

**THE CHEETAH (*Acinonyx jubatus*) IN NORTHERN AFRICA
A NON-INVASIVE GENETIC STUDY OF CARNIVORES FROM THE AHAGGAR
MOUNTAINS, SOUTHERN ALGERIA**

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ABSTRACT

The status of the cheetah, *Acinonyx jubatus*, in Northern Africa is unknown. Study of this species has concentrated on the two major populations of the Serengeti in Tanzania and in Namibia. A lack of detailed baseline data has led to an increasingly detached and unsure view of the present status of this animal in its most northern reaches of Africa. This paper aims to address this problem by assessing the status of the cheetah in Algeria.

A 2005 expedition to the Ahaggar region of the Algerian Sahara collected over 40 putative carnivore scat samples for further analysis. The first major objective of this analysis was to assign species identity to the scat. This was done through phylogenetic sequence analyses and through web-based sequence comparisons of 12S and cytochrome *b* genes. Among other carnivores, eight cheetahs and a leopard were found. This is the first time leopardsThus, this paper has an ancillary purpose in presenting a new way of using non-invasive molecular ecological techniques to compile a species list in remote areas where resources only allow for short, reconnaissance studies.

Having identified the species present, the second objective of this study was to analyse the genetic structure of the cheetah samples through microsatellite studies. Cheetah from Tanzania were used as reference samples and combined in the analysis with the Algerian cheetahs, and the number of unique genotypes and possible kinship relationships were ascertained. The cheetah samples were then geo-referenced on a map containing information gathered on the 2005 expedition.

This paper therefore conclusively proves the existence of cheetahs and leopards in Algeria and provides impetus for future work in this remote region.

1. Introduction

The cheetah, *Acinonyx jubatus*, is one of the most familiar and charismatic of all big cats. In the past its distribution spanned from the tip of southern Africa to the Mediterranean and into Asia (Marker 1998). Apart from a small pocket of approximately 100 cheetahs in Iran and possibly an even smaller population in Pakistan (Nowell and Jackson 1996; Marker 1998) the cheetah is now only found in Africa. Among the 32 African countries in which cheetah have been reported as historically present, it is now extinct in at least four of them (Marker 1998), all in North Africa. The cheetah is listed as Vulnerable on the 2006 IUCN Red List and Critically Endangered in Iran and North Africa¹. Because of small populations, remote home ranges, habitat fragmentation (Marker 2000) and a lack of research, the cheetah in North Africa is barely understood.

1.1. North African Cheetah

The status of cheetah in North Africa is unknown, although they predominantly inhabit the more mountainous regions of the Sahara where water and gazelle are more easily found (Kowalski and Rzebik-Kowalska 1991). This region is intensely arid which affects the availability of suitable prey species and therefore the numbers of cheetah that might occupy it.

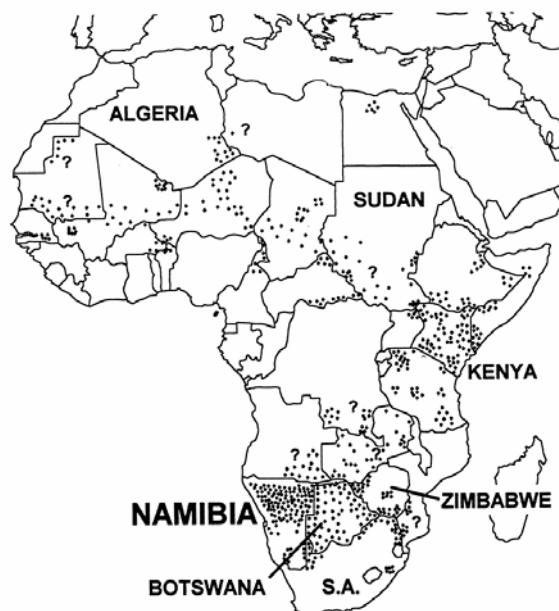


Figure 1.1. The distribution of cheetah in Africa

(from Marker 1998) the dots represent cheetah records

¹ www.iucnredlist.org

Figure 1.1. shows the distribution of cheetah in Africa (Marker 1998). As can be seen, the numbers in North Africa are uncertain and low. They also represent a subspecies of cheetah, *A. jubatus venaticus* about which little is known.

Figure 1.2. shows the collation of all reported cheetah sightings and signs in Algeria. In March 2005 a reconnaissance expedition to the Ahaggar Mountains of Algeria (boxed area in figure 1.2.) conducted by the Sahelo-Saharan Interest Group (SSIG) aimed to collect preliminary data on the desert wildlife of the area through fieldwork and sample collection (Wacher et al 2005). Habitat, weather and wildlife observations (such as large mammal signs) were made and an extensive collection of unknown carnivore scat samples was assembled. Field observations made at the collection sites provided good reason to believe that at least some of the scat samples came from Algerian cheetahs.

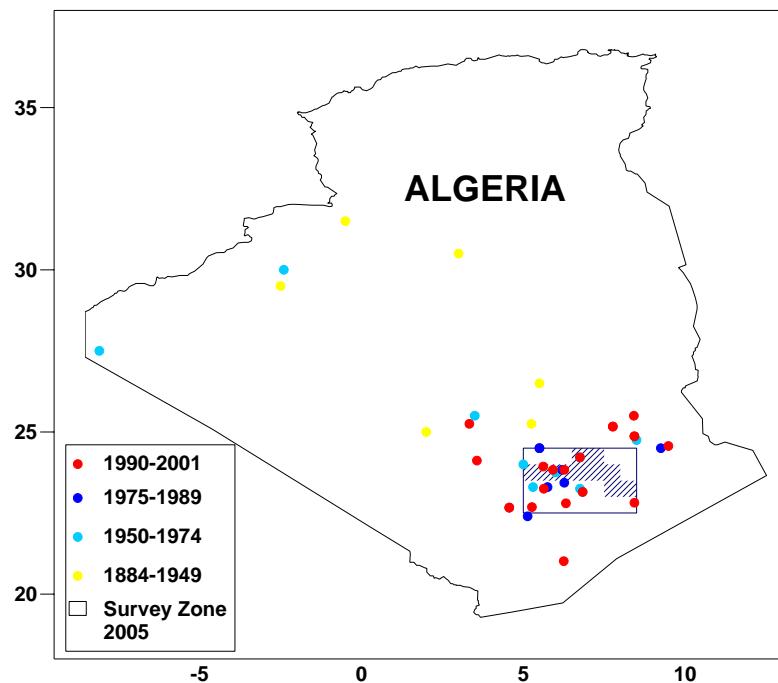


Figure 1.2. Positions of all cheetah records in Algeria from 1884 to present day

The boxed area is the survey zone of the 2005 SSIG expedition to the Ahaggar National Park (shown in hashed lines) The dots represent records collected before the expedition from the literature (Kowalski and Rzebik-Kowalska 1991; Hamdine et al 2003)

1.2. Non-invasive genetic sampling

Genetic data can be obtained with increasing ease from animal hair and scat samples. Data of this kind is advancing the field of conservation genetics. Studies using genetic information, especially with regard to

mammal conservation, collected through non-invasive means, abound in the literature (eg on Iberian lynx, Palomares et al 2002; wombats, Banks et al 2003; black bears, Triant et al 2004; Japanese carnivores, Kurose et al 2005; dholes lyengar et al 2005). However, these studies tend to use samples collected from known individuals or species. With the growing database of sequence data now available online² it should also be possible to obtain gene sequences from unknown samples, and assign specific identity to them, through comparison searches and phylogenetic analyses.

Molecular ecological techniques are becoming increasingly powerful and have been key to gaining a better understanding of, among other things, evolution, paternity, kinship, mating systems and phylogenetic relationships. Specifically, gene sequence data can be used to infer phylogenetic and phylogeographic relationships both within and between species. Microsatellites can also be used to assess genetic variation of different populations (eg Driscoll 1992; Gottelli et al 2004).

Through work on cheetahs from Eastern and Southern Africa, it has been known for a long time that cheetahs are genetically impoverished (O'Brien et al 1983; O'Brien et al 1985; Wayne et al 1986; O'Brien et al 1987), probably due to inbreeding following at least two population bottlenecks; one in the late Pleistocene (O'Brien et al 1987; Menotti-Raymond and O'Brien 1993) and one within the last century or so (O'Brien et al 1987). Although this might not necessarily be a problem (Caro and Laurenson 1994) it is of vital interest to examine the genetics of all cheetah populations if necessary conservation management decisions are to be made. Therefore, given that the world population is Vulnerable, and that cheetahs are genetically depauperate, studies on the more marginal and smaller populations of cheetah are necessary. This paper therefore provides emphasis for future genetic studies of cheetah from North Africa.

1.3. Aims

This project aims to investigate the faecal samples collected in Algeria in order to:

1. Identify some of the species of carnivore present in the Parc National de l'Ahaggar in Saharan Algeria, through genomic analysis.
2. Use all positive cheetah DNA samples to investigate the Ahaggar population structure.
3. Present a novel use for non-invasive sampling to type samples of an unknown origin.
4. Highlight the need for further, long term work in Algeria and its importance in felid conservation.

² www.ncbi.nlm.nih.gov

2. Materials and Methods

2.1. Samples

The samples used in this study were collected during the 2005 ZSL-SSIG expedition to the Ahaggar Mountains of Southern Algeria (figure 1.2.; Wacher et al 2005). 49 faecal samples were collected from different areas of the massif, of which 40 were available for analysis. A further two samples were collected from Algeria by Tim Wacher early in 2006. DNA was also extracted from the tissue of 7 animals from the Zoological Society of London (ZSL) tissue bank to be used as references. These samples belong to animals bred at London Zoo therefore no geographic data of their origin was available. ZSL reference samples included a cheetah, *Acinonyx jubatus*, two sand cats, *Felis margarita*, a Persian leopard, *Panthera pardus*, a dwarf mongoose *Helogale parvula*, a banded mongoose, *Mungos mungo*, and a fennec fox, *Vulpes zerda*. Sequences were also collected from the literature (table 2.1.) via GenBank (Benson et al 2005). In order to compare between North and East African populations, seven cheetah samples from Serengeti National Park in Tanzania were included in this study. These individuals were randomly selected from samples collected over the last 9 years by the Serengeti Cheetah Project (Gottelli et al under review).

2.2. DNA extraction and amplification

DNA was extracted from the samples using the QIAGEN QIAmp Stool and QIAGEN Tissue extraction kits according to the manufacturer's guidelines. The polymerase chain reaction (PCR) was then used to amplify the mitochondrial target region of the 12S and cytochrome *b* genes, using the primer combinations 12S1/12S4 and cyt_b1/cyt_b2, as described in Janczewski et al (1995). PCR amplification was carried out in a total volume of 10 µL containing 1µL of genomic DNA (approximate concentration 2 - 10 ng/µL) in 50 mM KCl, 2.0 mM MgCl₂, 10 mM Tris HCl (pH = 8.8), 1 mM dNTP mix, 2.5 – 5 units of Taq DNA polymerase, and 2.5 pmoles of each primer. The product was run on an agarose gel to test for the positive amplification of sequences of the correct length. Following this test, the products were purified using the QIAGEN PCR purification kit according to the manufacturer's guidelines, and subsequently sequenced using the ABI BigDye chemistry and 3100 Automatic Sequencer (Applied Biosystems).

2.3. Sequence production

The program SEQUENCING ANALYSIS (Applied Biosystems) was used to assign bases to the output from the automatic sequencer, which were then inspected and corrected manually with SEQUENCER (Gene Codes Corporation). Sequences were aligned for comparison in BIOEDIT³.

³ Free download from: <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>

2.3.1. Phylogenetic analysis

The 12S and cytochrome *b* sequences were analysed separately in the computer program PAUP (Swofford 1998). MODELTEST (Posada and Crandall 1998) was used to find the best (maximum likelihood) model of nucleotide substitution to be used in the construction of trees for 12S and cytochrome *b*. Sequences were also BLASTED on the NCBI website⁴ to look for homologous sequences within the database. The identity of the sample was assigned from the combination of these two analyses.

Table 2.1. Shows the numbers and origins of the reference sequences used in the phylogenetic analyses.

Family	Scientific name	Common Name	12S N	Cyt <i>b</i> N	GenBank accession numbers	citation
Felidae	<i>Acinonyx jubatus</i>	Cheetah	2	2	AY463959	this study; Burger et al 2004
	<i>Felis margarita</i>	Sand cat	1	-		this study
	<i>Felis catus</i>	Domestic cat	1	1	D28892	Masuda et al 1994; Meece et al 2005
	<i>Panthera pardus</i>	Leopard	1	1	AB211405	this study ; Sugimoto et al 2005
Viverridae	<i>Panthera leo</i>	Lion	1	1	S79300; AY928670	Janczewski et al 1995; Koepfli et al 2006
	<i>Genetta maculata</i>	Blotched genet	-	1	AY241921	Gaubert et al 2004
Hyaenidae	<i>Hyena hyaena</i>	Striped hyaena	-	1	AY928678	Koepfli et al 2006
Herpestidae	<i>Herpestes javanicus</i>	Indian mongoose	1	1	AY873843; AY928675	Penny and McLenaghan 2005; Koepfli et al 2006
	<i>Mungos mungo</i>	Banded mongoose	1	1		this study
	<i>Helogale parvula</i>	Dwarf mongoose	1	1		this study
Canidae	<i>Vulpes zerda</i>	Fennec fox	1	1	AF028146	this study ; Wayne et al 1997
	<i>Vulpes vulpes</i>	Red fox	1	1	Y08508; AF028146	Ledje and Arnason 1996; Yoo et al 2006
	<i>Canis familiaris</i>	Domestic dog	1	1	DQ780500	Bjornerfeldt et al 2006
	<i>Canis latrans</i>	Coyote	1	1	DQ480511	Bjornerfeldt et al 2006
	<i>Canis aureus</i>	Common jackal	1	1	DQ102371; AF028138	Das et al 2005; Wayne et al 1997
Mustelidae	<i>Mustela nivalis</i>	weasel	1	1	Y08515; AB026106	Ledje and Arnason 1996; Kurose et al 2000

2.4. Microsatellites

The positively identified Algerian cheetah samples and 7 Serengeti cheetahs were screened for the presence of 9 dinucleotide microsatellite loci, following the tube approach of Taberlet et al (1996). The loci, characterized in the domestic cat *Felis catus* (Menotti-Raymond et al 1999), chosen on the basis of their high polymorphism and easy scorability, were Fca008, Fca023, Fca026, Fca045, Fca084, Fca126, Fca133, Fca193, Fca247. Genotyping was carried out using fluoro-labelled primers and performing 40 cycles of PCR amplification in a 6 µl reaction volume. This contained 1µl (\leq 10 ng) DNA, 0.5µl (0.2 µM) of each primer and 4µl of PCR QIAGEN Master Mix which provides a final concentration of 3mM MgCl₂. The primers labeled with specific dyes with different alleles sizes were amplified and run separately with a size standard (Liz 500) on a ABI 3100 Automatic Sequencer (Applied Biosystems) running GENEMAPPER (Applied Biosystems). The reaction was repeated at least three times for each sample.

⁴ <http://www.ncbi.nlm.nih.gov/blast/>

Reference samples were included on each PCR allowing the standardisation of allele size measurements across runs.

2.4.1. Microsatellite analysis

The results were analysed on the computer program GENEMAPPER. The program gave allele sizes for each of the cheetahs at each locus. GIMLET (Valiere 2002) can be used to produce consensus genotypes after multiple genotyping runs. The total number of unique alleles was ascertained. The mean average and expected heterozygosities were estimated from the data using GIMLET. Using the consensus genotypes, GIMLET can also estimate the number of unique individuals and can conduct parentage analyses. Further relatedness was investigated using MER (Wang 2004). Multiple analyses were conducted in order to find for consistency in the small sample size of the results size and to compensate for the lack of robustness of the statistical tests.

2.5. Spatial analysis

The geographical position for each of the cheetah samples was known and plotted onto a map of the area using SURFER (Golden Software Colorado). This was combined with various other geo-referenced data collated by the SSIG during the expedition, such as cheetah signs and reports, the positions of large scats and gazelle densities.

3. Results

3.1. Assigning species identity to the samples

30 of the 42 faecal samples analysed gave good sequences for 12S and/or cytochrome *b*. Table 3.1. shows the results. The species were diagnosed through a combination of phylogenetic analysis and BLASTING of the sequences. In total 8 cheetahs, *Acinonyx jubatus*, a wildcat *Felis* spp, 1 leopard, *Panthera pardus*, 5 genets, *Genetta* spp, 1 banded mongoose *Mungos mungo* and 14 dogs *Canis* spp. were identified.

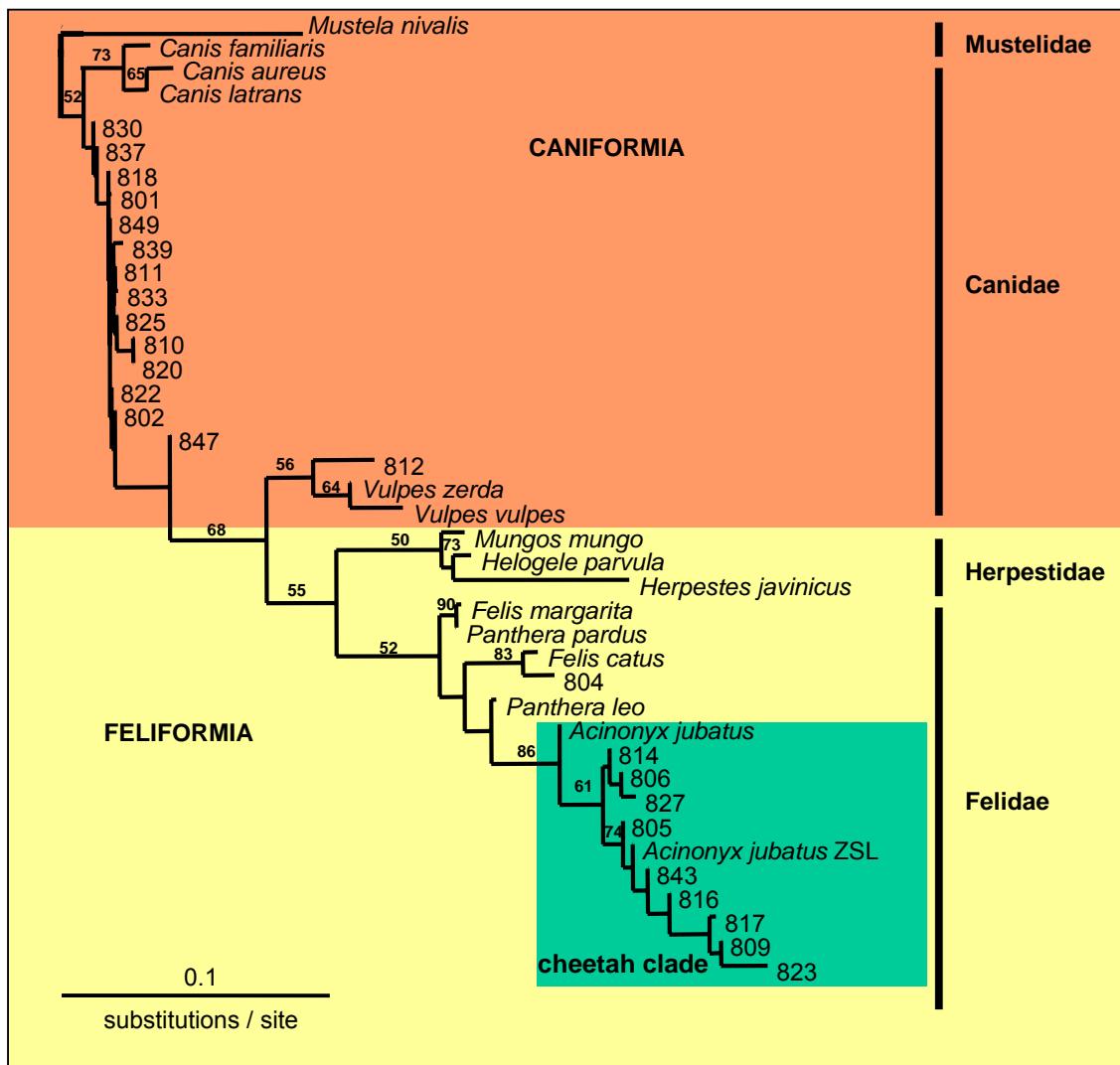
Appendix 1 gives the alignment of the 12S and cytochrome *b* sequences. Neighbour-joining trees were built using PAUP and are shown below (figures 3.1. and 3.2.). A Mustelid, *Mustela nivalis*, was used as the outgroup. Following the use of MODELTEST the 12S tree was built using the Tamura-Nei (1993) model for nucleotide substitution stating an estimate of the proportion of invariable sites, I, and a rate of substitution for variable sites, gamma, in advance. For cytochrome *b*, the General Time Reversible model (Tavare 1986) was used, also stating I and gamma in advance. Bootstrapping was used to assess the reliability of the branches and all bootstrap values over 50% are shown. The trees combine the Algerian samples with reference sequences from known ZSL animals and from GenBank (table 2.1.). The two trees differ slightly (see legend accompanying figures). Good sequence data for both 12S and cytochrome *b* was produced for 13 of the samples, six of which were putative cheetahs (individuals 805, 806, 809, 816, 817, 843). In general, both trees the major divisions in the clades are robustly supported.

The BLAST results for each of the sequences can be found in Appendix 2. On four occasions the inferences from 12S and cytochrome *b* differed. In these cases, the species identified from the cytochrome *b* gene was always used to diagnose the species, as these invariably had a higher E-value (see Appendix 2). Three of them belonged to a largely unresolved 'canid' group in the 12S analysis, but appeared to belong to a more robust genet group in the cytochrome *b* analysis. Only one of the ambiguous samples, individual 814, was of particular importance, as judged from 12S alone, because its 12S sequence BLASTED as a cheetah while its cytochrome *b* was that of a mongoose.

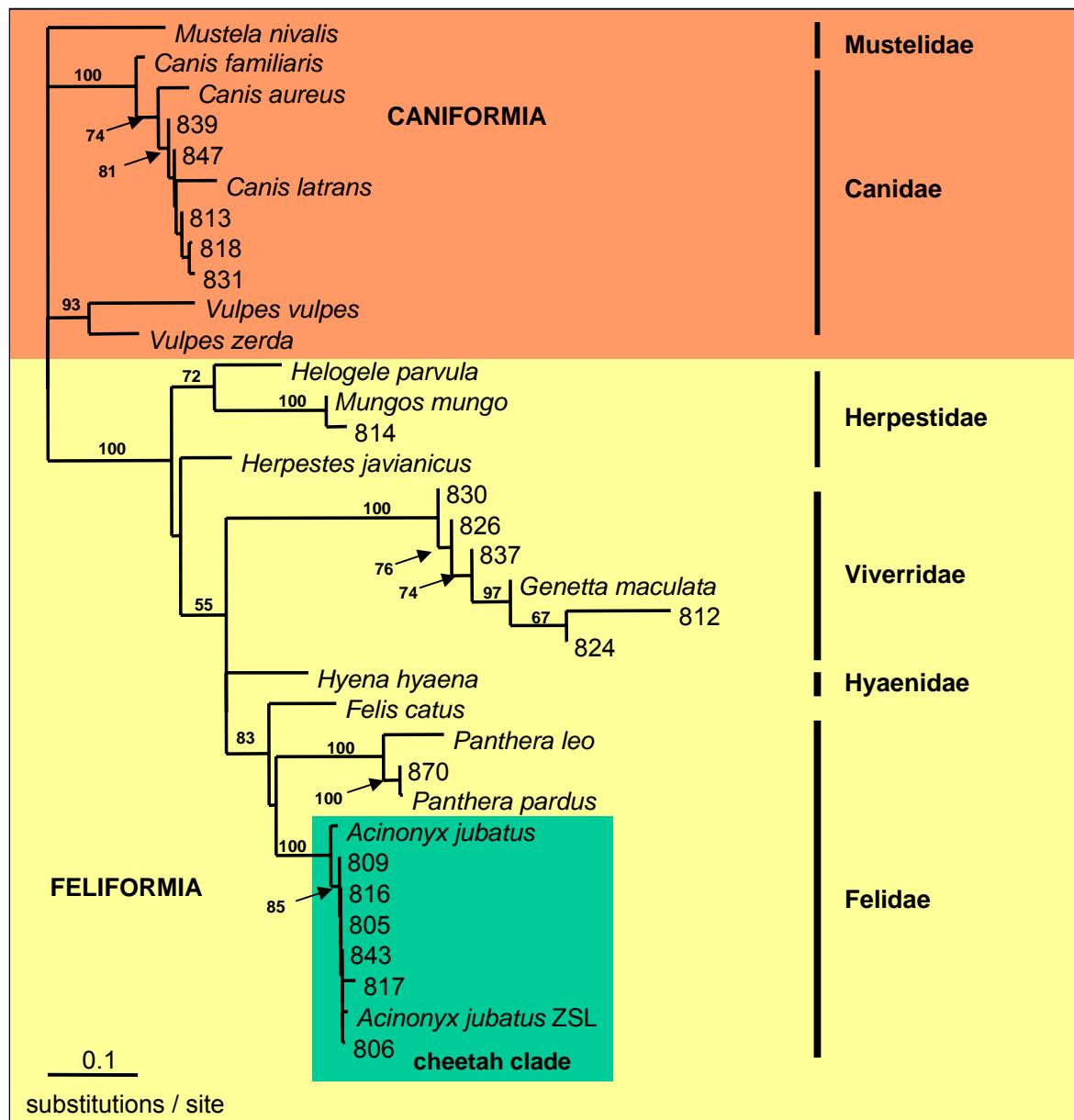
Table 3.1. Shows the species identity of the Algerian samples.

	Field ID	N	E	Grid	Sector	Tree Sp.	Ht / cm	Pos.	Lab ID	12S	Cytb	Species	Note
1	20050310/01	23.92	6.86	4	38.5	Tamarix	150	branch	814	Y	Y	<i>Mungos mungo</i>	12S = <i>Acinonyx</i>
2	20050311/02	23.93	6.77	4	39.5	Tamarix	-	-	801	Y	N	<i>Canis spp</i>	
3	20050311/03	23.93	6.77	4	39.5	Tamarix	-	-	812	Y	Y	<i>Genetta genetta</i>	12S = <i>Vulpes</i>
4	20050313/02	23.59	7.37	5	62	Acacia	-	-	810	Y	N	<i>Canis spp</i>	
5	20050313/04	23.59	7.37	5	62	Acacia	-	-	831	N	Y	<i>Canis spp</i>	
6	20050315/01	23.25	7.90	6	84	-	15	hole	811	Y	N	<i>Canis spp</i>	
7	20050315/02	23.25	7.90	6	84	-	-	-	839	Y	Y	<i>Canis spp</i>	
8	20050315/03	23.25	7.90	6	84	-	0	ground	847	Y	Y	<i>Canis spp</i>	
9	20050316/0?	23.32	8.19	7	99	Tamarix	0	ground	813	N	Y	<i>Canis spp</i>	
10	20050316/0?	23.33	8.13	7	98	-	-	-	849	Y	N	<i>Canis spp</i>	
11	20050316/04	23.32	8.19	7	99	Tamarix	0	ground	837	Y	Y	<i>Genetta spp</i>	12S = <i>Canis</i>
12	20050316/05	23.32	8.19	7	99	Tamarix	0	ground	804	Y	N	<i>Felis spp</i>	
13	20050316/06	23.42	7.92	6		Rock	0	ground	802	Y	N	<i>Canis spp</i>	
14	20050317/02	23.60	7.83	8	114	Tamarix	-	Ground	830	Y	Y	<i>Genetta spp</i>	12S = <i>Canis</i>
15	20050317/03	23.60	7.83	8	114	Tamarix	-	branch	816	Y	Y	<i>Acinonyx jubatus</i>	
16	20050317/04	23.60	7.83	8	114	Tamarix	0	ground	827	Y	N	<i>Acinonyx jubatus</i>	
17	20050317/05	23.61	7.74	8	116	Acacia	200	branch	817	Y	Y	<i>Acinonyx jubatus</i>	
18	20050317/08	23.65	7.72	8	117	Acacia	100	branch	809	Y	Y	<i>Acinonyx jubatus</i>	
19	20050318/02	23.75	7.58	8	121	-	-	Cave	833	Y	N	<i>Canis spp</i>	
20	20050318/07	23.75	7.58	8	121	-	-	Cave	818	Y	Y	<i>Canis spp</i>	
21	20050318/11	23.78	7.54	8	122	Tamarix	80	branch	820	Y	N	<i>Canis spp</i>	
22	20050319/01	24.05	7.44	9	129	Tamarix	160	branch	823	Y	N	<i>Acinonyx jubatus</i>	
23	20050319/03	24.05	7.44	9	129	Tamarix	80	branch	843	Y	Y	<i>Acinonyx jubatus</i>	
24	20050319/04	24.07	7.39	9	130	Tamarix	70	branch	805	Y	Y	<i>Acinonyx jubatus</i>	
25	20050319/04	24.06	7.34	9	131	Tamarix	-	-	870	N	Y	<i>Panthera pardus</i>	
26	20050319/06	24.07	7.39	9	130	Tamarix	-	-	822	Y	N	<i>Canis spp</i>	
27	20050319/07	24.07	7.39	9	130	Tamarix	80	branch	806	Y	Y	<i>Acinonyx jubatus</i>	
28	20050319/08	24.07	7.39	9	130	Tamarix	30	branch	825	Y	N	<i>Canis spp</i>	
29	20050320/01	24.15	7.06	9	139	Tamarix	0	ground	826	N	Y	<i>Genetta spp</i>	
30	20050322/01	23.92	6.20	3	24	Acacia	0	ground	824	N	Y	<i>Genetta spp</i>	

All cheetah samples are highlighted. The table also gives the information taken at the location in the Ahaggar Mountains where the sample was collected. Cheetah are known to climb trees and often scratch the bark. Samples were collected from trees and the height on the tree and the species of tree, where known, were also noted. The lab ID refers to the sample IDs that are used in this paper. In the final notes column the ambiguous samples are shown.

Figure 3.1. A phylogenetic tree made with 12S sequences from the unknown Algerian and carnivore reference samples

The numbers on the ends of the nodes refer to the identity of the samples as listed in table 3.1. The species are those listed in table 2.1. The numbers on the branches refer to the bootstrap value of that branch and include all branches supported by over 50% of the bootstrap consensus. A distinct clade of cheetah samples can be seen in green in the lower right corner, and is supported by a bootstrap value of 86. All of these 9 samples also BLASTED as cheetah 12S on the NCBI website. The positions Herpestidae and Felidae and the Canidae and Mustelidae families within the tree agree with Yu et al's (2004) carnivore phylogeny. The position of the foxes, *Vulpes* spp, is outwith of the main Canid clade however. The positions of the species within the family clades are both less resolved and against the current consensus (Yu et al 2004, Yu and Zhang 2005) although broadly agree with the classification of Carnivora from Ledje and Arnason's (1996) study on 12S sequences. Individual 814 appears in the cheetah clade, but this is not supported in the cytochrome *b* analysis. The large unresolved clade at the top of the tree is ambiguous. The most recent carnivore phylogenies (Yu et al 2004, Flynn et al 2005, Yu and Zhang 2006) suggest that Canids are the basal group within the Caniformia (dog-like carnivores) which is robustly supported. Mustelids are possibly one of the more diverse caniform taxa. The long branch between the Mustelid outgroup and the unresolved Algerian samples suggest that they represent a more closely related Caniform taxa. However there are no representatives of the other caniforms (sub-family Arctoidea) other than the Mustelidae and Lutriniae (otters) in Africa (Kingdon 1997). These individuals also BLASTED as *Canis* spp so it is likely that they are Canids.

Figure 3.2. A phylogenetic tree made with cytochrome b sequences from the unknown Algerian and carnivore reference samples

This neighbour-joining tree uses the same terminology as figure 3.1. The numbers on the branches indicate percentage bootstrap values. The analysis benefited from a larger number of reference samples from more detailed lineages. Here the cheetah clade, again in green, contains 6 of the Algerian samples all of which were present in the 12S analysis. The clade is robust with 100% bootstrap support. Individual 814, which appears in the cheetah clade in the 12S tree, now appears in the Herpestid clade, with 100% bootstrap support and is a putative mongoose. Individual 870 is a leopard. The presence of a genet sequence has resolved some of the discrepancies in the 12S tree, with a robustly supported group of 5 genets, two of which were in the large unresolved 'canid' clade in the 12S analysis, the other being a putative fox. The five Algerian canids samples are supported in a clade with the three *Canis* reference samples with a bootstrap value of 100. The Feliform clade is supported by bootstrap analysis. However, again the relationships within the clade are contrary to recent phylogenies (Yu et al 2004, Flynn et al 2005), which suggest that the Hyaenids and Herpestids are monophyletic. The Viverrid clade is notoriously contentious (Gaubert et al 2004).

3.2. Cheetah microsatellite analysis

3.2.1. Consensus genotypes

The consensus genotypes for the eight Algerian cheetahs and 7 Tanzanian cheetahs are shown below in table 3.2. Following Pompanon et al (2005) the error rate per locus from GIMLET is also reported. The mean error rate per locus was 0.17, or 17%, ostensibly due to allelic dropout.

Table 3.2. Genotypes for each of the Algerian and Tanzanian cheetah for 9 microsatellite loci

(an allele pairing of 000-000 refers to those samples where the typing did not work after three attempts)

Sample	Fca 8	Fca 23	Fca 26	Fca 45	Fca 84	Fca 126	Fca 133	Fca 193	Fca 247	
Tanzanian cheetahs	93	144-148	131-135	144-144	135-139	145-145	117-117	121-123	125-131	150-150
	94	148-154	131-135	144-144	135-139	139-145	117-131	123-129	125-137	150-152
	106	150-154	131-131	136-144	133-135	145-145	115-129	123-129	125-129	152-156
	107	150-154	131-131	144-144	133-135	145-145	115-129	129-133	125-129	154-154
	110	144-150	131-131	136-144	135-135	145-145	127-127	123-129	125-131	150-154
	116	148-154	131-131	144-144	133-135	139-145	119-127	123-133	137-141	152-156
	150	148-150	131-131	144-144	135-139	145-145	117-117	123-123	125-125	148-156
Algerian cheetahs	805	000-000	000-000	130-130	135-135	141-141	123-125	129-129	123-129	150-152
	806	150-158	131-131	136-136	135-135	143-145	121-133	129-129	133-133	000-000
	809	158-160	131-131	144-146	135-135	143-143	127-127	141-141	000-000	154-158
	816	158-160	131-131	144-146	133-135	143-143	127-127	119-129	131-139	154-154
	817	156-158	131-131	146-146	135-135	143-143	127-127	000-000	133-139	152-154
	823	000-000	131-135	142-142	135-135	000-000	121-127	119-129	123-129	152-154
	827	152-158	131-131	144-146	135-135	139-145	121-127	000-000	000-000	152-152
843	158-160	131-131	136-136	135-135	143-143	000-000	129-129	133-139	142-154	
Error rate / locus	0.12	0.5	0.4	0.05	0.17	0.00	0.08	0.12	0.12	

Table 3.3. shows the numbers of unique alleles for each population and the estimated expected (Hexp) and observed heterozygosities (Hobs) for the two populations. The genotypes were analysed further in GIMLET to determine whether the Algerian samples belonged to different individuals.

Table 3.3. Summary microsatellite data for the two cheetah populations

Population	No of alleles	No of unique alleles	Hexp	Hobs
Tanzanian	33	17 (32%)	0.55	0.64
Algerian	36	18 (34%)	0.53	0.41
TOTAL	53			

Table 3.4. below shows a matrix of the number (lower diagonal) and the percentage (upper diagonal) of identical loci between each of the 8 cheetahs. Missing data is treated as any allele. Even so, the highest percentage of identical loci is 67%. It can therefore be assumed that all of the Algerian samples come from different individuals.

Table 3.4. A pairwise matrix of Algerian cheetah genotype comparisons over the 9 microsatellite loci

The upper diagonal is the percentage of pairwise similarity and the lower is the actual number of identical loci

Sample	805	806	809	816	817	823	828	843
805	*	56	44	22	44	56	56	56
806	5	*	44	22	44	44	56	67
809	4	4	*	67	67	44	56	67
816	2	2	6	*	44	33	44	44
817	4	4	6	4	*	56	44	67
823	5	4	4	3	5	*	67	44
827	5	5	5	4	4	6	*	56
843	5	6	6	4	6	4	5	*

3.2.2. Kinship

Although in terms of number of loci amplified the sample size was small, kinship analysis of both populations was conducted with GIMLET through the exclusion method. The kinship of the Tanzanian cheetahs was known and as such could be used to test the reliability of any kinship inferences made about the Algerian individuals. The Tanzanian group contained two cubs from the same litter, individuals 106 and 107, and 94 is the parent of 93. GIMLET inspects the Mendelian inheritance of its alleles for each individual in turn, with each of the other individuals in the group. An individual is considered a parent if it shares at least one allele per locus with the potential offspring. GIMLET successfully identified individuals 93 and 94 to be parent/offspring. Analysis of the Algerian group is shown in table 3.5.

Table 3.5. Relatedness result from MER and GIMLET for the Algerian cheetahs

Individual 1	Individual 2	Estimates from MER		Possible Kinship		GIMLET
		Δ	SD	r	SD	
806	843	0.14	0.4	0.59	0.18	parent-offspring
809	816	0.55	0.3	0.54	0.35	full-sibs (siblings)
809	817	0.07	0.3	0.5	0.17	parent-offspring
816	817	-0.2	0.3	0.42	0.14	parent-offspring
817	843	0.39	0.3	0.22	0.33	half-sibs

The table shows the combined results for the two kinship analyses. Essentially, similar results were achieved for both analyses. GIMLET is unable to assign siblingship explicitly. The putative sibling relationship between 809 and 816 is as a result of both individuals sharing at least one allele over all 9 loci with individual 817, but not between each other.

These results indicate that individuals 806 and 843 are parent/offspring. 809 and 817 are also likely parent offspring, as are 816 and 817. 809 and 806 do not share at least one allele per locus but both do with 817. Therefore these three are possibly a family group of three, 817 being the parent with 809 and 816 offspring. In order to test these relationships further the same genotypes were run through another program, MER (Wang 2004). MER gives an estimate of three measures, phi, Φ , delta, Δ , and relatedness, r. Δ and r are used in the present analysis as Φ can only be used with reasonable confidence as a measure when the number of informative loci used is large (Wang 2002). Δ is the

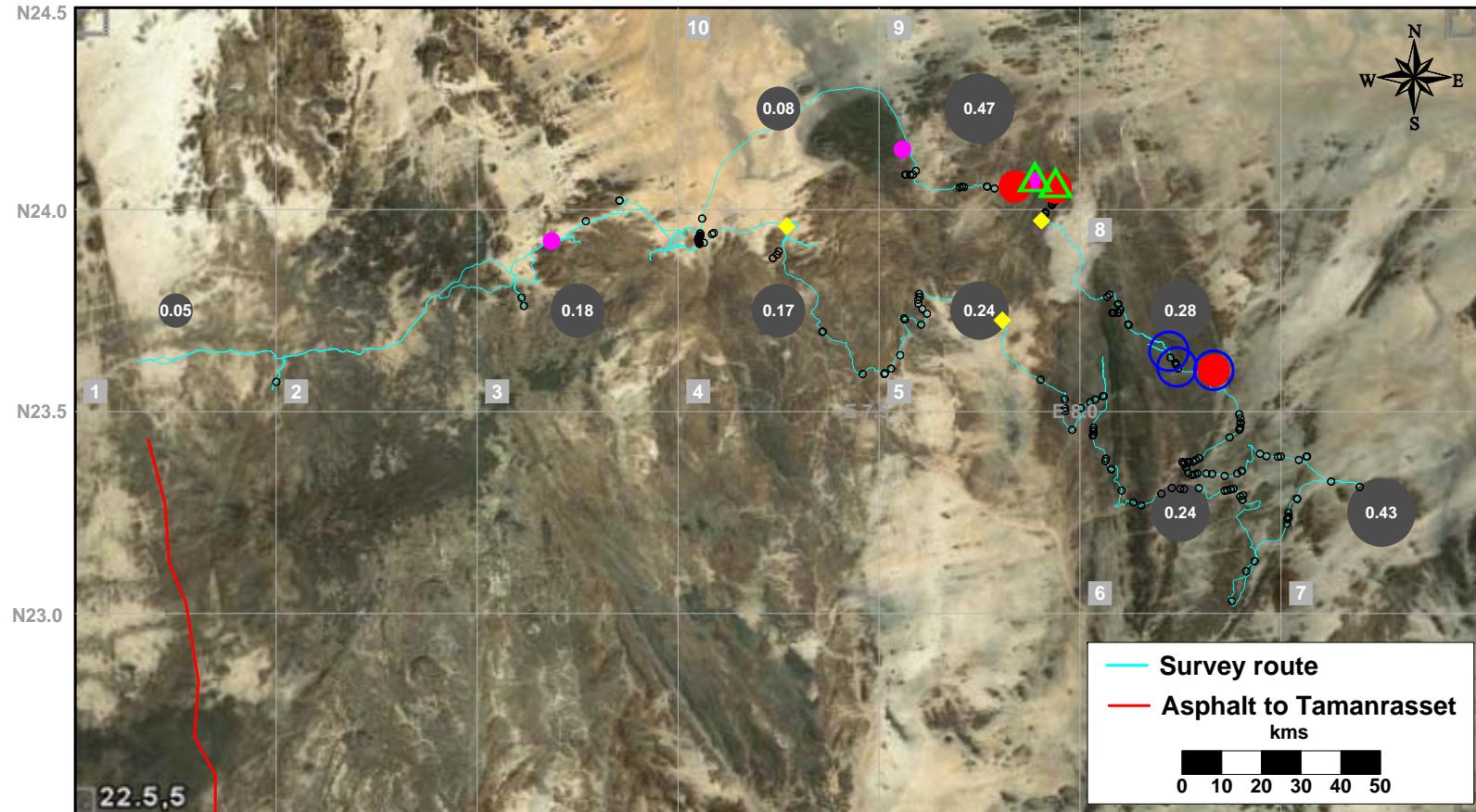
probability that both alleles at a locus are identical by descent and r is the standard measure of relatedness, measuring from 0 (completely un-related) to 1 (identical). Δ and r are accompanied by a bootstrap standard deviation (SD). In an out-breeding population, Δ , and r are 0, and 0.5, respectively, for parents and offspring; for full-siblings, 0.25, and 0.5, respectively, and 0, and 0.125, respectively, for half-siblings (Wang 2002).

For the Tanzanian cheetah, MER indicated that 94 and 93 were highly related with a very high $r = 0.86$ (+/- 0.13 SD) and $\Delta = 0.78$ +/- 0.19 SD, which implies more of a sibling relationship than parent-offspring. Individuals 106 and 107 had an $r = 0.47$ (0.32SD), but again had an inflated Δ value (= 0.61 +/- 0.23 SD) than the 0.25 expected for full sibs. This could be because of the known inbreeding in the Tanzanian population. The results for the Algerian cheetahs are shown in table 3.4. above. Pairs 806 and 843, 809 and 816, 809 and 817, and 816 and 817 all have r of around 0.5. Their Δ values are different however. Pairs 806 and 843, 809 and 817, and 816 and 817 all have lower scores expected to be attributable to parent-offspring relationships. 809 and 816 have a high Δ which is more like a putative full-sibling relationship. The final pair in table 3.5. have a low r but a higher Δ than the expected 0 for half-siblings. The SD on Δ is however large.

3.3. Mapping the cheetah samples

The majority of scat was found on trees, as these locations were targeted by the expedition. 6 out of the eight cheetah samples were found on, or around, *Tamarix* trees. Figure 3.3. shows the positions of the 8 cheetah samples on a map of the study area. All cheetah samples, and the leopard were found in grids 8 and 9. The putative families of cheetah are also highlighted in different colours. The family of three (809, 817, 816) were all found in close proximity of each other, as was the other parent-offspring pairing (806 and 843). The three remaining 'unrelated' cheetah were found dispersed but in the same two grids of the survey. The gazelle densities in this region were among the highest encountered, and this area was very remote (Tim Wacher pers comm.). The area was characterised by long wadis, interspersed with *Tamarix* trees, with high mountains on either side.

Cheetah reports and signs were found over a greater range than the genetic data alone would suggest. One of these, in grid 3, was a camel kill with definite cheetah tracks suggesting that cheetah roam at least that far west. Cheetah are also known to occur in the Tassili mountains to the north-east and also north of the study sight (Tim Wacher pers comm.).



CHEETAH OBSERVATIONS: SSIG/OPNA MARCH 2005

- Unrelated Cheetah 823, 805, 827
- ▲ Parent - Offspring 806, 843
- Family Group 817, 809, 816
- N Half degree grid reference number

- Gazelle seen / km
- ◆ Locations where guides reported previous cheetah encounters
- Tracks 2005
- Large scat on trees 2005

Figure 3.3. The positions of the cheetah samples in the Ahaggar Mountains, Southern Algeria. The survey route is shown in light green. The family group of three cheetahs, 817, 809, 816, are shown by blue circles and can be seen to be in the same area. The same is true for the two green triangles, another putative family group (parent-offspring). The three unrelated cheetah appear in both localities suggesting that cheetahs might be aggregating.

4. Discussion

4.1. A carnivore species list for the Ahaggar Mountains Southern Algeria

This report provides conclusive proof of the presence of six carnivore species in this region of Algeria.

4.1.1. Non-felid species

Genets are carnivores of the Viverridae family, belonging to the Feliformia branch of the lineage. According to Kingdon (1997) only one species is present in the Northern reaches of Africa, and it is probable that the species found here is *Genetta genetta*, the common genet. Indeed, genet have been reported in Algeria, surviving on small rodents and occasionally reptiles and insects (Lariviere and Calzada 2001). Kowalski and Rzebik-Kowalska (1991) report genet in Algeria, but only in the more coastal region and the Tell Atlas. In Spain, where most of the ecological fieldwork has been conducted, genet are a common prey species for owls and other birds of prey, as well as sympatric carnivores such as the Iberian lynx and domestic dogs (Lariviere and Calzada 2001). Given their relative abundance in this study (1/6 of all samples), their presence in the Ahaggar is novel. Although never documented, they might also present an opportunistic prey item for cheetah.

Individual 814 BLASTED as a banded mongoose, *Mungos mungo*. However, figure 3.2. shows that there is some difference in the cytochrome *b* of this sample and that of the banded mongoose cytochrome *b* sequenced for this study. Banded mongoose are not reported north of the Sahara and this species might in fact be the Egyptian mongoose, *Herpestes ichneumon* (Kingdon 1997). Kowalski and Rzebik-Kowalska (1991) report the Egyptian mongoose, as the only herpestid in Algeria. Proof of the presence of a small carnivore in this area is encouraging.

The fourteen dogs reported in this paper are likely domestic dogs kept by villagers and Toureg nomads. Golden jackals, *Canis aureus*, are found in the Ahaggar (Kowalski and Rzebik-Kowalska 1991; Wacher et al 2005), but the inclusion of a reference sample shows that the canids found are not jackals. Two other canids, Fennec foxes, *Vulpes zerda*, and Ruppells fox, *Vulpes rueppellii*, are also both found in the Ahaggar (Kowalski and Rzebik-Kowalska 1991; Wacher et al 2005), and Ruppells fox were caught in camera traps during the SSIG expedition (Wacher et al 2005). Although individual 812 had fox-like 12S (Appendix 2, figure 3.1.), cytochrome *b* showed it to be a genet. The canid species discovered here are unresolved. When BLASTED, most individuals appeared to be most similar to non-African canids in both cytochrome *b* and 12S sequences (Appendix 2). This might be the case if the dogs were mongrel domestic dogs. Therefore, although evidence of Ruppells fox and common jackals was found during the SSIG

expedition (Wacher et al 2005), none of the samples in the present study can be unambiguously assigned to these species.

4.1.2. Felid species

Perhaps the most intriguing result is the occurrence of a leopard sample, *Panthera pardus*. Kowalski and Rzebik-Kowalska (1991) report the leopard to be extinct in Algeria. They cite that during the 19th century the leopard was common in Northern Algeria but (following extensive hunting), other than possible migrants to Western Algeria from the Saharan Atlas in Morocco, the leopard is extinct in the country. Therefore this study presents the first proof that leopard are present in Algeria for more than 50 years. It also provides a note of its occurrence in an area of the country where it has never been seen before: the south-eastern Ahaggar massif.

A *Felis* sample was also uncovered in the present study. Unfortunately, sequencing for cytochrome b did not work for this sample, but the 12S analysis suggests that the cat found here was not the sand-cat, *Felis margarita*, which is likely to be widespread in the Algerian Sahara (Kowalski and Rzebik-Kowalska 1991). Wildcat tracks were found during the expedition (Wacher et al 2005). Records show that the wildcat, *Felis silvestris*, is present in much of the mountainous regions of Algeria (Kowalski and Rzebik-Kowalska 1991) and given that the African wildcat is often considered to be the ancestor of today's domestic cats (Alderton 1998) it is not surprising that it occurs in a clade with *F. catus* (figure 3.1.).

Eight different individual cheetah samples were found in the present study. Observations made during the 2005 expedition and reports collected by the team suggested that there were indeed cheetah in this area of Algeria. Conclusive proof of their presence is presented in this paper.

4.2. The present situation of cheetah in the Ahaggar Mountains

Figure 3.3. shows the cheetah samples plotted on a map of the region. All cheetah samples were found within two grids (8 and 9) in the study. Interestingly, the cheetah of the putative family groups (see figure 4.1.) map into similar positions. Grid 8, where half of the cheetah sample, and the leopard sample, were found, is also the grid with the highest density of gazelle. This area was characterised by long, deep wadis with patchy *Tamarix* bush (Tim Wacher pers comm). It was also one of the more remote areas of the survey. Together with the known records of cheetah in the Tassili and north of the Ahaggar, these data suggest that a population of cheetah is maintained in Algeria.

The microsatellite analysis of the cheetah samples suggest that at least two family groups were present, which might possibly part of one larger family (figure 4.1.).

Figure 4.1. The Algerian cheetah family

The picture that figure 4.1. shows is one of a related group of animals occupying a range in the north-eastern area of the SSIG expedition's study area. The analysis is based on the assumption that the allele frequencies of the population as a whole are equal to those displayed in the small sample investigated. This is unlikely to be the case. However it is highly likely that the cheetah are related in some way. The discovery of another 3 potentially unrelated individuals within a relatively small area suggests a larger total population than previously identified.

The number of unique alleles in both populations was roughly one third. Although this is based on a sample size too small to be tested statistically, it is potentially a very important result as it implies that the two populations are genetically dissimilar. The observed heterozygosity in the Algerian population is lower than the expected while in the Tanzanian population the inverse is true. However, the confidence in these results was small because of small sample sizes and this result suggests some demographic process might be occurring, but further more detailed work is needed.

4.3. Implications for the future

4.3.1. A novel technique for identifying unknown species in difficult conditions

Although non-invasive sampling is increasingly used in studying the genetics of known animals, this study presents a novel use of non-invasive techniques. Methods of the kind employed here can be used to evaluate the species composition in areas where time and resources are at a premium. Combined with other techniques, such as the use of domestic dogs to locate scat samples of defined species and even particular individuals, in large remote areas (see Wasser et al 2004), this study reports a powerful tool for assessing the species types within an area with the aim of providing proof of residence and therefore highlighting areas where endangered species might be present, in a quick and relatively cheap timeframe. Preliminary studies such as this can, and should, aid the direction of research and funding in the future and are of particular use in areas where animals are rare and the terrain is rough.

4.3.2. Future North African Cheetah studies

There were no 'recaptures' during this study: all of the cheetah individuals were unique. However, more extensive collection of scat in a systematic survey, with definite 'recaptures' will potentially lead to estimates of population size and range of individual cheetahs and the population as a whole. Geographic information of the sort collected by the SSIG expedition also helps to build a better picture of the lifestyle and habit of these Saharan cheetah.

Cheetah are infamously genetically similar (O'Brien et al 1983; O'Brien et al 1985; Wayne et al 1986; O'Brien et al 1987). Given that around one third of the alleles over the two populations are unique, this study highlights the need for further genetic work on a larger sample size in order to corroborate this result. It is also essential for the future management of this endangered species that these results be used to increase the conservation effort in this area of Africa.

4.4. Conclusions

This paper has conclusively proven the presence of cheetah in southern Algeria. The population is potentially larger than the several dozen assumed to survive in the area. It has also proven the existence of leopards in a previously unreported area of Africa. Therefore, this report provides impetus for further longer term and more intensive study into the felid species of this region of Africa. The eremitic desert habit of these carnivores is unlike any other areas where they are found. Nothing is known of the possible range, population numbers or adapted behaviours of these animals. It is in the interest of all conservationists that this lack of data be addressed now in the planning and proposing of continued research in Algeria.

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6. References

- Alderton, D.** *Wildcats of the World* Blandford Publ. Cassell Plc. UK. 1998
- Altschul, S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. 1997**
Gapped BLAST and PSI-BLAST: a new generation of protein database search programs
Nucleic Acids Research **25** 3389-3402
- Banks S.C., Hoyle, S.D., Horsup, S.D., Sunnock, P., Taylor, A.C. 2003** Demographic monitoring of an entire species (the northern hairy-nosed wombat, *Lasiorhinus krefftii*) by genetic analysis of non-invasively collected material *Animal Conservation* **6** 101-107
- Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Wheeler, D.L. 2005** GenBank
Nucleic Acids Research **33** – online issue www.ncbi.nlm.nih.gov/Genbank/index.html
- Bjornerfeldt, S., Webster, M.T., Vila,C. 2006** Relaxation of selective constraint on dog mitochondrial DNA following domestication *Genome Research* **16** 8 990-994
- Burger, P.A., Steinborn, R., Walzer, C., Petit, T., Mueller, M., Schwarzenberger,F. 2004**
Analysis of the mitochondrial genome of cheetahs (*Acinonyx jubatus*) with neurodegenerative disease *Gene* **338** 1 111-119
- Caro, T.M., Laurenson, M.K. 1994** Ecological and Genetic Factors in Conservation: a cautionary tale *Science* **263** 485-486
- Das, D.K., Saini, M., Gupta, A.R., Swarup, D., Yadav, M.P. Gupta, P.K. 2005** PCR amplification and sequencing of mitochondrial 12S rRNA gene fragment from Indian jackal *Canis aureus* – unpublished GenBank sequence - *DQ102371*
- Driscoll, C.A. 1992** A Characterization of Microsatellite Loci Variation in *Panthera Leo*, *Acinonyx jubatus* and *Felis concolor* Unpublished MSc Thesis. University of Maryland, Baltimore
- Flynn, J.J., Finarelli, J.A., Zehr, S., Hsu, J., Nedbal, M.A. 2005** Molecular Phylogeny of the Carnivora (Mammalia): Assessing the Impact of Increased Sampling on Resolving Enigmatic Relationships *Systematic Biology* **54** 2 317-337
- Gaubert, P., Fernandes, C.A., Bruford, M.W., Veron, G. 2004** Genets (Carnivora, Viverridae) in Africa: an evolutionary synthesis based on cytochrome b sequences and morphological characters *Biological Journal of the Linneus Society of London* **81** 4 589-610
- Gottelli, D., Marino, J., Sillero-Zubiri, C., Funk, S. M. 2004** The effect of the last glacial age on speciation and population genetic structure of the endangered Ethiopian wolf (*Canis simensis*) *Molecular Ecology* **13** 2275-2286.
- Gotelli, D., Wang, J., Bashir, S. Durant, S.M. under review** Genetic benefits of promiscuity among Serengeti female cheetah
- Hamdine, W., Meftah, T., Sekhi, A. 2003** Repartition et statut du guépard (*Acinonyx jubatus* Schreber, 1776) dans le Sahara central Algérien (Ahaggar et Tassili) *Mammalia* **69** 3 403-408

- Iyengar, A., Babu, V.N., Hedges, S., Venkataraman, A.B., Maclean, N., Morin, P.A. 2005**
Phylogeography, genetic structure and diversity in the Dhole (*Cuon alpinus*) *Molecular Ecology* **14** 2281-2297
- Janczewski, D., Modi, W., Stephens, J., O'Brien, S. 1995** Molecular Evolution of Mitochondrial 12S RNA and Cytochrome B sequences in the Pantherine Lineage of Felidae. *Molecular Biology and Evolution* **12** 4 690-707
- Kingdon, J.** *The Kingdon Field Guide to African Mammals* Academic Press, A&C Publishers, London. 1997
- Koepfli, K.-P., Jenks, S.M., Eizirik, E., Zahirpour, T., Van Valkenburgh, B., Wayne, R.K. 2006**
Molecular systematics of the Hyaenidae: Relationships of a relictual lineage resolved by a molecular supermatrix *Molecular Phylogenetic Evolution* **38** 3 603-620
- Kimura, M. 1980** A simple way for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences *Journal of Molecular Evolution* **16** 2 111-20
- Kowalski, K., Rzebik-Kowalska, B.** *Mammals of Algeria* Polish Academy of Sciences. Ossolineum. Krakow. Poland. 1991
- Kurose, N., Abramov, A.V., Masuda, R. 2000** Intrageneric diversity of the cytochrome b gene and phylogeny of Eurasian species of the genus mustela (Mustelidae, Carnivora) *Zoological Science* **17** 673-679
- Kurose, N., Masuda, R., Tatara, M. 2005** Fecal DNA Analysis for Identifying Species and Sex of Sympatric Carnivores: A Noninvasive Method for Conservation on the Tsushima Islands, Japan *Journal of Heredity* **96** 6 688-697
- Ledje, C. and Arnason, U. 1996** Phylogenetic relationships within caniform carnivores based on analyses of the mitochondrial 12S rRNA gene *Journal of Molecular Evolution* **43** 6 641-649
- Lariviere, S., Calzade, J. 2001** Genetta genetta *Mammalian Species* **680** 1-6
- Marker, L.** Proceedings of a Symposium on Cheetahs as Game Ranch Animal, Onderstepoort, 23-24 October 1998
- Marker, L. 2000** Aspects of the Ecology of the Cheetah *Journal of the Namibia Scientific Society* **48** 41-48
- Marker, L. 2002** Aspects of Cheetah (*Acinonyx jubatus*) Biology, Ecology and Conservation Strategies on Namibian Farmlands. DPhil Thesis. University of Oxford
- Masuda, R., Yoshida, M.C., Shinyashiki, F., Bando, G. 1994** Molecular phylogenetic status of the Iriomote cat, *Felis iriomotensis*, inferred from mitochondrial DNA sequence analysis *Zoological Science* **11** 4 597-604
- Meece, J.K., Reynolds, C.E., Stockwell, P.J., Christensen, J.E., Reed, K.D. 2005** Identification of mosquito blood meal source by terminal restriction fragment length polymorphism (T-RFLP) profile analysis of the cytochrome b gene. *Journal of Medical Entomology* **42** 4 657-667

- Menotti-Raymond, M. and O'Brien, S.** 1993 Dating the genetic bottleneck in Africa. *Proceedings of the National Academy of Science USA* **90** 3172-3176
- Menotti-Raymond, M., David, V.A., Lyons, L.A., Schäffer, A.A., Tomlin, J.F., Hutton, M.K., O'Brien, S.J.** 1999 A Genetic Linkage Map of Microsatellites in the Domestic Cat (*Felis catus*) *Genomics* **57** 1 9-23
- Nowell, K., Jackson, P.** 1996 Cheetah Conservation Action Plan. IUCN
- O'Brien, S. J., Wildt, D. E., Goldman, D., Merril, C., Bush, M.** 1983 The cheetah is depauperate in genetic variation *Science* **221** 459-462
- O'Brien, S. J., Roelke, M. E., Marker, L., Newman, A., Winkler, C. A., Meltzer, D., Colly, L., Evermann, J. F., Bush, M. & Wildt, D. E.** 1985 Genetic basis for species vulnerability in the cheetah *Science* **227** 1428-1434.
- O'Brien, S. J., Wildt, D. E., Bush, M., Caro, T. M., FitzGibbon, C., Aggundey, I. & Leakey, R. E.** 1987 East African cheetahs: evidence for two population bottlenecks? *Proceedings of the National Academy of Science USA* **84** 508-511
- Palomares, F., Godoy, J.A., Piriz, A. O'Brien S.J., Johnson, W.E.** 2002 Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx *Molecular Ecology* **11** 2171-2182
- Penny, D. McLenachan, P.** 2005 unpublished Genbank sequence - AY873843
- Pompanon, F., Bonin, A., Bellemain, E., Taberlet, P.** 2005 Genotyping errors: Causes, consequences and solutions *Nature Reviews Genetics* **6** 847-859
- Posada, D., Crandall, K.A.** 1998 Modeltest: testing the model of DNA substitution. *Bioinformatics* **14** 9 817-818
- Sugimoto, T., Nagata, J., Aramilev, V.V., Belozor, A., Higashi, S. McCullough, D.R.** 2005 Species and sex identification from faecal samples of sympatric carnivores, Amur Leopard and Siberian Tiger, in the Russian Far East – unpublished Gen Bank sample – AB211405
- Swofford, D.L.** 1998 PAUP Phylogenetic analysis using parsimony and other methods. Sinauer Associates. Sunderland MA
- Taberlet, P., Griffin, S., Gossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L.P., Bouvet, J.** 1996 Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acid Research* **24** (16) 3189 - 3194
- Tavare, S.** 1986 Some probabilistic and statistical problems in the analysis of DNA sequences. In *Some mathematical questions in biology – DNA sequence analysis* (ed. Miura, R.M.) pp 57-86
- Tamura, K., Nei, M.** 1993 Estimation of the number of nucleotide substitutions in the control region of the mitochondrial DAN of humans and chimpanzees *Molecular Biology and Evolution* **10** 512-526

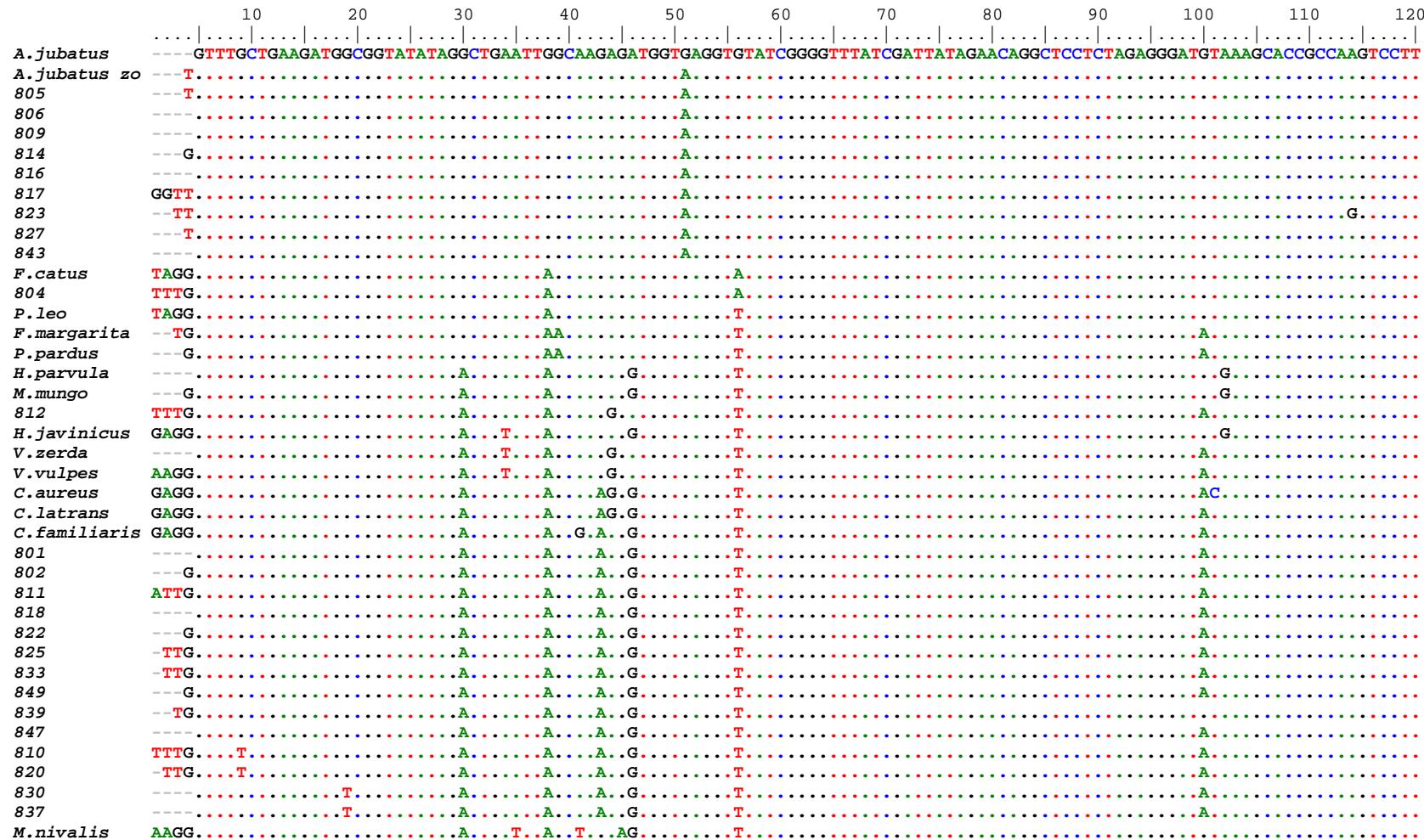
- Triant, D.A., Pace, R.M., Stine, M. 2004** Abundance, genetic diversity and conservation of Louisiana black bears (*Ursus americanus luteolus*) as detected through noninvasive sampling *Conservation Genetics* **5** 647-659
- Valiere, N. 2002** GIMLET: a computer program for analyzing genetic individual identification data. *Molecular Ecology Notes* **2** 377-379
- Wacher, T., De Met, K., Belbachir, F., Belbachir-Bazi, A., Fellous, A., Belghouf, M., Marker, L. 2005.** Sahelo-Saharan Interest Group Wildlife Surveys, Central Ahaggar Mountains March 2005
- Wang, J. 2002** An estimator for pairwise relatedness using molecular markers *Genetics* **160** 1203-1215
- Wang, J. 2004** Estimating pairwise relatedness from dominant genetic markers *Molecular Ecology* **13** 3169-3178
- Wasser, S.K., Davenport, B., Ramage, E.R., Hunt,K.E., Parker, M., Clarke,C., Stenhouse, G. 2004** Scat detection dogs in wildlife research and management: application to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada *Canadian Journal of Zoology* **82** 3 475-492
- Wayne, R. K., Modi, W. S. & O'Brien, S. J. 1986** Morphological variability and asymmetry in the cheetah (*Acinonyx jubatus*): a genetically uniform species. *Evolution* **40**, 78-85
- Wayne, R.K., Geffen, E., Girman, D.J., Koepfli, K.P., Lau, L.M., Marshall, C.R. 1997** Molecular systematics of the Canidae *Systematic Biology* **46** 4 622-653
- Wildt, D.E., O'Brien, S.J., Howard, J.G., Caro, T.M., Roelke, M.E., Brown, J.L., Bush, M. 1987** Similarity in ejaculate-endocrine characteristics in captive versus free-ranging cheetahs of two subspecies *Biology of Reproduction* **36** 351-360
- Yoo, M.H., Jeon, H.S., Kim, B.S., Rho,J.R. 2006** Genetic diversity of *Vulpes vulpes* unpublished GenBank sequence - DQ498127
- Yu, L., Li, Q., Ryder, O.A., Zhang, Y. 2004** Phylogenetic relationships within the order Carnivora indicated by sequences of two nuclear DNA genes *Molecular Phylogenetics and Evolution* **33** 694-705
- Yu, L. Zhang, Y. 2005** Phylogenetic studies of pantherine cats (Felidae) based on multiple genes, with novel application of nuclear nuclear β fibrinogen intron 7 to carnivores *Molecular Phylogenetics and Evolution* **35** 483-495
- Yu, L. Zhang, Y. 2006** Phylogeny of the caniform carnivoran: evidence from multiple genes *Genetica* **127** 65-79

APPENDIX 1

12S AND CYTOCHROME b SEQUENCES

The following sequences are the result of careful alignment of the sequences. They have been grouped together into family clades. Their similarity to the cheetah sequence is shown by dots. A dot in the sequence shows that that base is the same as the one in the top line, that of *Acinonyx jubatus*.

The first set of sequences are the 12S, the second are the cytochrome *b*. The names on the left of each sequence refer to the numbers of the samples or the species of reference sample (tables 2.1. and 3.1.).



12S SEQUENCE

	130	140	150	160	170	180	190	200	210	220
<i>A.jubatus</i>	TGAGTTTAAGCTGTTGC	TAGTAGTTCTGGCGGATAG	TTTGTTGAGTTAACTATCTAG	GGGCTAAGCA	TAGTGGGGTATCTAA	TCCCAGTTA				
<i>A.jubatus zo</i>			A.					A.		
805			A.					A.		
806										
809		G.	C.	A.					A.	
814										
816			A.					A.		AA
817				A.				A.		AA
823			C.	A.						TA
827										
843				A.				A.		AA
<i>F.catus</i>		A.			AG.A.	A.				
804		A.			AG.G.	A.				TA
<i>P.leo</i>					G.					GGA
<i>F.margarita</i>					G.C.					
<i>P.pardus</i>					G.C.					
<i>H.parvula</i>				A.	T.A..G.	A.				TC
<i>M.mungo</i>		A.	C.	A.	T.A..G.	G.				
812				A..T.	GC	T..T.				TA
<i>H.javinicus</i>					G.	A..A.	A.			GGG
<i>V.zerda</i>				A..T.	C.C.	T..T.				
<i>V.vulpes</i>				A..T.	AT..	T..T.				GGG
<i>C.aureus</i>		A.	C.	A..TA	TAA..T.	GT..				G..
<i>C.latrans</i>		A.	C.	A..TA	TAA..T.	T..				GGG
<i>C.familiaris</i>		A.	C.	A..TA	TAA..T.	T..				GGG
801		A.	C.	A..TA	TAA..T.	T..				CC
802		A.	C.	A..TA	TAA..T.	T..				
811		A.	C.	A..TA	TAA..T.	T..				
818		A.	C.	A..TA	TAA..T.	T..				
822		A.	C.	A..TA	TAA..T.	T..				
825		A.	C.	A..TA	TAA..T.	T..				
833		A.	C.	A..TA	TAA..T.	T..				TT
849		A.	C.	A..TA	TAA..T.	T..				
839		A.	C.	A..TA	TAA..T.	T..				
847		A.		TA..	G..					
810		A.	C.	A..TA	TAA..T.	T..				TA
820		A.	C.	A..TA	TAA..T.	T..				TA
830		A.	C.	A..TA	TAA..T.	T..				
837		A.	C.	A..TA	TAA..T.	T..				
<i>M.nivalis</i>		A.	AA..A.	AA..A..T.	AG..T..T..T..		G..			GGG

12S SEQUENCE

THE CHEETAH IN NORTHERN AFRICA

CYTOCHROME b SEQUENCE

THE CHEETAH IN NORTHERN AFRICA

	130	140	150	160	170	180	190	200	210	220	230	240						
<i>A.jubatus</i>						
<i>A.jubatus zo</i>	GTAGGAGCCG	TAGTATATT	CCTCGTCCTACATGCATGTATA	AGCAGATAAAGAA	TATAGGGCTCCGTTGGCGTGCA	TGTATCGAATT	CAGCCGTAGTTGAC	GTCGCGGCAGATGTG										
805						
806						
809						
816						
817						
843	T						
<i>F.catus</i>	C.	C.	T.	C.	T.	AA.	G.	A.	A.	A.	
<i>P.leo</i>	A.	A.	T.G.	C.	A.	T.	A.
870	A.	T.GA.	C.	A.	A.	T.	A.
<i>P.pardus</i>	A.	T.GA.	C.	A.	A.	T.	A.
<i>G.maculata</i>	A.T.	C.	G.	T.	AA.	A.	G.	A.	A.	A.
812	A.T.	C.	G.	T.	AA.	A.	G.	A.	A.	AT.
824	A.T.	C.	G.	T.	AA.	A.	G.	A.	A.	A.
826	A.T.	C.	G.	T.	AA.	A.	G.	A.	T.	A.
830	A.T.	C.	G.	T.	AA.	A.	G.	A.	A.	A.
837	A.T.	C.	G.	T.	AA.	A.	G.	A.	A.	A.
<i>M.mungo</i>	A..A..A.	G.	A.	TG.	T.	AA.	A.	G.	A.	A.	A..A..A.
814	A..A..A.	G.	A.	TG.	T.	AA.	A.	G.	A.	A.	A..A..A.
<i>H.parvula</i>	A..A..A.	G.	C.	T.	AA.	A.	G.	A.	G.
<i>H.javanicus</i>	A..A..A.	A.	G.	G.	T.	A.	A.	T.
<i>C.aureus</i>	A..A..T.	GG	G.	T.	A.	G.	A.	G.	T.	A..T.
818	A..A..T.	GG	T.	A.	A.	G.	T.	A..T.	A..G..G.
831	A..A..T.	GG	T.	A.	A.	G.	T.	A..T.	A..G..G.
847	A..A..T.	GG	T.	A.	A.	G.	T.	A..T.	A..G..G.
813	A..A..T.	GG	T.	A.	A.	G.	T.	A..T.	A..G..G.
<i>C.familiaris</i>	A..A..T.	A..G	T.	G.A.	G..A..G..A..T..A..G..G..	A..A..T..A..G..G..	A..T..A..G..G..
839	A..A..T.	GG	T.	A.	A..G..A..T..A..G..G..	A..A..T..A..G..G..	A..T..A..G..G..
<i>C.latrans</i>	A..A..T.	GG	C..T..A..C..A..A..	G..A..G..A..T..A..G..G..	A..A..T..A..G..G..	A..T..A..G..G..	
<i>V.vulpes</i>	A..A..T..A..A..	AG	G..A..G..A..T..A..G..G..	G..A..G..A..T..A..G..G..	A..A..T..A..G..G..	A..T..A..G..G..	
<i>V.zerda</i>	A..A..T..A..A..	AG	G..A..G..A..T..A..G..G..	G..A..G..A..T..A..G..G..	A..A..T..A..G..G..	A..T..A..G..G..	
<i>H.hyaena</i>	A..A..T..A..A..	C..G..GG..G..A..C..	G..A..C..G..C..	G..A..G..A..T..A..G..G..	A..A..T..A..G..G..	A..T..A..G..G..	
<i>M.nivalis</i>	A..A..A..A..A..	AG	G..T..G..A..	A..	G..A..G..A..T..A..G..G..	A..A..T..A..G..G..	A..T..A..G..G..	

CYTOCHROME b SEQUENCE

	250	260	270	280	290	300	310	320	330	340	350	360
<i>A.jubatus</i>
<i>A.jubatus zo</i>
805
806
809
816
817
843
<i>F.catus</i>	G.....
<i>P.leo</i>	G..G.....	G..A.....
870	G.....	G..A.....	G.....
<i>P.pardus</i>	G.....	G..A.....	G.....
<i>G.maculata</i>T.....G.....A.....A.....A.....A.....T.....T.....A.....T.....G.....A.....
812T.....G.....A.....A.....A.....A.....T.....T.....A.....T.....G.....C.....
824T.....G.....A.....A.....A.....A.....T.....T.....A.....T.....G.....C.....
826T.....G.....A.....A.....A.....A.....T.....T.....A.....T.....G.....C.....
830T.....G.....A.....A.....A.....A.....T.....T.....A.....T.....G.....C.....
837T.....G.....A.....A.....A.....A.....T.....T.....A.....T.....G.....C.....
<i>M.mungo</i>	G..T.....G.....A.....A.....A.....A.....T.....T.....A.....T.....G.....A.....
814	G..T.....G.....A.....A.....A.....A.....A.....T.....T.....A.....G.....G.....
<i>H.parvula</i>	T..T.....G.....A.....A.....A.....A.....T.....T.....A.....T.....AA.....G.....
<i>H.javanicus</i>	G..T.....G.....C.....C.....C.....C.....A.....T.....A.....T.....AG.....A.....
<i>C.aureus</i>	G..G.....T.A..T.....GGC.....C.....C.....C.....A.....A.....T.....A.....AC.AC.....A.....
818	G..G.....A..T.....GGC.....C.....A.....A.....A.....T.AA.....T.....A.....ATC.A.....A.....
831	G..G.....A..T.....GGC.....C.....A.....A.....A.....T.AA.....T.....A.....ATC.A.....A.....
847	G..G.....A..T.....GGC.....C.....A.....A.....A.....T.AA.....T.....A.....ATC.A.....A.....
813	G..G.....A..T.....GGC.....C.....A.....A.....A.....T.AA.....T.....A.....ATC.A.....A.....
<i>C.familiaris</i>	G..G.....A..T.....GGC.....C.....A.....A.....C.....A.....T.AA.....T.....ATC.A.....A.....
839	G..G.....A..T.....GGC.....C.....A.....A.....A.....T.AA.....T.....A.....ATC.A.....A.....
<i>C.latrans</i>	G..G.....A..T.....GGC.....C.....A.....A.....A.....T.AA.....T.....A.....ATC.A.....A.....
<i>V.vulpes</i>	...G..A.....G..A.....A.....A.....A.....A.....AA.....T..A.....GCA.....A..TA.....T..G.....C.....
<i>V.zerda</i>	T..CG..C.....T..GG.....G.....A.....C.....T.....A.....T..A.....CA.....A..TA.....T..G.....C.....
<i>H.hyaena</i>	T..T.....G.....A.....A.....A.....A.....T.....T.....C.....A..T.....T..A.....C.....
<i>M.nivalis</i>	G..G.....T..GGC.....A.....A.....A.....A.....AA.....T..AA.....T..A.....A..TA.....T..G.....A.....

CYTOCHROME b SEQUENCE

Sequence alignment showing a highly conserved region (AATGTAGGTGGGGTGGGTAATCGATGAATGAGTGATTAAACGATTTGATAAGGGGGTGTGATTTCGAATGTTGGTCATTAAGTCCTATAGTTGAAGTACAACCGATGGTTTTTCATG) across various species. The alignment is numbered from 370 to 480. Most species show identical or very similar sequences, indicated by dots. Some variations are present in F.catus, P.leo, G.maculata, M.mungo, H.parvula, H.javanicus, C.aureus, C.familiaris, and C.latrans.

CYTOCHROME b SEQUENCE

490

<i>A.jubatus</i>
<i>A.jubatus zo</i>	TCATTGGTCA
805T.GAG
806T.GA-
809T.G--
816T.G--
817	-----
843T.GA-
<i>F.catus</i>	-----
<i>P.leo</i>	-----
870GA-
<i>P.pardus</i>G-
<i>G.maculata</i>	-----
812	-----
824	-----
826
830
837A-
<i>M.mungo</i>A-
814	-----
<i>H.parvula</i>T--
<i>H.javanicus</i>	-----
<i>C.aureus</i>	-----
818	-----
831	-----
847	-----
813	-----
<i>C.familiaris</i>	-----
839	-----
<i>C.latrans</i>	-----
<i>V.vulpes</i>	-----
<i>V.zerda</i>T.GA-
<i>H.hyaena</i>	-----
<i>M.nivalis</i>	-----

CYTOCHROME b SEQUENCE

APPENDIX 2

BLAST SEQUENCES

Below is listed the top ten BLAST (Altschul et al 1997) results for each of the 12S sequences. The identity of the top sequence, which is the sequence on GenBank that is most similar to that of the sample, was used to identify the species of carnivore that produced the scat. Reference sequences obtained from analysis of ZSL tissue samples are also included. The results include a GenBank reference, the species of that reference, a bit score and an E value. The Expect value (E) is a parameter that describes the number of hits one can "expect" to see just by chance when searching a database of a particular size. It decreases exponentially with the Score that is assigned to a match between two sequences. Essentially, the E value describes the random background noise that exists for matches between sequences. For example, an E value of 1 assigned to a hit can be interpreted as meaning that in a database of the current size one might expect to see 1 match with a similar score simply by chance. This means that the lower the E-value, or the closer it is to "0" the more "significant" the match is⁵.

A typical BLAST result is shown below. It gives the percentage match in similarity between the query, and the subject as well as the number of gaps in the query sequence compared with the subject and the score in bits and E value. It also gives a diagram of showing where exactly the two sequences differ.

Score = 385 bits (208), Expect = 4e-104 Identities = 214/217 (98%), Gaps = 0/217 (0%)

Query	2	GTTTGCTGAAGATGGCGGTATAGGCTGAATTGGCAAGAGATGGTAAGGTGTATCGGGG	61
Sbjct	1563 G	1504
Query	62	TTTATCGATTATAGAACAGGCTCCTCTAGAGGGATGTAACGCACCGCAAGTCCTTGAG	121
Sbjct	1503	1444
Query	122	TTTTAAGCTGTTGCTAGTAGTTCTCTGGCGAATAGTTTGGAGTTAACTATCTAGGT	181
Sbjct	1443 G	1384
Query	182	TTAGGGCTAACATAGTGGGTATATAATCCCAGTT	218
Sbjct	1383 C	1347

Over the following pages the BLAST results are shown for each of the good sequences obtained from the Algerian carnivore scat samples. Where possible the results of both the 12S and Cytochrome B sequences from the same sample are shown together.

⁵ <http://www.ncbi.nlm.nih.gov/blast>

BLAST RESULTS**801 12S**

Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.1 	Canis latrans isolate 2 from Color...	418	3e-114
gi 91702675 gb DQ480509.1 	Canis latrans isolate 1 from Nebra...	418	3e-114
gi 91702661 gb DQ480508.1 	Canis lupus isolate 1 from Canada ...	418	3e-114
gi 91702647 gb DQ480507.1 	Canis lupus isolate 2 from Saudi A...	418	3e-114
gi 91702633 gb DQ480506.1 	Canis lupus isolate 1 from Saudi A...	418	3e-114
gi 91702619 gb DQ480505.1 	Canis lupus isolate 1 from Spain mito	418	3e-114
gi 91702605 gb DQ480504.1 	Canis lupus isolate 1 from Sweden ...	418	3e-114
gi 91702591 gb DQ480503.1 	Canis lupus isolate 1 from Russia ...	418	3e-114
gi 91702549 gb DQ480500.1 	Canis familiaris isolate 1 breed S...	418	3e-114
gi 91702535 gb DQ480499.1 	Canis familiaris isolate 1 breed S...	418	3e-114

802 12S

Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.1 	Canis latrans isolate 2 from Color...	420	8e-115
gi 91702675 gb DQ480509.1 	Canis latrans isolate 1 from Nebra...	420	8e-115
gi 91702661 gb DQ480508.1 	Canis lupus isolate 1 from Canada ...	420	8e-115
gi 91702647 gb DQ480507.1 	Canis lupus isolate 2 from Saudi A...	420	8e-115
gi 91702633 gb DQ480506.1 	Canis lupus isolate 1 from Saudi A...	420	8e-115
gi 91702619 gb DQ480505.1 	Canis lupus isolate 1 from Spain mito	420	8e-115
gi 91702605 gb DQ480504.1 	Canis lupus isolate 1 from Sweden ...	420	8e-115
gi 91702591 gb DQ480503.1 	Canis lupus isolate 1 from Russia ...	420	8e-115
gi 91702549 gb DQ480500.1 	Canis familiaris isolate 1 breed S...	420	8e-115
gi 91702535 gb DQ480499.1 	Canis familiaris isolate 1 breed S...	420	8e-115

804 12S

Genbank Reference	Species	score	E value
gi 1871557 emb Y08503.1 	F.domesticus mitochondrial 12S r	432	2e-118
gi 12700656 gb AY012149.1 	Felis catus 12S ribosomal RNA gene...	424	5e-116
gi 1098523 gb U20753.1 	Felis catus mitochondrion, compl	424	5e-116
gi 1098537 gb U20754.1 	Felis catus chromosome D2 mtDNA	392	2e-106
gi 1871567 emb Y08505.1 	P.leo mitochondrial 12S rRNA gen	392	2e-106
gi 1065402 gb U33495.1 	Felis concolor 12S rRNA gene,...	385	4e-104
gi 833949 gb S75064.1 	12S rRNA {cytoplasmic clone pCmt.12, t.	383	2e-103
gi 571525 dbj D28893.1 	Felis catus mitochondrial gene	383	2e-103
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrion, compl	377	1e-101
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrion, compl	377	1e-101

805 12S

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrion, compl	406	1e-110
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrion, compl	406	1e-110
gi 1871567 emb Y08505.1 	P.leo mitochondrial 12S rRNA gen	383	2e-103
gi 1065402 gb U33495.1 	Felis concolor 12S rRNA gene,...	383	2e-103
gi 12700658 gb AY012151.1 	Panthera onca 12S ribosomal RNA ge...	375	4e-101
gi 78709120 gb DQ151550.1 	Panthera tigris mitochondrion, partia	367	1e-98
gi 56406667 gb AY736583.1 	Panthera tigris haplotype COR3 12S...	367	1e-98
gi 56406666 gb AY736582.1 	Panthera tigris haplotype COR2 12S...	367	1e-98
gi 56406665 gb AY736581.1 	Panthera tigris haplotype AMO3/COR...	367	1e-98
gi 56406664 gb AY736580.1 	Panthera tigris haplotype AMO2 12S...	367	1e-98

805 Cytochrome B

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrion, compl	839	0.0
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrion, compl	839	0.0
gi 1098523 gb U20753.1 	Felis catus mitochondrion, compl	623	2e-175
gi 51339329 gb AY598487.1 	Puma concolor cytochrom...	580	1e-162
gi 20160336 emb AJ441329.1 	Felis silvestris partial...	560	1e-156
gi 78709120 gb DQ151550.1 	Panthera tigris mitochondrion, partia	556	2e-155
gi 20160342 emb AJ441332.1 	Lynx pardinus partial mi...	556	2e-155
gi 20160340 emb AJ441331.1 	Lynx pardinus partial mi...	556	2e-155
gi 693961 emb X82296.1 	F.domesticus mitochondrial cytoc	553	2e-154
gi 78709127 gb DQ151551.1 	Panthera tigris chromosome F2 nucl...	553	2e-154

806 12S

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrion, compl	422	2e-115
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrion, compl	422	2e-115
gi 1871567 emb Y08505.1 	P.leo mitochondrial 12S rRNA gen	398	3e-108
gi 1065402 gb U33495.1 	Felis concolor 12S rRNA gene,...	398	3e-108
gi 12700658 gb AY012151.1 	Panthera onca 12S ribosomal RNA ge...	391	7e-106
gi 78709120 gb DQ151550.1 	Panthera tigris mitochondrion, partia	383	2e-103
gi 56406667 gb AY736583.1 	Panthera tigris haplotype COR3 12S...	383	2e-103
gi 56406666 gb AY736582.1 	Panthera tigris haplotype COR2 12S...	383	2e-103
gi 56406665 gb AY736581.1 	Panthera tigris haplotype AMO3/COR...	383	2e-103
gi 56406664 gb AY736580.1 	Panthera tigris haplotype AMO2 12S...	383	2e-103

806 Cytochrome B

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrion, compl	783	0.0
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrion, compl	783	0.0
gi 51339329 gb AY598487.1 	Puma concolor cytochrom...	521	6e-145
gi 693961 emb X82296.1 	F.domesticus mitochondrial cytoc	482	5e-133
gi 42541651 gb AY499335.1 	Pardofelis marmorata voucher Pma4 ...	478	8e-132
gi 1098523 gb U20753.1 	Felis catus mitochondrion, compl	474	1e-130
gi 62870482 gb AY928671.1 	Lynx canadensis cytochrome b (CYTB...	472	5e-130
gi 42541653 gb AY499336.1 	Pardofelis marmorata voucher Pma5 ...	470	2e-129
gi 27762225 gb AY170102.1 	Felis silvestris specimen-voucher ...	466	3e-128
gi 2575784 dbj AB004238.1 	Felis catus mitochondrial DNA for cyt	466	3e-128

809 12S

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrion, compl	373	2e-100
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrion, compl	373	2e-100
gi 1871567 emb Y08505.1 	P.leo mitochondrial 12S rRNA gen	349	2e-93
gi 1065402 gb U33495.1 	Felis concolor 12S rRNA gene,...	349	2e-93
gi 12700658 gb AY012151.1 	Panthera onca 12S ribosomal RNA ge...	341	5e-91
gi 78709120 gb DQ151550.1 	Panthera tigris mitochondrion, partia	333	1e-88
gi 56406667 gb AY736583.1 	Panthera tigris haplotype COR3 12S...	333	1e-88
gi 56406666 gb AY736582.1 	Panthera tigris haplotype COR2 12S...	333	1e-88
gi 56406665 gb AY736581.1 	Panthera tigris haplotype AMO3/COR...	333	1e-88
gi 56406664 gb AY736580.1 	Panthera tigris haplotype AMO2 12S...	333	1e-88

809 Cytochrome B

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1	Acinonyx jubatus mitochondrial, compl	890	0.0
gi 38304374 gb AF344830.1	Acinonyx jubatus mitochondrial, compl	890	0.0
gi 1098523 gb U20753.1	Felis catus mitochondrial, compl	581	8e-163
gi 51339329 gb AY598487.1	Puma concolor cytochrom...	549	3e-153
gi 20160336 emb AJ441329.1	Felis silvestris partial...	523	2e-145
gi 20160342 emb AJ441332.1	Lynx pardinus partial mi...	517	1e-143
gi 20160340 emb AJ441331.1	Lynx pardinus partial mi...	517	1e-143
gi 693961 emb X82296.1	F.domesticus mitochondrial cytoc	509	2e-141
gi 20160338 emb AJ441330.1	Felis silvestris partial...	507	1e-140
gi 20160334 emb AJ441328.1	Felis catus partial mitocho	507	1e-140

810 12s

Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.1	Canis latrans isolate 2 from Color...	412	2e-112
gi 91702675 gb DQ480509.1	Canis latrans isolate 1 from Nebra...	412	2e-112
gi 91702661 gb DQ480508.1	Canis lupus isolate 1 from Canada ...	412	2e-112
gi 91702647 gb DQ480507.1	Canis lupus isolate 2 from Saudi A...	412	2e-112
gi 91702633 gb DQ480506.1	Canis lupus isolate 1 from Saudi A...	412	2e-112
gi 91702619 gb DQ480505.1	Canis lupus isolate 1 from Spain mito	412	2e-112
gi 91702605 gb DQ480504.1	Canis lupus isolate 1 from Sweden ...	412	2e-112
gi 91702591 gb DQ480503.1	Canis lupus isolate 1 from Russia ...	412	2e-112
gi 91702549 gb DQ480500.1	Canis familiaris isolate 1 breed S...	412	2e-112
gi 91702535 gb DQ480499.1	Canis familiaris isolate 1 breed S...	412	2e-112

811 12S

Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.1	Canis latrans isolate 2 from Color...	420	8e-115
gi 91702675 gb DQ480509.1	Canis latrans isolate 1 from Nebra...	420	8e-115
gi 91702661 gb DQ480508.1	Canis lupus isolate 1 from Canada ...	420	8e-115
gi 91702647 gb DQ480507.1	Canis lupus isolate 2 from Saudi A...	420	8e-115
gi 91702633 gb DQ480506.1	Canis lupus isolate 1 from Saudi A...	420	8e-115
gi 91702619 gb DQ480505.1	Canis lupus isolate 1 from Spain mito	420	8e-115
gi 91702605 gb DQ480504.1	Canis lupus isolate 1 from Sweden ...	420	8e-115
gi 91702591 gb DQ480503.1	Canis lupus isolate 1 from Russia ...	420	8e-115
gi 91702549 gb DQ480500.1	Canis familiaris isolate 1 breed S...	420	8e-115
gi 91702535 gb DQ480499.1	Canis familiaris isolate 1 breed S...	420	8e-115

812 12S

Genbank Reference	Species	score	E value
gi 1871573 emb Y08508.1	V.vulpes mitochondrial 12S rRNA	375	4e-101
gi 1871569 emb Y08504.1	P.tigris mitochondrial 12S rRNA	361	6e-97
gi 78709120 gb DQ151550.1	Panthera tigris mitochondrial, partia	353	2e-94
gi 12700658 gb AY012151.1	Panthera onca 12S ribosomal RNA ge...	353	2e-94
gi 56406667 gb AY736583.1	Panthera tigris haplotype COR3 12S...	353	2e-94
gi 56406666 gb AY736582.1	Panthera tigris haplotype COR2 12S...	353	2e-94
gi 56406665 gb AY736581.1	Panthera tigris haplotype AMO3/COR...	353	2e-94
gi 56406664 gb AY736580.1	Panthera tigris haplotype AMO2 12S...	353	2e-94
gi 56406663 gb AY736579.1	Panthera tigris haplotype TIG6 12S...	353	2e-94
gi 56406662 gb AY736578.1	Panthera tigris haplotype TIG4 12S...	353	2e-94

812 Cytochrome B			
Genbank Reference	Species	score	E value
gi 37932792 gb AY241921.11	Genetta maculata E35 cytochrome b ...	747	0.0
gi 39841327 gb AY397719.11	Genetta maculata isolate E30 cytoc... ...	747	0.0
gi 37932790 gb AY241920.11	Genetta maculata E32 cytochrome b ...	739	0.0
gi 37932786 gb AY241918.11	Genetta maculata E23 cytochrome b ...	739	0.0
gi 37932780 gb AY241915.11	Genetta maculata E22 cytochrome b ...	739	0.0
gi 37932778 gb AY241914.11	Genetta maculata E36 cytochrome b ...	739	0.0
gi 39841309 gb AY397710.11	Genetta maculata isolate E06 cytoc... ...	739	0.0
gi 33324639 gb AF511055.11	Genetta maculata cytochrome b (cyt... ...	731	0.0
gi 37932788 gb AY241919.11	Genetta maculata E20 cytochrome b ...	731	0.0
gi 37932784 gb AY241917.11	Genetta maculata E24 cytochrome b ...	731	0.0

813 Cytochrome B			
Genbank Reference	Species	score	E value
gi 34016937 gb AY291431.11	Canis lupus himalayensis haplotype... ...	761	0.0
gi 91702591 gb DQ480503.11	Canis lupus isolate 1 from Russia	743	0.0
gi 34016939 gb AY291432.11	Canis lupus indica haplotype GW-AP... ...	741	0.0
gi 91702619 gb DQ480505.11	Canis lupus isolate 1 from Spain mito ...	735	0.0
gi 91702605 gb DQ480504.11	Canis lupus isolate 1 from Sweden	735	0.0
gi 91702577 gb DQ480502.11	Canis familiaris isolate 2 breed J... ...	735	0.0
gi 91702563 gb DQ480501.11	Canis familiaris isolate 1 breed S... ...	735	0.0
gi 91702549 gb DQ480500.11	Canis familiaris isolate 1 breed S... ...	735	0.0
gi 91702521 gb DQ480498.11	Canis familiaris isolate 1 breed M... ...	735	0.0
gi 91702507 gb DQ480497.11	Canis familiaris isolate 1 breed W ...	735	0.0

814 12S			
Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.11	Acinonyx jubatus mitochondrial, compl ...	424	5e-116
gi 38304374 gb AF344830.11	Acinonyx jubatus mitochondrial, compl ...	424	5e-116
gi 1871567 emb Y08505.11	P.løo mitochondrial 12S rRNA gen ...	400	7e-109
gi 1065402 gb U33495.11	Felis concolor 12S rRNA gene,... ...	400	7e-109
gi 12700658 gb AY012151.11	Panthera onca 12S ribosomal RNA ge... ...	392	2e-106
gi 78709120 gb DQ151550.11	Panthera tigris mitochondrial, partia ...	385	4e-104
gi 56406667 gb AY736583.11	Panthera tigris haplotype COR3 12S... ...	385	4e-104
gi 56406666 gb AY736582.11	Panthera tigris haplotype COR2 12S... ...	385	4e-104
gi 56406665 gb AY736581.11	Panthera tigris haplotype AMO3/COR... ...	385	4e-104
gi 56406664 gb AY736580.11	Panthera tigris haplotype AMO2 12S... ...	385	4e-104

814 Cytochrome B			
Genbank Reference	Species	score	E value
gi 33326867 gb AF522348.11	Mungos mungo specimen-voucher C190... ...	783	0.0
gi 33326865 gb AF522347.11	Mungos mungo specimen-voucher C78	613	2e-172
gi 62870488 gb AY928674.11	Mungos mungo cytochrome b (CYTB) g... ...	589	3e-165
gi 27762211 gb AY170095.11	Mungos mungo specimen-voucher JCK-... ...	589	3e-165
gi 33326841 gb AF522335.11	Helogale hirtula specimen-voucher	454	1e-124
gi 57014054 gb AY873843.11	Herpestes javanicus mitochondrial, co ...	446	3e-122
gi 887666377 gb DQ391277.11	Herpestinae sp. PAML-2006 NADH deh... ...	438	7e-120
gi 887666374 gb DQ391276.11	Herpestinae sp. PAML-2006 NADH deh... ...	438	7e-120
gi 33324645 gb AF511058.11	Ichneumia albicauda cytochrome b (... ...	430	2e-117
gi 33326821 gb AF522325.11	Atilax paludinosus specimen-vouche... ...	424	1e-115

816 12S

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrial, compl	406	1e-110
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrial, compl	406	1e-110
gi 1871567 emb Y08505.1 	P.lxo mitochondrial 12S rRNA gen	383	2e-103
gi 1065402 gb U33495.1 	Felis concolor 12S rRNA gene,...	383	2e-103
gi 12700658 gb AY012151.1 	Panthera onca 12S ribosomal RNA ge...	375	4e-101
gi 78709120 gb DQ151550.1 	Panthera tigris mitochondrial, partia	367	1e-98
gi 56406667 gb AY736583.1 	Panthera tigris haplotype COR3 12S...	367	1e-98
gi 56406666 gb AY736582.1 	Panthera tigris haplotype COR2 12S...	367	1e-98
gi 56406665 gb AY736581.1 	Panthera tigris haplotype AMO3/COR...	367	1e-98
gi 56406664 gb AY736580.1 	Panthera tigris haplotype AMO2 12S...	367	1e-98

816 Cytochrome B

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrial, compl	890	0.0
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrial, compl	890	0.0
gi 1098523 gb U20753.1 	Felis catus mitochondrial, compl	581	8e-163
gi 51339329 gb AY598487.1 	Puma concolor cytochrom...	549	3e-153
gi 20160336 emb AJ441329.1 	Felis silvestris partial...	523	2e-145
gi 20160342 emb AJ441332.1 	Lynx pardinus partial mi...	517	1e-143
gi 20160340 emb AJ441331.1 	Lynx pardinus partial mi...	517	1e-143
gi 693961 emb X82296.1 	F.domesticus mitochondrial cytoc	509	2e-141
gi 20160338 emb AJ441330.1 	Felis silvestris partial...	507	1e-140
gi 20160334 emb AJ441328.1 	Felis catus partial mitocho	507	1e-140

817 12S

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrial, compl	406	1e-110
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrial, compl	406	1e-110
gi 1871567 emb Y08505.1 	P.lxo mitochondrial 12S rRNA gen	383	2e-103
gi 1065402 gb U33495.1 	Felis concolor 12S rRNA gene,...	383	2e-103
gi 12700658 gb AY012151.1 	Panthera onca 12S ribosomal RNA ge...	375	4e-101
gi 78709120 gb DQ151550.1 	Panthera tigris mitochondrial, partia	367	1e-98
gi 56406667 gb AY736583.1 	Panthera tigris haplotype COR3 12S...	367	1e-98
gi 56406666 gb AY736582.1 	Panthera tigris haplotype COR2 12S...	367	1e-98
gi 56406665 gb AY736581.1 	Panthera tigris haplotype AMO3/COR...	367	1e-98
gi 56406664 gb AY736580.1 	Panthera tigris haplotype AMO2	367	1e-98

817 Cytochrome B

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrial, compl	652	0.0
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrial, compl	652	0.0
gi 51339329 gb AY598487.1 	Puma concolor cytochrom...	422	3e-115
gi 42541651 gb AY499335.1 	Pardofelis marmorata voucher Pma4 ...	408	5e-111
gi 61655735 gb AY773082.1 	Catopuma temminckii cytochrome b g...	394	8e-107
gi 51339304 gb AY598475.1 	Lynx canadensis cytochr...	394	8e-107
gi 62870482 gb AY928671.1 	Lynx canadensis cytochrome b (CYTB...)	394	8e-107
gi 20160342 emb AJ441332.1 	Lynx pardinus partial mi...	394	8e-107
gi 20160340 emb AJ441331.1 	Lynx pardinus partial mi...	394	8e-107
gi 42541653 gb AY499336.1 	Pardofelis marmorata voucher Pma5 ...	392	3e-106

818 12S

Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.1 	Canis latrans isolate 2 from Color...	418	3e-114
gi 91702675 gb DQ480509.1 	Canis latrans isolate 1 from Nebra...	418	3e-114
gi 91702661 gb DQ480508.1 	Canis lupus isolate 1 from Canada ...	418	3e-114
gi 91702647 gb DQ480507.1 	Canis lupus isolate 2 from Saudi A...	418	3e-114
gi 91702633 gb DQ480506.1 	Canis lupus isolate 1 from Saudi A...	418	3e-114
gi 91702619 gb DQ480505.1 	Canis lupus isolate 1 from Spain mito	418	3e-114
gi 91702605 gb DQ480504.1 	Canis lupus isolate 1 from Sweden ...	418	3e-114
gi 91702591 gb DQ480503.1 	Canis lupus isolate 1 from Russia ...	418	3e-114
gi 91702549 gb DQ480500.1 	Canis familiaris isolate 1 breed S...	418	3e-114
gi 91702535 gb DQ480499.1 	Canis familiaris isolate 1 breed S...	418	3e-114

818 Cytochrome B

Genbank Reference	Species	score	E value
gi 34016937 gb AY291431.1 	Canis lupus himalayensis haplotype...	771	0.0
gi 34016939 gb AY291432.1 	Canis lupus indica haplotype GW-AP...	751	0.0
gi 91702591 gb DQ480503.1 	Canis lupus isolate 1 from Russia ...	739	0.0
gi 91702619 gb DQ480505.1 	Canis lupus isolate 1 from Spain mito	731	0.0
gi 91702605 gb DQ480504.1 	Canis lupus isolate 1 from Sweden ...	731	0.0
gi 91702577 gb DQ480502.1 	Canis familiaris isolate 2 breed J...	731	0.0
gi 91702563 gb DQ480501.1 	Canis familiaris isolate 1 breed S...	731	0.0
gi 91702451 gb DQ480493.1 	Canis familiaris isolate 1 breed B...	731	0.0
gi 91702437 gb DQ480492.1 	Canis familiaris isolate 1 breed J...	731	0.0
gi 91702395 gb DQ480489.1 	Canis familiaris isolate 1 breed G...	731	0.0

820 12S

Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.1 	Canis latrans isolate 2 from Color...	412	2e-112
gi 91702675 gb DQ480509.1 	Canis latrans isolate 1 from Nebra...	412	2e-112
gi 91702661 gb DQ480508.1 	Canis lupus isolate 1 from Canada ...	412	2e-112
gi 91702647 gb DQ480507.1 	Canis lupus isolate 2 from Saudi A...	412	2e-112
gi 91702633 gb DQ480506.1 	Canis lupus isolate 1 from Saudi A...	412	2e-112
gi 91702619 gb DQ480505.1 	Canis lupus isolate 1 from Spain mito	412	2e-112
gi 91702605 gb DQ480504.1 	Canis lupus isolate 1 from Sweden ...	412	2e-112
gi 91702591 gb DQ480503.1 	Canis lupus isolate 1 from Russia ...	412	2e-112
gi 91702549 gb DQ480500.1 	Canis familiaris isolate 1 breed S...	412	2e-112
gi 91702535 gb DQ480499.1 	Canis familiaris isolate 1 breed S...	412	2e-112

822 12s

Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.1 	Canis latrans isolate 2 from Color...	420	8e-115
gi 91702675 gb DQ480509.1 	Canis latrans isolate 1 from Nebra...	420	8e-115
gi 91702661 gb DQ480508.1 	Canis lupus isolate 1 from Canada ...	420	8e-115
gi 91702647 gb DQ480507.1 	Canis lupus isolate 2 from Saudi A...	420	8e-115
gi 91702633 gb DQ480506.1 	Canis lupus isolate 1 from Saudi A...	420	8e-115
gi 91702619 gb DQ480505.1 	Canis lupus isolate 1 from Spain mito	420	8e-115
gi 91702605 gb DQ480504.1 	Canis lupus isolate 1 from Sweden ...	420	8e-115
gi 91702591 gb DQ480503.1 	Canis lupus isolate 1 from Russia ...	420	8e-115
gi 91702549 gb DQ480500.1 	Canis familiaris isolate 1 breed S...	420	8e-115
gi 91702535 gb DQ480499.1 	Canis familiaris isolate 1 breed S...	420	8e-115

823 12S**Genbank Reference**

	Species	score	E value
gi 38373498 gb AY463959.1	Acinonyx jubatus mitochondrial, compl	398	3e-108
gi 38304374 gb AF344830.1	Acinonyx jubatus mitochondrial, compl	398	3e-108
gi 1871567 emb Y08505.1	P.lao mitochondrial 12S rRNA gen	375	4e-101
gi 1065402 gb U33495.1	Felis concolor 12S rRNA gene,...	375	4e-101
gi 12700658 gb AY012151.1	Panthera onca 12S ribosomal RNA ge...	367	1e-98
gi 78709120 gb DQ151550.1	Panthera tigris mitochondrial, partia	359	2e-96
gi 56406667 gb AY736583.1	Panthera tigris haplotype COR3 12S...	359	2e-96
gi 56406666 gb AY736582.1	Panthera tigris haplotype COR2 12S...	359	2e-96
gi 56406665 gb AY736581.1	Panthera tigris haplotype AMO3/COR...	359	2e-96
gi 56406664 gb AY736580.1	Panthera tigris haplotype AMO2 12S...	359	2e-96

824 Cytochrome B**Genbank Reference**

	Species	score	E value
gi 37932792 gb AY241921.1	Genetta maculata E35 cytochrome b ...	815	0.0
gi 39841327 gb AY397719.1	Genetta maculata isolate E30 cytoc...	815	0.0
gi 37932790 gb AY241920.1	Genetta maculata E32 cytochrome b ...	807	0.0
gi 37932786 gb AY241918.1	Genetta maculata E23 cytochrome b ...	807	0.0
gi 37932780 gb AY241915.1	Genetta maculata E22 cytochrome b ...	807	0.0
gi 37932778 gb AY241914.1	Genetta maculata E36 cytochrome b ...	807	0.0
gi 39841309 gb AY397710.1	Genetta maculata isolate E06 cytoc...	807	0.0
gi 33324639 gb AF511055.1	Genetta maculata cytochrome b (cyt...)	799	0.0
gi 37932788 gb AY241919.1	Genetta maculata E20 cytochrome b ...	799	0.0
gi 37932784 gb AY241917.1	Genetta maculata E24 cytochrome b ...	799	0.0

825 12S**Genbank Reference**

	Species	score	E value
gi 91702703 gb DQ480511.1	Canis latrans isolate 2 from Color...	420	8e-115
gi 91702675 gb DQ480509.1	Canis latrans isolate 1 from Nebra...	420	8e-115
gi 91702661 gb DQ480508.1	Canis lupus isolate 1 from Canada ...	420	8e-115
gi 91702647 gb DQ480507.1	Canis lupus isolate 2 from Saudi A...	420	8e-115
gi 91702633 gb DQ480506.1	Canis lupus isolate 1 from Saudi A...	420	8e-115
gi 91702619 gb DQ480505.1	Canis lupus isolate 1 from Spain mito	420	8e-115
gi 91702605 gb DQ480504.1	Canis lupus isolate 1 from Sweden ...	420	8e-115
gi 91702591 gb DQ480503.1	Canis lupus isolate 1 from Russia ...	420	8e-115
gi 91702549 gb DQ480500.1	Canis familiaris isolate 1 breed S...	420	8e-115
gi 91702535 gb DQ480499.1	Canis familiaris isolate 1 breed S...	420	8e-115

826 Cytochrome B**Genbank Reference**

	Species	score	E value
gi 37932792 gb AY241921.1	Genetta maculata E35 cytochrome b ...	835	0.0
gi 39841327 gb AY397719.1	Genetta maculata isolate E30 cytoc...	835	0.0
gi 37932790 gb AY241920.1	Genetta maculata E32 cytochrome b ...	827	0.0
gi 37932786 gb AY241918.1	Genetta maculata E23 cytochrome b ...	827	0.0
gi 37932780 gb AY241915.1	Genetta maculata E22 cytochrome b ...	827	0.0
gi 37932778 gb AY241914.1	Genetta maculata E36 cytochrome b ...	827	0.0
gi 39841309 gb AY397710.1	Genetta maculata isolate E06 cytoc...	827	0.0
gi 33324639 gb AF511055.1	Genetta maculata cytochrome b (cyt...)	819	0.0
gi 37932788 gb AY241919.1	Genetta maculata E20 cytochrome b ...	819	0.0
gi 37932784 gb AY241917.1	Genetta maculata E24 cytochrome b ...	819	0.0

827 12S

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.11	Acinonyx jubatus mitochondrial, compl	422	2e-115
gi 38304374 gb AF344830.11	Acinonyx jubatus mitochondrial, compl	422	2e-115
gi 1871567 emb Y08505.11	P.lxo mitochondrial 12S rRNA gen	398	3e-108
gi 1065402 gb U33495.11	Felis concolor 12S rRNA gene,...	398	3e-108
gi 12700658 gb AY012151.11	Panthera onca 12S ribosomal RNA ge...	391	7e-106
gi 78709120 gb DQ151550.11	Panthera tigris mitochondrial, partia	383	2e-103
gi 56406667 gb AY736583.11	Panthera tigris haplotype COR3 12S...	383	2e-103
gi 56406666 gb AY736582.11	Panthera tigris haplotype COR2 12S...	383	2e-103
gi 56406665 gb AY736581.11	Panthera tigris haplotype AMO3/COR...	383	2e-103
gi 56406664 gb AY736580.11	Panthera tigris haplotype AMO2 12S...	383	2e-103

830 12S

Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.11	Canis latrans isolate 2 from Color...	381	7e-103
gi 91702675 gb DQ480509.11	Canis latrans isolate 1 from Nebra...	381	7e-103
gi 91702661 gb DQ480508.11	Canis lupus isolate 1 from Canada ...	381	7e-103
gi 91702647 gb DQ480507.11	Canis lupus isolate 2 from Saudi A...	381	7e-103
gi 91702633 gb DQ480506.11	Canis lupus isolate 1 from Saudi A...	381	7e-103
gi 91702619 gb DQ480505.11	Canis lupus isolate 1 from Spain mito	381	7e-103
gi 91702605 gb DQ480504.11	Canis lupus isolate 1 from Sweden ...	381	7e-103
gi 91702591 gb DQ480503.11	Canis lupus isolate 1 from Russia ...	381	7e-103
gi 91702549 gb DQ480500.11	Canis familiaris isolate 1 breed S...	381	7e-103
gi 91702535 gb DQ480499.11	Canis familiaris isolate 1 breed S...	381	7e-103

830 Cytochrome B

Genbank Reference	Species	score	E value
gi 37932792 gb AY241921.11	Genetta maculata E35 cytochrome b ...	837	0.0
gi 39841327 gb AY397719.11	Genetta maculata isolate E30 cytoc...	837	0.0
gi 37932790 gb AY241920.11	Genetta maculata E32 cytochrome b ...	829	0.0
gi 37932786 gb AY241918.11	Genetta maculata E23 cytochrome b ...	829	0.0
gi 37932780 gb AY241915.11	Genetta maculata E22 cytochrome b ...	829	0.0
gi 37932778 gb AY241914.11	Genetta maculata E36 cytochrome b ...	829	0.0
gi 39841309 gb AY397710.11	Genetta maculata isolate E06 cytoc...	829	0.0
gi 33324639 gb AF511055.11	Genetta maculata cytochrome b (cyt...)	821	0.0
gi 37932788 gb AY241919.11	Genetta maculata E20 cytochrome b ...	821	0.0

831 Cytochrome B

Genbank Reference	Species	score	E value
gi 34016937 gb AY291431.11	Canis lupus himalayensis haplotype...	773	0.0
gi 34016939 gb AY291432.11	Canis lupus indica haplotype GW-AP...	753	0.0
gi 91702591 gb DQ480503.11	Canis lupus isolate 1 from Russia ...	745	0.0
gi 91702619 gb DQ480505.11	Canis lupus isolate 1 from Spain mito	737	0.0
gi 91702605 gb DQ480504.11	Canis lupus isolate 1 from Sweden ...	737	0.0
gi 91702577 gb DQ480502.11	Canis familiaris isolate 2 breed J...	737	0.0
gi 91702563 gb DQ480501.11	Canis familiaris isolate 1 breed S...	737	0.0
gi 91702451 gb DQ480493.11	Canis familiaris isolate 1 breed B...	737	0.0
gi 91702437 gb DQ480492.11	Canis familiaris isolate 1 breed J...	737	0.0
gi 91702395 gb DQ480489.11	Canis familiaris isolate 1 breed G...	737	0.0

833 12S

Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.1 	Canis latrans isolate 2 from Color...	420	8e-115
gi 91702675 gb DQ480509.1 	Canis latrans isolate 1 from Nebra...	420	8e-115
gi 91702661 gb DQ480508.1 	Canis lupus isolate 1 from Canada ...	420	8e-115
gi 91702647 gb DQ480507.1 	Canis lupus isolate 2 from Saudi A...	420	8e-115
gi 91702633 gb DQ480506.1 	Canis lupus isolate 1 from Saudi A...	420	8e-115
gi 91702619 gb DQ480505.1 	Canis lupus isolate 1 from Spain mito	420	8e-115
gi 91702605 gb DQ480504.1 	Canis lupus isolate 1 from Sweden ...	420	8e-115
gi 91702591 gb DQ480503.1 	Canis lupus isolate 1 from Russia ...	420	8e-115
gi 91702549 gb DQ480500.1 	Canis familiaris isolate 1 breed S...	420	8e-115
gi 91702535 gb DQ480499.1 	Canis familiaris isolate 1 breed S...	420	8e-115

837 12S

Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.1 	Canis latrans isolate 2 from Color...	383	2e-103
gi 91702675 gb DQ480509.1 	Canis latrans isolate 1 from Nebra...	383	2e-103
gi 91702661 gb DQ480508.1 	Canis lupus isolate 1 from Canada ...	383	2e-103
gi 91702647 gb DQ480507.1 	Canis lupus isolate 2 from Saudi A...	383	2e-103
gi 91702633 gb DQ480506.1 	Canis lupus isolate 1 from Saudi A...	383	2e-103
gi 91702619 gb DQ480505.1 	Canis lupus isolate 1 from Spain mito	383	2e-103
gi 91702605 gb DQ480504.1 	Canis lupus isolate 1 from Sweden ...	383	2e-103
gi 91702591 gb DQ480503.1 	Canis lupus isolate 1 from Russia ...	383	2e-103
gi 91702549 gb DQ480500.1 	Canis familiaris isolate 1 breed S...	383	2e-103
gi 91702535 gb DQ480499.1 	Canis familiaris isolate 1 breed S...	383	2e-103

837 Cytochrome B

Genbank Reference	Species	score	E value
gi 37932792 gb AY241921.1 	Genetta maculata E35 cytochrome b ...	835	0.0
gi 39841327 gb AY397719.1 	Genetta maculata isolate E30 cytoc...	835	0.0
gi 37932790 gb AY241920.1 	Genetta maculata E32 cytochrome b ...	827	0.0
gi 37932786 gb AY241918.1 	Genetta maculata E23 cytochrome b ...	827	0.0
gi 37932780 gb AY241915.1 	Genetta maculata E22 cytochrome b ...	827	0.0
gi 37932778 gb AY241914.1 	Genetta maculata E36 cytochrome b ...	827	0.0
gi 39841309 gb AY397710.1 	Genetta maculata isolate E06 cytoc...	827	0.0
gi 33324639 gb AF511055.1 	Genetta maculata cytochrome b (cyt...)	819	0.0
gi 37932788 gb AY241919.1 	Genetta maculata E20 cytochrome b ...	819	0.0
gi 37932784 gb AY241917.1 	Genetta maculata E24 cytochrome b ...	819	0.0

839 12S

Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.1 	Canis latrans isolate 2 from Color...	412	2e-112
gi 91702675 gb DQ480509.1 	Canis latrans isolate 1 from Nebra...	412	2e-112
gi 91702661 gb DQ480508.1 	Canis lupus isolate 1 from Canada ...	412	2e-112
gi 91702647 gb DQ480507.1 	Canis lupus isolate 2 from Saudi A...	412	2e-112
gi 91702633 gb DQ480506.1 	Canis lupus isolate 1 from Saudi A...	412	2e-112
gi 91702619 gb DQ480505.1 	Canis lupus isolate 1 from Spain mito	412	2e-112
gi 91702605 gb DQ480504.1 	Canis lupus isolate 1 from Sweden ...	412	2e-112
gi 91702591 gb DQ480503.1 	Canis lupus isolate 1 from Russia ...	412	2e-112
gi 91702549 gb DQ480500.1 	Canis familiaris isolate 1 breed S...	412	2e-112
gi 91702535 gb DQ480499.1 	Canis familiaris isolate 1 breed S...	412	2e-112

839 Cytochrome B			
Genbank Reference	Species	score	E value
gi 91702591 gb DQ480503.1 	Canis lupus isolate 1 from Russia ...	779	0.0
gi 91702619 gb DQ480505.1 	Canis lupus isolate 1 from Spain mito	771	0.0
gi 91702605 gb DQ480504.1 	Canis lupus isolate 1 from Sweden ...	771	0.0
gi 91702577 gb DQ480502.1 	Canis familiaris isolate 2 breed J...	771	0.0
gi 91702563 gb DQ480501.1 	Canis familiaris isolate 1 breed S...	771	0.0
gi 91702549 gb DQ480500.1 	Canis familiaris isolate 1 breed S...	771	0.0
gi 91702521 gb DQ480498.1 	Canis familiaris isolate 1 breed M...	771	0.0
gi 91702507 gb DQ480497.1 	Canis familiaris isolate 1 breed W...	771	0.0
gi 91702493 gb DQ480496.1 	Canis familiaris isolate 1 breed I...	771	0.0
gi 91702479 gb DQ480495.1 	Canis familiaris isolate 1 breed C...	771	0.0

843 12S			
Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrion, compl	406	1e-110
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrion, compl	406	1e-110
gi 1871567 emb Y08505.1 	P.leo mitochondrial 12S rRNA gen	383	2e-103
gi 1065402 gb U33495.1 	Felis concolor 12S rRNA gene,...	383	2e-103
gi 12700658 gb AY012151.1 	Panthera onca 12S ribosomal RNA ge...	375	4e-101
gi 78709120 gb DQ151550.1 	Panthera tigris mitochondrion, partia	367	1e-98
gi 56406667 gb AY736583.1 	Panthera tigris haplotype COR3 12S...	367	1e-98
gi 56406666 gb AY736582.1 	Panthera tigris haplotype COR2 12S...	367	1e-98
gi 56406665 gb AY736581.1 	Panthera tigris haplotype AMO3/COR...	367	1e-98
gi 56406664 gb AY736580.1 	Panthera tigris haplotype AMO2 12S...	367	1e-98

843 Cytochrome B			
Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrion, compl	890	0.0
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrion, compl	890	0.0
gi 1098523 gb U20753.1 	Felis catus mitochondrion, compl	581	8e-163
gi 51339329 gb AY598487.1 	Puma concolor cytochrom...	549	3e-153
gi 20160336 emb AJ441329.1 	Felis silvestris partial...	523	2e-145
gi 20160342 emb AJ441332.1 	Lynx pardinus partial mi...	517	1e-143
gi 20160340 emb AJ441331.1 	Lynx pardinus partial mi...	517	1e-143
gi 693961 emb X82296.1 	F.domesticus mitochondrial cytoc	509	2e-141
gi 20160338 emb AJ441330.1 	Felis silvestris partial...	507	1e-140
gi 20160334 emb AJ441328.1 	Felis catus partial mitoch	507	1e-140

847 12S			
Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.1 	Canis latrans isolate 2 from Color...	299	1e-78
gi 91702675 gb DQ480509.1 	Canis latrans isolate 1 from Nebra...	299	1e-78
gi 91702661 gb DQ480508.1 	Canis lupus isolate 1 from Canada ...	299	1e-78
gi 91702647 gb DQ480507.1 	Canis lupus isolate 2 from Saudi A...	299	1e-78
gi 91702633 gb DQ480506.1 	Canis lupus isolate 1 from Saudi A...	299	1e-78
gi 91702619 gb DQ480505.1 	Canis lupus isolate 1 from Spain mito	299	1e-78
gi 91702605 gb DQ480504.1 	Canis lupus isolate 1 from Sweden ...	299	1e-78
gi 91702591 gb DQ480503.1 	Canis lupus isolate 1 from Russia ...	299	1e-78
gi 91702549 gb DQ480500.1 	Canis familiaris isolate 1 breed S...	299	1e-78
gi 91702535 gb DQ480499.1 	Canis familiaris isolate 1 breed S...	299	1e-78

847 Cytochrome B			
Genbank Reference	Species	score	E value
gi 34016937 gb AY291431.11	Canis lupus himalayensis haplotype...	769	0.0
gi 34016939 gb AY291432.11	Canis lupus indica haplotype GW-AP...	749	0.0
gi 91702591 gb DQ480503.11	Canis lupus isolate 1 from Russia ...	735	0.0
gi 91702619 gb DQ480505.11	Canis lupus isolate 1 from Spain mito	728	0.0
gi 91702605 gb DQ480504.11	Canis lupus isolate 1 from Sweden ...	728	0.0
gi 91702577 gb DQ480502.11	Canis familiaris isolate 2 breed J...	728	0.0
gi 91702563 gb DQ480501.11	Canis familiaris isolate 1 breed S...	728	0.0
gi 91702549 gb DQ480500.11	Canis familiaris isolate 1 breed S...	728	0.0
gi 91702521 gb DQ480498.11	Canis familiaris isolate 1 breed M...	728	0.0
gi 91702507 gb DQ480497.11	Canis familiaris isolate 1 breed W...	728	0.0

849 12S			
Genbank Reference	Species	score	E value
gi 91702703 gb DQ480511.11	Canis latrans isolate 2 from Color...	420	8e-115
gi 91702675 gb DQ480509.11	Canis latrans isolate 1 from Nebra...	420	8e-115
gi 91702661 gb DQ480508.11	Canis lupus isolate 1 from Canada ...	420	8e-115
gi 91702647 gb DQ480507.11	Canis lupus isolate 2 from Saudi A...	420	8e-115
gi 91702633 gb DQ480506.11	Canis lupus isolate 1 from Saudi A...	420	8e-115
gi 91702619 gb DQ480505.11	Canis lupus isolate 1 from Spain mito	420	8e-115
gi 91702605 gb DQ480504.11	Canis lupus isolate 1 from Sweden ...	420	8e-115
gi 91702591 gb DQ480503.11	Canis lupus isolate 1 from Russia ...	420	8e-115
gi 91702549 gb DQ480500.11	Canis familiaris isolate 1 breed S...	420	8e-115
gi 91702535 gb DQ480499.11	Canis familiaris isolate 1 breed S...	420	8e-115

870 CytB			
Genbank Reference	Species	score	E value
gi 77799815 dbj AB211405.11	Panthera pardus orientalis mitoch...	791	0.0
gi 77799813 dbj AB211404.11	Panthera pardus orientalis mitoch...	791	0.0
gi 77799811 dbj AB211403.11	Panthera pardus orientalis mitoch...	791	0.0
gi 77799807 dbj AB211401.11	Panthera pardus orientalis mitoch...	791	0.0
gi 77799819 dbj AB211407.11	Panthera pardus orientalis mitoch...	783	0.0
gi 77799817 dbj AB211406.11	Panthera pardus orientalis mitoch...	783	0.0
gi 77799809 dbj AB211402.11	Panthera pardus orientalis mitoch...	783	0.0
gi 693977 emb X82300.11	P.lxo mitochondrial cytochrome	613	2e-172
gi 62870480 gb AY928670.11	Panthera leo cytochrome b (CYTB) g...	613	2e-172
gi 2995794 gb AF053052.11	Panthera leo cytochrome b (...)	613	2e-172

ZOO REFERENCE SAMPLES***Acinonyx jubatus* 12S**

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrial	385	4e-104
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrial	385	4e-104

***Acinonyx jubatus* Cytochrome B**

Genbank Reference	Species	score	E value
gi 38373498 gb AY463959.1 	Acinonyx jubatus mitochondrial, compl	882	0.0
gi 38304374 gb AF344830.1 	Acinonyx jubatus mitochondrial, compl	882	0.0

***Panthera pardus orientalis* 12S**

Genbank Reference	Species	score	E value
gi 12700658 gb AY012151.1 	Panthera onca 12S ribosomal RNA ge...	416	1e-113
gi 78709120 gb DQ151550.1 	Panthera tigris mitochondrial, partia	408	3e-111

***Vulpes zerda* 12S**

Genbank Reference	Species	score	E value
gi 1871573 emb Y08508.1 	V.vulpes mitochondrial 12S rRNA	385	4e-104
gi 12700658 gb AY012151.1 	Panthera onca 12S ribosomal RNA ge...	367	1e-98

***Mungos mungo* 12S**

Genbank Reference	Species	score	E value
gi 57014054 gb AY873843.1 	Herpestes javanicus mitochondrial, co	359	2e-96
gi 1871558 emb Y08506.1 	H.europunctatus mitochondrial 1	359	2e-96

***Mungos mungo* Cytochrome B**

Genbank Reference	Species	score	E value
gi 33326867 gb AF522348.1 	Mungos mungo specimen-voucher C190...	801	0.0
gi 33326865 gb AF522347.1 	Mungos mungo specimen-voucher C78 ...	636	2e-179

***Helogale parvula* 12S**

Genbank Reference	Species	score	E value
gi 57014054 gb AY873843.1 	Herpestes javanicus mitochondrial, co	373	2e-100
gi 1871558 emb Y08506.1 	H.europunctatus mitochondrial 1	373	2e-100

***Helogale parvula* Cytochrome B**

Genbank Reference	Species	score	E value
gi 33326837 gb AF522333.1 	Helogale parvula specimen-voucher ...	779	0.0
gi 33326839 gb AF522334.1 	Helogale parvula specimen-voucher ...	728	0.0