

Phylogeographic relationships and shallow mitochondrial divergence
of Algerian populations of *Salamandra algira*

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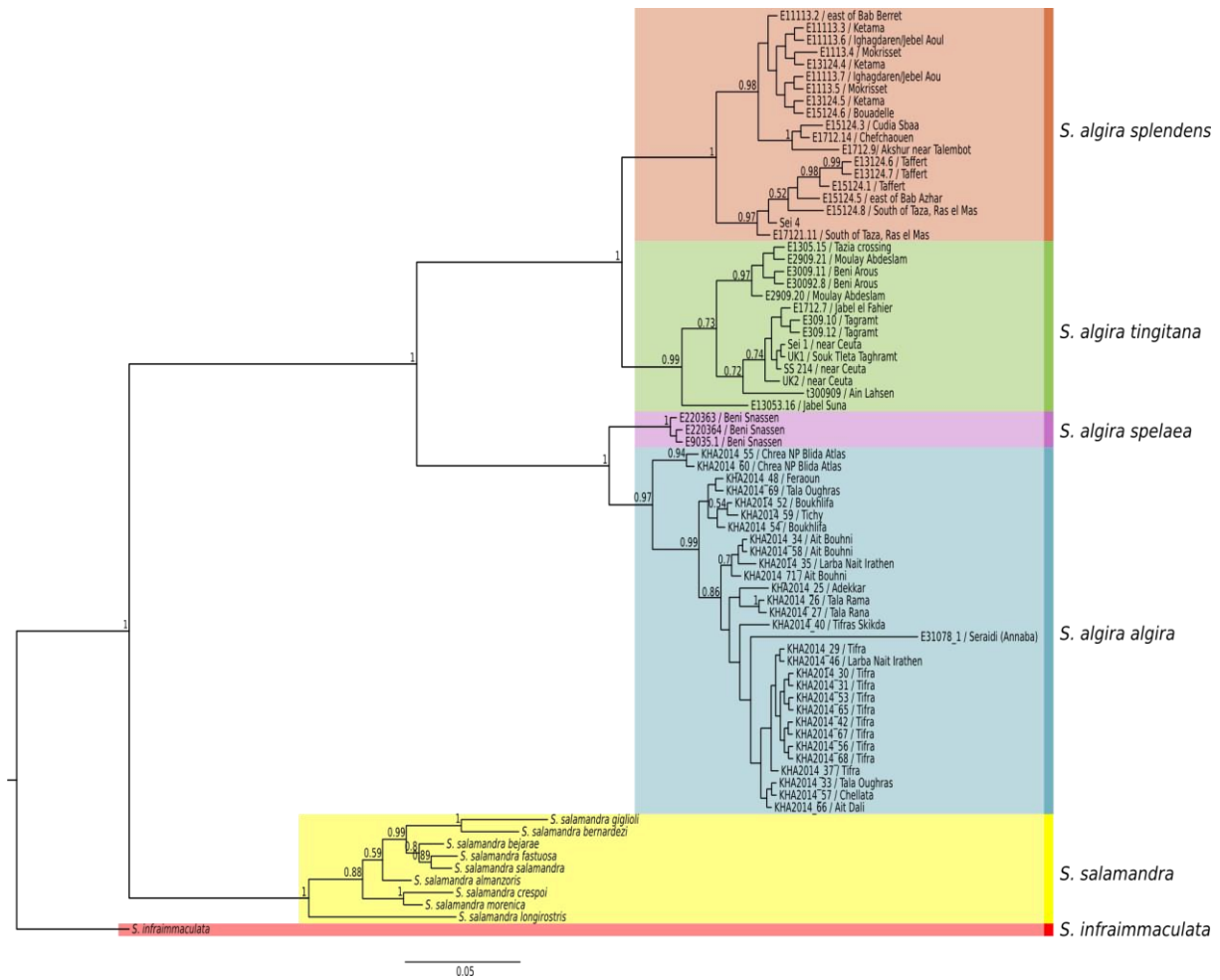
2 - Zoological Institute, Technical University of Braunschweig, Mendelssohnstr. 4, 38106
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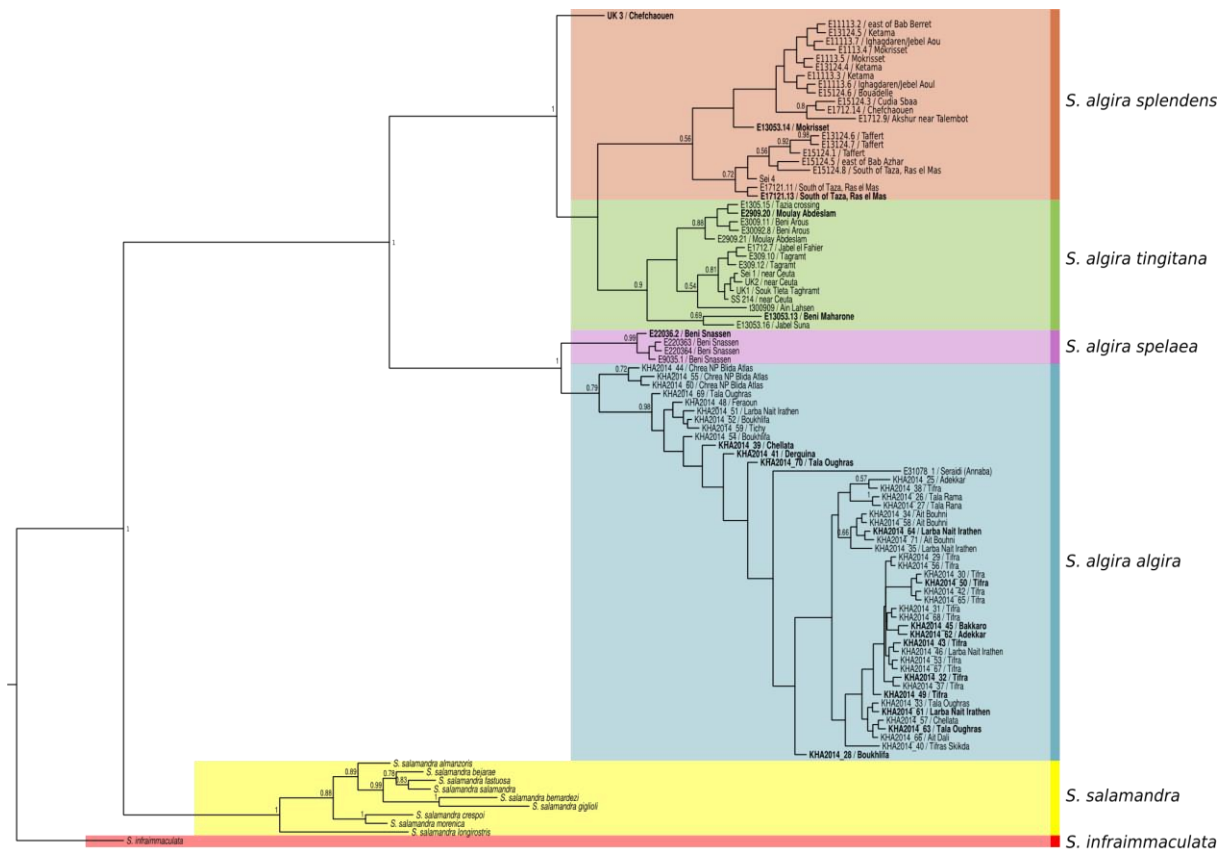
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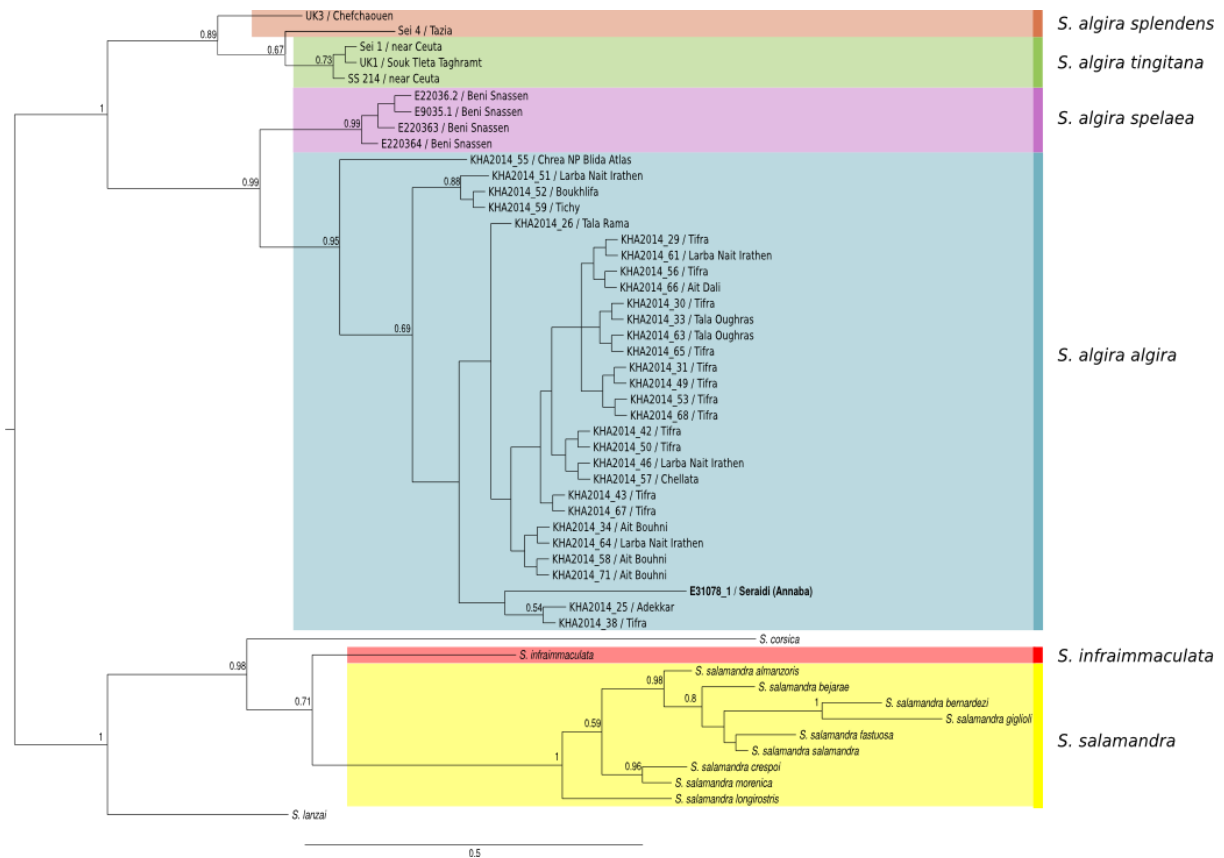
Supplementary Material



Supplementary Figure S1. Majority-rule consensus tree from a Bayesian Inference analysis of *Salamandra algira* sequences of segments of the mitochondrial 12S, cytochrome b and D-loop genes (length of total concatenated alignment: 1683 bp). This is the same tree as shown in the main paper, but including all outgroups used.



Supplementary Figure S2. Majority-rule consensus tree from a Bayesian Inference analysis of *Salamandra algira* sequences of segments of the mitochondrial 12S, cytochrome b and D-loop genes (length of total concatenated alignment: 1683 bp). This tree includes all Algerian samples for which a sequence of at least one of the three genes was available. Unusual placement of some samples are caused by missing data, and by the inclusion of samples sequenced only for single gene segments that are not available from their probable closest relatives.



Supplementary Figure S3. Majority-rule consensus tree from a Bayesian Inference analysis of *Salamandra algira* sequences of segments of the mitochondrial D-loop genes (length of total concatenated alignment: 769 bp).

Supplementary Table S1. Geographical coordinates of sampling locations, and sample numbers, for *S. a. tingitana* and *S. a. splendens* for which DNA sequences were used in the phylogenetic analyses (all taken from Genbank).

Samples	Locality	Country	Latitude	Longitude
<i>S. a. splendens</i>				
E15124.8, E17121.11	South of Taza, Ras el Ma	Morocco	34.14737 N	4.0093 W
Sei 4	locality uncertain, possibly near Taza	Morocco	NA	NA
E15124.1, E13124.6, E13124.7	Taffert (Middle Atlas)	Morocco	33.64342 N	4.14124 W
E15124.6	Bouadelle	Morocco	34.53353 N	4.50492 W
E11113.3, E13124.4, E13124.5	West of Ketama	Morocco	34.96072 N	4.68056 W
E11113.6, E11113.7	Ighagdaren/Jebel Aoul	Morocco	34.80931 N	4.66936 W
E11113.2	east of Bab Berred	Morocco	34.99327 N	4.82277 W
E15124.3	Cudia Sbaa (= Sebaa)	Morocco	35.02344 N	5.01544 W
E1712.9	Akchur (near Talembot)	Morocco	35.24344 N	5.18919 W
E1712.14	Chefchaouen (= Xauen)	Morocco	35.17914 N	5.24633 W
E15124.5	east of Bab Azhar	Morocco	34.05111 N	4.22028 W
E1113.4, E1113.5	Mokrisset	Morocco	34.95161 N	5.36322 W
<i>S. a. tingitana</i>				
E1712.7	Jabel el Fahies	Morocco	35.09163 N	5.40318 W
E13053.16	Yebel Sugna (= Jbel Soukna)	Morocco	35.12967 N	5.36517 W
Sei 1, SS 214, UK2	Beliunech (near Ceuta)	Morocco	35.908 N	5.398 W
E309.10, E309.12	Tagramt (= Taghramt)	Morocco	35.79564 N	5.45964 W
UK1	Souk Tleta Taghramt	Morocco	35.79694 N	5.45980 W
E2909.20, E2909.21	South of Moulay Abdeslam	Morocco	35.3 1741 N	5.49759 W
E3009.11, E30092.8	Jbel Hamziouat (near Beni Arous)	Morocco	35.437035 N	5.55413 W
E3009.9	Ain Lahsen (Ain el Hesn)	Morocco	35.5497 N	5.5641 W
E1305.15	Tazia crossing	Morocco	35.36458 N	5.56639 W

Supplementary Table S2. Genbank accession numbers of DNA sequences used in this study. numbers in columns subsequent to accession numbers indicate the sources of the sequences: (1) New sequences obtained in this study; (2) Steinfartz et al. (2000), (3) Escoriza & Comas (2007), (4) Escoriza et al. (2006), (5) Beukema et al. (2010); (6) Vences et al. (2014). NA, not available.

Taxon	Samples	Locality	12S	Cytb	D-loop
<i>S. algira</i>					
<i>algira</i>	KHA2014_44	Chrea NP Blida Atlas	NA	- KT335642	1 NA -
<i>algira</i>	KHA2014_55	Chrea NP Blida Atlas	KT335686	1 KT335643	1 KT335613 1
<i>algira</i>	KHA2014_60	Chrea NP Blida Atlas	KT335687	1 KT335644	1 NA -
<i>algira</i>	KHA2014_35	Larba Nait Irathen	KT335688	1 KT335645	1 NA -
<i>algira</i>	KHA2014_46	Larba Nait Irathen	KT335689	1 KT335646	1 KT335614 1
<i>algira</i>	KHA2014_51	Larba Nait Irathen	NA	- KT335647	1 KT335615 1
<i>algira</i>	KHA2014_61	Larba Nait Irathen	NA	- KT335648	1 KT335616 1
<i>algira</i>	KHA2014_64	Larba Nait Irathen	NA	- KT335649	1 KT335617 1
<i>algira</i>	KHA2014_26	Tala Rana	KT335690	1 KT335650	1 KT335618 1
<i>algira</i>	KHA2014_27	Tala Rana	KT335691	1 KT335651	1 NA -
<i>algira</i>	KHA2014_34	Ait Bouhni	KT335692	1 KT335652	1 KT335619 1
<i>algira</i>	KHA2014_58	Ait Bouhni	KT335693	1 KT335653	1 KT335620 1
<i>algira</i>	KHA2014_71	Ait Bouhni	KT335694	1 KT335654	1 KT335621 1
<i>algira</i>	KHA2014_25	Adekkar	KT335695	1 KT335655	1 KT335622 1
<i>algira</i>	KHA2014_62	Adekkar	NA	- KT335656	1 NA -
<i>algira</i>	KHA2014_39	Chellata	NA	- KT335657	1 NA -
<i>algira</i>	KHA2014_57	Chellata	KT335696	1 KT335658	1 KT335623 1
<i>algira</i>	KHA2014_45	Bakkaro	NA	- KT335682	1 NA -
<i>algira</i>	KHA2014_29	Tifra	KT335697	1 KT335659	1 KT335624 1
<i>algira</i>	KHA2014_30	Tifra	KT335698	1 KT335660	1 KT335625 1
<i>algira</i>	KHA2014_31	Tifra	KT335699	1 KT335661	1 KT335626 1
<i>algira</i>	KHA2014_32	Tifra	NA	- KT335662	1 NA -
<i>algira</i>	KHA2014_37	Tifra	KT335700	1 KT335663	1 NA -
<i>algira</i>	KHA2014_38	Tifra	NA	- KT335664	1 KT335627 -
<i>algira</i>	KHA2014_42	Tifra	KT335701	1 KT335665	1 KT335628 1
<i>algira</i>	KHA2014_43	Tifra	NA	- KT335666	1 KT335629 1
<i>algira</i>	KHA2014_50	Tifra	NA	- KT335667	1 KT335631 1
<i>algira</i>	KHA2014_53	Tifra	KT335702	1 KT335668	1 KT335632 1
<i>algira</i>	KHA2014_56	Tifra	KT335703	1 KT335669	1 KT335633 1
<i>algira</i>	KHA2014_65	Tifra	KT335704	1 KT335670	1 KT335634 1
<i>algira</i>	KHA2014_67	Tifra	KT335705	1 KT335671	1 KT335635 1
<i>algira</i>	KHA2014_68	Tifra	KT335706	1 KT335672	1 KT335636 1
<i>algira</i>	KHA2014_48	Feraoun	KT335707	1 KT335673	1 NA -
<i>algira</i>	KHA2014_66	Ait Dali	KT335708	1 KT335674	1 KT335637 1
<i>algira</i>	KHA2014_33	Tala Oughras	KT335709	1 KT335675	1 KT335638 1
<i>algira</i>	KHA2014_63	Tala Oughras	NA	- KT335676	1 KT335639 1
<i>algira</i>	KHA2014_69	Tala Oughras	KT335710	1 KT335677	1 NA -
<i>algira</i>	KHA2014_70	Tala Oughras	NA	- KT335678	1 NA -
<i>algira</i>	KHA2014_28	Boukhelifa	NA	- KT335681	1 NA -
<i>algira</i>	KHA2014_52	Boukhelifa	KT335711	1 KT335679	1 KT335640 1
<i>algira</i>	KHA2014_54	Boukhelifa	KT335712	1 KT335680	1 NA -
<i>algira</i>	KHA2014_59	Tichy	KT335713	1 KT335683	1 KT335641 1
<i>algira</i>	KHA2014_41	Derguina	NA	- KT335684	1 NA -
<i>algira</i>	KHA2014_40	Tifras Skikda	KT335714	1 KT335685	1 NA -
<i>algira</i>	E31078_1	Seraidi (near Annaba)	NA	- NA	- KT335852 2
<i>spelaea</i>	E220364	Ouartass (Beni Snassen)	HQ190867	3 DQ901444	5 DQ901447 3
<i>spelaea</i>	E9035.1	Ouartass (Beni Snassen)	DQ221231	4 DQ221247	4 DQ221248 4
<i>spelaea</i>	E220363	(Ouartass) Beni Snassen	HQ190866	3 DQ901443	5 DQ901446 3

<i>splendens</i>	E15124.8	South of Taza, Ras el Ma	HQ190865	5	HQ190896	5	NA	-
<i>splendens</i>	E17121.11	South of Taza, Ras el Ma	HQ190859	5	HQ190890	5	NA	-
<i>splendens</i>	E13124.6	Taffert	HQ190861	5	HQ190892	5	NA	-
<i>splendens</i>	E15124.1	Taffert	HQ190863	5	HQ190894	5	NA	-
<i>splendens</i>	E13124.7	Taffert	HQ190862	5	HQ190893	5	NA	-
<i>splendens</i>	E15124.6	Bouadelle	HQ190871	5	HQ190900	5	NA	-
<i>splendens</i>	E11113.3	Ketama	HQ190869	5	HQ190898	5	NA	-
<i>splendens</i>	E13124.4	Ketama	HQ190874	5	HQ190903	5	NA	-
<i>splendens</i>	E13124.5	Ketama	HQ190875	5	HQ190904	5	NA	-
<i>splendens</i>	E11113.6	Ighagdaren/Jebel Aoul	HQ190872	5	HQ190901	5	NA	-
<i>splendens</i>	E11113.7	Ighagdaren/Jebel Aoul	HQ190873	5	HQ190902	5	NA	-
<i>splendens</i>	E11113.2	east of Bab Berred	HQ190868	5	HQ190897	5	NA	-
<i>splendens</i>	E15124.3	Cudia Sbaa	HQ190870	5	HQ190899	5	NA	-
<i>splendens</i>	E1712.9	Akshur near Talembot	DQ221229	4	AY247734	4	NA	-
<i>splendens</i>	E1712.14	Chefchaouen	DQ221230	4	AY247735	4	NA	-
<i>splendens</i>	E1113.4	Mokrisset	HQ190858	5	HQ190889	5	NA	-
<i>Splendens</i>	E1113.5	Mokrisset	HQ190856	5	HQ190887	5	NA	-
<i>splendens</i>	E15124.5	east of Bab Azhar	HQ190864	5	HQ190895	5	NA	-
<i>tingitana</i>	E1712.7	Jebel el Fahies	DQ221227	4	AY247732	4	NA	-
<i>tingitana</i>	E13053.16	Jebel Suna	HQ190852	5	HQ190882	5	NA	-
<i>tingitana</i>	Sei 1	near Ceuta	KF645381	6	KF645934	6	KF645611	6
<i>tingitana</i>	SS 214	near Ceuta	KF645378	6	KF645931	6	KF645609	6
<i>tingitana</i>	UK2	near Ceuta	KF645380	6	KF645933	6	NA	-
<i>tingitana</i>	E309.10	Tagramt	HQ190853.	5	HQ190884	5	NA	-
			1					
<i>tingitana</i>	E309.12	Tagramt	HQ190854	5	HQ190885	5	NA	-
<i>tingitana</i>	UK1	Souk Tleta Taghramt	KF645379	6	KF645932	6	KF645610	6
<i>tingitana</i>	E2909.20	Moulay Abdeslam	HQ190846	5	HQ190876	5	NA	-
<i>tingitana</i>	E2909.21	Moulay Abdeslam	HQ190847	5	HQ190877	5	NA	-
<i>tingitana</i>	E3009.11	Nr. Beni Arous	HQ190849	5	HQ190879	5	NA	-
<i>tingitana</i>	E30092.8	Nr. Beni Arous	HQ190850	5	HQ190880	5	NA	-
<i>tingitana</i>	E3009.9	Ain Lahsen	HQ190855	5	HQ190886	5	NA	-
<i>tingitana</i>	E1305.15	Tazia crossing	HQ190848	5	HQ190878	5	NA	-
<i>splendens</i>	sei 4	Taza (?)	KF645377	6	KF645930	6	KF645608	6

S.

infraimmaculata

-	-	-	DQ221222	4	DQ221242	3	KF645593	6
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S. salamandra

<i>almanzoris</i>	-	-	DQ221219	4	DQ221239	4	SS64	2
<i>bejarae</i>	-	-	DQ221216	4	DQ221236	4	SS125	2
<i>bernardezi</i>	-	-	DQ221220	4	DQ221240	4	SS54	2
<i>crespoi</i>	-	-	DQ221217	4	DQ221237	4	SS48	2
<i>fastuosa</i>	-	-	DQ221214	4	DQ221234	4	SS59	2
<i>giglioli</i>	-	-	DQ221221	4	DQ221241	4	SS81	2
<i>longirostris</i> *	-	-	DQ221223	4	DQ221243	3	KF645598	6
<i>morenica</i>	-	-	DQ221218	4	DQ221238	4	KF645600	6
<i>salamandra</i>	-	-	DQ221213	4	DQ221232	4	KF645596	6

* The taxon *longirostris* is alternatively seen as subspecies of *S. salamandra* or as separate species in the literature.

References in table S2

(2) Steinfartz, S., Veith, M., Tautz, D. (2000): Mitochondrial sequence analysis of *Salamandra* taxa suggests old splits of major lineages and postglacial recolonizations of

- Central Europe from distinct source populations of *Salamandra salamandra*. *Mol. Ecol.* **9**: 397-410.
- (3) Escoriza, D., Comas, M.M. (2007): Description of a new subspecies of *Salamandra algira* Bedriaga, 1883 (Amphibia: Salamandridae) from the Beni Snassen massif (Northeast Morocco). *Salamandra* **43**: 77-90.
 - (4) Escoriza, D., Comas, M.M., Donaire, D., Carranza, S. (2006): Rediscovery of *Salamandra algira* Bedriaga, 1883 from the Beni Snassen massif (Morocco) and phylogenetic relationships of North African *Salamandra*. *Amphibia-Reptilia* **27**: 448-455.
 - (5) Beukema, W., de Pous, P., Donaire, D., Escoriza, D., Bogaerts, S., Toxopeus, A.G., De Bie, C.A.J.M., Roca, J., Carranza, S. (2010): Biogeography and contemporary climatic differentiation among Moroccan *Salamandra algira*. *Biol. J. Linn. Soc.* **101**: 626-641.
 - (6) Vences, M., Sanchez, E., Hauswaldt, J.S., Eikelmann, D., Rodríguez, A., Carranza, S., Donaire, D., Gehara, M., Helfer, V., Lötters, S., Werner, P., Schulz, S., Steinfartz, S. (2014): Nuclear and mitochondrial multilocus phylogeny and survey of alkaloid content in true salamanders of the genus *Salamandra* (Salamandridae). *Mol. Phylogenet. Evol.*, **73**: 208-216.

Supplementary Table S3. Approximate fragment sizes (bp), primers and PCR conditions applied for the amplification of mitochondrial DNA fragments. Thermal cycling schemes start with temperature (in °C) of each step, followed by the time in seconds between parentheses; cycling repetitions are indicated within brackets. References: (1) Kocher et al. (1989), (2) Hrbek & Larson (1999); (3) Steinfartz et al. (2000); (4) Beukema et al. (2010).

Marker	Primer	Primer sequence (5'-3')	PCR conditions	Reference
12S	12SAL Fwd (5'-3')	AAACTGGGATTAGATACCCCACTAT	94(90), [94(45), 52(45), 72(90) x 33], 72(300)	1
	16SR3 Rev (3'-5')	TTTCATCTTTCCCTTGCGGTAC		2
D-loop	L-Pro-ML Fwd (5'-3')	GGCACCCAAGGCCAAAATTCT	94(120), [94(30), 60(45), 72(80) x 36], 72(300)	3
	H-12S1-ML Rev (3'-5')	CAAGGCCAGGACCAAACCTTTA		3
Cytb	SalaCytbF Fwd (5'-3')	CTAATGACCCACATCCTTCGAAAAACA C	94(300), [94(30), 48(45), 72(60) x 35], 72(300)	4
	SalaCytbR Rev (3'-5')	TGGGAGTACGTATCCTACAAAGGCTG		4

References in table S3

- (1) Kocher, T.D., Thomas, W.K., Meyer, A. Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. (1989): Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci.* **86**: 6196-6200.
- (2) Hrbek, T., Larson, A. (1999): The evolution of diapause in the killifish family Rivulidae (Atherinomorpha, Cyprinodontiformes): a molecular phylogenetic and biogeographic perspective. *Evolution* **53**: 1200-1216.
- (3) Steinfartz, S., Veith, M., Tautz, D. (2000): Mitochondrial sequence analysis of *Salamandra* taxa suggests old splits of major lineages and postglacial recolonizations of Central Europe from distinct source populations of *Salamandra salamandra*. *Mol. Ecol.* **9**: 397-410.
- (4) Beukema, W., de Pous, P., Donaire, D., Escoriza, D., Bogaerts, S., Toxopeus, A.G., De Bie, C.A.J.M., Roca, J., Carranza, S. (2010): Biogeography and contemporary climatic differentiation among Moroccan *Salamandra algira*. *Biol. J. Linn. Soc.* **101**: 626-641.

Supplementary Table S4. Substitution models and partitions applied for the phylogenetic reconstruction, as suggested by Partitionfinder software.

Dataset	Subset	Best Model	Subset Partitions	Subset Sites
Concatenated 12S, Cytb and D-loop	1	GTR+G	12S, 1stPos_Cytb	1-389, 390-914\3
	2	HKY+G	2ndPos_Cytb	391-914\3
	3	GTR+G	3rdPos_Cytb	392-914\3
	4	HKY+I+G	1stPos_Dloop	915-1683