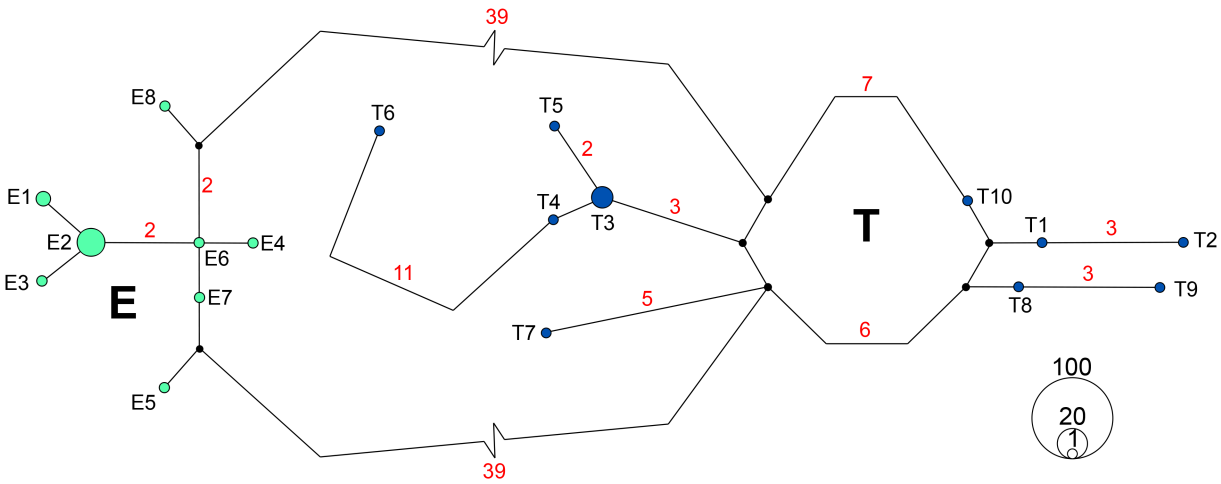

Figure S2. Parsimony network for 38 ND4+tRNAs sequences of *Natrix tessellata*. Symbol sizes reflect haplotype frequencies. Small black circles are missing node haplotypes; each line connecting two haplotypes corresponds to one mutation step, if not otherwise indicated by red numbers along lines. Haplotype colors correspond to lineages.

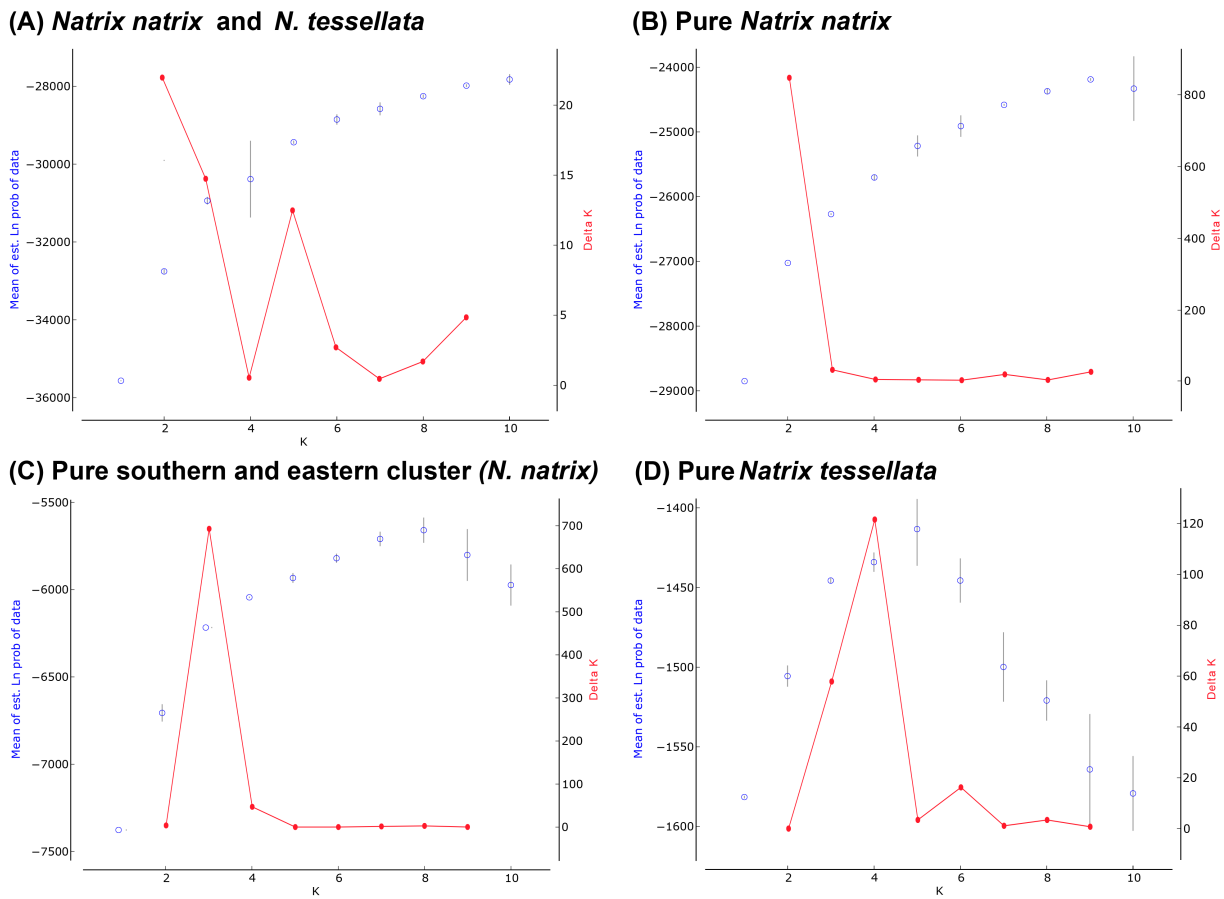

Figure S3. Δ*K* values and posterior probabilities for STRUCTURE runs for the four datasets derived from STRUCTURE HARVESTER (Earl and vonHoldt 2012). *K* = 1 could be excluded for all runs because of higher posterior probabilities for higher *K*s.

Table S1. Dice snake and grass snake samples used in the present study. → Excel spreadsheet

Table S2. Primers used for amplification and sequencing of mitochondrial genes (cyt *b,* ND4+tRNAs), amplicon lengths, and thermocycling conditions. Primers with asterisks were also used for sequencing.

mtDNA fragment	Primer	Direction	Primer sequence (5' to 3')	Reference
cyt *b*	L14724NAT*	Forward	GACCTGCGGTCCGAAAAACCA	Guicking et al. (2006)
cyt *b*	Thrsnr2*	Reverse	CTTTGGTTTACAAGAACAATGCTTTA	Guicking et al. (2006)
cyt *b*	Natrix_Cyt_b_For3	Forward	CTAGGAAAYACCCTCACAACC	Kindler et al. (2013)
cyt *b*	Natrix_Cyt_b_Rev3	Reverse	TTAATGTGTTGYGGGGTTACTA	Kindler et al. (2013)
ND4+tRNAs	ND4ab*	Forward	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Guicking et al. (2006)
ND4+tRNAs	tRNA-Leu*	Reverse	CATTACTTTTACTTGGATTTGCACCA	Guicking et al. (2006)
ND4+tRNAs	Natrix_ND4_For4	Forward	TCATCAGCACTCTTYTGCCTAGC	Schultze et al. (2020)
ND4+tRNAs	Natrix_ND4_Rev4	Reverse	AAATTAATGCTGGGRGGRGT	Schultze et al. (2020)

		Thermocycling conditions					
Primer combination	**Amplicon length (without primers)**	**Initial Denaturation**	**Cycles**	**Denaturation**	**Annealing**	**Extension**	**Final Extension**
L1472NAT + Thrsnr2	1155 bp	94°C, 5 min	40	94°C, 45 s	53°C, 45 s	72°C, 60 s	72°C, 10 min
L1472NAT + Natrix_Cyt_b_Rev3	782 bp	94°C, 5 min	40	94°C, 45 s	53°C, 45 s	72°C, 60 s	72°C, 10 min
Natrix_Cyt_b_For3 + Thrsnr2	671 bp	94°C, 5 min	40	94°C, 45 s	53°C, 45 s	72°C, 60 s	72°C, 10 min
ND4ab + tRNA-Leu	866 bp	94°C, 5 min	40	94°C, 45 s	55°C, 45 s	72°C, 60 s	72°C, 10 min
ND4ab + Natrix_ND4_Rev4	436 bp	94°C, 5 min	40	94°C, 45 s	55°C, 45 s	72°C, 60 s	72°C, 10 min
Natrix_ND4_For4 + tRNA-Leu	528 bp	94°C, 5 min	40	94°C, 45 s	55°C, 45 s	72°C, 60 s	72°C, 10 min

Table S3. Used microsatellite loci and multiplex sets. Forward primers are fluorescent-labeled. Allele size range and number of alleles correspond to whole dataset used for the first STRUCTURE run.

Locus	Primer sequences (5' to 3')	Repeat motif	Allele size range [bp]	Number of alleles	Multiplex set	Fluorescent label	Annealing temperature [°C]	Original reference
Natnat09	for – TGTAAATAACACTGTACCATTTTGG rev – TGACTGGGCAACAGAAAAGC	$(AC)_{22}$	80 – 130	21	1	FAM	55	Meister et al. (2009)
Natnat05	for – TCTGCACTGGGGATAGGAAG rev – GTCCCTTTTTCAGTGCTGTTG	$(GT)_{16}$	134 – 194	30	1	HEX	55	Meister et al. (2009)
µNt8new	for – GTATCGTCCTTCCAGACAAG rev – GCAAATCAAATAAATCTCACTGG	$(AC)_{15}$	73 – 105	17	1	Atto565	55	Meister et al. (2009)
Nsµ3	for – CTGACTCACTTCTGACCCTAAT rev – AATATTTGCTTGGCTCAAAC	$(ATCT)_{14}ATC$ $(CA)_{20}$	137 – 457	46	1	Atto550	55	Prosser et al. (1999)
µNt3	for – GGCAGGCTATTGGAGAAATG rev – GGCAAAACTCCAGGTGCTAC	$(AC)_{16}$	121 – 171	25	2	FAM	60	Gautschi et al. (2000)
µNt7	for – TTTGAAAGGAGAATGAATCGTG rev – CGCGAGGAATCAGAATGAAC	$(AC)_{17}$	164 – 212	25	2	HEX	60	Gautschi et al. (2000)
Hb30	for – CCCACTGGCTCATTTCAAGT rev – CCACATTTGCATCGGAGTG	$(CA)_{14}$	229 – 265	19	2	Atto565	60	Burns and Houlden (1999)
Natnat11	for – GGCTGTTTTCCCAGTGAAGC rev – GGTCTGGGGAAAAAGAAAGG	$(GA)_{13}$	104 – 228	25	3	FAM	55	Meister et al. (2009)
Natnat06	for – AATGGCATTCTCTCCAGCTC rev – ACCCATATCCGTATCCATATCC	$(GT)_{21}$	145 – 185	19	3	HEX	55	Meister et al. (2009)
TbuA09	for – CATCTCAACCAAAGTCGCTTC rev – GGATGTTGTGGGGTGTTTTC	$(AC)_{7}$	104 – 130	14	3	Atto565	55	Sloss et al. (2012)
3TS	for – GGTCACTTAAATACAACGAAATTGGTTAGCT rev – CGGACAGCTCTGGCTCCCTTG	$(GATA)_{19}$	178 – 298	27	3	Atto550	55	Garner et al. (2002)
Eobµ1	for – ATCAGTAGGAGTGAGAGCAACT rev – CTGCATACTCTTCCAGAACC	$(TG)_{21}$	120 – 142	10	-	FAM	51	Blouin-Demers and Gibbs (2003)
Eobµ13	for – TGATCTGAGTCTCTTTCTGG rev – CAATTCAAATCCATTGGTTT	$(AC)_{20}$	128 – 162	18	-	FAM	51	Blouin-Demers and Gibbs (2003)

Table S4. Simulated data for inferring hybrid status and pure ancestry using HYBRIDLAB (Nielsen et al. 2006) for all samples. Twenty-five samples each of *Natrix natrix* and *N. tessellata* were chosen as pure parental genotypes. Using this data set, 25 genotypes of each hybrid class (F_1, F_2, two backcrosses) were modeled in HYBRIDLAB and analyzed with STRUCTURE. Individuals with *Q* values ≥ 93% were reliably identified as pure *N. natrix*, individuals with *Q* values ≥ 94% as pure *N. tessellata*.

	Natrix natrix	***Natrix tessellata***	**F_1 (referred to *natrix* cluster)**	**F_1 (referred to *tessellata* cluster)**	**F_2 (referred to *natrix* cluster)**	**F_2 (referred to *tessellata* cluster)**	**Backcross *natrix* × F_1**	**Backcross *tessellata* × F_1**
Average *Q* score	0.970	0.974	0.503	0.497	0.509	0.491	0.799	0.782
SD	0.012	0.009	0.043	0.043	0.130	0.130	0.095	0.091
Minimum *Q*	0.932	0.935	0.382	0.428	0.309	0.217	0.574	0.555
Maximum *Q*	0.979	0.981	0.572	0.618	0.783	0.691	0.957	0.914
Misassignment	if $Q \geq 93\%$: 0%	if $Q \geq 94\%$: 0%	if $Q \geq 93\%$: 0%	if $Q \geq 94\%$: 0%	if $Q \geq 93\%$: 0%	if $Q \geq 94\%$: 0%	if $Q \geq 93\%$: 12%	if $Q \geq 94\%$: 0%

Table S5. Simulated data for inferring hybrid status and pure ancestry using HYBRIDLAB (Nielsen et al. 2006) for *Natrix natrix* only. Twenty-five samples each of the southern and eastern cluster and of the western cluster of *N. natrix* were chosen as pure parental genotypes. Using this data set, 25 genotypes of each hybrid class (F_1, F_2, two backcrosses) were modeled in HYBRIDLAB and analyzed with STRUCTURE. Individuals with *Q* values ≥ 92% were reliably identified as pure representatives of the southern and eastern cluster, individuals with *Q* values ≥ 87% as pure representatives of the western cluster.

	Southern and eastern cluster	**Western cluster**	**F_1 (referred to southern and eastern cluster)**	**F_1 (referred to western cluster)**	**F_2 (referred to southern and eastern cluster)**	**F_2 (referred to western cluster)**	**Backcross southern and eastern cluster × F_1**	**Backcross western cluster × F_1**
Average *Q* score	0.953	0.939	0.565	0.435	0.521	0.479	0.818	0.764
SD	0.010	0.027	0.113	0.113	0.152	0.152	0.113	0.109
Minimum *Q*	0.923	0.858	0.385	0.246	0.129	0.178	0.541	0.518
Maximum *Q*	0.970	0.968	0.754	0.615	0.822	0.871	0.945	0.934
Misassignment	if $Q \geq 92\%$: 0%	if $Q \geq 87\%$: 0%	if $Q \geq 92\%$: 0%	if $Q \geq 87\%$: 0%	if $Q \geq 92\%$: 0%	if $Q \geq 87\%$: 0%	if $Q \geq 92\%$: 8%	if $Q \geq 87\%$: 8%

Table S6. European Nucleotide Archive (ENA) accession numbers for all haplotypes.

cyt *b*			ND4+tRNAs		
Haplotype	**Accession number**	**Reference**	**Haplotype**	**Accession number**	**Reference**
***Natrix natrix* haplotypes**					
Lineage 1 of Kindler et al. (2013)					
gr1	LT900468	Kindler et al. (2018)	**gr1**	LT900416	Kindler et al. (2018)
gr2	LT900469	Kindler et al. (2018)	**gr2**	LT900417	Kindler et al. (2018)
gr3	LT900470	Kindler et al. (2018)	**gr3**	LT900418	Kindler et al. (2018)
gr4	LT900471	Kindler et al. (2018)			
gr5	LT900472	Kindler et al. (2018)			
gr6	LT900473	Kindler et al. (2018)			
Lineage 2 of Kindler et al. (2013)					
p1	LT900474	Kindler et al. (2018)	**p1**	LT900419	Kindler et al. (2018)
p2	LT900475	Kindler et al. (2018)	**p2**	LT900420	Kindler et al. (2018)
p3	LT900476	Kindler et al. (2018)	**p3**	LT900421	Kindler et al. (2018)
p4	LT900477	Kindler et al. (2018)	**p4**	LT900422	Kindler et al. (2018)
p5	LT900478	Kindler et al. (2018)			
Lineage 3 of Kindler et al. (2013)					
y1	LT839258	Kindler et al. (2017)	**y1**	LT839104	Kindler et al. (2017)
y2	LT839259	Kindler et al. (2017)	**y2**	LT839105	Kindler et al. (2017)
y3	LT839260	Kindler et al. (2017)	**y3**	LT839106	Kindler et al. (2017)
y4	LT839261	Kindler et al. (2017)	**y4**	LT839107	Kindler et al. (2017)
y5	LT839262	Kindler et al. (2017)	**y5**	LT839108	Kindler et al. (2017)
y6	LT839263	Kindler et al. (2017)	**y6**	LT839109	Kindler et al. (2017)
y7	LT839264	Kindler et al. (2017)	**y7**	LT839110	Kindler et al. (2017)
y8	LT839265	Kindler et al. (2017)	**y8**	LT839111	Kindler et al. (2017)
y9	LT839266	Kindler et al. (2017)	**y9**	LT839112	Kindler et al. (2017)
y10	LT839267	Kindler et al. (2017)	**y10**	LT839113	Kindler et al. (2017)
y11	LT839268	Kindler et al. (2017)	**y11**	LT839114	Kindler et al. (2017)
y12	LT839269	Kindler et al. (2017)	**y12**	LT839115	Kindler et al. (2017)
y13	LT839270	Kindler et al. (2017)	**y13**	LT839116	Kindler et al. (2017)
y14	LT839271	Kindler et al. (2017)	**y14**	LT839117	Kindler et al. (2017)
y15	LT839272	Kindler et al. (2017)	**y15**	LT839118	Kindler et al. (2017)
y16	LT839273	Kindler et al. (2017)	**y16**	LT839119	Kindler et al. (2017)
y17	LT839274	Kindler et al. (2017)	**y17**	LT839120	Kindler et al. (2017)
y18	LT839275	Kindler et al. (2017)	**y18**	LT839121	Kindler et al. (2017)
y19	LT839276	Kindler et al. (2017)	**y19**	LT839122	Kindler et al. (2017)
y20	LT839277	Kindler et al. (2017)	**y20**	LT839123	Kindler et al. (2017)
y21	LT839278	Kindler et al. (2017)	**y21**	LT839124	Kindler et al. (2017)
y22	LT839279	Kindler et al. (2017)	**y22**	LT839125	Kindler et al. (2017)
y23	LT839280	Kindler et al. (2017)	**y23**	LT839126	Kindler et al. (2017)
y24	LT839281	Kindler et al. (2017)	**y24**	LT839127	Kindler et al. (2017)
y25	LT839282	Kindler et al. (2017)	**y25**	LT839128	Kindler et al. (2017)
y26	LT839283	Kindler et al. (2017)	**y26**	LT839129	Kindler et al. (2017)
y27	LT839284	Kindler et al. (2017)	**y27**	LT839130	Kindler et al. (2017)
y28	LT839285	Kindler et al. (2017)	**y28**	LT839131	Kindler et al. (2017)

■Table S6 continued

cyt *b*			ND4+tRNAs		
Haplotype	**Accession number**	**Reference**	**Haplotype**	**Accession number**	**Reference**
Lineage 3 of Kindler et al. (2013)					
y29	LT839286	Kindler et al. (2017)	**y29**	LT839132	Kindler et al. (2017)
y30	LT839287	Kindler et al. (2017)	**y30**	LT839133	Kindler et al. (2017)
y31	LT839288	Kindler et al. (2017)	**y31**	LT839134	Kindler et al. (2017)
y32	LT839289	Kindler et al. (2017)	**y32**	LT839135	Kindler et al. (2017)
y33	LT839290	Kindler et al. (2017)	**y33**	LT839136	Kindler et al. (2017)
y34	LT839291	Kindler et al. (2017)	**y34**	LT839137	Kindler et al. (2017)
y35	LT839292	Kindler et al. (2017)	**y35**	LT839138	Kindler et al. (2017)
y36	LT839293	Kindler et al. (2017)	**y36**	LT839139	Kindler et al. (2017)
y37	LT839294	Kindler et al. (2017)	**y37**	LT839140	Kindler et al. (2017)
y38	LT839295	Kindler et al. (2017)	**y38**	LT839141	Kindler et al. (2017)
y39	LT839296	Kindler et al. (2017)	**y39**	LT839142	Kindler et al. (2017)
y40	LT839297	Kindler et al. (2017)	**y40**	LT839143	Kindler et al. (2017)
y41	LT839298	Kindler et al. (2017)	**y41**	LT839144	Kindler et al. (2017)
y42	LT839299	Kindler et al. (2017)	**y42**	LT839145	Kindler et al. (2017)
y43	LR694442	Schultze et al. (2019)	**y43**	LT839146	Kindler et al. (2017)
y44	LR991918	Asztalos et al. (2021a)	**y44**	LR694443	Schultze et al. (2019)
y45	LR991919	Asztalos et al. (2021a)	**y45**	LR694444	Schultze et al. (2019)
y46	LR991920	Asztalos et al. (2021a)	**y46**	LR694445	Schultze et al. (2019)
y47	LR991921	Asztalos et al. (2021a)	**y47**	LR991928	Asztalos et al. (2021a)
y48	LR991922	Asztalos et al. (2021a)	**y48**	LR991929	Asztalos et al. (2021a)
y49	LR991923	Asztalos et al. (2021a)			
Lineage 4 of Kindler et al. (2013)					
r1	LT839300	Kindler et al. (2017)	**r1**	LT839147	Kindler et al. (2017)
r2	LT839301	Kindler et al. (2017)	**r2**	LT839148	Kindler et al. (2017)
r3	LT839302	Kindler et al. (2017)	**r3**	LT839149	Kindler et al. (2017)
r4	LT839303	Kindler et al. (2017)	**r4**	LT839150	Kindler et al. (2017)
r5	LT839304	Kindler et al. (2017)	**r5**	LT839151	Kindler et al. (2017)
r6	LT839305	Kindler et al. (2017)	**r6**	LT839152	Kindler et al. (2017)
r7	LT839306	Kindler et al. (2017)	**r7**	LT839153	Kindler et al. (2017)
r8	LT839307	Kindler et al. (2017)	**r8**	LT839154	Kindler et al. (2017)
r9	LT839308	Kindler et al. (2017)	**r9**	LT839155	Kindler et al. (2017)
r10	LT839309	Kindler et al. (2017)	**r10**	LT839156	Kindler et al. (2017)
r11	LT839310	Kindler et al. (2017)	**r11**	LT839157	Kindler et al. (2017)
r12	LT839311	Kindler et al. (2017)	**r12**	LT839158	Kindler et al. (2017)
r13	LT839312	Kindler et al. (2017)	**r13**	LT839159	Kindler et al. (2017)
r14	LT839313	Kindler et al. (2017)	**r14**	LT839160	Kindler et al. (2017)
r15	LT839314	Kindler et al. (2017)	**r15**	LT839161	Kindler et al. (2017)
r16	LT839315	Kindler et al. (2017)	**r16**	LT839162	Kindler et al. (2017)
r17	LT839316	Kindler et al. (2017)	**r17**	LT839163	Kindler et al. (2017)
r18	LT839317	Kindler et al. (2017)	**r18**	LT839164	Kindler et al. (2017)
r19	LT839318	Kindler et al. (2017)	**r19**	LT839165	Kindler et al. (2017)
r20	LT839319	Kindler et al. (2017)	**r20**	LT839166	Kindler et al. (2017)
r21	LT839320	Kindler et al. (2017)	**r21**	LT839167	Kindler et al. (2017)

■Table S6 continued

cyt *b*			ND4+tRNAs		
Haplotype	**Accession number**	**Reference**	**Haplotype**	**Accession number**	**Reference**
Lineage 4 of Kindler et al. (2013)					
r22	LT839321	Kindler et al. (2017)	**r22**	LT839168	Kindler et al. (2017)
r23	LT839322	Kindler et al. (2017)	**r23**	LT839169	Kindler et al. (2017)
r24	LT839323	Kindler et al. (2017)	**r24**	LT839170	Kindler et al. (2017)
r25	LT839324	Kindler et al. (2017)	**r25**	LT839171	Kindler et al. (2017)
r26	LT839325	Kindler et al. (2017)	**r26**	LT839172	Kindler et al. (2017)
r27	LT839326	Kindler et al. (2017)	**r27**	LT839173	Kindler et al. (2017)
r28	LT839327	Kindler et al. (2017)	**r28**	LT839174	Kindler et al. (2017)
r29	LT839328	Kindler et al. (2017)	**r29**	LT839175	Kindler et al. (2017)
r30	LT839329	Kindler et al. (2017)	**r30**	LT839176	Kindler et al. (2017)
r31	LT839330	Kindler et al. (2017)	**r31**	LT839177	Kindler et al. (2017)
r32	LT839331	Kindler et al. (2017)	**r32**	LT839178	Kindler et al. (2017)
r33	LT839332	Kindler et al. (2017)	**r33**	LT839179	Kindler et al. (2017)
r34	LT839333	Kindler et al. (2017)	**r34**	LR721660	Schultze et al. (2020)
r35	LT839334	Kindler et al. (2017)	**r35**	LR721661	Schultze et al. (2020)
r36	LT839335	Kindler et al. (2017)	**r36**	LR991926	Asztalos et al. (2021a)
r37	LT839336	Kindler et al. (2017)	**r37**	LR991927	Asztalos et al. (2021a)
r38	LT839337	Kindler et al. (2017)	**r38**	LR983952	Ahnelt et al. (2021)
r39	LT839338	Kindler et al. (2017)	**r39**	OU862605	This study
r40	LT839339	Kindler et al. (2017)			
r41	LT839340	Kindler et al. (2017)			
r42	LT839341	Kindler et al. (2017)			
r43	LT839342	Kindler et al. (2017)			
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)			

■Table S6 continued

cyt *b*			ND4+tRNAs		
Haplotype	**Accession number**	**Reference**	**Haplotype**	**Accession number**	**Reference**
Lineage 4 of Kindler et al. (2013)					
r65	LT839364	Kindler et al. (2017)			
r66	LT839365	Kindler et al. (2017)			
r67	LR991914	Asztalos et al. (2021a)			
r68	LR991915	Asztalos et al. (2021a)			
r69	LR991916	Asztalos et al. (2021a)			
r70	LR991917	Asztalos et al. (2021a)			
r71	AY487731	Guicking et al. (unpubl.)			
Lineage 5 of Kindler et al. (2013)					
l1	LT839366	Kindler et al. (2017)	**l1**	LT839180	Kindler et al. (2017)
l2	LT839367	Kindler et al. (2017)	**l2**	LT839181	Kindler et al. (2017)
l3	LT839368	Kindler et al. (2017)	**l3**	LT839182	Kindler et al. (2017)
l4	LT839369	Kindler et al. (2017)	**l4**	LT839183	Kindler et al. (2017)
l5	LT839370	Kindler et al. (2017)	**l5**	LT839184	Kindler et al. (2017)
l6	LT839371	Kindler et al. (2017)	**l6**	LT839185	Kindler et al. (2017)
l7	LT839372	Kindler et al. (2017)	**l7**	LT839186	Kindler et al. (2017)
l8	LT839373	Kindler et al. (2017)	**l8**	LT839187	Kindler et al. (2017)
l9	LT839374	Kindler et al. (2017)	**l9**	LT839188	Kindler et al. (2017)
l10	LT839375	Kindler et al. (2017)	**l10**	LT839189	Kindler et al. (2017)
l11	LT839376	Kindler et al. (2017)	**l11**	LT839190	Kindler et al. (2017)
l12	LT839377	Kindler et al. (2017)	**l12**	LT839191	Kindler et al. (2017)
l13	LT839378	Kindler et al. (2017)	**l13**	LT839192	Kindler et al. (2017)
l14	LT839379	Kindler et al. (2017)	**l14**	LT839193	Kindler et al. (2017)
l15	LT839380	Kindler et al. (2017)	**l15**	LT839194	Kindler et al. (2017)
l16	LT839381	Kindler et al. (2017)	**l16**	LT839195	Kindler et al. (2017)
l17	LT839382	Kindler et al. (2017)	**l17**	LT839196	Kindler et al. (2017)
l18	LT839383	Kindler et al. (2017)	**l18**	LT839197	Kindler et al. (2017)
l19	LT839384	Kindler et al. (2017)	**l19**	LT839198	Kindler et al. (2017)
l20	LT839385	Kindler et al. (2017)	**l20**	LT839199	Kindler et al. (2017)
l21	LT839386	Kindler et al. (2017)			
l22	LT839387	Kindler et al. (2017)			
l23	OU862648	This study			
l24	AY487742	Guicking et al. (unpubl.)			
l25	AY487746	Guicking et al. (unpubl.)			
l26	AY487725	Guicking et al. (unpubl.)			
Lineage 6 of Kindler et al. (2013)					
rs1	LT900479	Kindler et al. (2018)	**rs1**	LT900423	Kindler et al. (2018)
rs2	LT900480	Kindler et al. (2018)			
Lineage 7 of Kindler et al. (2013)					
gy1	LT839388	Kindler et al. (2017)	**gy1**	LT839200	Kindler et al. (2017)
gy2	LT839389	Kindler et al. (2017)	**gy2**	LT839201	Kindler et al. (2017)
gy3	LT839390	Kindler et al. (2017)	**gy3**	LT839202	Kindler et al. (2017)
gy4	LT839391	Kindler et al. (2017)	**gy4**	LT839203	Kindler et al. (2017)
gy5	LT839392	Kindler et al. (2017)	**gy5**	LT839204	Kindler et al. (2017)

■Table S6 continued

cyt *b*			ND4+tRNAs		
Haplotype	**Accession number**	**Reference**	**Haplotype**	**Accession number**	**Reference**
Lineage 7 of Kindler et al. (2013)					
gy6	LT839393	Kindler et al. (2017)	**gy6**	LT839205	Kindler et al. (2017)
gy7	LT839394	Kindler et al. (2017)	**gy7**	LT839206	Kindler et al. (2017)
gy8	LT839395	Kindler et al. (2017)	**gy8**	LT839207	Kindler et al. (2017)
gy9	LT839396	Kindler et al. (2017)	**gy9**	LT839208	Kindler et al. (2017)
gy10	LT839397	Kindler et al. (2017)	**gy10**	LT839209	Kindler et al. (2017)
gy11	LT839398	Kindler et al. (2017)	**gy11**	LT839210	Kindler et al. (2017)
gy12	LT839399	Kindler et al. (2017)	**gy12**	LR963483	Asztalos et al. (2021b)
gy13	LT839400	Kindler et al. (2017)	**gy13**	OU862606	This study
gy14	LR963484	Asztalos et al. (2021b)	**gy14**	OU862607	This study
gy15	OU862649	This study	**gy15**	OU862608	This study
gy16	OU862650	This study	**gy16**	OU862609	This study
gy17	OU862651	This study	**gy17**	OU862610	This study
gy18	OU862652	This study	**gy18**	OU862611	This study
gy19	OU862653	This study	**gy19**	OU862612	This study
gy20	OU862654	This study	**gy20**	OU862613	This study
gy21	OU862655	This study	**gy21**	OU862614	This study
gy22	OU862656	This study	**gy22**	OU862615	This study
gy23	OU862657	This study	**gy23**	OU862616	This study
gy24	OU862658	This study	**gy24**	OU862617	This study
gy25	OU862659	This study	**gy25**	OU862618	This study
gy26	OU862660	This study	**gy26**	OU862619	This study
gy27	OU862661	This study	**gy27**	OU862620	This study
gy28	OU862662	This study	**gy28**	OU862621	This study
gy29	OU862663	This study	**gy29**	OU862622	This study
gy30	OU862664	This study	**gy30**	OU862623	This study
gy31	OU862665	This study	**gy31**	OU862624	This study
Lineage 8 of Kindler et al. (2013)					
gn1	LT839401	Kindler et al. (2017)	**gn1**	LT839211	Kindler et al. (2017)
gn2	LT839402	Kindler et al. (2017)	**gn2**	LT839212	Kindler et al. (2017)
gn3	LT839403	Kindler et al. (2017)	**gn3**	LT839213	Kindler et al. (2017)
gn4	LT839404	Kindler et al. (2017)	**gn4**	LT839214	Kindler et al. (2017)
gn5	LT839405	Kindler et al. (2017)	**gn5**	LT839215	Kindler et al. (2017)
gn6	LT839406	Kindler et al. (2017)	**gn6**	LT839216	Kindler et al. (2017)
gn7	LT839407	Kindler et al. (2017)	**gn7**	LT839217	Kindler et al. (2017)
gn8	LT839408	Kindler et al. (2017)	**gn8**	LT839218	Kindler et al. (2017)
gn9	LT839409	Kindler et al. (2017)	**gn9**	LT839219	Kindler et al. (2017)
gn10	LT839410	Kindler et al. (2017)	**gn10**	LT839220	Kindler et al. (2017)
gn11	LT839411	Kindler et al. (2017)	**gn11**	LT839221	Kindler et al. (2017)
gn12	LT839412	Kindler et al. (2017)	**gn12**	LT839222	Kindler et al. (2017)
gn13	LT839413	Kindler et al. (2017)	**gn13**	LT839223	Kindler et al. (2017)
gn14	LT839414	Kindler et al. (2017)	**gn14**	LT839224	Kindler et al. (2017)
gn15	LT839415	Kindler et al. (2017)	**gn15**	LT839225	Kindler et al. (2017)
gn16	LT839416	Kindler et al. (2017)	**gn16**	LT839226	Kindler et al. (2017)

■Table S6 continued

cyt *b*			ND4+tRNAs		
Haplotype	**Accession number**	**Reference**	**Haplotype**	**Accession number**	**Reference**
Lineage 8 of Kindler et al. (2013)					
gn17	LT839417	Kindler et al. (2017)	**gn17**	OU862625	This study
gn18	LT839418	Kindler et al. (2017)	**gn18**	OU862626	This study
gn19	LT839419	Kindler et al. (2017)	**gn19**	OU862627	This study
gn20	LT839420	Kindler et al. (2017)	**gn20**	OU862628	This study
gn21	LT839421	Kindler et al. (2017)	**gn21**	OU862629	This study
gn22	OU862666	This study			
gn23	OU862667	This study			
gn24	OU862668	This study			
gn25	OU862669	This study			
gn26	OU862670	This study			
gn27	OU862671	This study			
gn28	AF471059	Lawson et al. (2005)			
gn29	AY487723	Guicking et al. (unpubl.)			
gn30	AY487730	Guicking et al. (unpubl.)			
gn31	AY487735	Guicking et al. (unpubl.)			
gn32	AY487736	Guicking et al. (unpubl.)			
gn33	AY487740	Guicking et al. (unpubl.)			
gn34	AY487749	Guicking et al. (unpubl.)			
gn35	AY487750	Guicking et al. (unpubl.)			
gn36	AY487754	Guicking et al. (unpubl.)			
***Natrix tessellata* haplotypes**					
Lineage A of Guicking et al. (2009)					
A1	AY487620	Guicking et al. (2009)			
A2	AY487623	Guicking et al. (2009)			
A3	AY487624	Guicking et al. (2009)			
A4	AY487621	Guicking et al. (2009)			
A5	AY487622	Guicking et al. (2009)			
A6	AY866532	Guicking et al. (2006)			
A7	AY487626	Guicking et al. (2009)			
A8	AY487629	Guicking et al. (2009)			
A9	AY487627	Guicking et al. (2009)			
A10	AY487628	Guicking et al. (2009)			
A11	AY487625	Guicking et al. (2009)			
A12	EU119167	Guicking et al. (2009)			
Lineage C of Guicking et al. (2009)					
C1	AY487630	Guicking et al. (2009)			
C2	AY487631	Guicking et al. (2009)			
C3	JX315485	Kyriazi et al. (2013)			
C4	JX315486	Kyriazi et al. (2013)			
Lineage E of Guicking et al. (2009)					
E1	AY487632	Guicking et al. (2009)	**E1**	OU862630	This study
E2	AY487633	Guicking et al. (2009)	**E2**	OU862631	This study
E3	AY487637	Guicking et al. (2009)	**E3**	OU862632	This study

■Table S6 continued

cyt *b*			ND4+tRNAs		
Haplotype	**Accession number**	**Reference**	**Haplotype**	**Accession number**	**Reference**
Lineage E of Guicking et al. (2009)					
E4	AY487634	Guicking et al. (2009)	**E4**	OU862633	This study
E5	AY487635	Guicking et al. (2009)	**E5**	OU862634	This study
E6	AY487636	Guicking et al. (2009)	**E6**	OU862635	This study
E7	AY486533	Guicking et al. (2009)	**E7**	OU862636	This study
E8	AY486534	Guicking et al. (2009)	**E8**	OU862637	This study
E9	AY487638	Guicking et al. (2009)			
E10	AY487639	Guicking et al. (2009)			
E11	AY487640	Guicking et al. (2009)			
E12	AY487641	Guicking et al. (2009)			
E13	AY487642	Guicking et al. (2009)			
E14	AY487643	Guicking et al. (2009)			
E15	AY487644	Guicking et al. (2009)			
E16	AY487649	Guicking et al. (2009)			
E17	AY487651	Guicking et al. (2009)			
E18	AY487652	Guicking et al. (2009)			
E19	AY487646	Guicking et al. (2009)			
E20	AY487647	Guicking et al. (2009)			
E21	AY487650	Guicking et al. (2009)			
E22	AY487645	Guicking et al. (2009)			
E23	AY487648	Guicking et al. (2009)			
E24	AY487653	Guicking et al. (2009)			
E25	AY487655	Guicking et al. (2009)			
E26	AY487660	Guicking et al. (2009)			
E27	AY487661	Guicking et al. (2009)			
E28	AY487656	Guicking et al. (2009)			
E29	AY487668	Guicking et al. (2009)			
E30	AY487665	Guicking et al. (2009)			
E31	AY487662	Guicking et al. (2009)			
E32	AY487663	Guicking et al. (2009)			
E33	AY487664	Guicking et al. (2009)			
E34	AY487666	Guicking et al. (2009)			
E35	AY487667	Guicking et al. (2009)			
E36	AY487654	Guicking et al. (2009)			
E37	AY487657	Guicking et al. (2009)			
E38	AY487658	Guicking et al. (2009)			
E39	AY487659	Guicking et al. (2009)			
E40	AY487669	Guicking et al. (2009)			
E41	AY487670	Guicking et al. (2009)			
E42	AY487672	Guicking et al. (2009)			
E43	AY487671	Guicking et al. (2009)			
E44	AY487674	Guicking et al. (2009)			
E45	AY487673	Guicking et al. (2009)			
E46	AY487678	Guicking et al. (2009)			

■Table S6 continued

cyt *b*			ND4+tRNAs		
Haplotype	**Accession number**	**Reference**	**Haplotype**	**Accession number**	**Reference**
Lineage E of Guicking et al. (2009)					
E47	AY487679	Guicking et al. (2009)			
E48	AY487680	Guicking et al. (2009)			
E49	AY487675	Guicking et al. (2009)			
E50	AY487676	Guicking et al. (2009)			
E51	AY487677	Guicking et al. (2009)			
E52	OU862672	This study			
E53	OU862673	This study			
E54	OU862674	This study			
E55	OU862675	This study			
E56	OU862676	This study			
E57	OU862677	This study			
E58	OU862678	This study			
E59	OU862679	This study			
E60	OU862680	This study			
E61	OU862681	This study			
E62	JX315493	Kyriazi et al. (2013)			
E63	JX315491	Kyriazi et al. (2013)			
E64	JX315494	Kyriazi et al. (2013)			
E65	JX315495	Kyriazi et al. (2013)			
E66	JX315496	Kyriazi et al. (2013)			
Lineage G of Guicking et al. (2009)					
G1	AY487577	Guicking et al. (2009)			
G2	AY487578	Guicking et al. (2009)			
G3	AY487579	Guicking et al. (2009)			
G4	AY487580	Guicking et al. (2009)			
G5	AY487581	Guicking et al. (2009)			
G6	AY487582	Guicking et al. (2009)			
G7	AY487583	Guicking et al. (2009)			
G8	AY487587	Guicking et al. (2009)			
G9	AY487588	Guicking et al. (2009)			
G10	AY487584	Guicking et al. (2009)			
G11	AY487585	Guicking et al. (2006)			
G12	AY487586	Guicking et al. (2009)			
G13	JX315490	Kyriazi et al. (2013)			
G14	JX315489	Kyriazi et al. (2013)			
G15	JX315488	Kyriazi et al. (2013)			
Lineage I of Guicking et al. (2009)					
I1	AY487575	Guicking et al. (2009)			
I2	AY487574	Guicking et al. (2006)			
I3	AY487576	Guicking et al. (2009)			
Lineage J of Guicking et al. (2009)					
J1	AY487589	Guicking et al. (2009)			
J2	AY487591	Guicking et al. (2006)			

■Table S6 continued

cyt *b*			ND4+tRNAs		
Haplotype	**Accession number**	**Reference**	**Haplotype**	**Accession number**	**Reference**
Lineage J of Guicking et al. (2009)					
J3	AY487590	Guicking et al. (2006)			
Lineage K of Guicking et al. (2009)					
K1	AY487602	Guicking et al. (2009)			
K2	AY487607	Guicking et al. (2009)			
K3	AY487603	Guicking et al. (2009)			
K4	AY487604	Guicking et al. (2009)			
K5	AY487606	Guicking et al. (2009)			
K6	AY487605	Guicking et al. (2009)			
K7	AY487608	Guicking et al. (2009)			
K8	AY487609	Guicking et al. (2009)			
K9	AY487612	Guicking et al. (2009)			
K10	AY487613	Guicking et al. (2009)			
K11	AY487610	Guicking et al. (2009)			
K12	AY487611	Guicking et al. (2009)			
Lineage T of Guicking et al. (2009)					
T1	AY487592	Guicking et al. (2009)	**T1**	OU862638	This study
T2	AY487593	Guicking et al. (2009)	**T2**	OU862639	This study
T3	AY487594	Guicking et al. (2009)	**T3**	OU862640	This study
T4	AY487595	Guicking et al. (2009)	**T4**	OU862641	This study
T5	AY487596	Guicking et al. (2009)	**T5**	OU862642	This study
T6	AY487597	Guicking et al. (2009)	**T6**	OU862643	This study
T7	AY866531	Guicking et al. (2006)	**T7**	OU862644	This study
T8	AY487598	Guicking et al. (2009)	**T8**	OU862645	This study
T9	AY487599	Guicking et al. (2009)	**T9**	OU862646	This study
T10	AY487600	Guicking et al. (2009)	**T10**	OU862647	This study
T11	AY487601	Guicking et al. (2009)			
T12	EU119169	Guicking et al. (2009)			
T13	EU119171	Guicking et al. (2009)			
T14	EU119170	Guicking et al. (2009)			
T15	EU119168	Guicking et al. (2009)			
T16	OU862682	This study			
T17	OU862683	This study			
T18	OU862684	This study			
T19	OU862685	This study			
T20	OU862686	This study			
T21	OU862687	This study			
T22	OU862688	This study			
T23	OU862689	This study			
T24	OU862690	This study			
T25	JX315499	Kyriazi et al. (2013)			
T26	JX315498	Kyriazi et al. (2013)			
T27	JX315501	Kyriazi et al. (2013)			
T28	JX315502	Kyriazi et al. (2013)			

■Table S6 continued					
cyt *b*			ND4+tRNAs		
Haplotype	**Accession number**	**Reference**	**Haplotype**	**Accession number**	**Reference**
Lineage T of Guicking et al. (2009)					
T29	JX315504	Kyriazi et al. (2013)			
T30	JX315492	Kyriazi et al. (2013)			
T31	JX315500	Kyriazi et al. (2013)			
T32	JX315503	Kyriazi et al. (2013)			
Lineage U of Guicking et al. (2009)					
U1	AY487614	Guicking et al. (2009)			
U2	AY487616	Guicking et al. (2009)			
U3	AY487617	Guicking et al. (2009)			
U4	AY487618	Guicking et al. (2009)			
U5	AY487619	Guicking et al. (2009)			
U6	AY487615	Guicking et al. (2009)			

Table S7. Mean uncorrected *p* distances (percentages) between and within dice snakes and grass snakes calculated with MEGA 10.2.3 (Kumar et al. 2018) based on the number of haplotypes (n_h). Between-group value for ND4+tRNAs above the diagonal; for cyt *b* below the diagonal.

	Between-group values		**Within-group values**			
	N. natrix	***N. tessellata***	n_h	**cyt *b***	n_h	**ND4+tRNAs**
N. natrix	-	12.10	266	2.68	166	3.34
N. tessellata	11.50	-	153	3.65	19	5.21

Table S8. Mean uncorrected *p* distances (percentages) for the mitochondrial lineages in the study region, based on distinct haplotypes calculated with MEGA 10.2.3 (Kumar et al. 2018). Between-group values for ND4+tRNAs above the diagonal; for cyt *b* below the diagonal. The mtDNA fragment containing the partial ND4 gene plus tRNA genes (866 bp) had 288 variable sites and 191 parsimony-informative sites across all haplotypes; for the cyt *b* gene (1117 bp) the representative values were 403 and 352. Black-coded values correspond to interspecific divergences; orange and blue-coded values correspond to intraspecific divergences of *Natrix natrix* and *N. tessellata*, respectively.

	Between-group values																	Within-group values			
Lineage	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**A**	**C**	**E**	**G**	**I**	**J**	**K**	**T**	**U**	n_h	**cyt *b***	n_h	**ND4+tRNAs**
1	-	0.76	4.25	4.62	4.48	4.30	3.60	4.71	-	-	12.67	-	-	-	-	12.53	-	6	0.27	3	0.32
2	0.96	-	4.42	4.74	4.62	4.50	3.81	4.85	-	-	12.31	-	-	-	-	12.25	-	5	0.30	4	0.19
3	3.37	3.44	-	5.17	4.94	3.93	3.96	5.02	-	-	12.65	-	-	-	-	12.58	-	49	0.19	48	0.33
4	3.42	3.61	4.03	-	0.60	4.16	3.55	4.78	-	-	12.61	-	-	-	-	11.67	-	71	0.39	39	0.41
5	3.15	3.33	3.74	1.03	-	4.13	3.41	4.74	-	-	12.48	-	-	-	-	11.63	-	26	0.21	20	0.33
6	3.62	3.85	4.21	3.82	3.58	-	2.67	3.58	-	-	12.79	-	-	-	-	11.79	-	2	0.07	1	n/c
7	2.80	2.79	3.30	3.10	2.94	2.76	-	2.24	-	-	11.52	-	-	-	-	11.41	-	31	0.51	30	0.56
8	3.65	3.69	3.90	3.51	3.67	3.01	2.35	-	-	-	12.61	-	-	-	-	11.42	-	36	0.40	21	0.42
A	11.33	11.42	11.81	10.80	10.82	10.76	11.18	11.98	-	-	-	-	-	-	-	-	-	12	0.30	-	-
C	11.80	11.79	12.55	11.10	11.20	11.47	11.93	12.53	4.97	-	-	-	-	-	-	-	-	4	0.23	-	-
E	11.81	11.77	12.53	10.97	11.23	11.56	11.91	12.30	5.05	1.93	-	-	-	-	-	6.93	-	66	0.34	8	3.51
G	10.78	10.65	11.43	10.64	10.70	10.48	10.72	11.20	6.04	5.80	5.66	-	-	-	-	-	-	15	0.66	-	-
I	10.12	10.05	10.50	9.82	9.73	9.31	10.11	10.19	5.82	6.15	5.98	5.78	-	-	-	-	-	3	0.29	-	-
J	10.43	10.60	11.43	10.78	10.52	10.23	10.69	11.38	5.69	5.35	5.20	5.82	5.63	-	-	-	-	3	0.12	-	-
K	11.95	11.87	12.14	11.56	11.51	11.19	11.68	12.32	1.70	4.93	5.08	5.78	5.76	5.58	-	-	-	12	0.37	-	-
T	11.39	11.34	11.99	10.74	10.79	10.78	11.26	11.72	2.84	4.40	4.69	5.59	5.35	5.44	2.87	-	-	32	1.12	11	3.33
U	11.19	11.22	11.52	10.89	10.90	10.69	11.18	11.92	1.34	5.07	5.10	5.72	5.93	5.58	1.89	2.78	-	6	0.23	-	-

n_h – number of haplotypes

Table S9. Genetic diversity of grass snakes harboring mitochondrial lineage 7 from Bulgaria, Cyprus, Greece (East Macedonia and Thrace) and Turkey (western Anatolia) based on 13 microsatellite loci.

	Microsatellites						
Cluster	n	n_A	$n_{\bar{A}}$	AR	H_O	H_E	F_{IS}
Bulgaria	18	108	8.31	7.979	0.667	0.717	0.085*
Cyprus	27	65	5.00	4.895	0.336	0.485	0.288*
Greece (East Macedonia and Thrace)	17	77	5.92	5.835	0.672	0.697	0.084
Turkey (western Anatolia)	70	177	13.62	8.727	0.548	0.699	0.250*

*$p<0.05$; n: number of individuals; n_A: number of alleles; $n_{\bar{A}}$: average number of alleles per locus; AR: allelic richness; H_O: average observed heterozygosity; H_E: average expected heterozygosity; F_{IS}: inbreeding coefficient.

AR was calculated in FSTAT 2.9.4 (Goudet 1995); the remaining values were calculated locus-by-locus using ARLEQUIN 3.5.2.2 (Excoffier and Liescher 2010) with 1,000,000 Markov chain and 100,000 dememorization steps.

Table S10. Alleles per microsatellite locus for pure grass snakes (*n* = 103), *N. natrix* x *N. tessellata* hybrids (*n* = 26) and pure dice snakes (*n* = 52) from Turkey. Private alleles and alleles shared between hybrids and parental species are color-coded.

Locus/cluster	**Alleles**																									
Natnat09	80	82	86	90	94	96	98	100	102	104	106	108	110	112	114	116	118	120	122	130						
N. natrix	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+						
N. natrix* x *N. tessellata	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-						
N. tessellata	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-						
Natnat05	134	136	138	140	142	144	146	148	150	152	154	156	158	160	162	164	172	174	176	178	180	182	184	186	188	192
N. natrix	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+
N. natrix* x *N. tessellata	+	+	+	-	+	-	+	-	-	+	-	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-
N. tessellata	-	-	-	+	+	-	+	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
µNt8new	73	75	77	79	81	83	85	87	89	91	93	95	97	99	101	103	105									
N. natrix	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+									
N. natrix* x *N. tessellata	+	-	+	+	-	+	+	+	+	+	+	+	-	+	-	-	-									
N. tessellata	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	-	-									
Nsµ3	137	139	141	145	147	149	151	153	155	157	159	161	163	165	167	169	171	173	175	177	179	181	183	185	187	189
N. natrix	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N. natrix* x *N. tessellata	+	-	-	-	-	-	+	-	-	+	+	-	+	-	-	+	-	-	+	+	-	+	-	+	-	+
N. tessellata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	+
Nsµ3 (continued)	193	195	197	201	205	207	209	211	213	215	217	221	225	229	233											
N. natrix	-	+	-	-	-	+	+	-	-	+	-	-	-	-	-											
N. natrix* x *N. tessellata	+	-	-	+	+	-	-	+	+	-	+	-	-	-	-											
N. tessellata	+	-	+	+	+	-	+	-	+	-	+	+	+	+	+											
µNt3	121	123	125	127	129	131	133	135	139	141	143	145	147	149	151	153	155	157	163	167	169	171				
N. natrix	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	+	-	+	-	-	-				
N. natrix* x *N. tessellata	-	+	-	+	+	+	+	-	+	-	+	+	-	+	-	+	+	+	+	-	-	-				
N. tessellata	-	-	-	-	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+				

■Table S10 continued																					
Locus/cluster	**Alleles**																				
µNt7	170	172	174	178	180	182	184	186	188	190	192	194	196	198	200	202	204	206	208	212	
N. natrix	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
N. natrix* x *N. tessellata	+	-	-	+	-	+	+	+	+	+	-	-	+	+	+	-	-	+	-	-	
N. tessellata	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	
Hb30	229	233	235	237	239	241	243	245	247	249	251	253	255	257	259	261	263	265			
N. natrix	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-			
N. natrix* x *N. tessellata	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-			
N. tessellata	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+			
Natnat11	104	106	108	110	112	126	168	172	174	182	184	190	194	198	202						
N. natrix	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+						
N. natrix* x *N. tessellata	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-						
N. tessellata	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-						
Natnat06	145	147	153	155	157	159	161	163	165	167	169	177	181								
N. natrix	+	+	+	+	-	-	-	-	-	-	+	+	-								
N. natrix* x *N. tessellata	-	-	+	+	-	+	+	+	+	+	+	-	+								
N. tessellata	-	+	-	+	+	+	+	+	+	+	-	-	-								
3TS	178	182	186	202	206	210	218	226	230	234	238	242	246	250	254	258	270	282	286	290	294	298
N. natrix	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
N. natrix* x *N. tessellata	-	-	+	+	-	-	+	+	-	+	+	+	+	+	+	+	-	+	+	-	-	-
N. tessellata	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TbA09	104	106	108	112	114	116	118	120	122	124	126	128	130								
N. natrix	-	-	-	+	+	+	-	+	+	+	+	+	+								
N. natrix* x *N. tessellata	+	+	-	+	+	+	+	+	+	+	-	-	-								
N. tessellata	+	+	+	-	-	-	-	-	-	-	-	-	-								

■Table S10 continued																	
Locus/cluster	**Alleles**																
Eobμ1	120	122	124	128	130	134	136	138	140	142							
N. natrix	+	+	+	+	+	+	+	+	+	+							
N. natrix* x *N. tessellata	+	+	+	+	+	-	+	+	-	-							
N. tessellata	-	+	+	-	-	-	-	-	-	-							
Eob13	128	130	132	134	136	138	140	142	144	146	148	150	152	154	156	158	160
N. natrix	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
N. natrix* x *N. tessellata	-	-	+	+	-	-	-	+	+	+	+	+	-	+	+	-	-
N. tessellata	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+

Table S11. Alleles per microsatellite locus for pure grass snakes harboring mitochondrial lineage 7 from Bulgaria (*n* = 18), Cyprus (*n* = 27), Greece (East Macedonia and Thrace; *n* = 17) and Turkey (western Anatolia; *n* = 70) and pure *N. tessellata* (*n* = 52). Private alleles are color-coded. The only allele of the 3TS locus exclusively shared by Cypriot grass snakes and *N. tessellata* is highlighted in red.

Locus/group	**Alleles**																							
Natnat09	80	82	86	90	94	96	98	100	102	104	106	108	110	112	114	116	120	122						
Bulgaria	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+	-	-	-						
Cyprus	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	+	-						
Greece (East Macedonia and Thrace)	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	-	+	-						
Turkey (western Anatolia)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
N. tessellata	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+						
Natnat05	136	138	140	142	144	146	148	152	154	156	158	160	162	164	172	174	176	178	180	182	184	186	188	192
Bulgaria	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+	-	-	+	-	-
Cyprus	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-
Greece (East Macedonia and Thrace)	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-
Turkey (western Anatolia)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N. tessellata	-	-	+	+	-	+	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
µNt8new	73	75	81	83	85	87	89	91	93	95	97	99	101	103	105									
Bulgaria	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-									
Cyprus	-	-	-	+	-	-	+	+	-	+	-	+	-	-	-									
Greece (East Macedonia and Thrace)	-	-	-	+	-	+	+	+	-	+	-	+	-	-	-									
Turkey (western Anatolia)	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+									
N. tessellata	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-									
Nsµ3	139	141	147	149	151	153	155	157	159	161	163	165	167	169	171	173	175	177	183	185	187	189	193	197
Bulgaria	-	-	-	-	+	-	+	+	+	-	+	+	+	-	+	-	+	+	+	+	-	-	-	-
Cyprus	-	-	-	-	+	+	-	-	+	-	+	+	-	+	-	+	-	-	-	-	-	-	-	-
Greece (East Macedonia and Thrace)	-	-	-	-	+	-	+	-	+	-	+	+	+	+	-	+	-	-	+	-	-	-	-	-
Turkey (western Anatolia)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-
N. tessellata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	+	+

■**Table S11 continued**

Locus/group	**Alleles**																					
Nsμ3 (continued)	201	205	209	211	213	215	217	221	225	229	233	445	457									
Bulgaria	-	-	-	-	-	-	-	-	-	-	-	+	+									
Cyprus	-	-	-	-	-	-	-	-	-	-	-	-	-									
Greece (East Macedonia and Thrace)	-	-	-	-	-	-	-	-	-	-	-	-	-									
Turkey (western Anatolia)	-	-	-	+	+	+	-	-	-	-	-	-	-									
N. tessellata	+	+	+	-	+	-	+	+	+	+	+	-	-									
μNt3	121	123	125	127	129	131	133	135	139	141	143	145	147	149	151	153	155	157	163	167	169	171
Bulgaria	+	+	+	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	-	-	-	-
Cyprus	+	+	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
Greece (East Macedonia and Thrace)	+	+	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-
Turkey (western Anatolia)	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-	+	-	+	-	-	-
N. tessellata	-	-	-	-	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
μNt7	166	170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200	202	204	206	208	212
Bulgaria	+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	-	-	-	+
Cyprus	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-
Greece (East Macedonia and Thrace)	-	+	-	-	-	-	+	-	+	+	-	+	+	-	-	-	+	-	-	-	-	-
Turkey (western Anatolia)	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N. tessellata	-	-	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-
Hb30	233	235	237	239	241	243	245	247	249	251	253	255	257	259	261	263	265					
Bulgaria	+	-	+	+	+	+	-	+	+	-	+	+	-	-	-	-	-					
Cyprus	-	-	-	+	+	-	+	+	-	-	-	+	-	-	-	-	-					
Greece (East Macedonia and Thrace)	+	-	-	+	+	+	-	+	+	+	-	+	-	-	-	-	-					
Turkey (western Anatolia)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-					
N. tessellata	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+					

■**Table S11 continued**

Locus/group	**Alleles**																				
Natnat11	104	106	108	110	112	126	128	168	172	174	182	184	186	188	190	194	198	202	220	224	228
Bulgaria	-	-	-	+	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-
Cyprus	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Greece (East Macedonia and Thrace)	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey (western Anatolia)	-	-	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	-	-	-
N. tessellata	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Natnat06	145	147	153	155	157	159	161	163	165	167	169	171	173	177
Bulgaria	-	-	+	-	-	-	-	-	-	-	+	+	+	-
Cyprus	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Greece (East Macedonia and Thrace)	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Turkey (western Anatolia)	+	+	+	+	-	-	-	+	-	-	+	-	-	+
N. tessellata	-	+	-	+	+	+	+	+	+	+	-	-	-	-

3TS	178	182	186	202	206	210	218	222	226	230	234	238	242	**246**	250	254	258	270	282	286	290	294	298
Bulgaria	-	-	+	+	-	-	-	+	+	+	+	+	+	-	-	+	-	-	+	-	-	-	-
Cyprus	-	-	+	+	-	-	-	+	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-
Greece (East Macedonia and Thrace)	-	-	+	+	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Turkey (western Anatolia)	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-
N. tessellata	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TbµA09	104	106	108	112	114	116	118	120	122	124	126	128	130
Bulgaria	-	-	-	-	-	+	+	+	+	+	+	-	-
Cyprus	-	-	-	-	+	-	+	+	+	+	+	-	-
Greece (East Macedonia and Thrace)	-	-	-	+	+	+	+	+	+	+	+	-	-
Turkey (western Anatolia)	-	-	-	+	+	+	-	+	+	+	+	+	+
N. tessellata	+	+	+	-	-	-	-	-	-	-	-	-	-

■**Table S11 continued**

Locus/group	**Alleles**																
Eobμ1	120	122	124	128	130	136	138	140	142								
Bulgaria	-	+	+	+	-	+	+	+	+								
Cyprus	-	-	-	+	+	-	+	-	+								
Greece (East Macedonia and Thrace)	-	-	+	+	+	+	+	+	+								
Turkey (western Anatolia)	+	+	-	+	+	+	+	+	-								
N. tessellata	-	+	+	-	-	-	-	-	-								
Eobμ13	130	132	134	136	138	140	142	144	146	148	150	152	154	156	158	160	162
Bulgaria	+	-	+	+	+	+	-	+	-	-	-	+	-	-	-	-	-
Cyprus	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Greece (East Macedonia and Thrace)	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Turkey (western Anatolia)	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
N. tessellata	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-

References

Ahnelt H, Romanova T, Klinge A, Böhme W, Fritz U, Asztalos M (2021) The common grass snake (*Natrix natrix*) on Sylt: Human-mediated colonization of a North Sea island. Salamandra 57: 285-290.

Asztalos M, Glaw F, Franzen M, Kindler C, Fritz U (2021a) Transalpine dispersal: Italian barred grass snakes in southernmost Bavaria – This far but no further! Journal of Zoological Systematics and Evolutionary Research 59: 1136-1148. https://doi.org/10.1111/jzs.12471

Asztalos M, Wielstra B, Struijk RPJH, Ayaz D, Fritz U (2021b) Aliens in the Netherlands: Local genetic pollution of barred grass snakes (Squamata: Serpentes: Natricidae). Salamandra 57: 174-179.

Blouin-Demers G, Gibbs HL (2003) Isolation and characterization of microsatellite loci in the black rat snake (*Elaphe obsoleta*). Molecular Ecology Notes 3*:* 89-99. https://doi.org/10.1046/j.1471-8286.2003.00363.x

Burns EL, Houlden BA (1999) Isolation and characterization of microsatellite markers in the broad-headed snake *Hoplocephalus bungaroides*. Molecular Ecology 8: 520-521.

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4: 359-361. https://doi.org/10.1007/s12686-011-9548-7

Excoffier L, Lischer HEL (2010) ARLEQUIN suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Garner TWJ, Gregory PT, McCracken GF, Burghardt GM, Koop BF, McLain SE, Nelson RJ (2002) Geographic variation of multiple paternity in the common garter snake (*Thamnophis sirtalis*). Copeia 2002: 15-23. https://doi.org/10.1643/0045-8511(2002)002[0015:GVOMPI]2.0.CO;2

Gautschi B, Widmer A, Koella J (2000) Isolation and characterization of microsatellite loci in the dice snake (*Natrix tessellata*). Molecular Ecology 9: 2192-2193. https://doi.org/10.1046/j.1365-294X.2000.105320.x

Goudet J (1995) FSTAT (version 1.2): A computer program to calculate *F*-statistics. Journal of Heredity 86: 485-486. https://doi.org/10.1093/oxfordjournals.jhered.a111627

Guicking D, Joger U, Wink M (2009) Cryptic diversity in a Eurasian water snake (*Natrix tessellata*, Serpentes: Colubridae): Evidence from mitochondrial sequence data and nuclear ISSR-PCR fingerprinting. Organisms, Diversity & Evolution 9: 201-214. https://doi.org/10.1016/j.ode.2009.03.001

Guicking D, Lawson R, Joger U, Wink M (2006) Evolution and phylogeny of the genus *Natrix* (Serpentes: Colubridae). Biological Journal of the Linnean Society 87: 127-143. https://doi.org/10.1111/j.1095-8312.2006.00561.x

Kindler C, Böhme W, Corti C, Gvoždík V, Jablonski D, Jandzik D, Metallinou M, Široký P, Fritz U (2013) Mitochondrial phylogeography, contact zones and taxonomy of grass snakes (*Natrix natrix*, *N. megalocephala*). Zoologica Scripta 42: 458-472. https://doi.org/10.1111/zsc.12018

Kindler C, Chèvre M, Ursenbacher S, Böhme W, Hille A, Jablonski D, Vamberger M, Fritz U (2017) Hybridization patterns in two contact zones of grass snakes reveal a new Central European snake species. Scientific Reports 7: 7378. https://doi.org/10.1038/s41598-017-07847-9

Kindler C, de Pous P, Carranza S, Beddek M, Geniez P, Fritz U (2018) Phylogeography of the Ibero-Maghrebian red-eyed grass snake (*Natrix astreptophora*). Organisms Diversity & Evolution 18: 143-150. https://doi.org/10.1007/s13127-017-0354-2

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35: 1547-1549. https://doi.org/10.1093/molbev/msy096

Kyriazi P, Kornillos P, Nagy ZT, Poulakakis N, Kumlutaş Y, Ilgaz Ç, Avcı A, Göçmen B, Lymberakis P (2013) Comparative phylogeography reveals distinct colonization patterns of Cretan snakes. Journal of Biogeography 40: 1143-1155. https://doi.org/10.1111/jbi.12057

Lawson R, Slowinski JB, Crother BI, Burbrink FT (2005) Phylogeny of the Colubroidea (Serpentes): New evidence from mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution 37: 581-601. https://doi.org/10.1016/j.ympev.2005.07.016

Meister B, Armbruster FJ, Frauenfelder N, Bauer B (2009) Novel microsatellite loci in the grass snake (*Natrix natrix*) and cross-amplification in the dice snake (*Natrix tessellata*). Molecular Ecology Resources 9: 604-606. https://doi.org/10.1111/j.1755-0998.2008.02460.x

Nielsen EE, Bach LA, Kotlicki P (2006) HYBRIDLAB (version 1.0): A program for generating simulated hybrids from population samples. Molecular Ecology Notes 6: 971-973. https://doi.org/10.1111/j.1471-8286.2006.01433.x

Prosser MR, Gibbs HL, Weatherhead PJ (1999) Microgeographic population genetic structure in the northern water snake, *Nerodis sipedon sipedon* detected using microsatellite DNA loci. Molecular Ecology 8: 329-333. https://doi.org/10.1046/j.1365-294X.1999.00530.x

Schultze N, Laufer H, Kindler C, Fritz U (2019) Distribution and hybridisation of barred and common grass snakes (*Natrix helvetica*, *N. natrix*) in Baden-Württemberg, South-western Germany. Herpetozoa 32: 229-236. https://doi.org/10.3897/herpetozoa.32.e38897

Schultze N, Spitzweg C, Corti C, Delaugerre M, Di Nicola MR, Geniez P, Lapini L, Liuzzi C, Lunghi E, Novarini N, Picariello O, Razzetti E, Sperone E, Stellati L, Vignoli L, Asztalos M, Kindler C, Fritz U (2020) Mitochondrial ghost lineages blur phylogeography and taxonomy of *Natrix helvetica* and *N. natrix* in Italy and Corsica. Zoologica Scripta 49: 395-411. https://doi.org/10.1111/zsc.12417

Sloss BL, Schuurman GW, Paloski RA, Boyle OD, Kapfer JM (2012) Novel microsatellite loci for studies of *Thamnophis* gartersnake genetic identity and hybridization. Conservation Genetics Resources 4: 383-386. https://doi.org/10.1007/s12686-011-9555-8