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BIODIVERSITY RESEARCH

Genetic diversity of endangered brown bear (*Ursus arctos*) populations at the crossroads of Europe, Asia and Africa

Sébastien Calvignac†, Sandrine Hughes and Catherine Hänni*

Paleogenetics and Molecular Evolution, Institut de Génomique Fonctionnelle de Lyon, Université de Lyon, Université Lyon 1, CNRS, INRA, Ecole Normale Supérieure de Lyon, 46 allée d'Italie 69364 Lyon cedex 07, France

ABSTRACT

Aim Middle East brown bears (*Ursus arctos syriacus* Hemprich and Ehrenberg, 1828) are presently on the edge of extinction. However, little is known of their genetic diversity. This study investigates that question as well as that of Middle East brown bear relationships to surrounding populations of the species.

Location Middle East region of south-western Asia.

Methods We performed DNA analyses on 27 brown bear individuals. Twenty ancient bone samples (Late Pleistocene to 20th century) from natural populations and seven present-day samples obtained from captive individuals were analysed.

Results Phylogenetic analyses of the mitochondrial sequences obtained from seven ancient specimens identify three distinct maternal clades, all unrelated to one recently described from North Africa. Brown bears from Iran exhibit striking diversity (three individuals, three haplotypes) and form a unique clade that cannot be linked to any extant one. Individuals from Syria belong to the Holarctic clade now observed in Eastern Europe, Turkey, Japan and North America. Specimens from Lebanon surprisingly appear as tightly linked to the clade of brown bears now in Western Europe. Moreover, we show that *U. a. syriacus* in captivity still harbour haplotypes closely linked to those found in ancient individuals.

Main conclusion This study brings important new information on the genetic diversity of brown bear populations at the crossroads of Europe, Asia and Africa. It reveals a high level of diversity in Middle East brown bears and extends the historical distribution of the Western European clade to the East. Our analyses also suggest the value of a specific breeding programme for captive populations.

Keywords
Ancient DNA, brown bear, genetic diversity, Middle East, mtDNA, *Ursus arctos syriacus*.

*Correspondence: Catherine Hänni, Ecole Normale Supérieure de Lyon, 46 allée d'Italie 69364 Lyon cedex 07, France.
E-mail: catherine.hanni@ens-lyon.fr
†Present address: Subterranean Hydrobiology and Ecology, Université de Lyon, LEHF UMR 5023, UCB Lyon 1, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France.

INTRODUCTION

To assess the genetic diversity of brown bears (*Ursus arctos*), mitochondrial variation has been surveyed in European and North American populations, generally revealing the existence of allopatric clades (Taberlet & Bouvet, 1992, 1994; Randi *et al.*, 1994; Kohn *et al.*, 1995; Talbot & Shields, 1996; Waits *et al.*, 1998; Saarma *et al.*, 2007). Relatively few studies have concentrated on Asian populations (Masuda *et al.*, 1998; Matsuhashi *et al.*, 1999). Middle East brown bears, which are often referred to a single subspecies, the Syrian brown bear [*Ursus arctos syriacus* Hemprich & Ehrenberg, 1828; Kurten (1965)], represented one of these neglected populations. Until historical times, brown bears occupied a large portion of the Middle East, going from the Sinaï Desert to mountainous regions of Iran (Boitani *et al.*, 2008; Fig. 1). *Ursus arctos* has since been extirpated from Egypt, Israel, Lebanon and, more recently, Syria, while its range was dramatically reduced in other countries (Ridings, 2006; Boitani *et al.*, 2008; Fig. 1). Thus, it tended to survive as small populations (for which no reliable estimates are available; Can & Togan, 2004) in Iraq, Iran and Turkey, where forest fragmentation and direct persecution by humans have resulted in population declines during the last 50 years (Can & Togan, 2004). To date, only three individuals

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Figure 1 Historical (grey) and present-day (dark grey) distributions of brown bears (*Ursus arctos*) in Europe and the Middle East. The approximate distributions have been simplified from the IUCN report (Boitani *et al.*, 2008). Clades are numbered according to Miller *et al.* (2006). Clade 1: Western European clade *sensu* Taberlet & Bouvet (1994); clade 3a: Eastern European clade *sensu* Taberlet & Bouvet (1994). Individual 49, coming from Iran and described in Miller *et al.* (2006), is also represented. Blue crosses stand for the localities of North African brown bears analysed by Calvignac *et al.* (2008). The dotted rectangle refers to Fig. 2.

originating from Turkey and Iran have been genetically characterized, yielding mitochondrial DNA control region (CR) or cytochrome *b* (cyt *b*) sequences (Talbot & Shields, 1996; Miller *et al.*, 2006). As Middle East brown bears are rare in the wild, elusive, and live in remote areas, extensive genetic studies of extant populations are precluded. To document recent Middle East brown bear genetic diversity, we targeted CR and cyt *b* fragments in twenty ancient individuals, representing palaeolithic to historical populations in present-day Iran, Lebanon and Syria. We also compared these 'wild' haplotypes with those maintained in captive populations. The new sequences we describe here shed light on past genetic diversity of Middle East brown bears, on their relationships with adjacent European and North African populations and provide the basis for efficient conservation guidelines.

METHODS

Description of samples

A total of twenty *Ursus arctos* bone fragments belonging to 20 different individuals coming from the Middle East and spanning a period from the Upper Pleistocene to very recent times were analysed. These samples had either been collected from the zoological collection of the Museum National d'Histoire Naturelle (MNHN), Paris, corresponding to remains of wild animals prepared for museum storage (UAS9 to 12), or in the field, mostly from archaeological sites (UAS8 and UAS13 to 27). Seven captive individuals were also sampled (UAS1 to 7): two blood samples were obtained from individuals housed in Paris (recorded as individuals T1079 and T1145 in the European Regional Brown Bear Studbook, ERBBS) and five faecal samples from brown bears kept in Montpellier (T2142 and T2257), Heidelberg (T2230 and 2231) and Ostrava (T1066) (for genealogies deduced from the ERBBS; see Appendix S5). The 27 samples are described in Table 1. Finally, to complete the brown bear cyt *b* dataset, one flesh sample from an individual belonging to the clade 1 – Balkan subclade was also provided for analysis by the Laboratoire d'Ecologie Alpine (LECA), Grenoble.

DNA extraction, amplification and sequencing

Treatment of ancient samples

Extraction, primary amplification, cloning and sequencing were performed as described in Calvignac *et al.* (2008). Depending on the DNA quality of samples, either ca. 270 base pairs (bp) fragments of CR and cyt *b*, or shorter, *c.* 90–140 bp fragments, were targeted. The following primer pairs were used: H3/H16299 (267–271 bp) and H3/H4 (154 bp) (Loreille *et al.*, 2001; Orlando *et al.*, 2002) or CB2670MP/CBH45 (97 bp) (Hofreiter *et al.*, 2004; Pagès *et al.*, 2008) for CR; SCcytb1F/SCcytb2R (278 bp) and SCcytb1F/SCcytb1R (145 bp) or SCcytb2F/SCcytb2R (134 bp; Calvignac *et al.*, 2008) for cyt *b*.

Ancient DNA controls

Contaminations were monitored during the extraction and PCR processes by blank controls (one blank for a maximum of five samples) and aerosol controls, which were kept open throughout the manipulation (Loreille *et al.*, 2001). Moreover, during each brown bear extraction, additional ancient samples from other species (monkeys, lemurs, sheep and equids) were treated and the extracts used in amplification attempts with bear-specific primers to serve as cross-contamination tests. No contamination was detected in these various controls.

At least two independent PCR products/fragment/individual were obtained and subsequently cloned to allow the identification of artifactual substitutions (three to eight clones per product/fragment/individual were analysed). Consensus sequences were then determined for each fragment and for each individual from the clone alignment.

Table 1 Brown bear samples taken from captive individuals and from ancient bones. Ancient samples are arranged according to their dating. Approximate age ranges of archaeological periods are given in years before present (BP). UAS1 and UAS2 are descendants of Syrian brown bears coming from Tabriz (Iran).

Present-day individuals (zoo samples)	Initial provenance	Studbook	Type of sample	Sample provider	Provider
UAS1	Tabriz (Iran)	T1079	Blood	Paris Zoo, France	J.-L. Berthier
UAS2	Tabriz (Iran)	T1145	Blood	Paris Zoo, France	J.-L. Berthier
UAS3	?	T2257	Faeces	Montpellier Zoo, France	C. Libert
UAS4	?	T2142	Faeces	Montpellier Zoo, France	C. Libert
UAS5	?	T2231	Faeces	Heidelberg Zoo, Germany	I. Figura
UAS6	?	T2230	Faeces	Heidelberg Zoo, Germany	I. Figura
UAS7	?	T1066	Faeces	Ostrava Zoo, Czesch Republic	P. Bendova

Ancient individuals (bone samples)	Sample provenance*	Age or date of sampling†	Sample provider	Provider
UAS8	Iran	?	Bones collected in the field outside any archaeological context	J. Michaux
UAS9	Iran	1972	MNHN, Paris	C. Lefèvre
UAS10	Syria	1890	MNHN, Paris	C. Lefèvre
UAS11	Lebanon	1870	MNHN, Paris	C. Lefèvre
UAS12	Lebanon	1861	MNHN, Paris	C. Lefèvre
UAS13	Becharre, North Lebanon	< 1900	Bones collected in the field outside any archaeological context	J. Loiselet and H. Abdul-Nour
UAS14	Mar Challita cave, North Lebanon	< 1900	Bones collected in the field outside any archaeological context	J. Loiselet and H. Abdul-Nour
UAS15	Qatna, Syria	Iron age (3200–2300 BP)	MOM, Lyon	E. Vilà
UAS16	Sidon, Syria	Early Bronze age (5500–4000 BP)	MOM, Lyon	E. Vilà
UAS17				
UAS18	Ras Shamra, Syria	Neolithic to Bronze age (12,000–3100 BP)	MOM, Lyon	D. Helmer
UAS19				
UAS20	Byblos, Lebanon	Neolithic (12,000–5500 BP)	MOM, Lyon	D. Helmer
UAS21	Wezmeh, Iran	Late Pleistocene to Holocene (128,000–12,000 BP)	MNHN, Paris	M. Mashkour
UAS22				
UAS23				
UAS24				
UAS25				
UAS26				
UAS27				

*When precise locations are unknown, only country names are specified.
†The dates are the year of collection, the samples could be older.

Present-day sample treatment

DNA extraction of flesh, blood or fresh faeces samples as well as amplification attempts and sequencing reactions were performed independently of ancient DNA experiments, as described in Calvignac *et al.* (2008). For both CR and cyt *b*, only the longest fragments were targeted using the primer pairs described above.

Datasets

Control region sequences from modern brown bears covering our entire fragment of 267–271 bp were obtained from Taberlet & Bouvet (1994), Waits *et al.* (1998), Masuda *et al.* (1998) and Matsuhashi *et al.* (1999; see Appendix S1 in Supporting Information for accession numbers). Cyt *b* sequences from modern brown bears were obtained from Talbot & Shields (1996), Matsuhashi *et al.* (1999), Delisle & Strobeck (2002) and Calvignac *et al.* (2008; Appendix S1). North African brown bear sequences (CR and cyt *b*), shown to belong to an extinct and very divergent clade (Calvignac *et al.*, 2008), were also included in these alignments. Two separate datasets (one for CR and one for cyt *b*) gathering present-day sequences, North African sequences and sequences determined in this study were generated. A third dataset of concatenated CR and cyt *b* sequences was generated from individuals

represented by both CR and cyt *b* sequences (540-bp-long alignment available upon request). Only ancient individuals UAS8, UAS9 and UAS11 were included in this last dataset, having yielded both 267- to 271-bp-long CR and 278-bp-long cyt *b* fragments (Ha1, 2 and 4; Table 2). Considering that: (1) UAS1 and UAS2 exhibited the same CR and cyt *b* haplotypes found in UAS8 (Ha1; Table 2), (2) UAS4 sequences matched perfectly those of UAS3, and (3) UAS6 and UAS7 sequences were identical to those of UAS5, the only sequences from captive specimens included in this dataset were those from UAS3 and UAS5 (Ha3 and 5; Table 2). Sequences of brown bears belonging to either clade 5 or 6 were only considered in the CR dataset because cyt *b* sequences were unavailable. Each one of these datasets included *Ursus americanus* (Stone & Cook, 2000; Marshall & Ritland, 2002) and *Ursus spelaeus* (Loreille *et al.*, 2001) sequences that served as outgroups for the analyses. Finally, we generated a fourth dataset containing all concatenated sequences, but no outgroup sequence. Sequences obtained in this study were deposited in EMBL under accession numbers FN292970–FN292996.

Observed divergence and phylogenetic analyses

Kimura-2-Parameters (K2P) distances were calculated using PAUP v4.0b10 (Swofford, 2000). Phylogenetic analyses of the four datasets – CR, cyt *b*, CR + cyt *b* and CR + cyt *b* without outgroup – were performed to infer relationships between the different brown bear sequences. The appropriate model of evolution was determined using MrModeltest 2.0 (Nylander, 2004), a modified version of MODELTEST (Posada & Crandall, 1998) adapted to MrBayes v3.1 (Ronquist & Huelsenbeck, 2003). According to MrModeltest and following the Akaike information criterion, the best probabilistic models of sequence evolution were, respectively, HKY+I+G, HKY+G, HKY+I+G and HKY+I+G. For each dataset, both maximum likelihood (ML) and Bayesian analyses (BA) were performed as described in Calvignac *et al.* (2008) except that BA were run for 2,500,000 generations. ML and BA runs produced similar tree topologies and yielded consistent bootstrap support (Bp) and posterior probability (pp) values.

Table 2 Results of amplification for brown bear samples taken from zoo-kept individuals and from ancient bones. The size of the fragments obtained is indicated in base pairs. We got a positive result for UAS15, but on a different cyt *b* fragment (see text) whose length is indicated in italics. UAS14 yielded sequences, but we were unable to replicate them (underlined) and so it was not considered in the following analyses. A dash indicates that no amplification was obtained, even for very short fragments (see text).

Sample name	CR	cyt *b*	Clade	Haplotype
Present-day individuals: samples from zoos				
UAS1	271	278	New	Ha1
UAS2	271	278	New	Ha1
UAS3	271	278	3a	Ha3
UAS4	271	278	3a	Ha3
UAS5	272	278	3a	Ha5
UAS6	273	278	3a	Ha5
UAS7	273	278	3a	Ha5
Ancient individuals: bone samples				
UAS8	271	278	New	Ha1
UAS9	271	278	New	Ha2
UAS10	104	145	3a	Ha3
UAS11	267	278	1	Ha4
UAS12	100	145	1	Ha4
UAS13	100	145	1	Ha4
UAS14	105	145	–	–
UAS15	–	*134*	*3a*	*Ha3*
UAS16–UAS27	–	–	–	–

Intra-clade diversity analysis

A maximum parsimony network (with a 0.95 parsimony criterion) was generated with TCS1.21 (Clement *et al.*, 2000) to infer relationships between the CR sequences of brown bears belonging to the 3a clade (see Appendix S1 and Table 2).

Bayesian estimation of dates of divergence

To determine times of divergence, we performed Bayesian analyses using BEAST (Drummond & Rambaut, 2007). To apply an estimate of the CR evolutionary rate that had been obtained previously from a 193 bp alignment [39% per site per million year (Myr), standard deviation (SD) 8.3% per site per Myr; Ho *et al.*, 2008], the CR matrix described above was trimmed to the same length and haplotyped. The resulting matrix thus consisted of 60 haplotypes, among which a single Iranian one (instead of 62 sequences, among which two Iranian ones). In all cases, monophyly was enforced following the results of phylogenetic (clade 1, clade 1 plus Lebanon, clade 3a plus 3b, all brown bears) or tokogenic (Romania2 plus UAS3 and UAS5) analyses. A total of six analyses was run for 10,000,000 generations using a relaxed (uncorrelated log-normal) molecular clock and placing a strong prior on the evolutionary rate, which was described as a normal distribution of mean 39 ± 8.3% per site per Myr (Ho *et al.*, 2008). Three demographic models were applied: constant population size, exponential growth of the population size and logistic growth of the population size. In each case, two runs were performed, appropriate convergence and mixing of the Monte Carlo-Markov Chains being checked with Tracer v1.4 (Rambaut & Drummond, 2007).

RESULTS

DNA recovery from ancient specimens

Of the 20 ancient specimens, only eight provided amplifiable DNA. The four specimens sampled at MNHN allowed the recovery of DNA sequences spanning 245–549 bp (CR and cyt *b*; Appendix S2; Table 2), while only four of the remaining sixteen ancient bones (all collected in the field) yielded sequences from 100 to 549 bp in length (CR and/or cyt *b;*

UAS8, UAS13, UAS14 and UAS15; Appendix S2; Table 2). As UAS14 sequences could not be replicated and thus authenticated, they were discarded from all datasets.

Divergence, phylogeny and dates of divergence

For both markers, four haplotypes (Ha1–Ha4) could be identified from ancient specimens (Table 2): two in Iran (Ha1 and Ha2), one in Lebanon (Ha4) and one in Syria (Ha3). Genetic distances between these haplotypes were relatively high (from 5.8 to 7.7% in the CR and from 1.4 to 6% in the cyt *b*; Appendix S3), except for those observed between Ha1 and Ha2 (0.7% in the CR and 0.4% in the cyt *b*; Appendix S3). When compared with other brown bear sequences of the same length (representing most of the genetic diversity of this species), Ha1, Ha2 and Ha4 were most similar to sequences from clade 1 (Western European brown bears), without exactly matching any of them (Appendix S4). Meanwhile, Ha3 was little divergent from clade 3a (Eastern European) sequences (1.1% for the CR and 0% for the cyt *b*; Appendix S4).

Seven Syrian brown bears in four different zoos were also sequenced for both markers (Table 2), which yielded three CR haplotypes and two cyt *b* haplotypes (Appendix S4). UAS1 and UAS2 perfectly matched Ha1 in both CR and cyt *b*, as did UAS3 and UAS4 to Ha3 (Table 2). The remaining three bears (UAS5, UAS6 and UAS7) were identical to Ha3 in cyt *b* sequences, but differed from it by a single substitution in CR, defining a distinct haplotype, Ha5 (Table 2).

Based on CR or cyt *b* sequences alone, both ML and BA methods failed to support grouping sequences from Iran or Lebanon with any other brown bear sequence (not shown). To place these samples, we analysed a concatenated dataset of CR and cyt *b* sequences (total length 540 bp). In reconstructions based on the concatenated dataset, all known clades were well-supported (Fig. 2). The Lebanese sequence was placed with clade 1 (Western Europe) with high support values (Bp: 90; pp: 0.96, Fig. 2). Sequences from Iran were not placed in any brown bear clade (Fig. 2). The remaining sequences found in some ancient Syrian and captive individuals were nested within clade 3a (Holarctic). Analyses of this dataset excluding the outgroup (i.e. theoretically minimizing homoplasy) yielded similar results in terms of both topology and branch supports.

The relationships of captive individuals UAS3 and UAS5 with other brown bears belonging to clade 3a (Appendix S1) were further investigated through network analysis (Fig. 3). The latter showed a distribution centred on the Russian haplotype and haplotypes tended to be clustered according to geography. Proximity of UAS3 and UAS5 with brown bears from Eastern Europe, most notably with Romanian ones, was observed (Fig. 3), echoing similar placement in CR phylogenetic analyses (not shown).

To place Middle East brown bear sequences in the temporal history of the species, Bayesian analyses were run to determine times of divergence (Table 3). The time to the most recent common ancestor (TMRCA) of all brown bears was calculated to be around 200 thousand years (kyr) (184–242 kyr according to the model used, Table 3). The use of different demographic models had little impact on the remaining estimates (Table 3). Present-day clade 1 and grouping of clade 3a and 3b were found to be respectively *c.* 48 and 68 kyr old, while the TMRCA of modern sequences belonging to clade 1 and Ha4 (Lebanon) was estimated to be *c.* 65 kyr old. Finally, the TMRCA of haplotypes Romania2, Ha3 and Ha5 was determined to be *c.* 17–19 kyr (Table 3).

DISCUSSION

Genetic diversity of wild Middle East brown bears

Until historical times, small-bodied, blond brown bears were commonly found all throughout the Middle East. They have now been extirpated from most of their historical range, eventually surviving as small populations in Iraq, Iran and Turkey (Ridings, 2006; Boitani *et al.*, 2008; Fig. 1). Previously, only three individuals native to the region had been sampled for mtDNA sequences (Talbot & Shields, 1996; Miller *et al.*, 2006). We have added CR and cyt *b* sequences isolated from seven ancient specimens representing wild historical populations. Taken together, these ten individuals harbour at least six different mtDNA haplotypes [Ha1–Ha4 (this study); GB28 (Talbot & Shields, 1996); Individual 49 (Miller *et al.*, 2006)]. In comparison, analyses of 317 North American brown bears revealed the existence of at least 28 matrilineages (Waits *et al.*, 1998). Middle East brown bears thus present a high level of genetic diversity.

Brown bears originating in Syria appear to be closely related to clade 3a (including populations from Eastern Europe, Asia and North America; Appendix S4; Taberlet & Bouvet, 1994), as were the two Turkish individuals analysed by Talbot & Shields (1996). Network analyses suggest that these bears are closest to Romanian ones (Fig. 3). Romanian bears are among the closest European neighbours of Middle East brown bears (Fig. 1). Brown bears native to the Lebanon seem to be related to the clade 1 (Western Europe *sensu* Taberlet & Bouvet, 1994), while brown bears from Iran form a relatively diverse clade (three individuals, three haplotypes) distinct from any known clade. Befitting their distribution at a biogeographical nexus, Middle East brown bears belong to three distinct clades and are polyphyletic with respect to their mtDNA (Fig. 2; Appendix S4).

This genetic pattern contradicts the classical taxonomy of *Ursus arctos* in the region, which recognizes a single subspecies (*U. a. syriacus*) characterized by small body size, small molars and the blondness of its coat (Kurten, 1965). However, very few of the many subspecies defined to date are monophyletic with respect to their mtDNA (Talbot & Shields, 1996; Waits *et al.*, 1998). On the basis of mtDNA alone (which is maternally inherited), it is difficult to assess patterns of gene flow or determine whether shared states of characters reflect common ancestry or adaptations to similar environments.

In any case, occupation of the Middle East by brown bears has involved at least three of the extant clades of brown bears, gathering characteristic haplotypes in close proximity. To our knowledge, the only comparable case is that of Hokkaido

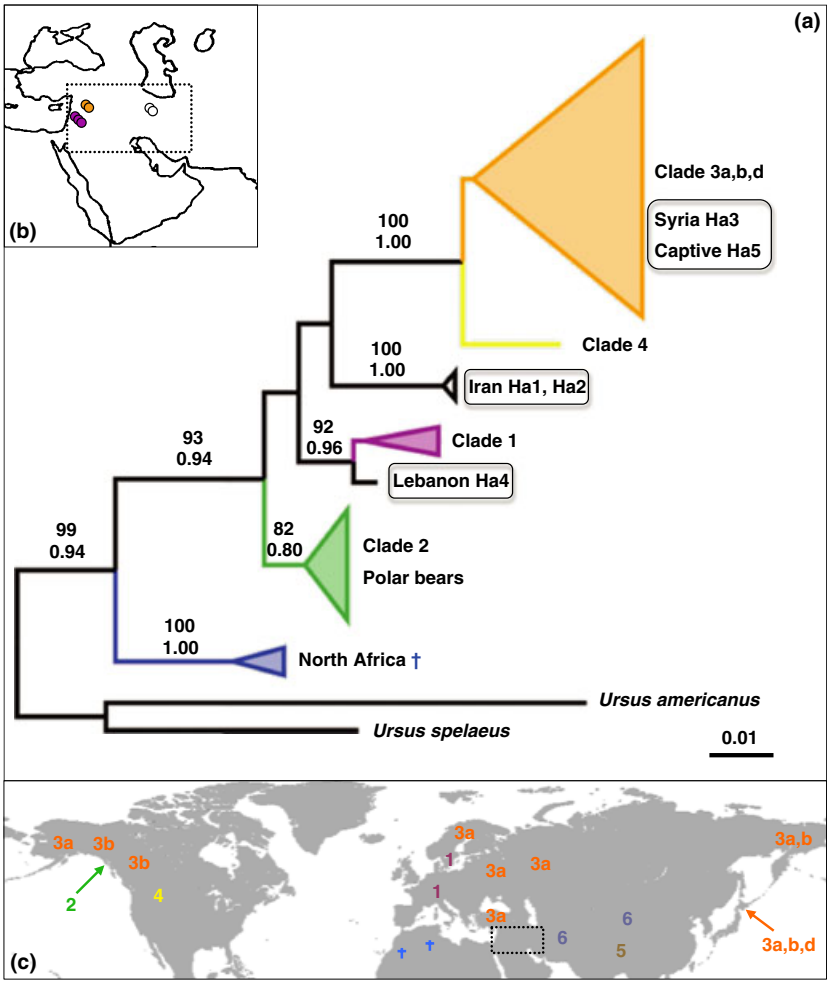

Figure 2 Phylogenetic relationships of *Ursus arctos syriacus* with other brown bears (a) and maps showing (b) approximate locations of the seven ancient samples that yielded DNA and (c) the geographical distribution of present-day brown bear clades. Panel a: Phylogenetic tree from the ML analysis of the combined CR and cyt *b* dataset (≈270 bp CR plus 278 bp cyt *b*). Bayesian analyses yielded similar topologies. Bootstrap values (above) and posterior probabilities (below) are above branches. Support values are indicated only when bootstrap > 50 and posterior probability > 0.80. See Table 2 for the correspondence between haplotypes and samples. Panels b/c: The dotted rectangle refers to the historical distribution of *U. a. syriacus* (see also Fig. 1). Panel c is modified from Miller *et al.* (2006). The eight identified CR clades are numbered according to Miller *et al.* (2006).

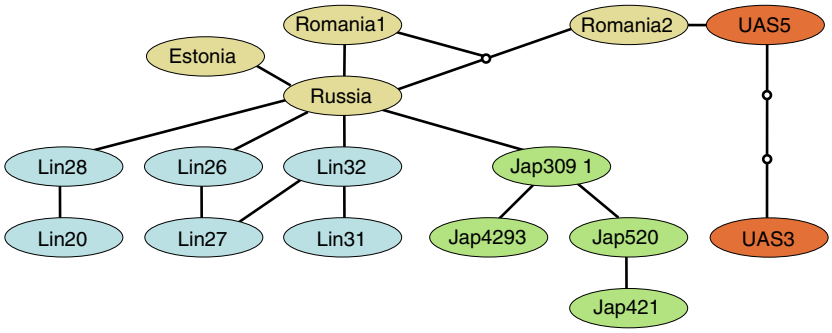

Figure 3 Parsimony network of brown bear CR haplotypes belonging to clade 3a. A connecting line between haplotypes represents one mutation and a small circle, a missing haplotype. Geographical origins are indicated by a colour code: light blue (North America), light green (Japan), light brown (Eastern Europe) and orange (Middle East). All sequences are described in text (in the case of Middle East ones) or in Appendix S1 (all other sequences).

brown bears, which also belong to three different clades (Matsuhashi *et al.*, 1999), albeit closer geographically and phylogenetically than their Middle Eastern counterparts (Matsuhashi *et al.*, 1999, 2001).

Analysis of Middle East brown bear sequences in the context of brown bear evolution yields additional information about the colonization of the region by two of the clades. The sample from Lebanon seems to have diverged from clade 1 (Western European sequences) *c.* 65 ka, contemporaneous with the start of diversification of Beringian brown bears, i.e. 68 ka. Colonizations of the two regions from North China, the proposed core range of the species (Kurten, 1965) might have been more-or-less synchronous. On the other hand, UAS3/UAS5 sequences and the modern haplotype Romania2 from clade 3a (Holarctic bears) shared a common ancestor just 17–19 ka, an indication of a recent colonization of the Middle East or of the

Table 3 Bayesian estimates of times of divergence under different demographic models.

	Dates of monophyly in thousands of years (median with 95% confidence intervals)				
Demographic model	All brown bears	Clade 1 and Lebanon	Clade 1	Clade 3a and clade 3b	Romania2 and UAS3/UAS5
Constant size	242 (115–449)	65 (25–130)	48 (21–97)	69 (31–126)	17 (3–44)
Exponential growth	184 (91–318)	64 (29–119)	49 (22–91)	68 (33–116)	19 (4–46)
Logistic growth	216 (98–398)	65 (26–127)	48 (20–93)	68 (33–126)	17 (3–44)

reciprocal scenario, a colonization of present-day Romania from a Middle East glacial refugium (Hansson *et al.*, 2008).

Relationships of Middle East brown bears to West European brown bears

European brown bears underwent distinctive histories: Western and Eastern European populations belong to the two very divergent clades 1 and 3a (Randi *et al.*, 1994; Taberlet & Bouvet, 1994; Kohn *et al.*, 1995; Saarma *et al.*, 2007). As recently as 1500 years ago, the situation was strikingly different as European populations were an admixture of both clades, the Western European one being markedly more diverse than today (Valdiosera *et al.*, 2007, 2008). Members of both clades 1 and 3a can be found in the Middle East (Fig. 2). Interestingly, the Lebanese sequence (Ha4; Table 2; Fig. 2) is different from all other clade 1 haplotypes (data not shown; Valdiosera *et al.*, 2007), making the Western European clade even more diverse than previously thought. It also had a broader distribution area than today. The historical distribution of clade 1 encompassed Western Europe (Fig. 1), North Africa (Calvignac *et al.*, 2008) and the Middle East (this study). The presence of brown bears belonging to clade 1 in North Africa was suggested to be human-driven importation (Calvignac *et al.*, 2008), but the relatively ancient divergence of Lebanese sequences and the absence of this haplotype from Western Europe refute this hypothesis for Middle East brown bears. Thus, a large part of the Mediterranean area may have been occupied naturally by clade 1 brown bears, but in a discontinuous manner. Lebanese brown bears were indeed, at least in recent times, isolated from Western European brown bears by Eastern European populations (clade 3a), as shown by Turkish (Talbot & Shields, 1996) and Syrian (Table 2, Fig. 2) samples.

Relationships of Middle East brown bears to North African brown bears

The brown bear succeeded in colonizing the Northern and Eastern shores of the Mediterranean Sea, but also its Southern shores. *Ursus arctos* was found in North Africa until *c.* 1200 years ago (Hamdine *et al.*, 1998; Calvignac *et al.*, 2008). The region shares closer zoogeographical affinities with the Middle East than with any of the other surrounding areas, when considering non-flying mammals as a whole (Dobson & Wright, 2000) or the particular case of another large mobile carnivore, *Panthera leo* (Barnett *et al.*, 2006). This makes it reasonable to hypothesize some kind of phylogenetic vicinity between Middle East and Maghreb brown bears. However, none of the North African haplotypes was found in our samples of Middle East brown bears (Calvignac *et al.*, 2008). This might suggest that ancestors of North African brown bears did not reach Africa via the Middle East, but used another migration route. The alternative hypothesis, migration to North Africa through Europe, would imply crossing considerable water barriers, which effectively isolate brown bears (Paetkau *et al.*, 1998). However, we may have failed to detect a North African signature in Middle East brown bears because of the limited number of individuals we sampled and/or a very ancient colonization of North Africa, which is supported by the basal position of these bears, followed by a population replacement. It seems nevertheless plausible that Middle East and North African brown bears have been linked at some point in their histories.

Genetic diversity of captive Middle East brown bears

Given the high genetic diversity observed in the subspecies, the quasi-extinction of Middle East brown bears is synonymous with the loss of a number of endemic haplotypes. This adds to the growing body of evidence suggesting a continuous loss of mitochondrial diversity in the species as a whole from the end of the Pleistocene (Leonard *et al.*, 2000; Barnes *et al.*, 2002; Valdiosera *et al.*, 2007, 2008) to very recent times (Miller *et al.*, 2006; Valdiosera *et al.*, 2007, 2008; Calvignac *et al.*, 2008). For conservation purposes, it was important to examine captive populations to determine whether ancestral maternal lineages had been preserved. A large number of Syrian brown bears are housed in zoological gardens. The European Regional Brown Bear Studbook lists as many as 50 individuals in 17 different European institutions while seven others can be found in five institutions located in North America or Asia (C. Libert, Montpellier; pers. com.). Our analyses reveal that at least two of the mitochondrial clades identified from ancient specimens are retained in these captive populations. However, only four of a possible 15 maternal lineages in European zoos were sampled (Appendix S5). It would be highly desirable to analyse additional ancient specimens as well as individuals representing these unsampled zoo maternal lineages. Such work offers a more accurate assessment of the mitochondrial diversity of endangered Middle East brown bears, clarification of its biogeographical history and possibly, more efficient guidelines for captive breeding programmes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Accession numbers of all sequences used in this study.

Appendix S2 Aligned *Ursus arctos syriacus* CR and cyt *b* sequences.

Appendix S3 K2P-distances computed between CR or cyt *b* sequences obtained from ancient specimens.

Appendix S4 Minimum K2P-distances observed between brown bear sequences for CR and cyt *b*.

Appendix S5 Maternal lineages of captive Syrian brown bears analysed in this study.

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