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Unexpected early extinction of the European pond turtle (*Emys orbicularis*) in Sweden and climatic impact on its Holocene range

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Abstract

Using ancient DNA sequences of subfossil European pond turtles (*Emys orbicularis*) from Britain, Central and North Europe and accelerator mass spectrometry radiocarbon dating for turtle remains from most Swedish sites, we provide evidence for a Holocene range expansion of the pond turtle from the southeastern Balkans into Britain, Central Europe and Scandinavia, according to the 'grasshopper pattern' of Hewitt. Northeastern Europe and adjacent Asia were colonized from another refuge located further east. With increasing annual mean temperatures, pond turtles reached southern Sweden approximately 9800 years ago. Until approximately 5500 years ago, rising temperatures facilitated a further range expansion up to Östergötland, Sweden (approximately 58°30′N). However, around 5500 years ago pond turtle records suddenly terminate in Sweden, some 1500 years before the Holocene thermal maximum ended in Scandinavia and distinctly earlier than previously thought. This extinction coincides with a temporary cooling oscillation during the Holocene thermal maximum and is likely related to lower summer temperatures deteriorating reproductive success. Although climatic conditions improved later again, recolonization of Sweden from southern source populations was prevented by the Holocene submergence of the previous land connection via the Danish Straits that occurred approximately 8500 years ago.

Keywords: ancient DNA, climate, Holocene, phylogeography, radiocarbon dating

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Introduction

During the Late Quaternary, global climate changes strongly affected the biogeography and species diversity in Europe (Hewitt 2000; Stuart *et al*. 2004; Guthrie 2006). In the last glacial, thermophilous species were forced to retreat to southern refugia from where more northerly parts of Europe were recolonized with Holocene warming (Hewitt 2000, 2004; Schmitt 2007). Modelling and predicting such climate-triggered range fluctuations (e.g. Sala *et al*. 2000; Phillips *et al*. 2006) is of particular importance for the current debate on the impact of climate change on biodiversity (e.g. Thomas *et al*. 2004). Within this framework, data on Holocene, postglacial, recolonization of central and northern Europe yielded important insights (e.g. Bennett *et al*. 1991; Taberlet & Bouvet 1994; Petit *et al*. 2003; Hewitt 2004; Joger *et al*. 2007; Schmitt 2007; Valdiosera *et al*. 2007). However, at the continental scale the dynamics of changing environments often appear too complex to easily disentangle the reasons for range expansion and extinction (e.g. Barnosky *et al*.

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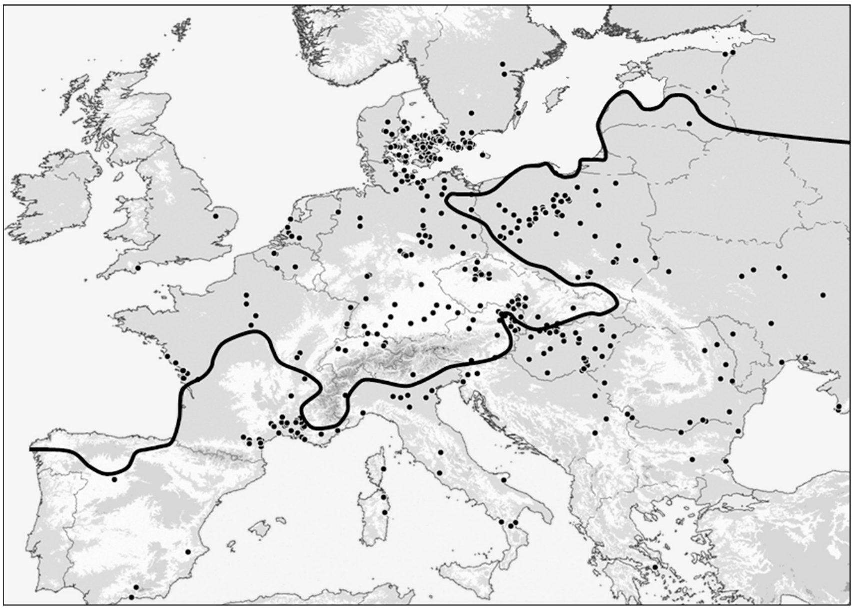

Fig. 1 Holocene subfossil records of *Emys orbicularis* in Europe (black circles). Line indicates northern range border of extant native populations (Sommer *et al*. 2007).

2004; Pastor & Moen 2004; Stuart *et al*. 2004; Guthrie 2006), a situation caused in part by the lack of detailed spatio-temporal data.

The European pond turtle, *Emys orbicularis* (L., 1758), is the sole chelonian species in the northern parts of the Western Palaearctic. The species is thermophilous and temperature sensitive, and its distribution and long-term survival clearly correlates with climate (Kurck 1917; Isberg 1929; Degerbøl & Krog 1951; Stuart 1979; Fritz 1996, 2001, 2003; Schneeweiß 2004; Sommer *et al*. 2007). Key factors for survival of northern populations are sufficient summer temperatures and insolation rates, enabling successful incubation of clutches (Schneeweiß 2004). The turtle's current range is only a remnant of a much wider Holocene distribution. More than 500 finds demonstrate its earlier occurrence in many northern and Central European regions from which it is absent today, for instance Great Britain and Scandinavia (Fritz 1996; Sommer *et al*. 2007; Fig. 1). These Holocene records attest to a rapid post-glacial range expansion that was favoured by significantly increased mean temperatures during the Early and Middle Holocene (Kurck 1917; Isberg 1929; Degerbøl & Krog 1951; Stuart 1979; Sommer *et al*. 2007). It has been put forward that the European pond turtle entered the northern European lowlands approximately 11 100–10 800 years ago, when the temperature reached a level similar to today, and that the species arrived in coastal northern Germany some 10 000 years ago (Sommer *et al*. 2007), when annual mean temperatures exceeded current values (Kaufman *et al*. 2004).

At present, the northernmost Central European relic populations occur in northeastern Germany (Brandenburg) and Poland (Fritz 1996, 2001, 2003; Fritz *et al*. 2004). While central and eastern Polish pond turtles harbour a haplotype belonging to a mitochondrial lineage distributed all around the Black Sea and north of the Caspian Sea (lineage I), the haplotype of German and western Polish turtles represents a distinct lineage with a patchy range in the Danube Basin, the southeastern Balkans, France, and northern Spain (lineage II). These mitochondrial data suggest range expansions of *E. orbicularis* from two distinct glacial refuges, one located in the southeastern Balkans and the other further east in the Black Sea region (Lenk *et al*. 1999; Fritz *et al*. 2007). Until now, it is unknown whether the extinct Scandinavian and British pond turtles have immigrated from the southern Balkans or from the second, more easterly refuge.

In the current paper, we aim at (i) tracking the species' Holocene immigration route from glacial refugia into Scandinavia and Britain through ancient DNA analyses using 16 subfossil specimens from England, Central and northern Europe; and (ii) assessing the spatio-temporal range dynamics of Scandinavian pond turtles by accelerator mass spectrometry (AMS) radiocarbon dating of 35 Holocene bone fragments, representing 25 out of the 29 known Swedish sites for *E. orbicularis*.

Materials and methods

Sampling

Sixteen subfossil specimens of *Emys orbicularis* (Table 1) were used for ancient DNA analyses in order to assign their mitochondrial haplotype to previously characterized lineages (Lenk *et al*. 1999; Fritz *et al*. 2007). Furthermore, the northernmost Holocene pond turtle records in Scandinavia were comprehensively sampled. Thirty-five subfossil specimens from 25 localities in southern Sweden were subjected to AMS radiocarbon dating (Fig. 2; Table 2).

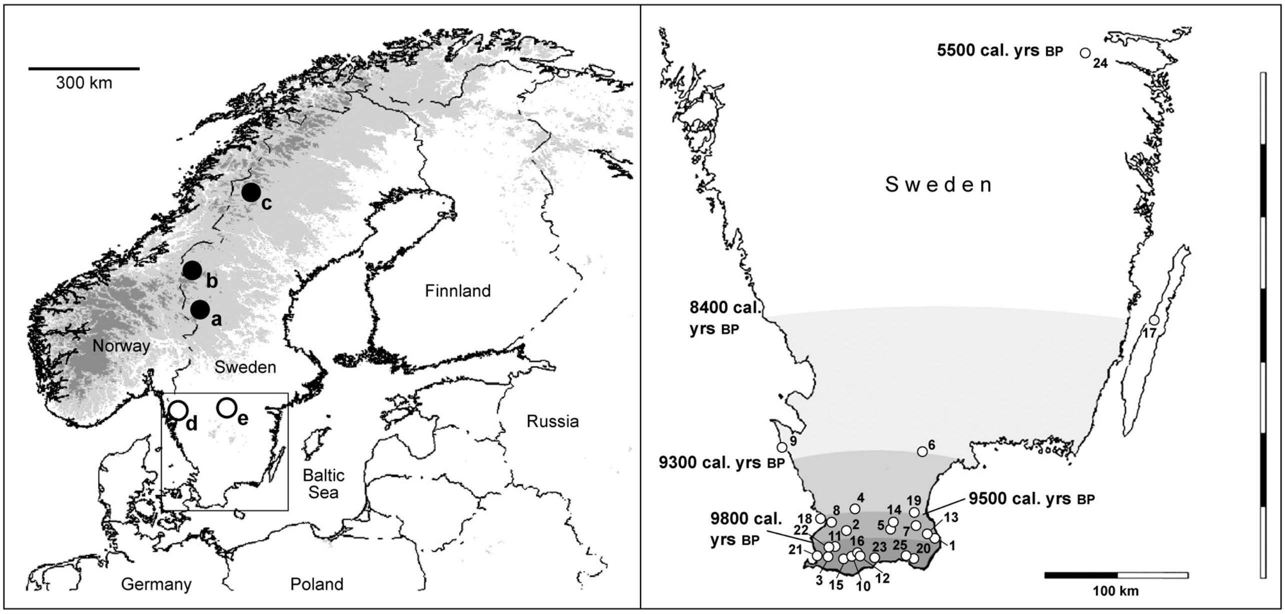

Fig. 2 Left, sampling sites of proxy data for palaeoclimatic reconstruction. Black circles indicate sampling sites for radiocarbon dates of pines from Scandes Mountains (Kullman & Kjällgren 2006): province of Dalarna (a), province of Jämtland (b), and province of Västerbotten (c). Open circles indicate lakes of Trehörningen (d) and Flarken (e) that were sampled for pollen-based reconstruction of annual mean temperature (Antonsson & Seppä 2007). Box corresponds to enlarged map on the right, collection sites of AMS radiocarbon-dated subfossil bone samples of *Emys orbicularis*. Site numbers refer to Table 2. Shading indicates chronologically successive northern range borders at 9800, 9500, 9300, and 8400 cal. yrs BP. The northernmost record (24) dates to 5500 cal. yrs BP (for further details, see text and Table 2).

Table 1 Subfossil *Emys orbicularis* samples used for genetic analyses. Locality numbers refer to Fig. 4. Haplotype IIa, identified from a Danish, German and Swedish subfossil turtle each, survives today only in France and the southern parts of the distribution area of lineage II. Extant pond turtles from the east German and west Polish relic population exclusively harbour haplotype IIb (Fig. 4)

			Successfully amplified fragment				
Coll. no./Sample ID	Locality (country code)	Period	1	2	3	Mitochondrial lineage	Haplotype
CUMZ R3467†	1 — East Wretham (GB)	Atlantic	+	–	–	II	a or b
NMB 5563x423‡	2 — Agernæs, Fyn (DK)	Atlantic	+	+	+	II	a
LMR 9747:14§	3 — Ristinge Nor, Langeland (DK)	?	+	+	+	II	b
LZZ 3750¶	4 — Östraby, Sniberups mosse (S)	Atlantic	–	+	+	II	b
LZZ 3751¶	5 — Sankt Olof, Vitesjö mosse (S)	Atlantic	–	+	+	II	a
LZZ 3792¶	5 — Sankt Olof, Vitesjö mosse (S)	Atlantic	+	+	+	II	b
LZZ 3797¶	6 — Brösarp, Holmana mosse (S)	Atlantic	+	–	–	II	a or b
A 8/88††	7 — Schwieloch Lake near Goyatz (D)	?	+	+	–	II	a or b
H. v. 1165‡‡	8 — Hohen Viecheln (D)	Boreal	-	+	+	II	a
POL-1-01§§	9 — Żuławka Mała (PL)	Bronze Age	+	+	+	I	a
ARUP:DK 50-1/8¶¶	10 — Denemark near Kutná Hora (CZ)	Copper Age	+	–	+	II	a
ARUP:1975-O.148-S.621¶¶	11 — Mlékojedy (CZ)	Copper Age	+	–	+	II	a
NMP 6V 73477†††	12 — Čejč (CZ)	Iron Age	–	+	–	II	a or b
NMP 6V 72798†††	13 — Brankovice (CZ)	Middle Ages	+	+	+	II	a
NMP 6V 73476†††	14 — Šakvice (CZ)	Middle Ages	+	+	+	II	a
ARUP:1975-O.480-S.869¶¶	15 — Dolní Beřkovice (CZ)	Neolithic	–	–	+	II	a

†Cambridge University Museum of Zoology; ‡Nordfyns Museum, Bogense; §Langelands Museum, Rudkøbing; ¶Museum of Zoology of the University of Lund; ††Natural History Museum Cottbus; ‡‡State Office of Archaeology of Mecklenburg-Western Pomerania; §§District Museum Piła; ¶¶Institute of Archaeology, Czech Academy of Sciences; †††National Museum Prague.

Table 2 AMS radiocarbon dates of Holocene subfossil *Emys orbicularis* from Sweden sorted according to age. Locality numbers refer to Fig. 2. The calendaric age (cal. yrs BP) was calculated from raw radiocarbon data (^{14}C yrs BP) using the program CalPal 2007 (Weninger *et al.* 2008). Further explanation may be found under www.calpal.de

Locality	Local description/bog	Coll. no./Sample ID	Dated bone	Laboratory code	^{14}C yrs BP	cal. yrs BP
1 — Smedstorp*	Skräppeboda mosse	LZZ 3809†	plastron	Ua-3278	8780 ± 95	9861 ± 193
2 — Genarp	Genarps mosse	LZZ 3791†	plastron	LuA-4707	8720 ± 90	9755 ± 145
3 — Lilla Slågarp	Mosse vid Haglösa	LZZ 3790†	plastron	LuA-4706	8570 ± 100	9590 ± 96
4 — Revinge*	Revinge mosse	CGB†	plastron	LuA-4705	8560 ± 90	9572 ± 81
5 — Vanstad*	Vanstad mosse	LGM1†	plastron	Ua-2272	8470 ± 100	9438 ± 93
6 — Råbelöv	Torvmosse	—‡	plastron	Ua-2273	8390 ± 130	9341 ± 148
7 — Sankt Olof*	—	ShM 7486§	plastron	Ua-11911	8285 ± 85	9273 ± 125
7 — Sankt Olof**	Vitjesjö mosse	LZZ 3795†	plastron	Ua-11906	8215 ± 95	9204 ± 135
1 — Smedstorp***	Mosse i Pinneboda	LZZ 3738†	plastron	Ua-11913	8195 ± 70	9168 ± 102
8 — Brågarp	Gullåkra mosse	LZZ 3737†	carapace	Ua-11903	8185 ± 80	9165 ± 109
9 — Allerum*	Allerums mosse	LZZ 3807†	plastron	Ua-2267	8110 ± 100	9028 ± 184
9 — Allerum***	Allerums mosse	LZZ 3808†	plastron	Ua-11901	8075 ± 85	8961 ± 154
10 — Östraby*	Sniberups mosse	LZZ 3789†	plastron	Ua-2655	8000 ± 120	8860 ± 168
11 — Svedala	Mosse på Vannmådan	LZZ 3811†	plastron	Ua-3277	7995 ± 73	8850 ± 121
12 — Hassle-Bösarp	Mosse på Hassle-Bösarp	LZZ 3794†	plastron	Ua-11905	7890 ± 75	8769 ± 141
13 — Östra Vemmerlöv	Näbbe mosse	LZZ 3367†	plastron	Ua-11909	7880 ± 75	8759 ± 141
14 — Lövestad	Sjön	LZZ 3798†	shoulder girdle	Ua-11910	7875 ± 75	8753 ± 141
4 — Revinge***	Revinge i kalkgyttjy	LZZ 4515 (105a)†	plastron	Ua-2654	7815 ± 90	8656 ± 142
10 — Östraby***	Sniberups mosse	LZZ 3040†	pelvic bone	Ua-11912	7775 ± 80	8569 ± 94
5 — Vanstad***	Vanstad mosse	LZZ 4512†	carapace	Ua-11914	7745 ± 80	8531 ± 75
15 — Grönby	Öbersjö mosse	LZZ 3787†	pelvic bone	Ua-2270	7620 ± 100	8436 ± 91
16 — Skurup*	Saritslövs mosse	LZZ 3812†	plastron	Ua-1793	7570 ± 120	8371 ± 126
17 — Köping, Öland	Öij mosse	LZZ 3771†	carapace	Ua-1792	7530 ± 150	8338 ± 145
7 — Sankt Olof***	Vitjesjö mosse	LZZ 3796†	plastron	Ua-11907	7465 ± 85	8281 ± 78
18 — Segebro	—	Segebro-Emys1¶	carapace	Ua-3291	7240 ± 110	8076 ± 100
19 — Brösarp	Holmana mosse	LZZ 3797†	plastron	Ua-11904	7085 ± 75	7907 ± 67
16 — Skurup**	Sandåkra, i mosse	LZZ 3753†	plastron	Ua-2269	6520 ± 100	7426 ± 90
20 — Löderup	Hagstad mosse	LZZ 3793†	plastron	Ua-2271	6500 ± 100	7409 ± 89
21 — Fuglie	Mosse inom socknen	LZZ 3772†	plastron	Ua-11902	6130 ± 75	7030 ± 107
22 — Krageholm	—	LZZ 3813†	plastron	Ua-11908	5210 ± 70	6015 ± 94
23 — Sjörup	Södra Vallösa mosse	LYM 1226††	plastron	Ua-2268	5120 ± 110	5872 ± 127
24 — Norsholm*	—	R1744‡‡	carapace	Ua-1794	5080 ± 110	5824 ± 117
24 — Norsholm***	—	R1743 b‡‡	plastron	Ua-1796	4820 ± 100	5530 ± 123
16 — Skurup***	Munkholmens mosse	—§§	plastron	Ua-1795	4780 ± 110	5490 ± 123
25 — Vallby	Glivarps mosse	ShM 2820D§	plastron	Ua-3276	4785 ± 65	5489 ± 94

*Oldest, **second oldest, and ***youngest sample from a particular locality. †Museum of Zoology of the University of Lund; ‡Österängsskolan, Kristianstad; §Museum of Österlen; ¶Museum of Malmö; ††Läroverkets i Ystad Museum; ‡‡Swedish Museum of Natural History Stockholm; §§GeoBiosphere Science Centre of Lund.

Analysis of subfossil DNA

DNA extractions of samples (Table 1) were performed at the Natural History Museum, University of Oslo, in a laboratory dedicated to ancient DNA work that is physically isolated from other DNA processing facilities. No pond turtle samples have been processed earlier in either the ancient DNA laboratory or in the contemporary DNA laboratory. About 50–300 mg of turtle remains were ground to powder and DNA was extracted following established protocols (Höss & Pääbo 1993; Borge *et al.* 2007).

Three short fragments of the mitochondrial cytochrome *b* gene were targeted that have been previously shown to be informative for haplotype discrimination of the two lineages and haplotypes in question. Haplotype nomenclature follows Lenk *et al.* (1999) and Fritz *et al.* (2007) in that mitochondrial lineages are indicated by Roman numerals and individual haplotypes by consecutive letters. Accordingly, the lineage currently distributed from central Poland eastwards all around the Black Sea and north of the Caspian Sea is lineage I; the other lineage with a patchy range in east Germany and west Poland, the Danube Basin, the southeastern Balkans, France, and northern Spain is lineage II. Haplotypes occurring in extant northern populations are Ia, IIa, and IIb (Lenk *et al.* 1999; Fritz *et al.* 2007). Fragment 1 of 85 bp (43 bp informative) includes two positions that

Table 3 Primer pairs used for amplification of aDNA fragments of subfossil bone fragments of *Emys orbicularis*. Positions correspond to the complete cytochrome *b* sequence GenBank Accession no. AF258868 (Feldman & Parham 2002)

Fragment	Length	Primer pair — forward (F)/reverse (R)	Position
1	85 bp	1F 5′-ACTTCTCATACAAAGACCTATT-3′ 1R 5′-CCTAGAAGGTTAGGAGAGAA-3′	671–755
1	99 bp	1Fa 5′-CCATCCCTACTTCTCATACAAAGA-3′ 1Ra 5′-TGGGTCTCCTAGAAGGTTAGG-3′	663–762
2	78 bp	2F 5′-GCCTCTATTCTCCACTTCTCAATC-3′ 2R 5′-TTAAGTTAGTATTTTGTTTTCGAT-3′	1063–1140
3	106 bp	3F 5′-CAGTAGATAACGCAACCCTAACC-3′ 3R 5′-CCGGTTTCGTGTAGGAAGAG-3′	509–614

discriminate mitochondrial lineages I and II. Fragment 1 was amplified either through primer pair 1F and 1R or using the primers 1Fa and 1Ra, targeting a 14-bp longer fragment. Fragment 2 of 78 bp (30 bp informative) includes two further positions that discriminate mitochondrial lineages I and II. It was amplified using primer pair 2F and 2R. Fragment 3 of 106 bp (63 bp informative) includes one position discriminating the haplotypes IIa and IIb and was amplified using primer pair 3F and 3R (Table 3). Polymerase chain reaction (PCR) was carried out for 40 cycles using Pfu polymerase (Stratagene) and an annealing temperature of 48 °C. Amplification products of the correct size were cleaned with exoSAP-IT (USB Corporation) and either sequenced directly on an ABI 3100 capillary sequencer (Applied Biosystems), or cloned using the TOPO TA Cloning kit (Invitrogen), and individual clones sequenced subsequently. Sequencing followed the instructions of the BigDye chemistry (Applied Biosystems). Sequences were edited and aligned to reference sequences using the software Sequencher 4.1.4. (Gene Codes Corporation).

To illustrate mutational relationships of *Emys* haplotypes, we used a previously published data set of 1107 cytochrome *b* sequences of extant pond turtles (Fritz *et al.* 2007) and applied the median joining network reconstruction algorithm (Bandelt *et al.* 1999) as implemented in Network 4.5.0.0 (sparse network: equal weighting, epsilon = 0; http://www.fluxus-engineering.com). In such networks, interiorly located haplotypes, having more than one mutational connection, are thought to be ancestral to and older than tip haplotypes (Posada & Crandall 2001).

AMS ^{14}C dating

The ^{14}C dating was carried out in the AMS laboratories of the universities Uppsala and Lund, Sweden. ^{14}C analyses were performed following standard protocols for dating bone samples by collagen extraction and AMS determination of the ^{14}C content with the Uppsala 6MV Tandem van de Graaf Accelerator System. A standard HCl-gelatine pretreatment procedure (Taylor 1983) was applied since the samples were of high quality (plastic, light coloured, and released a distinct organic smell upon drilling) and well preserved. The surface of small pieces of the bone samples was mechanically (scraping, and in some cases sand blasting) and ultrasonically cleaned in boiled distilled water, adjusted to pH = 3.0, followed by grinding in a mortar. For removal of the apatite fraction, 0.8 M HCl was added and the sample stirred at 10 °C for 30 min. Distilled water adjusted to pH = 3.0 was added to the insoluble fraction, which was subsequently stirred for 6–8 h at 90 °C. Since the soluble fraction contains most of the organic part (the collagen fraction) of the original sample material, this was used for AMS ^{14}C dating. The fraction to be ^{14}C-dated was combusted by CuO at 800 °C for 10 min to CO_2, and then converted to graphite using an iron-catalyst reaction with hydrogen.

Relation to palaeoclimatic record

Radiocarbon dating measures the decay of the naturally occurring radioisotope ^{14}C. The resulting raw data are reported in '^{14}C years before present' (^{14}C yrs BP), corresponding to the number of so-called radiocarbon years before 1950. These raw data are based on the content of ^{14}C in the atmosphere equal to the 1950 level. Because of fluctuations of the level of atmospheric ^{14}C over time, such raw data deviate, in part considerably, from calendaric dates and need to be calibrated against standard curves that are obtained using other proxies.

Our AMS raw data of Swedish *E. orbicularis* were calibrated and plotted against the oxygen isotope ratios $\delta^{18}O$ (‰) from Greenland ice core data (Stuiver *et al.* 1995) using the CalPal 2007 software (Weninger *et al.* 2008; Table 2). The obtained calibrated data (calibrated years BP = cal. yrs BP; present = AD 1950) were then related with pollen-based reconstructed annual mean temperatures from the lakes of Trehörningen and Flarken, southern Sweden (Antonsson & Seppä 2007), as well as with the uppermost pine samples from the Scandes Mountains, Swedish provinces of Dalarna, Jämtland, and Västerbotten relative to the elevation of the pine limit at the end of the 19th century (Kullman & Kjällgren 2006). The sampling sites of the proxy data for palaeoclimatic reconstruction are depicted in Fig. 2, the relation of pond turtle finds and proxy data in Fig. 3.

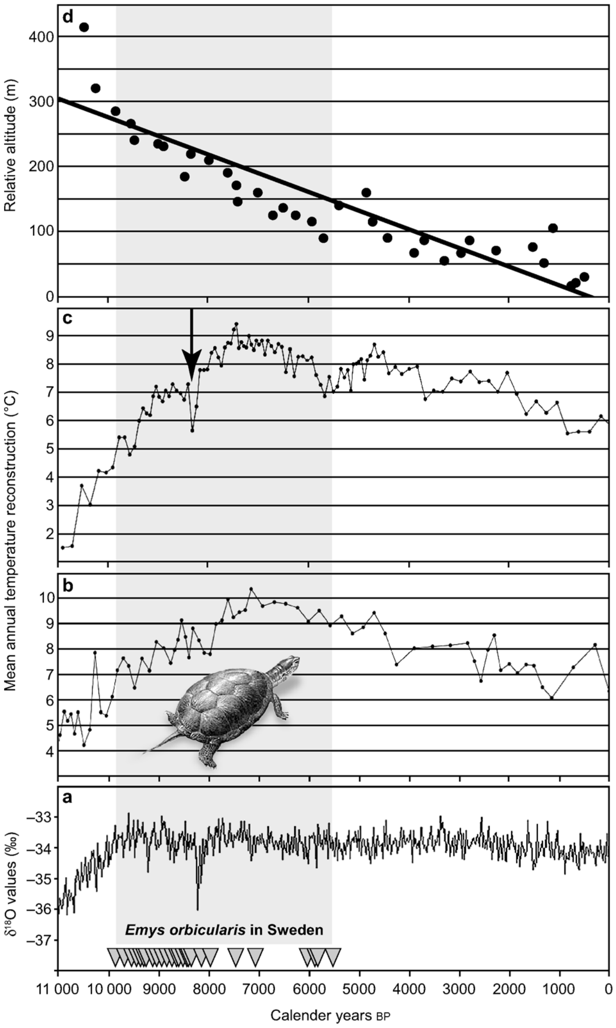

Fig. 3 Radiocarbon-dated subfossil *Emys orbicularis* from Sweden (shaded; approximately 9800–5500 cal. yrs BP) related to palaeoclimatic proxies from northern Europe. (a) Calibrated radiocarbon dates of pond turtle records (grey triangles) and oxygen isotope ratios $\delta^{18}O$ (‰) from the GRIP ice core record (Stuiver *et al.* 1995); pollen-based reconstructed annual mean temperature from the lakes of Trehörningen (b) and Flarken (c) in southern Sweden (Antonsson & Seppä 2007); (d) uppermost pine samples from the Scandes Mountains, Swedish provinces of Dalarna, Jämtland, and Västerbotten relative to the elevation of the pine limit at the end of the 19th century in metres (zero line, data adjusted to allow for glacio-isostatic land uplift; Kullman & Kjällgren 2006). For sampling sites of literature data, see Fig. 2. Arrow indicates 8.2 ka event (see Discussion).

Results

Subfossil DNA data

One to three of the targeted cytochrome *b* regions were successfully amplified and sequenced for our 16 subfossil specimens of *Emys orbicularis* (Table 1). The obtained sequences allowed for all specimens an unambiguous assignment to a mitochondrial lineage (Tables 1 and 4). Only sample POL-1-01 from western central Poland yielded a haplotype belonging to lineage I (Ia); all other 15 samples bore haplotypes of lineage II. Out of these samples, eight were identified as haplotype IIa and three as haplotype IIb; for four samples further specification of the haplotype was not possible.

In the median-joining network, haplotype Ia is separated by six mutational steps from haplotype IIa; haplotype IIb is a tip haplotype and differs by one mutational step from its ancestral haplotype IIa (Fig. 4). While haplotype IIa occurs nearly all over the current range of lineage II pond turtles, haplotype IIb is in extant pond turtles restricted to the populations occurring in east Germany and neighbouring western Poland and is in these populations the only haplotype (Fritz *et al.* 2004, 2007). Our records of haplotype IIa from subfossil turtles from northeast Germany, Denmark, and Sweden (Table 1), in part together with haplotype IIb, demonstrate that these northern populations once harboured both haplotypes and that IIa was lost in the surviving German and Polish populations.

AMS data

The comprehensive AMS radiocarbon chronology of 35 subfossil remains is summarized in Table 2. The data provide evidence for the occurrence of *E. orbicularis* in Sweden from *c.* 9800 to 5500 years ago (Figs 2–3). Two plastra from Skräppeboda bog (Ua-3278; near Smedstorp, Skåne) and Genarp (LuA-4707; Skåne) constitute the oldest records, dating to about 9800 years ago. The pond turtle had crossed the 56° parallel some 500 years later as indicated by a plastron from the peat bog of Råbelöv (Ua-2273). The only record from Öland (Ua-1792) dates to approximately 8350 years ago. The northernmost records from Norsholm (Ua-1794 and Ua-1796; Östergötland, approx. 58°30′N) represent two of the most recent pond turtle records in Sweden, dating to approximately 5800 and 5500 years ago.

The first records of *E. orbicularis* in southern Sweden approximately 9800 years ago correspond well to increased annual mean temperatures. Further rising temperatures match with a range expansion within Sweden, with the most northerly records in Norsholm (Östergötland) being among the youngest finds. However, entirely unexpected is the sudden termination of records around 5500 years ago, that is, some 1500 years before the end of the Holocene thermal maximum in Scandinavia. This striking disappearance of *E. orbicularis* coincides with a declining pine tree line in the Scandes Mountains and a decrease of mean temperatures by nearly 1 °C in southern Sweden from 6000 to 5500 years ago, as indicated by pollen data (Fig. 3).

Table 4 Nucleotide sequences determined for three mtDNA fragments obtained from subfossil bones of *Emys orbicularis*. Primer sequences in italics; informative sequences, bold. Sample codes of sequences confirmed by cloning bear asterisks. For sample codes, datings and localities, see Table 1

Accession/Sample Number	Sequence
Fragment 1 (85 bp, 43 bp informative)	
AJ131407 (haplotype Ia)	_AC TTCTCATACA AAGACCTATT_ **AGGACTCATC CTAATACTAG CCTTCCTACT AACCCTAACA CTA**_TTCTCTC CTAACCTTCT AGG_
AJ131411 (haplotype IIa)	----T----- ---------- ---------- ------G--- ---
AJ131412 (haplotype IIb)	----T----- ---------- ---------- ------G--- ---
CUMZ R3467	----T----- ---------- ---------- ------G--- ---
NMB 5563 × 423	----T----- ---------- ---------- ------G--- ---
LMR 9747:14	----T----- ---------- ---------- ------G--- ---
LZZ 3792	----T----- ---------- ---------- ------G--- ---
LZZ 3797	----T----- ---------- ---------- ------G--- ---
A 8/88*	----T----- ---------- ---------- ------G--- ---
POL-1-01*	---------- ---------- ---------- ---------- ---
ARUP:DK 50-1/8	----T----- ---------- ---------- ------G--- ---
ARUP:1975-O.148-S.621	----T----- ---------- ---------- ------G--- ---
NMP 6V 72798	----T----- ---------- ---------- ------G--- ---
NMP 6V 73476	----T----- ---------- ---------- ------G--- ---
Fragment 2 (78 bp, 30 bp informative)	
AJ131407 (haplotype Ia)	_GCCTCTATTC TCCACTTCTC AATC_**CTATTA ATCCTAATAC CTATCGCAGG GGCA**_ATCGAA AACAAAATAC TAACTTAA_
AJ131411 (haplotype IIa)	------ -----C---- ---------- -A--
AJ131412 (haplotype IIb)	------ -----C---- ---------- -A--
NMB 5563 × 423	------ -----C---- ---------- -A--
LMR 9747:14	------ -----C---- ---------- -A--
LZZ 3750*	------ -----C---- ---------- -A--
LZZ 3751	------ -----C---- ---------- -A--
LZZ 3792	------ -----C---- ---------- -A--
A 8/88*	------ -----C---- ---------- -A--
H. v. 1165	------ -----C---- ---------- -A--
POL-1-01	------ ---------- ---------- ----
ARUP:DK 50-1/8	------ -----C---- ---------- -A--
ARUP:1975-O.148-S.621	------ -----C---- ---------- -A--
NMP 6V 73477*	------ -----C---- ---------- -A--
NMP 6V 72798	------ -----C---- ---------- -A--
NMP 6V 73476	------ -----C---- ---------- -A--
Fragment 3 (106 bp, 63 bp informative)	
AJ131407 (haplotype Ia)	_CAGT AGATAACGCA ACCCTAACC_**C GATTCTTCAC CTTCCATTTC TTACTGCCAT TTACCATTAT AGGCCTAACA ATAGTACACC TA**_CTCTTCCT ACACGAAACC GG_
AJ131411 (haplotype IIa)	- ---------- ---------- ---------- ---------- ---------- ---------- --
AJ131412 (haplotype IIb)	- ---------- ---------- -----A---- ---------- ---------- ---------- --
NMB 5563 × 423	- ---------- ---------- ---------- ---------- ---------- ---------- --
LMR 9747:14	- ---------- ---------- -----A---- ---------- ---------- ---------- --
LZZ 3750*	- ---------- ---------- -----A---- ---------- ---------- ---------- --
LZZ 3751	- ---------- ---------- ---------- ---------- ---------- ---------- --
LZZ 3792	- ---------- ---------- -----A---- ---------- ---------- ---------- --
H. v. 1165	- ---------- ---------- ---------- ---------- ---------- ---------- --
POL-1-01*	- ---------- ---------- ---------- ---------- ---------- ---------- --
ARUP:DK 50-1/8	- ---------- ---------- ---------- ---------- ---------- ---------- --
ARUP:1975-O.148-S.621	- ---------- ---------- ---------- ---------- ---------- ---------- --
NMP 6V 72798	? ?????----- ---------- ---------- ---------- ---------- ---------- --
NMP 6V 73476	- ---------- ---------- ---------- ---------- ---------- ---------- --
ARUP:1975-O.480-S.869	- ---------- ---------- ---------- ---------- ---------- ---------- --

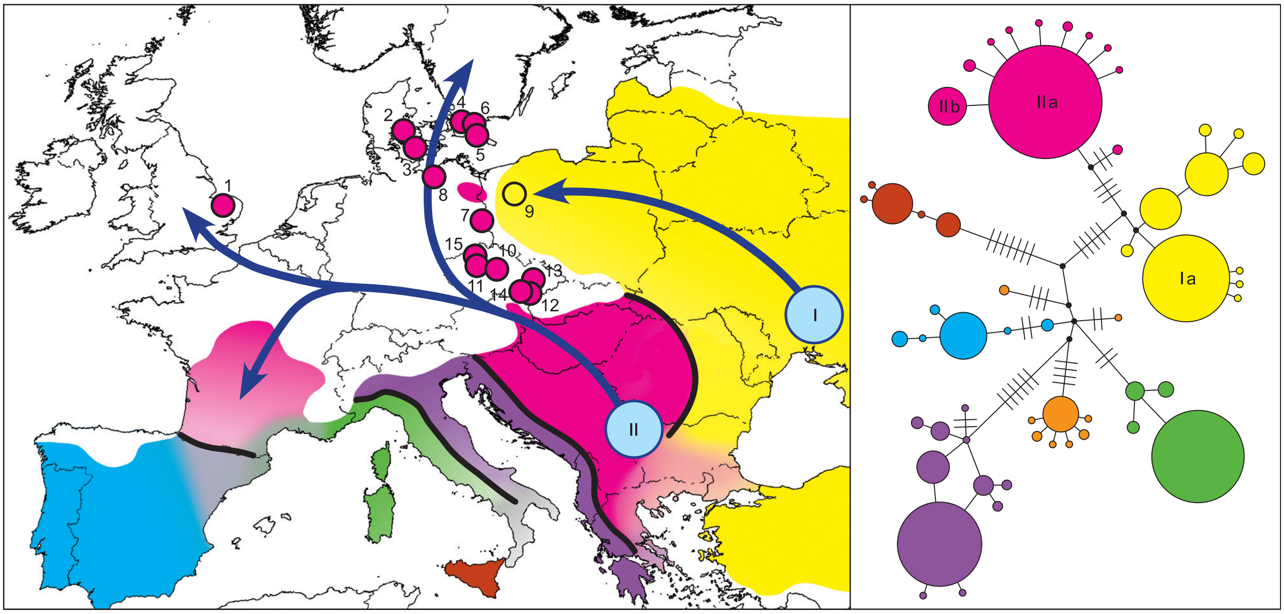

Fig. 4 Left, sampling sites of subfossil *Emys orbicularis* used for ancient DNA haplotype determination (for details, see Table 1). Extant distribution of mitochondrial lineages and haplotypes of subfossil samples are colour coded; colours correspond to haplotype network on the right. Merging colours indicate hybrid zones; black bars, mountain barriers. Arrows indicate inferred Holocene colonization routes for mitochondrial lineages I and II. The other mitochondrial lineages occurring in the south of the distribution range did not contribute to the Holocene colonization of Central and northern Europe (Lenk *et al*. 1999; Fritz *et al*. 2007). Some lineages occur beyond the map sector. Right, median-joining network for haplotypes of extant European pond turtles (*E. orbicularis*, *E. trinacris*) based on data set from Fritz *et al*. (2007). Circle size is a rough approximation to haplotype frequency. Small black circles indicate missing node haplotypes. Each line joining haplotypes corresponds to one nucleotide substitution except when hatches across lines are present; then each hatch indicates one step. Haplotypes Ia, IIa, and IIb occurring in the north of the range of extant *E. orbicularis* indicated.

Discussion

The extant range of European pond turtles is characterized by a wide distribution gap in Central Europe, separating French from Central and eastern European populations (Figs 1 and 4). This biogeographical gap has been explained as the result of Holocene extinction (Fritz 1996, 2001, 2003; Sommer *et al*. 2007). While in Central and South Europe, humans significantly fuelled this extirpation process from prehistoric times onwards (Fritz 1996, 2001, 2003; Cheylan 1998), anthropogenic impact on Scandinavian turtle populations was negligible. Turtles were only exceptionally eaten or otherwise utilized by early Scandinavians, as reflected by the very few turtle remains unearthened in prehistoric settlements; the vast majority of the rich Swedish and Danish finds originates from peat-bogs (Kurck 1917; Isberg 1929; Degerbøl & Krog 1951; Lepiksaar 1982; Richter & Noe-Nygaard 2003).

Nearly all extant pond turtle populations east of the Central European distribution gap represent mitochondrial lineage I that is occurring also all around and north of the Black Sea and north of the Caspian Sea. However, the east German and westernmost Polish populations belong to mitochondrial lineage II. This lineage occurs otherwise in the Danube Basin, the southeastern Balkans, France and northern Spain (Lenk *et al*. 1999; Fritz *et al*. 2004, 2007). In the present study, we successfully amplified and sequenced informative fragments of the mitochondrial cytochrome *b* gene of subfossil samples from extinct populations along the western distribution border of lineage I, from Great Britain and from southern Scandinavia, ranging in age from 10 000 to 500 years ago. In contrast to samples from the Czech Republic, northeastern Germany, Great Britain, Denmark, and Sweden that yielded all haplotypes of lineage II, only a specimen from western central Poland harboured a lineage I haplotype (Tables 1 and 4). These data provide firm evidence for a historic range expansion of *Emys orbicularis* from the southeastern Balkans towards Central Europe, southern Britain, France, and Scandinavia, that is, according to the 'grasshopper pattern' as defined by Hewitt (2000), while northeastern Europe and adjacent parts of Asia were colonized from another refuge further east (Fig. 4). The extant pond turtle populations in the German-Polish Oder River Basin represent the last northern survivors of this range expansion and, therefore, deserve special conservational attention.

The Holocene climate of the northern hemisphere and the spatio-temporal temperature changes are crucial for understanding the immigration and extinction patterns of the European pond turtle in the northernmost parts of its Holocene distribution range. Numerous proxies, including chironomid-, pollen- and diatom-inferred temperatures and stable isotopic records from the northern hemisphere, as well as ice core data from Greenland, are available for the period 10 000 to 4000 years ago (Stuiver *et al*. 1995; Dahl-Jensen *et al*. 1998; Seppä & Birks 2001; Bigler *et al*. 2002, 2003; Kaufman *et al*. 2004; Masson-Delmotte *et al*. 2005; Antonsson *et al*. 2006). When *E. orbicularis* colonized southern Sweden approximately 9800 years ago, the annual mean temperatures had reached present-day values (Fig. 3). Further range expansion was facilitated by increasing temperatures. About 9000 years ago, the annual mean temperatures in southern Sweden were already 1 °C higher than today (Seppä *et al*. 2005; Antonsson *et al*. 2006; Antonsson & Seppä 2007), which corresponds well to estimates for southern Finland and Estonia (Heikkilä & Seppä 2003; Seppä & Poska 2004) and the rise of the $\delta^{18}O$ values in the GISP2 and GRIP Greenland ice cores (Stuiver *et al*. 1995; Dahl-Jensen *et al*. 1998; Masson-Delmotte *et al*. 2005). Most importantly, mean July temperatures, crucial for the incubation of the turtle's eggs (Schneeweiß 2004), were higher than today at 9800 years ago for different regions of Scandinavia (Seppä & Birks 2001, 2002; Bigler *et al*. 2002; Hammarlund *et al*. 2004), as indicated by diatom-, chironomid- and pollen-inferred reconstructions of Holocene temperature patterns. According to temperature simulations using a coupled atmosphere-sea ice-ocean-vegetation model, the July temperatures in Scandinavia reached a Holocene maximum some 9000 years ago (Renssen *et al*. 2005).

The most recent records of *E. orbicularis* in Sweden testify its sudden extinction about 5500 years ago (Table 2), however, which is surprising because this happened some 1500 years before the Holocene thermal maximum ended in Scandinavia (according to palynological and isotopic data) and at least 500 years before the end of the assumed climatic optimum of the northern hemisphere as inferred from Greenland ice core data (Dahl-Jensen *et al*. 1998; Antonsson & Seppä 2007). Previously it has been suggested that the extinction of *E. orbicularis* coincides in Sweden with the end of the Holocene thermal optimum (Sommer *et al*. 2007). Its much earlier extinction relates to a decline of the pine tree line in the Scandes Mountains. By the time *E. orbicularis* colonized Scandinavia, the pine tree line was 300 m higher than today (Kullman & Kjällgren 2006), undoubtedly reflecting significantly warmer summer temperatures. Such conditions were favourable for the reproduction of pond turtles (see below). The sudden termination of pond turtle records 5500 years ago is paralleled by the temporary decline of the pine tree line below the 100-m level, indicative of cooler summers, and a decrease of the mean temperature by nearly 1 °C between 6000 and 5500 years ago (pollen data; Fig. 3). A climatic deterioration at 5500 years ago is evident from proxy records of the aquatic environment in Scandinavia, too (Bergmann *et al*. 2005). Episodic climatic cooling is also well documented in Central Europe 5600–5300 years ago by variations of ancient lake levels (Magny *et al*. 2006), by a distinct change in the GISP2 ice core accumulation and glaciochemical records (O'Brien *et al*. 1995), as well as by the carbon-isotope compositions ($\delta^{13}C$) of the North Atlantic deepwater (Oppo *et al*. 2003). This suggests that a temporary cooling oscillation during the Holocene thermal maximum, affecting mainly summer temperatures, drove Swedish pond turtles extinct. Although the climate improved later again, re-immigration from further south was prevented by the Holocene rise of the sea level, causing the submergence of the previous land connection via the Danish Straits approximately 8500 years ago (Björck 1995).

However, it is stunning that the pond turtle previously survived another, even more marked cold event in northern Europe, which occurred 8200 years ago, the so-called 8.2 ka event (Alley *et al*. 1997; Johnsen *et al*. 2001; Tinner & Lotter 2001) (Fig. 3). This is supportive of the view that the 8.2 ka event was mostly, or completely, caused by lower winter temperatures (Rohling & Pälike 2005) and underlines that the cooling oscillation 5500 years ago rather affected summer temperatures. Summer temperature and insolation rate are the key factors for reproductive success in extant northern pond turtle populations, enabling incubation of clutches (Schneeweiß 2004), and a lack of recruitment of offspring will necessarily result in the extinction of local populations. By contrast, *E. orbicularis* is quite cold resistant during hibernation, as evinced by extant populations in the province of Moscow and the southern Urals, Russia (Fritz 2001, 2003).

Our data demonstrate the power of relating AMS radiocarbon datings, ancient DNA data and palaeoclimatic records for disentangling the impact of Holocene climate fluctuations on biogeographical patterns. The colonization and extinction dynamics of the pond turtle in Sweden reflects the complex history and sensitive reactions of a thermophilous species to climatic changes. Such temperature-sensitive species may even react to short-term deviations from their thermal optimum by suffering local extinction. Also less temperature-sensitive species such as the wildcat (*Felis silvestris*) may respond accordingly. Wildcat records from the pleniglacial are known from Spain, Italy, southwestern France, and Moldavia. Wildcats also colonized Scandinavia during the early or middle Holocene, and became extinct in Scandinavia approximately 3000 years ago (Sommer & Benecke 2006), when the last Danish turtle populations already had disappeared (Sommer *et al*. 2007). In contrast to the pond turtle, the wildcat did not go extinct in Great Britain but survived until today (Sommer & Benecke 2006). This difference could be, at least in part, related with a greater sensitivity of the wildcat to colder winter temperatures, while *E. orbicularis* is more influenced by summer conditions. The red deer (*Cervus elaphus*) exemplifies another pattern. This species persisted throughout the late glacial maximum on the Iberian Peninsula, in adjacent regions of southwestern France, on the Italian Peninsula, in the Balkans and Greece, and east of the Carpathians in Moldavia. It colonized Britain more than 5000 years earlier than wildcats and European pond turtles, but surprisingly reached Scandinavia (Sommer *et al*. 2008) only at the same time as the turtle, a species often considered as symbol of slowness.

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RS Sommer is interested in Holocene range fluctuations of European vertebrates; he shares his interest for phylogeography and the application of aDNA methods with C Lindqvist and L Bachmann; one of the main interests of A Persson and H Bringsøe is the extinction history of Scandinavian pond turtles; AGJ Rhodin is focusing on the conservation of chelonians and acts as director of the Chelonian Research Foundation and chair of the IUCN Tortoise and Freshwater Turtle Specialist Group; N Schneeweiß works in nature conservation, among others in the EU project 'Protection of *Emys orbicularis* and Amphibians in the North European Lowlands'; P Široký works on parasites and the phylogeography of chelonians; the main interests of U Fritz are evolutionary biology and diversity of chelonians and the application of aDNA methods for phylogeographic purposes.