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Distribution update

DNA analysis confirms African wolf in Morocco

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Abstract

We collected hair samples from two road-killed animals known as golden jackals *Canis aureus* in Bouhachem forest in northern Morocco. Analysis of 353bp of cytochrome B and 296 bp of d-loop of the mitochondrial genome placed these two individuals in the same lineage of African wolf *Canis lupus lupaster* identified by Gaubert et al. (2012). This is the first DNA evidence for the presence of this taxon in Morocco.

Article

In 2011, the canid formerly known as the golden jackal *Canis aureus* was recognised as a new sub-species, the African wolf *C. lupus lupaster* in western Egypt and Ethiopia (Rueness et al. 2011). Further genetic work has since recognised the presence of this taxon in Algeria, Mali and Senegal (Gaubert et al. 2012). We present evidence here that the canid we observed in Bouhachem forest in northern Morocco is the African wolf, confirming this distribution update with the first DNA evidence for the presence of the species in Morocco.

Bouhachem forest (Figure 1) is an area of approximately 142km^2 of mixed oak forest. It is included in the Moroccan protected areas network as a Site of Biological and Ecological Interest. Local villages depend on the forest for their livelihoods using it to pasture their goats and collect non-timber forest products such as mushrooms.

No systematic mammal surveys have taken place in the area, apart from those for the Endangered Barbary macaque *Macaca sylvanus* (Fa 1982, Waters et al. 2007). Shepherds in Bouhachem refer to the canid they observe as the *dib* (literally "wolf" in Arabic) and we use this term hereon. We made brief observations of the *dib* whilst conducting survey and monitoring work on Barbary macaques. We also encountered road-killed *dib* along the only paved road in Bouhachem. In 2012, we noted that the dead animals bore a strong resemblance to photographs of the African wolf in neighbouring Algeria (Gaubert et al. 2012). The species is cryptic and difficult to observe and photograph in the forest, so we concentrated on opportunistic hair sample collection from road-killed specimens (Figure 2).

In October 2012 and February 2014 we found two road-killed *dib*, collected hair samples, and extracted DNA using the Dneasy Blood & Tissue kit (QIAGEN) according to standard protocols recommended by the manufacturer. Two regions of the mitochondrial genome were amplified, 353bp of cytochrome B and 296 bp of d-loop (control region), using the following primers: Dloop (5' to 3'): F-GCACCCAAAGCTGAAATTCT, R-ATGGGCCCGGAGCGAGAAGAG, CytB(5' to 3'): F-TTGTATTTCAACTATAAGAACAT, R-GCAAAGAATCGTGTTAGGGTTG. DNA extraction, PCR amplification and subsequent sequence analysis were conducted following Senn et al. (2014).

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Figure 1. Location of Bouhachem in Morocco

Figure 2. *Dib* killed by a vehicle in Bouhachem – October 2012

A single haplotype for the two individuals was obtained at cytochrome B but both had different control-region haplotypes, differing by a 1bp mutation. The sequences for each animal were concatenated and aligned against publically available data for *Canis* species obtained from Genbank (from Aggarwal et al. 2007; Bjornefeldt et al. 2006, Rueness et al. 2011, Gaubert et al. 2012). The resulting alignment was 579bp long. The alignment file is at http://www.rzss.org.uk/staff/dr-helen-senn and the sequences have been deposited in Genbank under the accession numbers KM670012-KM670014.

One animal had a haplotype identical to the sequence JQ088661.1 *Canis lupus lupaster* sampled in Algeria (Algeria 3, see Figure 2), in the coastal region between Skikda and El-Kala (Gaubert et al. 2012). Phylogenetic trees were built in two ways: a simple distance-matrix method using neighbour joining with the Tamura-Nei model of genetic distance and Bayesian inference carried out with MrBayes v3.1.2 [38]. For details of analyses see Senn et al. (2014). Both analyses clearly place these two individuals in the same lineage of "African wolf" identified by Gaubert et al. (2012) with 100% posterior probability/100% bootstrap support (Figure 3). Unfortunately direct comparison with the "African wolf" sequences for western Egypt detailed in the paper by Rueness et al. (2011) was not possible because the relevant data had not been published at the time of writing. However the sequences from the samples of "*Canis aureus lupaster*" collected in Ethiopia, from the same paper, were included and fell in the "African wolf" cluster defined by Gaubert et al. (2012).

This finding in Morocco, albeit from only two samples, increases the geographic range of this putative subspecies. It is clear that there is much about the evolutionary history of *Canis* species in North Africa that is unknown and more detailed analysis with fuller sampling and the addition of nuclear data would be required to obtain a proper understanding of the role that hybridisation between species may have played in the evolution of the wolf-jackal complex (Gaubert et al. 2012).

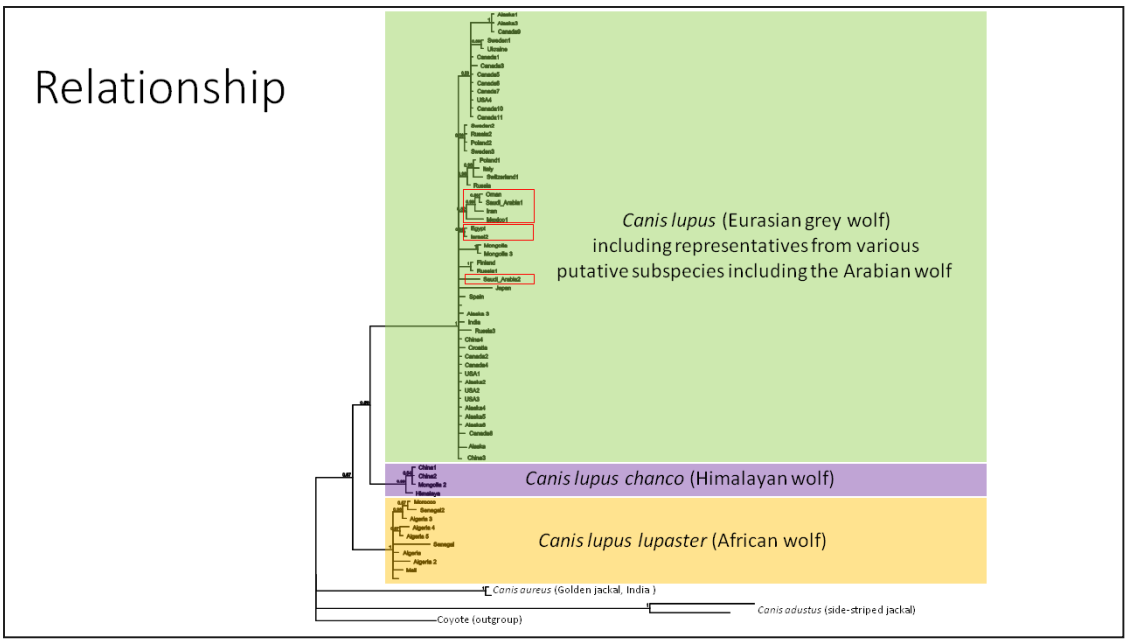

Figure 3. Bayesian inference of the phylogenetic relationship between the Moroccan samples and other *Canis* specimens from around the world at control region and cytochrome b genes of the mitochondrial genome (579bp). The Moroccan samples sit clearly within the African wolf cluster defined by Gaubert et al. (2012). Further details of the analysis can be found in the text.

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Biographical sketch

Siân Waters is a conservation social scientist and the founder and director of Barbary Macaque Awareness and Conservation. Her interests focus on the human dimension of wildlife conservation.

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