

Phylogeography of grass snakes (*Natrix natrix*) all around the Baltic Sea: implications for the Holocene colonization of Fennoscandia

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Abstract. We show that Fennoscandia was invaded in the Holocene by three distinct mitochondrial lineages of *Natrix natrix*. Two of these lineages arrived from the south, and one from the east. One of the 'southern lineages' is confined to Gotland, where also the second 'southern lineage' is found. The latter is widely distributed in the southwestern Baltic region, western Fennoscandia and on the Åland Islands, while the other lineage present on Gotland is only known from a few sites in the Baltic region. In addition, we recorded a third mitochondrial lineage in southern continental Finland, which was previously unknown from Fennoscandia. This lineage also occurs in the southeastern Baltic region and further east, suggesting that southern Finland was colonized from the east. Thus, the phylogeography of *N. natrix* matches a general paradigm for Fennoscandia, with Holocene invasions from the south and east.

Keywords: colonization history, Fennoscandia, mitochondrial DNA, reptiles, snakes.

The grass snake, *Natrix natrix* (Linnaeus, 1758), belongs to the snake species with the largest distribution ranges in the Palaearctic (Kabisch, 1999). Following Kabisch (1999), *N. natrix* is one of the few reptiles which colonized Fennoscandia up to the Arctic Circle. However, according to Fog, Schmedes and Rosenørn de Lasson (1997) the distribution range does not extend so far northwards (fig. 1). Using mitochondrial DNA sequences, Kindler et al. (2013) demonstrated that grass snakes are genetically much differentiated. However, the current subspecies classification does not match genetic differentiation and needs revision. Therefore, we use in the following genetic lineages instead of subspecies for characterizing different grass snakes.

Previous studies discovered three mitochondrial lineages in Scandinavia and the Baltic region (Guicking et al., 2006; Kindler et al., 2013). One lineage was found in northern Germany, Denmark, Norway and Sweden (includ-

ing Gotland); another lineage in northern Germany, central Poland and Gotland (Sweden); and a third lineage in Kaliningrad Oblast (Russia), Lithuania, and eastern Poland. However, due to incomplete sampling, it could not be examined which lineage occurs in Finland. It is well known for several other species that Fennoscandia was colonized after the retreat of the glacial ice shield from two different source regions. While northeastern Fennoscandia, in particular Finland, was reached from the east, the more western parts were invaded from the south via Denmark. Both colonization waves may have met in northern Fennoscandia, leading to a contact or suture zone there (Taberlet et al., 1998).

The present study aims at clarifying whether such a double colonization pattern may also refer to Fennoscandian grass snakes. For doing so, we use phylogenetic analyses of mtDNA sequences of 97 *N. natrix* and discuss the results in the context of the range-wide phylogeography provided by Kindler et al. (2013).

Fifty grass snake samples (shed skins, saliva samples, tails tips or tissues from roadkills) were processed for the present study. The samples originated from Denmark, Norway, Sweden, Finland, Poland, and northern Germany. For each sample, two mitochondrial genes were sequenced, the partial ND4 gene plus adjacent DNA coding for tRNAs and the cytochrome *b* (*cyt b*) gene. Laboratory procedures

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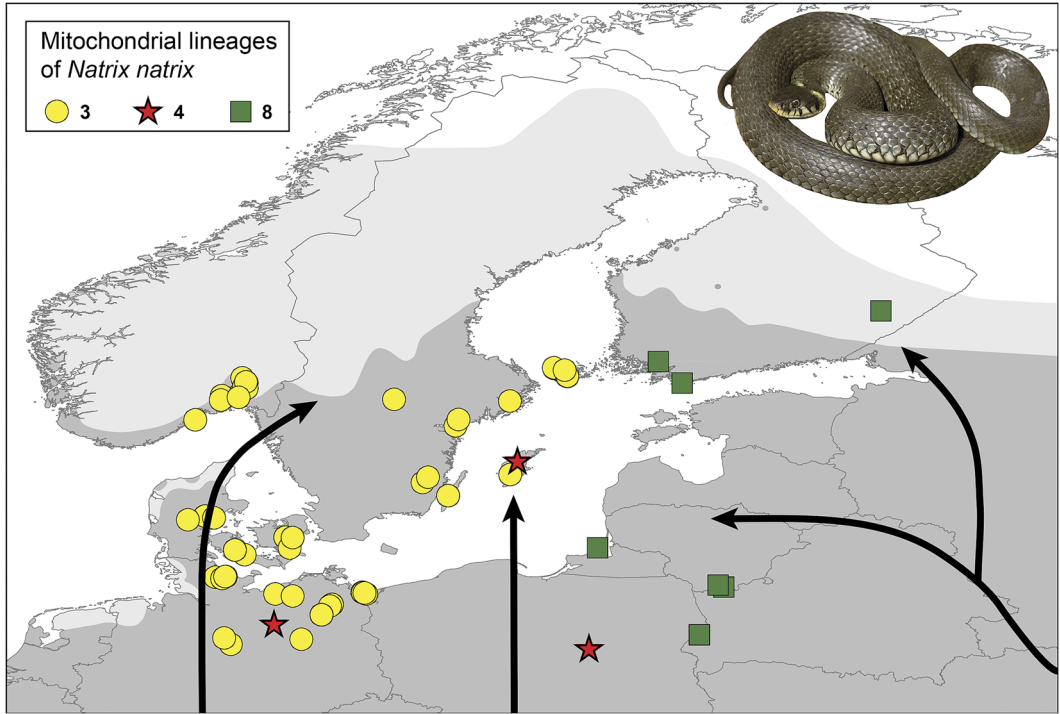


Figure 1. Distribution of mitochondrial lineages of *Natrix natrix* in the Baltic region. Light grey: distribution range of *N. natrix* according to Kabisch (1999); dark grey: distribution range according to Fog et al. (1997). Inset: *N. natrix* from Öland, Sweden (photo: H. Bringsøe). Arrows show putative immigration routes. This figure is published in colour in the online version.

were described in detail by Kindler et al. (2013). The mtDNA sequences containing the partial ND4 gene plus DNA coding for tRNAs varied in length between 688 and 867 bp, and the *cyt b* sequences were between 988 and 1099 bp long. For phylogenetic analyses, both mtDNA fragments were concatenated and merged with sequences from 81 grass snakes from previous studies (Guicking et al., 2006; Fritz, Corti and Päckert, 2012; Kindler et al., 2013), resulting in a 1984-bp-long alignment of 131 *N. natrix* sequences. Homologous sequences of *N. maura*, *N. tessellata* and *Nerodia sipedon* were added as outgroups (for exact locality data and GenBank accession numbers see Appendix 1). The sequences from previous studies included one to three samples of each mitochondrial lineage of *Natrix natrix* and all 47 samples from the Baltic region.

For finding out the optimal partitioning for phylogenetic inference, the alignment was examined using the software PARTITIONFINDER 1.1.1 (Lanfear et al., 2012) and the Bayesian Information Criterion. The following partition schemes were examined: (a) unpartitioned, (b) partitioned by gene with DNA coding for tRNAs merged in one partition, and (c) maximum partitioning, i.e. using each codon of protein-coding genes and the merged tRNAs as a distinct partition. Scheme (c) was selected as the best one and phylogenetic trees were calculated applying this scheme and two different approaches. Using MRBAYES 3.2.1 (Ronquist et

al., 2012) and the best-fit models for each partition (see Appendix 2), two parallel runs were computed, each with four chains. The chains ran for 10 million generations, with every 500th generation sampled. Convergence was verified by examining the standard deviations of split frequencies. For generating the final 50% majority rule consensus, a burn-in of 2.5 million generations was used. In addition, Maximum Likelihood analyses were conducted using RAxML 7.2.8 (Stamatakis, 2006) and the default GTR + G model for each partition. Five independent ML searches were run with different starting conditions and the fast bootstrap algorithm to explore the robustness of the branching patterns by comparing the best trees. Then, 1000 nonparametric thorough bootstrap values were calculated and plotted against the best tree.

Maximum Likelihood and Bayesian analyses yielded identical tree topologies with well-supported branching patterns, except for some deeper nodes (fig. 2). The trees reflect the 16 mitochondrial clades or lineages of Kindler et al. (2013). Our 50 new samples represent lineage 3 (47 samples) and lineage 8 (3 samples) of Kindler et al. (2013). These two lineages were

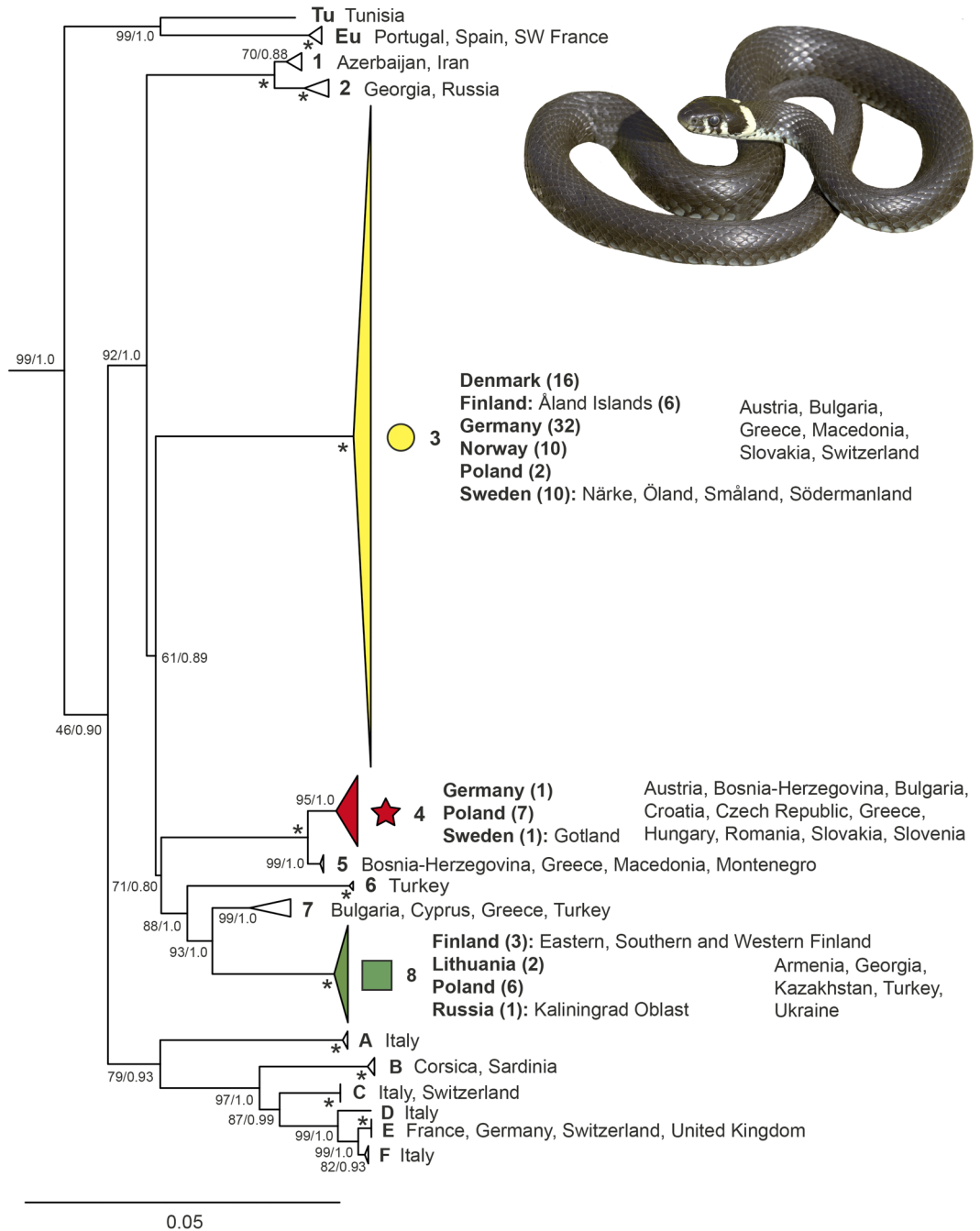


Figure 2. Mitochondrial phylogeny of grass snakes inferred by Maximum Likelihood analyses using 1984-bp mtDNA (ND4+tRNAs, cyt b) of 131 samples of *Natrix natrix*. Terminal clades collapsed to cartoons. Outgroups (*N. maura*, *N. tessellata*, *Nerodia sipedon*) removed for clarity. Numbers along nodes indicate branch support under Maximum Likelihood (1000 bootstrap replicates) and Bayesian analyses (posterior probabilities). Asterisks indicate maximum support under both methods. Clade symbols correspond to fig. 1. Countries indicate distribution range of lineages (Kindler et al., 2013). Samples from the Baltic region in bold (number of studied samples in brackets). Inset: *N. natrix* from eastern Zealand, Denmark (photo: H. Bringsøe). This figure is published in colour in the online version.

already known from the Baltic region. However, with three records in the southern Finnish mainland (fig. 1), lineage 8 was discovered for the first time for Fennoscandia. Previously, this lineage was in the Baltic region only known from Lithuania, eastern Poland and Russia (Kindler et al., 2013). Lineage 3 is widely distributed in Denmark, Norway, Sweden (including Gotland) and on the Finnish Åland Islands (fig. 1). These regions are inhabited exclusively by snakes harbouring haplotypes of lineage 3, except Gotland, from where Kindler et al. (2013) reported also lineage 4. Lineage 3 has also a wide distribution in northern Germany (and probably Poland), while lineage 4 seems to be rare in the Baltic region, with only one record in northern Germany and another one in central Poland. Thus, there is evidence for a triple Holocene invasion of the Baltic region and Fennoscandia (fig. 1).

It has been suggested that lineages 3 and 4 survived the last glacial in refuges on the Balkan Peninsula (Kindler et al., 2013), from whence they spread northwards. For terrestrial species, it was easy in the early Holocene to reach Fennoscandia from the south, because Sweden and Central Europe were connected via Denmark (Björck, 1995). However, in contrast to mammals (e.g. Sommer et al., 2008, 2009a, 2011) or the European pond turtle (Sommer et al., 2009b), the fossil record is not helpful for determining the arrival date of grass snakes in Fennoscandia. There are only few Holocene records in Denmark and Sweden. One find from 6310 ± 105 to 5180 ± 95 years before present was discovered in northern Zealand, Denmark (Aaris-Sørensen, 1980). Further records from Denmark and Sweden date to the Atlantic period (approximately 9200 to 5800 years before present; Wieseke, 2007). Yet, the absence of lineage 4 in Denmark, Norway and Sweden (except Gotland) and its rareness in northern Germany and Poland suggests that lineage 3 had reached the Baltic region first and that lineage 4 arrived later. Obviously, lineage 4 was either not able to invade southern Scandinavia or to estab-

lish there because the habitats were already occupied by grass snakes harbouring haplotypes of lineage 3.

Lineage 4 was more successful on Gotland. This island started to emerge out of the Baltic Ice Lake 10 300 years ago. It was never connected by land bridges to the Scandinavian Peninsula or the southern Baltic coast (Björck, 1995). Thus, *N. natrix* must have reached Gotland either by natural or human-mediated overseas dispersal. For natural overseas dispersal speaks that a similar case is known from the closely related dice snake (*N. tessellata*). It lives in the Black Sea on the famous 'snake island' (Ukraine), 40 km off the Danube estuary (Mertens, 1957). However, it is also possible that grass snakes were introduced to Gotland, either by chance or deliberately, and both options are not mutually exclusive. That human activity could play a role is underlined by the recently reported accidental introductions of grass snakes to the German island of Sylt (Böhme and Grell, 2013).

In any case, grass snakes harbouring haplotypes of lineage 4 must have arrived on Gotland from the south, because this lineage is lacking in Denmark and mainland Sweden. The geographic origin of the Gotland snakes harbouring lineage 3, widely distributed in Scandinavia and along the south coast of the Baltic Sea, cannot be inferred.

The discovery of lineage 8 in Finland provides evidence that another colonization wave reached Fennoscandia from the east, repeating a phylogeographic pattern known from other species which invaded Fennoscandia from the south and east (*Vipera berus*: Ursenbacher et al., 2006; *Sorex araneus*: Fredga and Nawrin, 1977; *Castor fiber*: Horn et al., 2014; *Microtus agrestis*: Jaarola and Tegelström, 1995; *Microtus oeconomus*: Brunhoff et al., 2003; *Myodes glareolus*: Tegelström, 1987; *Meles meles*: Marmi et al., 2006; *Ursus arctos*: Taberlet et al., 1995). Lineage 8 is thought to have spread from a glacial refuge in the Caucasus region (Kindler et al., 2013).

In some of the other species, distinct genetic lineages established contact zones in Fennoscandia (Taberlet et al., 1998), and this could be also true for *N. natrix*. For *Vipera berus*, such a contact zone has been inferred for northeastern Fennoscandia (Urnenbacher et al., 2006). For beavers (Horn et al., 2014), brown bears (Taberlet et al., 1995), root and field voles (Jaarola and Tegelström, 1995; Brunhoff et al., 2003) and common shrews (Fredga and Nawrin, 1977), the contact zones are located further southwestwards, in central Norway and central Sweden. However, with respect to the grass snake, it is unclear whether such a contact zone exists at all. Many old grass snake records from central Norway are most probably wrong or refer to confusions with *V. berus* (D. Dolmen, pers. comm. 2014), and many northern records in Sweden and Finland are doubtful as well (cf. Fog et al., 1997 and Kabisch, 1999). Thus, the Fennoscandian ranges of grass snakes harbouring haplotypes of lineages 3 and 8 could be fully allopatric (fig. 1).

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Appendix 1. Used samples with GenBank accession numbers. Names of mitochondrial lineages follow Kindler et al. (2013). Abbreviations for vouchers: MNCN – Museo Nacional de Ciencias Naturales, Madrid; MTD – Museo für Tierkunde, Senckenberg Dresden; MZUF – Museo di Storia Naturale dell’Università di Firenze, Sezione di Zoologia “La Specola”; ROM – Royal Ontario Museum, Department of Natural History, Toronto, Ontario; ZFMK – Zoologisches Forschungsmuseum Alexander Koenig; ZMB – Museum für Naturkunde, Berlin; ZMUO – Zoologisk museum, Universitetet i Oslo.

Voucher	Collecting site	Accession numbers			Reference
		Clade	ND4+tRNAs	cyt <i>b</i>	
Samples from study regions:					
MTD T 9652	Denmark: Funen: NNW Svendborg	3	HF679697	HF680000	Kindler et al. (2013)
MTD T 9653	Denmark: Funen: NNW Svendborg	3	HF679698	HF680001	Kindler et al. (2013)
MTD T 9654	Denmark: Funen: NNW Svendborg	3	HF679699	HF680002	Kindler et al. (2013)
MTD T 9655	Denmark: Funen: NNW Svendborg	3	HF679700	HF680003	Kindler et al. (2013)
–	Denmark: Jutland	3	AY873712	AY866539	Guicking et al. (2006)
MTD T 9913	Denmark: Jutland: SE Gammel Rye	3	HF679701	HF680004	Kindler et al. (2013)
MTD T 9914	Denmark: Jutland: SE Gammel Rye	3	HF679702	HF680005	Kindler et al. (2013)
MTD T 9915	Denmark: Jutland: SE Gammel Rye	3	HF679703	HF680006	Kindler et al. (2013)
MTD T 9916	Denmark: Jutland: SE Skanderborg	3	HF679704	HF680007	Kindler et al. (2013)
MTD T 9651	Denmark: Langeland: S Tranekær	3	HF679705	HF680008	Kindler et al. (2013)
MTD T 10918	Denmark: Zealand: 6 km W Køge	3	LL999848	LL999898	This study
MTD T 9649	Denmark: Zealand: N Præstø; Feddet	3	HF679706	HF680009	Kindler et al. (2013)
MTD T 9269	Denmark: Zealand: S Borup	3	HF679707	HF680010	Kindler et al. (2013)
MTD T 9270	Denmark: Zealand: S Borup	3	HF679708	HF680011	Kindler et al. (2013)
MTD T 9271	Denmark: Zealand: S Borup	3	HF679709	–	Kindler et al. (2013)
MTD T 9648	Denmark: Zealand: W Køge	3	HF679710	HF680012	Kindler et al. (2013)
MTD T 11583	Finland: Åland	3	LL999849	LL999899	This study
MTD T 11584	Finland: Åland	3	LL999850	LL999900	This study
MTD T 11586	Finland: Åland	3	LL999851	LL999901	This study
MTD T 11589	Finland: Åland: Finström	3	LL999852	LL999902	This study
MTD T 11587	Finland: Åland: Lemland: Bergö	3	LL999853	LL999903	This study
MTD T 11588	Finland: Åland: Lemland: Nätö	3	LL999854	LL999904	This study
MTD T 11591	Finland: Eastern Finland: Savonlinna: Hernemäki	8	LL999855	LL999905	This study
MTD T 11585	Finland: Southern Finland: Hanko	8	LL999856	LL999906	This study
MTD T 11590	Finland: Western Finland: near Kaarina	8	LL999857	LL999907	This study
MTD D 29503	Germany: Brandenburg: Cumlosen	4	HF679715	HF680017	Kindler et al. (2013)

Appendix 1. (Continued.)

Voucher	Collecting site	Clade	Accession numbers		Reference
			ND4+IRNAs	cyt <i>b</i>	
ZMB 66570	Germany: Brandenburg: Nennhausen: Spolierenberg near Bammie	3	LL999858	LL999908	This study
ZFMK 86134	Germany: Lower Saxony: Lachendorf	3	HF679722	HF680025	Kindler et al. (2013)
ZFMK 89088	Germany: Lower Saxony: Leiferde	3	HF679723	HF680026	Kindler et al. (2013)
ZFMK 61035	Germany: Mecklenburg-Western Pomerania: Güstrow	3	–	HF680027	Kindler et al. (2013)
ZFMK 47435	Germany: Mecklenburg-Western Pomerania: Neukloster	3	HF679724	–	This study
MTD T 11465	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999859	LL999909	This study
MTD T 11466	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999860	LL999910	This study
MTD T 11467	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999861	LL999911	This study
MTD T 11468	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999862	LL999912	This study
MTD T 11469	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999863	LL999913	This study
MTD T 11470	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999864	LL999914	This study
MTD T 11471	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999865	LL999915	This study
MTD T 11472	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999866	LL999916	This study
MTD T 11473	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999867	LL999917	This study
MTD T 11474	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999868	LL999918	This study
MTD T 11476	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999869	LL999919	This study
MTD T 11477	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999870	LL999920	This study
MTD T 11478	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999871	LL999921	This study
MTD T 11479	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999872	LL999922	This study
MTD T 11480	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999873	LL999923	This study
MTD T 11481	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999874	LL999924	This study
MTD T 11482	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999875	LL999925	This study
MTD T 11462	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	3	LL999876	LL999926	This study
MTD T 11463	Germany: Mecklenburg-Western Pomerania: Neubrandenburg	3	LL999877	LL999927	This study
MTD T 11464	Germany: Mecklenburg-Western Pomerania: Neubrandenburg	3	LL999878	LL999928	This study
MTD T 11475	Germany: Mecklenburg-Western Pomerania: Neubrandenburg: Lake Tollense	3	LL999879	LL999929	This study
MTD T 11483	Germany: Mecklenburg-Western Pomerania: Usedom	3	LL999880	LL999930	This study
MTD T 11484	Germany: Mecklenburg-Western Pomerania: Usedom	3	LL999881	LL999931	This study
ZFMK 62405	Germany: Schleswig-Holstein: Kiel: Landwehr	3	HF679759	HF680066	Kindler et al. (2013)
ZFMK 85184	Germany: Schleswig-Holstein: Kiel-Elmschenhagen	3	HF679760	HF680067	Kindler et al. (2013)

Appendix 1. (Continued.)

Voucher	Collecting site	Clade	Accession numbers		Reference
			ND4+tRNAs	cyt <i>b</i>	
ZFMK 92535	Germany: Schleswig-Holstein: Probstei: Hagener Moor	3	HF679761	HF680068	Kindler et al. (2013)
ZFMK 73639	Germany: Schleswig-Holstein: Probsteierhagen	3	HF679762	HF680069	Kindler et al. (2013)
MTD T 8967	Lithuania: Marcinkonys	8	HF679772	HF680078	Kindler et al. (2013)
MTD T 8968	Lithuania: Trasninkas	8	HF679773	HF680079	Kindler et al. (2013)
MTD T 11568	Norway: Akershus: Ski	3	LL999932	LL999932	This study
MTD T 11569	Norway: Akershus: Ski	3	LL999883	LL999933	This study
ZMUO 54-96	Norway: Akershus: Son	3	HF679774	HF680080	Kindler et al. (2013)
MTD T 11567	Norway: Andebu: Ilesiadvannet	3	LL999884	LL999934	This study
MTD T 11566	Norway: Holmestrand: Hallingsrud	3	LL999885	LL999935	This study
ZMUO 31-83	Norway: Oslo: Grønmo: Sølvdobla	3	HF679775	HF680081	Kindler et al. (2013)
ZMUO 26-85	Norway: Oslo: Maridalen: Skjervensaga	3	HF679776	HF680082	Kindler et al. (2013)
MTD T 11571	Norway: Østfold: Moss	3	LL999886	LL999936	This study
MTD T 11570	Norway: southern Oslo	3	LL999887	LL999937	This study
ZMUO IH 10-2000	Norway: Telemark: Levangshalvøya	3	HF679777	HF680083	Kindler et al. (2013)
MTD T 10318	Poland: Mazovia: Kampinos National Park	4	HF679784	HF680087	Kindler et al. (2013)
MTD T 9965	Poland: Mazovia: Kampinos National Park	4	HF679778	-	Kindler et al. (2013)
MTD T 9966	Poland: Mazovia: Kampinos National Park	4	HF679779	-	Kindler et al. (2013)
MTD T 9968	Poland: Mazovia: Kampinos National Park	4	HF679780	HF680084	Kindler et al. (2013)
MTD T 9969	Poland: Mazovia: Kampinos National Park	4	HF679781	HF680085	Kindler et al. (2013)
MTD T 9972	Poland: Mazovia: Kampinos National Park	4	HF679782	HF680086	Kindler et al. (2013)
MTD T 9974	Poland: Mazovia: Kampinos National Park	4	HF679783	-	Kindler et al. (2013)
MTD T 9975	Poland: Podlacia: Białowieża National Park	8	HF679785	-	Kindler et al. (2013)
MTD T 9976	Poland: Podlacia: Białowieża National Park	8	HF679786	HF680088	Kindler et al. (2013)
MTD T 9977	Poland: Podlacia: Białowieża National Park	8	HF679787	HF680089	Kindler et al. (2013)
MTD T 10314	Poland: Podlacia: Białowieża National Park	8	-	HF680090	Kindler et al. (2013)
MTD T 10315	Poland: Podlacia: Białowieża National Park	8	HF679788	HF680091	Kindler et al. (2013)
MTD T 10316	Poland: Podlacia: Białowieża National Park	8	HF679789	HF680092	Kindler et al. (2013)
MTD T 11485	Poland: Usedom	3	LL999888	LL999938	This study

Appendix I. (Continued.)

Voucher	Collecting site	Clade	ND4+ rRNAs	Accession numbers		Reference
				cyt <i>b</i>		
MTD T 11486	Poland: Usedom	3	LL999889	LL99939	This study	
–	Russia: Kaliningrad Oblast: Rybachy	8	AY487793	AY487741	Guicking et al. (2006)	
ZFMK 36114	Sweden: Gotland	3	HF679605	HF679926	Kindler et al. (2013)	
ZFMK 38356	Sweden: Gotland	4	HF679606	HF679927	Kindler et al. (2013)	
MTD T 11582	Sweden: Närke: Klockhammar	3	LL999890	LL999940	This study	
MTD T 10920	Sweden: Öland: Halltorps Hage	3	LL999891	LL999941	This study	
MTD T 10921	Sweden: Öland: Halltorps Hage	3	LL999892	LL999942	This study	
MTD T 10922	Sweden: Öland: Halltorps Hage	3	LL999893	LL999943	This study	
–	Sweden: Småland: Högsby	3	AY487799	AY487755	Guicking et al. (2006)	
MTD T 10919	Sweden: Småland: Kråksmåla	3	LL999894	LL999944	This study	
MTD T 11580	Sweden: Södermanland: near Boo	3	LL999895	LL999945	This study	
MTD T 11579	Sweden: Södermanland: near Nyköping	3	LL999896	LL999946	This study	
MTD T 11581	Sweden: Södermanland: near Nyköping	3	LL999897	LL999947	This study	
Samples from other regions:						
ZFMK 67196	Tunisia: Cap Serrat	Tu	HF679598	HF679918	Kindler et al. (2013)	
ZFMK 87516	Portugal: S Setúbal: Torre	Eu	HF679591	HF679908	Kindler et al. (2013)	
MTD T 9650	Spain: Aragón: Nerín	Eu	HF679593	HF679912	Kindler et al. (2013)	
ZFMK 60734	Spain: Catalonia: Elbro Delta	Eu	HF679596	HF679916	Kindler et al. (2013)	
MTD T 8956	Azerbaijan: Calilabad	1	HF679821	HF680120	Kindler et al. (2013)	
MTD T 3680	Azerbaijan: Istisu: near Astara	1	–	HF680121	Kindler et al. (2013)	
MTD T 8954	Iran: Nowshahr	1	HF679880	HF680176	Kindler et al. (2013)	
MTD T 9338	Georgia: Sakdrioni	2	HF679856	HF680156	Kindler et al. (2013)	
MTD T 8959	Georgia: Telavi	2	HF679858	HF680158	Kindler et al. (2013)	
MTD T 8958	Georgia: Telavi	2	HF679857	HF680157	Kindler et al. (2013)	
MTD T 8644	Bosnia and Herzegovina: Hutovo Blato	5	HF679822	HF680123	Kindler et al. (2013)	
MTD T 8645	Bosnia and Herzegovina: Hutovo Blato	5	HF679823	HF680124	Kindler et al. (2013)	

Appendix 1. (Continued.)

Voucher	Collecting site	Clade	Accession numbers		Reference
			ND4+rRNAs	cyt <i>b</i>	
MTD T 8633	Former Yugoslav Republic of Macedonia: Stenje	5	HF679847	HF680146	Kindler et al. (2013)
ZFMK 71176	Turkey: Mediterranean Region: Burmaz (near Botas)	6	HF679903	HF680201	Kindler et al. (2013)
–	Turkey: Mediterranean Region: SW Osmaniye	6	KC570263	KC570308	Kindler et al. (2013)
MTD T 9319	Bulgaria: Ropotamo	7	HF679835	HF680137	Kindler et al. (2013)
MTD T 9321	Bulgaria: Malko Gradšte (pass)	7	HF679830	HF680131	Kindler et al. (2013)
ZFMK 76753	Cyprus: Larnaka: Paralimni	7	HF679604	HF679922	Kindler et al. (2013)
MTD T 2116	Italy: Sicily: Agrigento: Siculiana: Biosphere Reserve Torre Salsa	A	HF679901	–	Kindler et al. (2013)
MTD T 8464	Italy: Sicily: Siracusa: Vendicari	A	HF679902	–	Kindler et al. (2013)
–	Italy: Calabria: Serro San Bruno	A	KC570265	HF680200	Kindler et al. (2013)
MTD D 35388	France: Corsica: Porto-Vecchio	A	KC570265	KC570305	Kindler et al. (2013)
MTD D 42489	France: Corsica: Santa Giulia	B	HE584623	HE584624	Fritz et al. (2012)
ZFMK 60737	Italy: Sardinia: Limbara Mountains	B	HE584627	HE584628	Fritz et al. (2012)
MTD T 9656	Italy: Trentino: Vela	B	HF679600	HF679920	Kindler et al. (2013)
–	Italy: Venezia: NW Asiago	C	HF679619	HF679936	Kindler et al. (2013)
–	Switzerland: Ticino: Astano	C	KC570255	KC570299	Kindler et al. (2013)
–	Italy: Apulia: Torre San Gennaro (Brindisi)	C	AY487795	AY487751	Guicking et al. (2006)
ZFMK 70420	Germany: North Rhine-Westphalia: between Hürtgenwald and Gey	D	AY873715	AY487733	Guicking et al. (2006)
ZFMK 92536	Germany: North Rhine-Westphalia: Bonn	E	HF679636	HF679944	Kindler et al. (2013)
ZFMK 92193	Germany: North Rhine-Westphalia: Bad Münstereifel	E	HF679639	HF679946	Kindler et al. (2013)
ZFMK 90574	Italy: Calabria	E	HF679634	HF679943	Kindler et al. (2013)
MZUF 31620	Italy: Lazio: Monti della Tolfa	F	HF679900	HF680198	Kindler et al. (2013)
MZUF 39870	Italy: Tuscany: Florence	F	HF679654	HF679959	Kindler et al. (2013)
–	–	F	HF679656	HF679961	Kindler et al. (2013)
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

Appendix 2. Evolutionary models for MRBAYES calculated with PARTITIONFINDER.

Gene	Partition	Evolutionary model
ND4	1-696:	
	Codon position 1	GTR + G
	Codon position 2	F81 + I
tRNAs	Codon position 3	GTR
	697-867	K80 + I
cyt <i>b</i>	868-1984:	
	Codon position 1	HKY + G
	Codon position 2	HKY + I
	Codon position 3	GTR + G