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Monitoring of the Mongolian Wild Camel (*Camelus ferus*) Population using Mitochondrial Markers for Non-invasively Collected Hair Samples

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1. Introduction

Until recently it has been widely unrecognized that the tribe of the Old World camelids (*Camelini*) is represented by three different species, the one-humped dromedary or Arabian camel (*Camelus dromedarius*), the two-humped Bactrian camel or Asiatic camel (*Camelus bactrianus*), and the two-humped wild camel (*Camelus ferus*). The introduction provides essential background information on the last wild two-humped camels and their threat to extinction as well as the aims of this diploma thesis.

1.1 Evolutionary background of the wild two-humped camel

The wild camel (*Camelus ferus*) was first described by Przewalski in 1878 and its naming follows the ruling of the International Commission on Zoological Nomenclature (opinion 2027, 2003), which fixes the first available name based on a wild population (GENTRY et al., 2004).

There are seven different species in the family of Camelidae (s. fig. 1), which are divided into two tribes, known as the New World camelids (*Lamini*), occurring only in Southern America, and the Old World camelids (*Camelini*). These two tribes diverged from each other around 11 (STANLEY et al., 1994) to 25 (JI et al., 2009) million years ago (mya). Within the Old World camelids the Bactrian camel was separated from the Dromedary 5 to 8 mya (STANLEY et al., 1994; JI et al., 2009). In the past the wild camels have been considered to be either feral domestic Bactrian camels or even their ancestor species. However, recent studies have shown that the wild camel is a separate species (JIRIMUTU et al., 2012, SILBERMAYR et al., 2010, CUI et al., 2007) and therefore the last extant wild representative of the Old World camelids today. It is very likely that the two species diverged around 0.7 mya (JI et al., 2009, CUI et al., 2007). This is long before domestication, which took place 4.000 to 5.000 years ago (reviewed in BURGER, 2012). Hence, we are dealing with two different species and it could be clearly disproved, that the wild Bactrian camel is feral (SILBERMAYR et al, 2010; JI et al., 2009). These phylogenetic data make conservation efforts even more important and were considered during the preparation of a conservation action plan within the framework of the “International Meeting on Wild Bactrian Camel Conservation Strategy of Mongolia” in 2010 (YADAMSUREN et al., 2012).

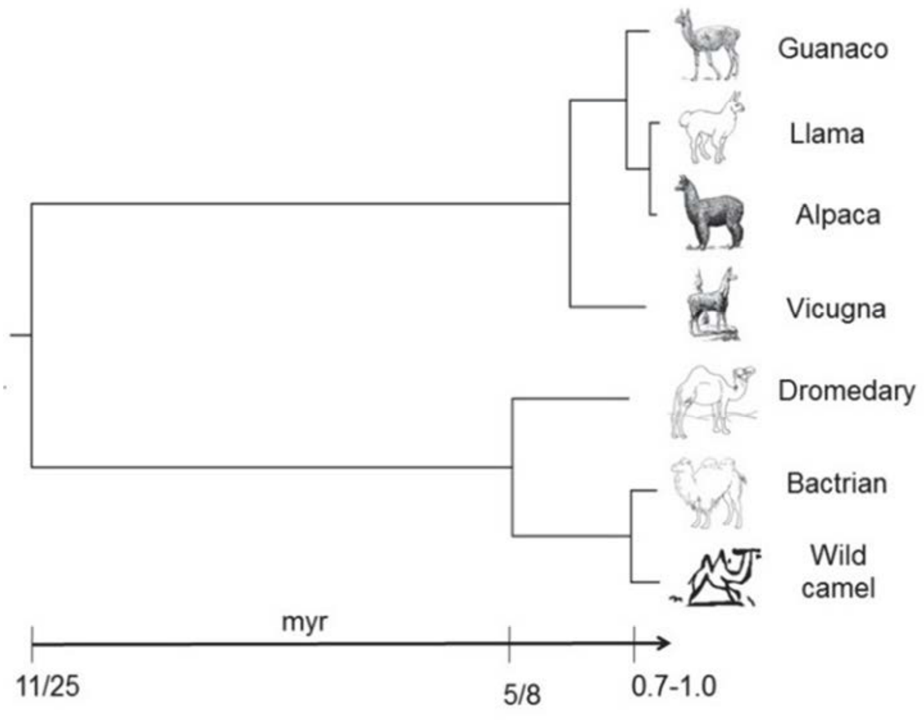


Figure 1. Maximum likelihood tree of the *Camelidae* complete mitochondrial genomes (Burger, 2012)

1.2 Conservation Status of wild camels (*Camelus ferus*)

This chapter gives an overview of the distribution of the wild camel and of the threats to the Mongolian camel population in the Great Gobi Strictly Protected Area “A” (GGSPAA).

1.2.1 Distribution of the wild camel

The wild camel is listed in the Mongolian Red Book (SHIIREVDAMBA et al., 1997) and is classified as critically endangered by the International Union for Conservation of Nature (IUCN; Red list of endangered species, 2010). Momentarily, there is evidence of four smaller subpopulations. Three are located in China, in Gansu province, Lop Nur and Taklamakan, and one in Mongolia in the GGSPAA (s. fig. 2). In spite of subsequent surveillance efforts, the actual population numbers remain uncertain. Recent reports provide estimates of around one thousand individuals in total, around 650 in China and 350 in Mongolia (YADAMSUREN et al., 2012; LEI et al., 2012; READING et al, 2005; READING et al. 2002; READING et al, 1999; HARE, 1997).

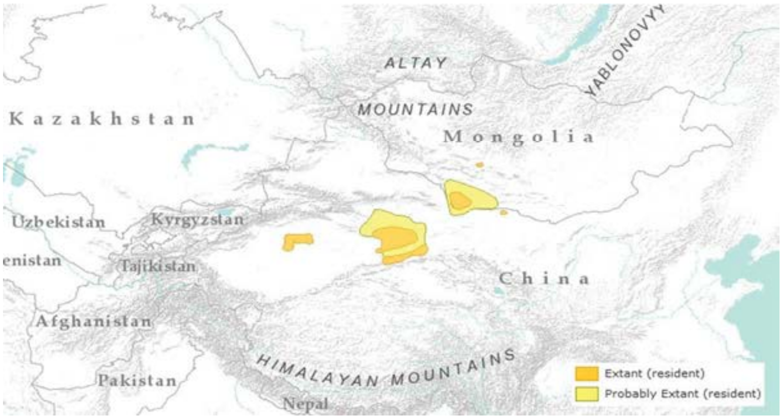


Figure 2. This map shows the current distribution of *Camelus ferus* (IUCN, 2012; http://maps.iucnredlist.org/map.html?id=63543, consulted on 2012-10-10)

1.2.2 Threats to the Mongolian wild population

Today, wild populations in general are confronted with many different dangers, such as habitat degradation, overexploitation, the effects of global climate change and introgressive hybridization with invasive organisms (RANDI, 2008). Referring to the Mongolian wild camel population, we can again identify several of these threats, which are mostly caused by humans.

A serious harassment is the upcoming branch of gold mining. Illegal miners disturb the timid animals by their presence in the GGSPAA and keep them away from the rare water points. Especially risky seems the use of potassium cyanide for gold-extraction, which is classified as highly toxic and may have fatal consequences on the wild camel and its habitat (LEI et al., 2012).

Global climate change has worldwide impact on flora and fauna. Thus, it also affects the fragile ecosystem in the Gobi desert. The continuous reduction of humidity leads to the ongoing loss of important springs and forces the camels to cover longer distances on their search for food and water (YADAMSUREN et al., 2012).

Another important factor is the buffer zone, which surrounds the GGSPAA. About 400 families with approximately 10.000 domestic camels in their livestock are living along this boundary. Numerous problems arise from this tight wildlife-livestock interface such as the competition for food and water sources, the transmission of diseases and hybridization (ENKHBILEG et. al, 2006; WALZER et al., 2006). Recently, the inter-breeding with domestic animals has been identified to be an additional threat to wildlife and may also contribute to the decline of extant populations. Moreover, it could possibly lead to the irreversible loss of their genetic distinctiveness and mechanisms of local adaption (RANDI, 2008; OLIVEIRA et al., 2008).

1.3 Genetic mechanisms of hybridization

The following explanations are based on the phylogenetic species concept (PSC), which describes a species as a monophyletic group sharing the same evolutionary history (COYNE and ORR, 2004).

Hybridization is defined as the interbreeding of individuals, which belong to populations of different species and produce viable offspring. If the progeny of members of two different species is fertile, as it applies to all representatives of the Old World camelids (Camelini), new genetic information can invade the population. This gene flow between populations is referred to as introgression (ALLENDORF and LUIKART, 2007).

1.3.1 Danger of hybridization to a wild population

Hybridization between wild species and their domestic relatives has been identified as a serious threat to their genetic integrity in many cases. The European wildcat (*Felis sylvestris*; BEAUMONT et al., 2001), the gray wolf (*Canis lupus*; RANDI, 2008) and the bison (*Bison bison*; HALBERT and DERR, 2007) are just a few examples for this specific interaction between wildlife and domestic livestock.

The wild camel (*Camelus ferus*) has perfectly adapted to the harsh and dry conditions in the Gobi. If its unique genetic information gets lost by hybridization, it could lead to a significant fitness reduction in the population. This process, which is described as outbreeding depression, could then contribute to the further decline of the population (FREDRICKSON et al., 2006).

1.3.2 Anthropogenic hybridization

Human influence often increases the risk of hybridization. This phenomenon can also be seen in the Mongolian wild camel population.

Deliberate hybridization

It is known, that the tradition of crossbreeding of camels has a very long history. In Kazakhstan, for instance, hybrids between Bactrian camels and dromedaries are famous for their increased milk and wool production (FAYE and KONSUPAYEVA, 2012), as well as for their better performance in camel races (POTTS, 2004).

In Mongolia deliberate hybridization between the wild camel (*Camelus ferus*) and the domestic Bactrian camel (*Camelus bactrianus*) has become a considerable issue as well. Among Mongolian herders hybrids in the first generation are known to be stronger and faster than their purebred relatives. They are primarily used as racing camels. Thus, the locals in the buffer zone area of the GGSPAA accept the interbreeding of wild Bactrian camels with their domestic herd to a certain degree. Male hybrids are usually castrated, because second generation hybrids show weak condition and more aggressive behaviour. As a first reaction to the occurrence of hybrids the Mongolian government recently decided to completely forbid their use in camel races (YADAMSUREN et al.,2012; ENKHBILEG et al., 2006).

Accidental hybridization

The presence of hybrid camels in the GGSPAA has been observed occasionally on scientific expeditions (ENKHBILEG et al. 2006; WALZER et al., 2006). Especially, the temporarily release of domestic camels during the winter season along the buffer zone may enforce the problem of cross-breeding. It is known that domestic camels, once they are separated from their herd, invade the protected area and willingly join wild camels on their search for grazing areas. On the other hand, wild camel bulls may advance into the buffer zone in order to collect domestic females for their harem, when they come into rut (YADAMSUREN et al., 2012).

1.3.3 Detection of hybridization

The identification of hybrids based on their phenotype usually requires long-time observation and has some potential for error because of their similar appearance with wild camels. However, genetic methods require less time in the field and also provide interesting data on population structure, effective population size, inbreeding and genetic diversity. Hence, nowadays genetic monitoring is additionally used to detect hybridization and also for other questions (SILBERMAYR and BURGER, 2012).

Mitochondrial DNA as a molecular marker in Conservation Genetics

Mitochondrial DNA (mtDNA) serves as a useful marker in conservation genetics, e. g. for the analysis of the phylogenetic status and the survey of genetic variance of a wild population. In this study it was also used as a tool for screening a wild population for maternal domestic hybrids, because of its particular characteristics, especially when it comes to forensic samples, which are gathered non-invasively (SILBERMAYR and BURGER, 2012; SILBERMAYR, 2010).

Depending on the cell type a mammalian cell contains up to 1.000 mitochondria, each equipped with two to three molecules of mtDNA (ROBIN and WONG, 1988; s. fig. 3). This allows extracting high quantities of mtDNA. It is therefore very appropriate for forensic analysis of non-invasively collected samples, which are normally exposed to DNA degrading conditions such as sunlight, heat or humidity. Especially, shed hair collected from bushes, as used in this study, is particularly tricky to work with, because of the little amount of hair roots, so that it may not provide enough nuclear DNA (nDNA; HOLLAND and PARSON, 1999).

In addition, mtDNA is mostly maternally inherited and the offspring generally receives the mtDNA genotype (haplotype) from its mother. This highly facilitates genetic analysis and the construction of phylogenetic trees (ALLENDORF and LUIKART, 2007). Regarding the fact, that interbreeding of domestic and wild camels mostly takes place between domestic females and wild bulls (YADAMSUREN et al., 2012), the mtDNA analysis alone appears to be sufficient to give at least a rough overview of the present situation. However, nuclear markers, e.g. microsatellites or single nucleotide polymorphism (SNP) data, should always be included, to infer nuclear levels of admixture and introgression (SILBERMAYR and BURGER, 2012; BALLARD and WHITLOCK, 2004).

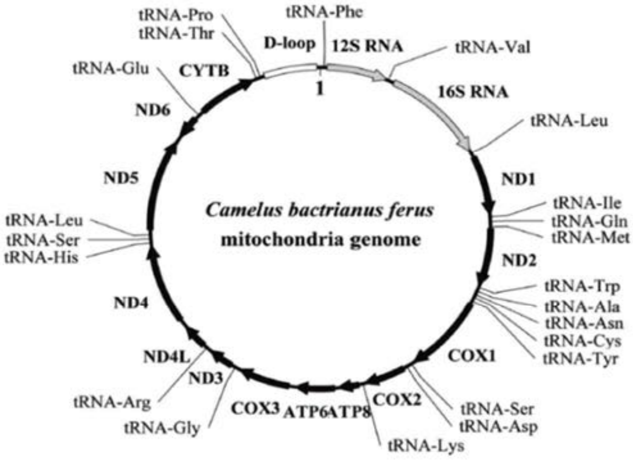


Figure 3. Map of the mammalian mitochondrial genome. It is organised as a circular molecule and consists of ca. 16,000-17,000 base pairs. Most of the coding regions are highly conserved, whereas the non-coding region, which mainly consists of the Control Region (D-Loop, 700 - 1.000bp), can be described as highly variable between different taxa (CUI et al., 2007).

1.4 Aims of this work

In this diploma study I searched for evidence of hybridization between wild and domestic Bactrian camels in the GGSPAA using mitochondrial markers. Furthermore I aimed to retrieve a general overview on the genetic diversity of the Mongolian wild camel population.

The results, developed in the framework of this diploma thesis, have been presented at the “International Meeting on Wild Bactrian Camel Conservation Strategy of Mongolia” (Hustai National Park, Mongolia, 2010) and contributed to the release of the “Mongolian National Conservation Strategy for the Wild Camel and its Desert Habitat”, which was presented to the Mongolian Ministry of Nature, Environment and Tourism for Official Endorsement (MNET). In this document the preservation of the genetic integrity of the wild camel is defined as a primary objective (YADAMSUREN et al., 2012).

2. Material and Methods

2.1 Material

I used 39 hair samples from Great Gobi Strictly Protected Area “A” (GGSPAA; s. fig. 4), that had been collected from bushes on a scientific trip to the Gobi Desert led by Chris Walzer and Petra Kaczensky in November 2009. For the statistical and phylogenetic analysis I added previously obtained sequences of both species, *Camelus ferus* and *Camelus bactrianus*, which had been published by SILBERMAYR et al. (2010) and some of the just completed FWF project (P1084-B17; PI: P. BURGER), as well as sequences published in GenBank (s. appendix table A1 and A2).

The hair samples had been collected from bushes around different water points in the middle of the GGSPAA (s. fig. 4). In order to retrieve samples from different individuals, only one floccus was taken per plant. Between the sampling areas there was minimum distance of several kilometres. However, the risk of sampling the same animal twice could not be excluded completely. Therefore, individual microsatellite profiles would be needed. The hair was stored at room temperature in a fresh envelope labelled with the date of sampling and the place of collection.

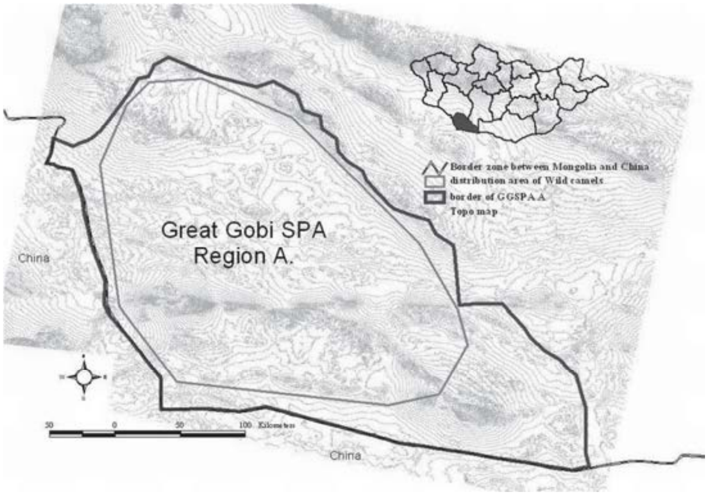


Figure 4. The map shows the current distribution (inner circle) of the Mongolian wild camel population in the GGSPAA (YADAMSUREN et al., 2012).

2.2. Methods

2.2.1 Hair lysis

In order to obtain DNA out of the collected hair samples I used the following procedure: An amount of approximately 15 to 30 single hairs mostly with roots were cut to a length of 15 to 20 mm and put into tubes. I then added 340µl of the Lysis buffer (PFEIFFER et al., 2004) containing 100mM Tris-HCl, 100mM NaCl, 3mM $CaCl_2$, 2% SDS, 40mM DTT (13.6 µl) and 250 µg/ml proteinase K (30 µl) . If required, some more Lysis buffer was added, so that the samples were completely covered. The camel hairs were then incubated with a thermo shaker at 56 ℃ overnight. The lysis buffer had been stored at room temperature without DTT (13.6µl) and Proteinase K (30µl) at 4 to 8 ℃ and was added just before use.

2.2.2 DNA extraction from hair samples

After at least 24 hours of incubation I continued with the DNA extraction. Therefore I used the Nucleospin®Tissue DNA extraction kit (MARCHEREY-NAGEL, Düren, Germany) and followed the instructions according to the protocol (s. appendix table A4) except for the following modifications. I added 210µl of cooled ethanol and stored the tubes up to one hour in the fridge in order to enhance precipitation and, finally, I added two times 30µl of water instead of 100µl the original elution buffer once so that the DNA concentration was higher in the end.

2.2.3 DNA amplification

For the analysis I used an 804bp nucleotide fragment of mtDNA (nt 15060 – 16034; CUI et al., 2007). This fragment contains the end of cytochrome b (MT-CYB), tRNAs Threonine (MT-TT) and Proline, and the beginning of the control region (MT-CR).

The used method is based on the previous work of SILBERMAYR et al. (2010) on detecting hybrids between the wild camel and the domestic Bactrian camel. Because of the poor DNA quality in forensic hair samples, I used four primer pairs in total (s. appendix table A1) for the amplification of the complete target region. The overlapping DNA fragments had a length from 311 to 394bp.

Amplification of the specific fragment was performed by Polymerase Chain Reaction (PCR; s. table 1). The contents of 15µl of PCR-solution were (final concentrations in brackets): 1.50µl DNA, 1.20µl of $MgCl_2$ (1.5mM), 0.09µl of each primer (600nM), 0.09µl BioTaq (1.5 U), 1.50µl dNTP-mix with dUTP (0.2 mM), 1.50µl Bovine Serum Albumin (1mg/ml), 1.50µl buffer (1x) and 7.53µl water. The cycling procedure consisted of an initialization step, then 38 cycles of denaturation, annealing and elongation, and a final elongation step. Initially, the PCR was heated up to 95 ℃ for 5 minutes, then one minute for primer binding at 55 to 59 ℃, depending on the melting temperature (Tm) of the primer pair (s. appendix table A3), and three minutes of elongation at 72 ℃. This first step was followed by 38 cycles of 30 seconds at 95 ℃, 45 seconds at 55 to 59 ℃ and 1.25 minutes at 72 ℃. The final step was at 72 ℃ for five minutes again to ensure that every single-stranded DNA was fully elongated. Afterwards, the PCR products were visualized by gel electrophoresis (0.8% agarose) to check, if the requested fragment had been amplified correctly.

Table 1. Example of a PCR protocol for 52 samples

Mastermix [µl]	15µl rxn	Stock solutions	Final concentration
-	1.50	DNA	
46.80	0.90	$MgCl_2$ (25mM)	1.5mM
4.68	0.09	tPRO15402_CD-f (100pmol/µl)	600.0nM
4.68	0.09	CR16034_CD-r (100pmol/µl)	600.0nM
4.68	0.09	BioTaq (10U/µl)	1.5U
78.00	1.50	dNTP mix with dUTP (2/4mM)	0.2/0.4
78.00	1.50	BSA (10mg/ml=100x)	10x
78.00	1.50	Buffer (10x)	1x
407.16	7.83	H_2O	
∑702.00	∑15.00		

2.2.4 PCR-product purification

The PCR-products were cleaned by using the ExoSAP® protocol. For every 5µl of PCR reaction I added 0.5µl of Exonuclease I (20U/µl) and 1µl of Shrimp Alkaline Phosphatase (1U/µl). Subsequently, the samples were incubated at 37 ℃ for 60 minutes and at 80 ℃ for 15 minutes in order to inactivate the enzymes.

2.2.5 Measurement of DNA concentration

The concentration of DNA was measured by gel electrophoresis (0.8% agarose) with Low DNA Mass Ladder (Invitrogen Corp.) as quantification standard. 0.5µl of bromophenol blue was added to each sample as loading dye. The first slot was filled with 2µlof Low DNA Mass Ladder. Then 2µl of each PCR product were added per slot. After the gel electrophoresis had been completed, the concentration of DNA was determined by comparing the density of each band to that of the standard (s. fig. 5).

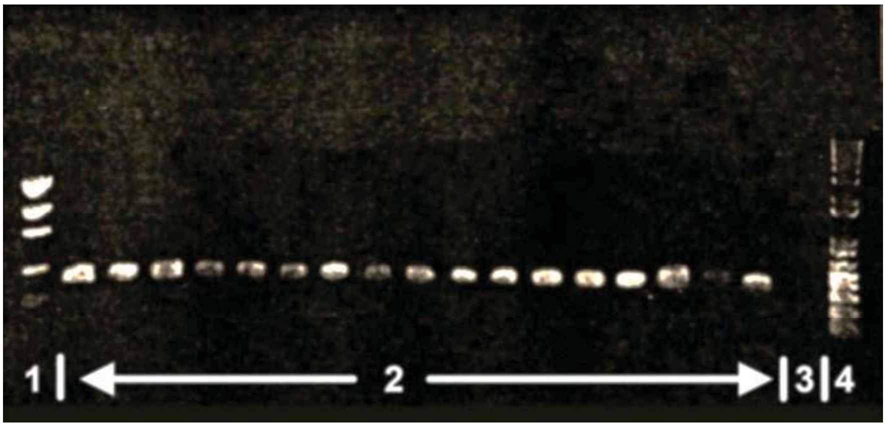


Figure 5. 0.8% agarose gel stained with ethidium bromide and loaded with 2µl of Low DNA Mass Ladder (1), PCR products WC123 – WC139 (2), negative control (3) and 1 Kb Plus DNA Ladder (4) (INVITROGEN CORPORATION, California, USA). The amount of the DNA fragments in this picture ranges from 20 to 60ng (2).

2.2.6 Sequencing reaction

The sequencing was conducted in both directions (5’ to 3’ and 3’ to 5’). The purified DNA templates were amplified by a sequencing PCR with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Invitrogen Corp.). Each well was filled with 1µl of buffer, 1µl of one specific primer (5pmol/ml), which had also been used in the previous PCR, and 1.5µl of BigDye®. Then 5 to 15ng of the DNA template were added. The used volume of DNA was calculated according to the concentration of the template.

Finally, deionized and distilled water was added to reach the total reaction volume of 10µl. The PCR temperature profile consisted of 28 cycles of 20 s at 96 ℃ for denaturation, 5 s at 45 - 50 ℃ for primer annealing and 4 min at 60 ℃ for extension.

2.2.7 Sequencing

The sequencing PCR products were purified by using the Multiscreen® 96-well filtration system (EMD Millipore Corp., MA, USA). The 96-well filtration plate was prepared by adding Sephadex G-50 with a Column Loader. Afterwards, 300µl of Milli-Q water were added to each well to swell the resin. The plate had then to be incubated at room temperature for three hours. Before adding the PCR products the HV plate was centrifuged at 1920 rpm for five minutes in order to pack the columns. One volume of PCR product (13µl) and one volume of binding buffer (13µl; pH 5.6) were mixed together by carefully pipetting the solution up and down at least five times. Then 10µl of the prepared sequencing dilution and 10µl Milli-Q water were put into each well, so that in the end the total volume of 20µl of diluted BigDye® solution could be obtained. Finally, the HV plate was put on top of a new 96-well v-bottom plate and centrifuged at 1500 rpm for 30 seconds and after five to ten minutes at 1920 rpm for four minutes.

For sequencing of the mtDNA fragment I used the BigDye® Terminator technology (Invitrogen Co.), a bidirectional automated sequencing method. A MEGABACE 1000 (GE-healthcare™) served as automated sequencer.

2.2.8 Controls to prevent contamination

I took the following controls to avoid possible DNA-contamination. Pre- and post-PCR working areas were strictly separated so that the risk of carry-over-contamination from one place to the other could be reduced. For the making of PCR mixes there was a special DNA-free room, which had been cleaned frequently with bleach. Moreover, everybody entering the room had to put on new gloves and a coat, which was only worn inside. Another aspect was the use of highly specific primers (s. appendix table A3), so that alien DNA would not be amplified. Finally, within all steps there were negative controls included.

2.2.9 Phylogenetic analysis

The sequences were visualized and aligned by using the software CODON CODE ALIGNER 3.0.2 (Codon Code Cooperation). Polymorphisms were only accepted, when they could be detected in both directions. The sequences established in this diploma thesis (s. appendix table A1) were added to a larger data set of 139 sequences of wild and domestic Bactrian camels, published by Silbermayr et al. (2010), Charruau (2012) and in GenBank (s. appendix table A2) for phylogenetic analysis. The 168 samples in total of wild and domestic Bactrian camels originated from Mongolia, China, Kazakhstan, Russia, Israel, Hungary, England and Austria (s. appendix table A2). For assessing parameters of genetic diversity within and between the two species I specifically compared the 29 wild camel samples established in this diploma thesis with 51 domestic Bactrian camel samples, originating from Mongolia (SILBERMAYR et al., 2010; s. appendix table A2).

The median joining network (MJN) was created with the software NETWORK 4.5 (BANDELT et al., 1999) with default parameters to demonstrate the phylogenetic relationship between the two camel species. Structure in the mitochondrial haplotypes of the wild and domestic Bactrian camels was investigated using a Bayesian method implemented in BAPS 5.2 (CORANDER et al. 2008). This method treats the number of populations as unknown parameter (without any prior) and clusters individuals using the information in the data set. I determined prior upper bound values for the number of clusters (i.e. 6-10) and performed ten independent runs for each value. All runs provided the same number of clusters.

The best fitting model of nucleotide substitution was determined by the Akaike information criterion (AIC) using the software JMODELTEST 2.1.1 (DARRIBA et al., 2012). I used the 2-parameter model of evolution according to KIMURA (K2P; 1980) with a gamma correction of 0.03 implemented in the software ARLEQUIN 3.5 (EXCOFFIER et al., 2010) to determine the parameters of genetic diversity in the Mongolian camel populations. I calculated the fixation index F_{ST} between the wild and the domestic camel populations (WEIR et al., 1984), the haplotype diversity H_d (NEI, 1987), the nucleotide diversity π and the mean number of pairwise differences (TAJIMA, 1983) as well as the estimators of nucleotide diversity Theta_S (θ_S; WATTERSON, 1975) and Theta_π ($\theta_{\pi;}$ TAJIMA, 1983). The mismatch distribution of pairwise differences (SCHNEIDER and EXCOFFIER, 1999) two neutrality tests Tajima’s *D* (TAJIMA, 1989) and Fu’s F_s (FU, 1997) were carried out with ARLEQUIN 3.5. Along with detecting deviation from neutrality these tests can also provide information on past demographic events, while especially Fu’s F_s is sensitive to population expansion (FU, 1997). Significance of the tests was assessed with 10.000 permutations.

2.2.10 Hands-on training in Mongolia

After the lab work for this diploma thesis I had the opportunity to assist on a scientific trip to the wild camel captive breeding centre in Zakhyn Us, Mongolia (Wild Camel Protection Foundation, *http://www.wildcamels.com*) in September 2010. There we took several blood samples from the captive camels in order to get information on the parentage of each individual and for veterinary health check.

Figure 6. Blood sampling at the Captive breeding Centre Zakhyn Us, Mongolia in 09/2010

The blood samples of the captive breeding herd were taken by punctuation of the External Jugular Vein. The procedure was done completely without anaesthesia of the camels. However, they were restrained in a metal frame, which had been specially designed for veterinary examination and treatment. Immediately after the withdrawal, the blood was transferred to EDTA-tubes, serum-tubes and FTA®cards. From then on the samples have been stored in a mobile freezer during the whole transport. The blood was taken for routine veterinary control and an aliquot was used for later DNA extraction.

In the genetic laboratory (head: Prof. Tumenasan) of the Institute of Biology, Mongolian Academy of Sciences I processed the samples together with two Mongolian students, who I instructed to do the DNA extraction. For the DNA extraction from blood samples we used again the Nucleospin®Tissue-Kit and followed the “support protocol for blood samples” (MARCHEREY-NAGEL, 2009). 25µl of Proteinase K and 200µl of blood were pipetted into 1.5ml microcentrifuge tubes. 200µl of Buffer B3 were added to the samples and vortexed vigorously for 10 to 20 seconds. The samples have been incubated at 70 ℃ for 15 min afterwards. In order to adjust DNA binding conditions 210µl of ethanol (96%) were added and vortexed again. Each sample was then transferred into a column with a matrix-membrane, which had been placed in a collection tube. After 1 min. of centrifugation at 11.000 x g, the flow-through was discarded. The silica membrane was then washed according to the “standard protocol for human or animal tissue” (MARCHEREY-NAGEL, 2009).

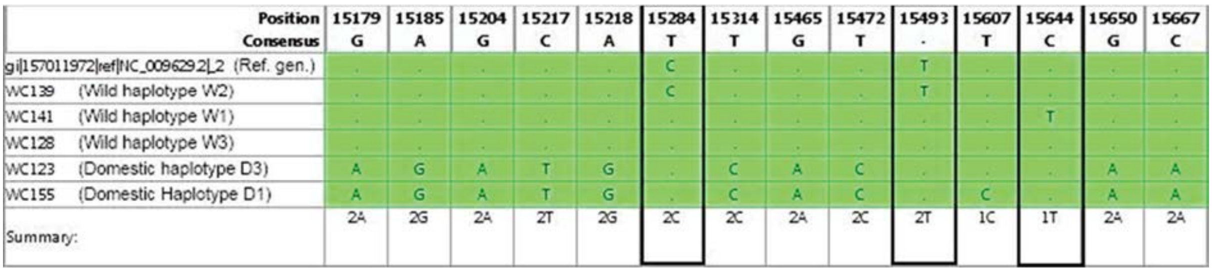
The obtained DNA samples from this trip were then shipped to Vienna and were included in later studies (CHARRUAU, 2012).

3. Results

In this study hair samples collected in the GGSPAA were investigated with mitochondrial markers and their profile was compared to the sequences of domestic Bactrian camels. The complete 804bp mtDNA fragment could be obtained from 29 of 39 hair samples. Within this group, five samples could be assigned to domestic mtDNA haplotypes (s. fig. 10). Diversity indices (s. chapter 3.1), and demographic inferences (s. chapter 3.2) were calculated within the Mongolian samples of wild camels (s. appendix table A2) and compared to their domestic relatives as well as phylogenetic analyses (s. chapter 3.3) were performed with the joint data set of all 168 sequences (s. appendix table A2).

3.1 Analysis of genetic diversity in the samples from GGSPAA

Five of the 29 new sequences showed domestic mtDNA haplotypes (s. appendix table A1) although they had been collected in the GGSPAA and were therefore excluded from the following calculations. Within the analysed 804bp mtDNA fragment the wild samples displayed three polymorphic sites (two transitions, one indel), whereas the domestic sample group of the 51 Mongolian samples showed seven (five transitions, two transversions).The substitutions in the wild camels were at the positions 15284 and 15644 the insertion-deletion (indel) at the position 15493 (numbering as per CUI et al., 2007) and they are displayed in figure 7.



Position	15179	15185	15204	15217	15218	15284	15314	15465	15472	15493	15607	15644	15650	15667
Consensus	G	A	G	C	A	T	T	G	T	-	T	C	G	C
gi|157011972|ref|NC_009629.2|_2 (Ref. gen.)	.	.	.	.	.	C	.	.	.	T	.	.	.	.
WC139 (Wild haplotype W2)	.	.	.	.	.	C	.	.	.	T	.	.	.	.
WC141 (Wild haplotype W1)	.	.	.	.	.	.	.	.	.	.	.	T	.	.
WC128 (Wild haplotype W3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.
WC123 (Domestic haplotype D3)	A	G	A	T	G	.	C	A	C	.	.	.	A	A
WC155 (Domestic Haplotype D1)	A	G	A	T	G	.	C	A	C	.	C	.	A	A
Summary:	2A	2G	2A	2T	2G	2C	2C	2A	2C	2T	1C	1T	2A	2A

Figure 7. Single nucleotide variations (SNV) among the 29 hair samples from GGSPAA. The sequences were aligned to a reference genome published in GenBank (gi157011972). The SNV in the wild group are framed. Dots refer to correspondence with the consensus.

In the following analysis I compared specifically the Mongolian populations (s. appendix table A2) of *C. ferus* (n=24) and *C. bactrianus* (n=51) by different parameters of genetic variability. In the wild camel population I identified three haplotypes, whereas the domestic sample group showed seven. The haplotype diversity (H_d), which is the likelihood of two randomly chosen mtDNA sequences being different, was very similar in both sample groups (*C. ferus*: 0.65 ± 0.05, *C. bactrianus*: 0.64 ± 0.06). The nucleotide diversity (π) defined as the number of pairwise nucleotide differences between randomly chosen sequences, was low within both populations (*C. ferus*: 0.0014 ± 0.0010, *C. bactrianus*: 0.0018 ± 0.0012). The mean number of pairwise nucleotide differences was calculated with 1.085 in the wild and 1.427 in the domestic group.

In order to assess a possible loss of genetic variation I compared the estimates of nucleotide diversity based on the segregating sites θ_S and the nucleotide diversity calculated with pairwise comparisons θ_π (WEBER et al., 2004). Assuming that θ_S is less resistant to the occurrence of rare alleles than θ_π, the difference of θ_π -θ_S should be zero in large populations, which are in stable equilibrium of mutation and genetic drift (WEBER et al., 2004). However, referring to this parameter, the relatively small wild camel population was suggestive of being in disequilibrium (θ_π -θ_S= 0.549), possibly because of an excess of intermediate-frequency mutations or loss of polymorphic sites due to a shrinking population size or recent bottleneck.

Table 2. Comparison of mtDNA diversity parameters in the Mongolian populations of *C. ferus* and *C. bactrianus* (s. appendix table A2)

Parameters	**Abbr.**	***C. ferus***	***C. bactrianus***
Sample size	n	24	51
No. of polymorphic sites	-	3	7
No. of genotypes	-	3	7
Haplotype diversity	H_d	0.649 ± 0.050	0.635 ± 0.063
Nucleotide diversity	π	0.0014 ± 0.0010	0.0018 ± 0.0012
Mean number of pairwise nucleotide differences	-	1.085 ± 0.739	1.427 ± 0.886
Estimates of nucleotide diversity (segregating sites)	θ_S	0.536 ± 0.398	1.556 ± 0.707
Estimates of nucleotide diversity (pairwise comp.)	θ_π	1.085 ± 0.824	1.427 ± 0.983
Harpending's raggedness index	r	0.218 (p>0.1)	0.067 (p>0.1)
Tajima's D	D	1.937 (p>0.1)	-0.427 (p>0.1)
Fu's F_S	F_S	2.587 (p>0.1)	-1.059 (p>0.1)

3.2. Inferences on the demographic history of wild camels in the GGSPAA

In order to evaluate a possible decline of the wild camel population I performed two neutrality tests, which, along with detecting selection in a gene, can indicate past demographic events. The Tajima's D- test (D= 1.94, p> 0.1) and the Fu's F_S- test (F_S= 2.587, p> 0.1) both provided considerably positive values, but not significant. These positive values could hint at a possible population decline (SCHNEIDER and EXCOFFIER, 1999), as a very rough rule of thumb to significance is that values greater than +2 or less than -2 are likely to be significant. In the Mongolian domestic Bactrian camel population both tests produced negative (not significant) values

(Tajima’s D = 0.427, $p > 0.1$; Fu’s Fs = -1.053, $p > 0.1$), which could indicate population expansion.

To further investigate the demographic history of the *C. ferus* population I constructed a mismatch distribution of pairwise differences (s. fig. 8, 9) and compared it to the *C. bactrianus* population. Although both distributions were multimodal, the Mongolian *C. bactrianus* population showed a smooth distribution, which was similar to the modelled curve (ROGERS and HARPENDING, 1992), while the *C. ferus* population had a more disrupted scatter. This could indicate structuring within a population or hint to a population that is stable or shrinking (EXCOFFIER and SCHNEIDER, 1999; ROGERS and HARPENING, 1992; ROGERS et al., 1996). This impression was also confirmed by the Harpending’s raggedness index (r), a statistical measure for the development of population size, which is higher in the wild camel ($r = 0.218$, $p > 0.1$) than in the domestic Bactrian camel ($r = 0.067$, $p > 0.1$). This index takes larger values for multimodal distributions commonly found in a stationary or shrinking population than for unimodal and smoother distributions typical of expanding populations. However, in both populations this value was not significant; therefore I could not reject the null hypothesis of population expansion under the sudden-expansion model (SCHNEIDER and EXCOFFIER, 1999).

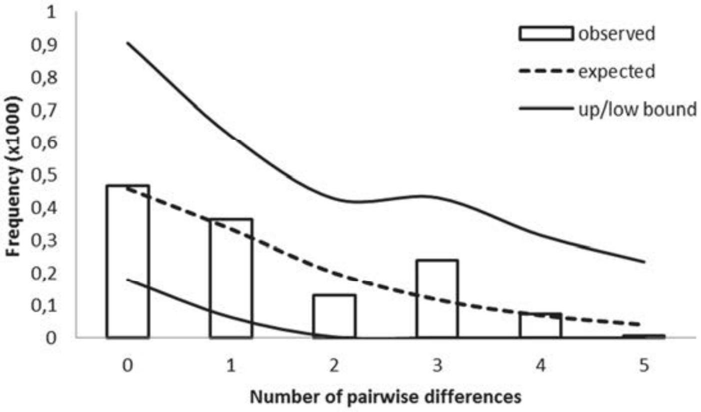


Figure 8. Mismatch distribution of the Mongolian ***C. bactrianus*** population (n=51; s. appendix table A2; CI = 95%)

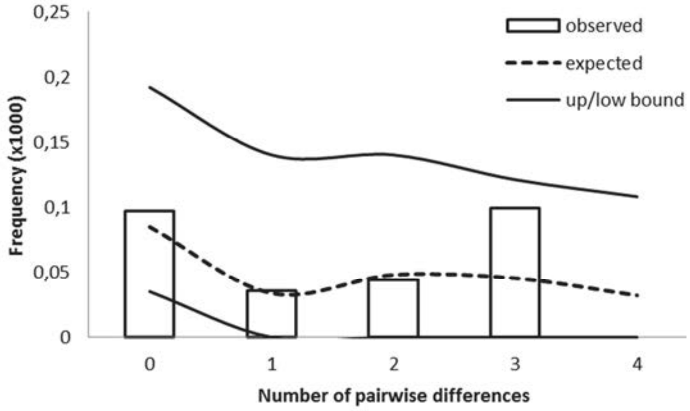


Figure 9. Mismatch distribution of the Mongolian ***C. ferus*** population (n=24; s. appendix table A2; CI = 95%)

3.3 Phylogenetic analysis of *C. ferus* and *C. bactrianus*

The phylogenetic analysis of the complete data set of 168 samples (s. appendix table A2) resulted in 18 different haplotypes of mtDNA, three belonging to the species *C. ferus* and 15 to *C. bactrianus.* For the first time a third wild haplotype (W3) was described. A minimum spanning network, also known as median joining network (MJN), which is based on the most parsimonious relation between haplotypes, was created. The MJN (s. figure 10) shows two different haplogroups, separated by 13 fixed single nucleotide polymorphisms (SNPs), illustrating once again the clear distinction between the both taxa.

By using a Bayesian model the 168 individuals were related to different clusters (CORANDER and TANG, 2007). There were four clusters in total, two domestic and two wild (s. fig. 11) with a posterior probability (pp) of 0.96. This result is also traceable by the MJN (s. fig. 10).

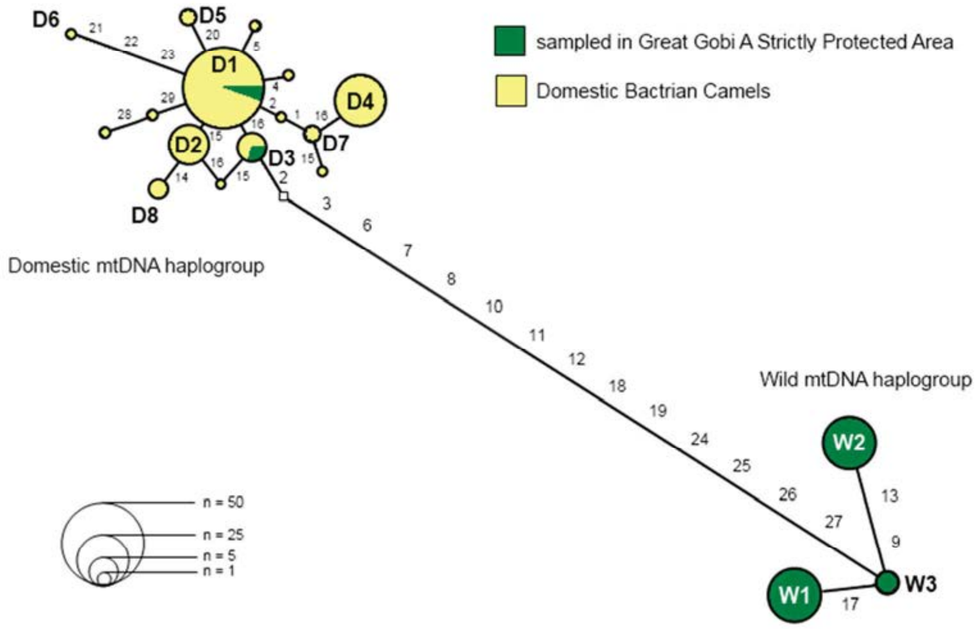


Figure 10. Median Joining Network (BANDELT et al., 2009) based on the analysis of the mtDNA fragment analysis illustrating the different haplotypes of *Camelus ferus* (W1-W3) and *Camelus bactrianus* (D1-D8). The circle size is proportional to the number of individuals showing the haplotype. The figures at the side of the lines represent the location of difference in nucleotide sequences between the haplotypes (numbering refers to table A5 in appendix). The four clusters of population structure refer to the Bayesian model (s. fig. 11)

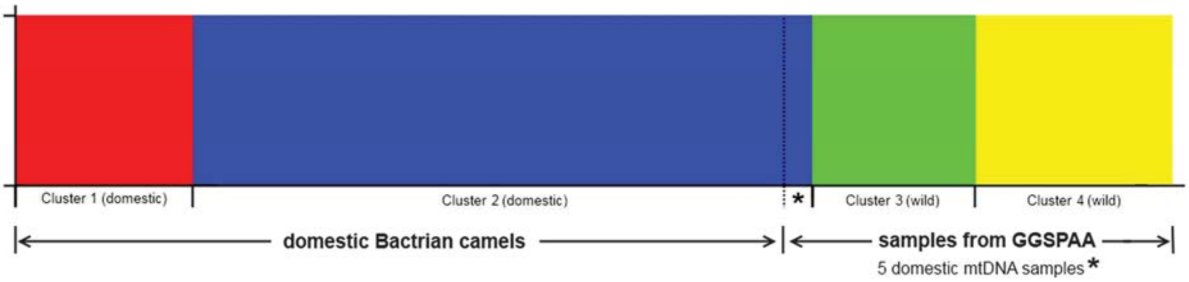


Figure 11. Bayesian Model showing 4 clusters (2 wild, 2 domestic)

4. Discussion

In this diploma study I applied mitochondrial markers for genetic monitoring and investigation of maternal hybridization between the wild camel (*C. ferus*) and the domestic Bactrian camel (*C. bactrianus*) in the GGSPAA by analysing non-invasively collected hair samples. Five (17%) of the 29 randomly collected hair samples in the GGSPAA showed domestic mtDNA and gave first genetic evidence for maternal hybridization. Taking into account that the analysis of mtDNA only reveals maternal hybrids, this could mean that the Mongolian wild camel population has already reached a considerable level of hybridization. Yet, the hair samples could also originate from feral camels (domestic camels gone wild) in the core zone or from widely roaming domestic camels, which had been deliberately released over the winter season. In any case, the results indicate that domestic camels are in close contact with the wild camel population. In order to clarify the origin (hybrid or domestic) of the suspicious samples the completed mtDNA analysis will be followed by an investigation with nuclear markers.

A deeper analysis of genetic diversity in the Mongolian domestic Bactrian camel and wild camel population provided several results, indicating a possible recent loss of genetic variability. The informative value of mitochondrial markers for demographic and genetic diversity analysis has been doubted in recent studies (BAZIN et al., 2006). Nevertheless, it can serve as a useful tool for first investigations at least in mammalian taxa (NABHOLZ et al., 2008), especially if nuclear markers are inapplicable because of poor DNA quality.

Although, the distribution of pairwise difference and the neutrality tests were non-significant, the comparison with the stable Mongolian domestic Bactrian camel population allows the conclusion that the wild camel has suffered from a recent population decline. These results are also confirmed by the latest surveys in the wild (YADAMSUREN et al., 2012). While Reading et al. (1999) provided estimates of 2000 individuals, Guoying et al. (2002) assumed only 880 remaining wild camels in China and Mongolia. Though they used different methods of population census, these studies give us at least a clue of the demographic history.

However, mitochondrial markers are not sufficient to thoroughly evaluate the demographic development of a population. Nuclear markers such as microsatellites and genome-wide SNPs, which are more sensitive, will provide more information on effective population size, genetic diversity and introgressive hybridization.

4.1 Hybridization

Recent studies showed that the wild camel (*C. ferus*) and the domestic Bactrian camel (*C. bactrianus*) are highly differentiated on a genetic level (SILBERMAYR et al., 2010; CUI et al., 2007) and, therefore, have to be regarded as separate species referring to the Phylogenetic Species Concept (PSC; COYNE and ORR, 2004). The latest phylogenetic analysis have confirmed that the wild camel is neither feral nor the wild ancestor of the domestic Bactrian camel (BURGER et al., 2012).

At the moment, the wild camel is classified as critically endangered by IUCN and, although estimates of population size vary widely, authors broadly agree, that there is a decline or at least a stagnation of population numbers over the last decade (READING et al., 2002; YADAMSUREN et al., 2012; HARE et al., 1997). In the framework of the “International Meeting on Wild Bactrian Camel National

Conservation Strategy of Mongolia” (Hustai National Park, Mongolia, 2010) several causes for this alarming development were discussed and led to the formulation of a conservation action plan.

Like in many other regions, there is a strong human influence even in the remote Gobi desert. First of all, men have direct impact on this fragile ecosystem in terms of exploitation, like illegal mining activities and poaching. However, there are also indirect mechanisms, like the global climate change, which leads, among other things, to the desiccation of rare water points. A high ecologic pressure also originates from the local herders with their livestock along the buffer zone of the GGSPAA. The development of a tight wildlife-livestock interface always goes along with problems for both sides. In the case of the wild camel, we can identify three main interactions: direct competition for food and water, transmission of diseases, like brucellosis or anthrax, and hybridization (FOUFOPOULOS et al., 2003).

Introgressive hybridization with closely related domestic species has led to the extinction of many taxa and is also described as a major threat to the genetic integrity of the wild camel population (RHYMER and SIMBERLOFF, 1996; ALLENDORF et al., 2001; SILBERMAYR and BURGER, 2012). Although, hybridization between two species can occur as a natural phenomenon, and may even be essential for evolutionary processes, it can also have negative effects, especially when it is anthropogenic. If formerly allopatric, but closely related species get sympatric by transport or human-caused habitat fragmentation, the risk of interbreeding rises significantly (RHYMER and SIMBERLOFF, 1996). If their offspring is fertile, it may have dramatic consequences for their gene pools and may even lead to their homogenization. Especially, small populations are known to be susceptible to this form of introgression of alien alleles (OLIVEIRA et al., 2008). Crossbreeding with domestic species has already been identified as a major threat to several other endangered mammalian species, like the wild yak (*Bos mutus*; SCHALLER and WULIN, 1996), the bison (*Bison bison*; HALBERT and DERR, 2007) or the Ethiopian wolf (*Canis simensis*, GOTTELLI et al., 1994), because of the loss of local adaption and fitness reduction.

The wild camel has perfectly adapted to a life in its forbidding habitat, whereas the domestic Bactrian camel has been bred for other traits that may not have any effect in captivity, but could turn out as critical disadvantage in the wild. Reduced seasonality in reproduction, higher growth rates, increased milk production are detrimental characteristics in regions with highly limited resources. Moreover, the introgression of domestic alleles can have negative impact on the fitness of a wild population by affecting its fertility and disease resistance, thus its reproduction and survival rate (FOUFOPOULOS et al., 2003; OLIVEIRA et al., 2007).

4.2 Wild camel conservation

In the framework of the last “International Meeting on the Wild Bactrian Camel National Conservation Strategy of Mongolia” (2010) the preservation of the genetic integrity was defined as primary objective. There are several approaches to this task such as the elevation of population numbers, the strict separation from domestic herds and the removal of existing hybrids. As a first step the Mongolian government has ordered the elimination of hybrid camels among domestic herds and in the GGSPAA. In addition, the use of hybrids for camel races has been forbidden to make them less attractive. However, to completely stop contact between the species further efforts must be made by the local administration.

Since 2004 the Wild Camel Protection Foundation (WCPF; *http://www.wildcamels.com*) has run a captive breeding centre in Zakhyn Us, Mongolia with around 20 wild camels in order to facilitate the research on the rare animal and to build up a stock for future reintroduction into the wild. The success of a captive breeding programme depends on its high management standards, including veterinary care and the correct keeping of a studbook. The latter is essential in order to prevent inbreeding and the introgression of domestic animals (HAMMER et al., 2008). Ex-situ conservation may save a highly threatened species, like the Przewalski's Horse (*Equus ferus przewalskii;* WALZER et al., 2012), which had already been extinct in the wild, before it was reintroduced in its original habitat again. However, if there are still extant populations in the wild, the first attempt must be the preservation of the animal in its original habitat, known as in-situ conservation.

Therefore, it seems to be important to continue negotiations between the Mongolian and the Chinese government to create corridors across the borders between the GGSPAA and China to enable genetic transfer between the wild camel populations (YADAMSUREN et al., 2012). This may be a suitable approach to increase the effective population size and prevent further loss of genetic diversity within the rather small wild camel populations by random genetic drift.

4.3 Outlook

In order to make the conservation efforts more efficient further information on the wild camel such as its population structure, genetic diversity and the extent of introgressive hybridization must be gathered. Therefore, I recommend intensifying the non-invasive sampling in the GGSPAA and along the boundary as well as starting a long-time survey of the wild camel population based on the previously described methods of mtDNA and microsatellite analysis (SILBERMAYR et al., 2010; SILBERMAYR and BURGER, 2012). The mtDNA analysis has proven to be a useful tool for the rapid screening of wild population for maternal hybridization and is also suitable for non-invasively collected samples such as hair flocci from bushes.

5. Summary

Current surveillance programmes indicate a continuous decrease of individuals in the Mongolian wild camel population (*Camelus ferus*). Lately, hybridization has been considered to be a possible cause for fitness-reduction in wildlife populations. Hence, I analysed 39 camel hair samples, collected in the Great Gobi Strictly Protected Area “A”. In order to get further genetic information on the remaining wild camel population in Mongolia I sequenced an 804bp fragment of mitochondrial DNA (mtDNA). I obtained 29 sequences and included them in a data set of altogether 168 samples, consisting of wild and domestic Bactrian camels, for phylogenetic analysis. As a surprising result we found five domestic mtDNA sequences in the core zone of the protected area. As possible reasons we opposed the presence of real maternal hybrids to the occurrence of feral domestic camels. Either way, there is need for further conservation efforts in order to prevent both species from cross-breeding. An analysis of mitochondrial genetic diversity in the Mongolian wild camel population gave signs of a recent decline of population size and loss of genetic variability. Finally, the described method of mtDNA extraction and analysis has proven to be a useful tool for genetic monitoring and the rapid screening of a wild population for maternal hybridization with non-invasively collected hair samples.

Zusammenfassung

Laufende Beobachtungen weisen auf einen fortschreitenden Rückgang der höchst bedrohten Mongolischen Wildkamelpopulation *(Camelus ferus)* hin. In letzter Zeit wurde die Hybridisierung mit zweihöckrigen domestizierten Kamelen (*Camelus bactrianus*) als potentieller Faktor für eine Reduktion der Fitness von Wildpopulationen erkannt. Daher untersuchte ich 39 Kamelhaarproben aus dem Schutzgebiet „Great Gobi A“. Um nähere Informationen über die verbleibende Mongolische Wildkamelpopulation zu erhalten wurde ein 804bp Fragment des mitochondrialen Genoms sequenziert. In 29 Proben konnte das Fragment erfolgreich sequenziert werden. Diese wurden zusammen mit bereits vorhandenen Sequenzdaten des *C. ferus* und *C. bactrianus* (gesamt 168 Sequenzen) für phylogenetische Untersuchungen verwendet. Überraschenderweise waren fünf der aus dem Schutzgebiet „Great Gobi A“ stammenden Proben dem mitochondrialen Genotyp des domestizierten *C. bactrianus* zuzuordnen. Als mögliche Ursachen stellte ich das Vorhandensein maternaler Hybriden der Anwesenheit verwilderter Hauskamele gegenüber. Jedenfalls gibt es Bedarf für weitere Schutzmaßnahmen, um ein Kreuzen der zwei Spezies zu verhindern. Eine Analyse der genetischen Vielfalt in der Mongolischen Wildkamelpopulation ließ auf einen kürzlichen Rückgang der Populationsgröße und einen Verlust der genetischen Variabilität schließen. In dieser Arbeit konnten mitochondriale Marker als hilfreiches Werkzeug des genetischen Monitorings und des raschen Screenings nach maternaler Hybridisierung mithilfe von nicht invasiv gesammelten DNA-Proben angewendet werden.

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Appendix

Table A1. List of the new samples from the core zone of the GGSPAA from 11/2009

No.	Sample ID	Species inferred from mtDNA haplotype	Haplotype (mtDNA)
1	WC123	*C. bactrianus*	D3
2	WC124	*C. ferus*	W1
3	WC125	*C. ferus*	W2
4	WC126	*C. ferus*	W2
5	WC128	*C. ferus*	W3
6	WC129	*C. ferus*	W1
7	WC130	*C. ferus*	W2
8	WC131	*C. ferus*	W2
9	WC132	*C. ferus*	W1
10	WC133	*C. bactrianus*	D3
11	WC134	*C. ferus*	W1
12	WC135	*C. ferus*	W1
13	WC136	*C. ferus*	W3
14	WC137	*C. ferus*	W2
15	WC139	*C. ferus*	W2
16	WC142	*C. ferus*	W1
17	WC143	*C. ferus*	W3
18	WC145	*C. ferus*	W2
19	WC146	*C. ferus*	W2
20	WC147	*C. ferus*	W1
21	WC149	*C. ferus*	W2
22	WC150	*C. ferus*	W3
23	WC152	*C. ferus*	W2
24	WC153	*C. bactrianus*	D1
25	WC154	*C. bactrianus*	D1
26	WC155	*C. bactrianus*	D1
27	WC157	*C. ferus*	W1
28	WC158	*C. ferus*	W2
29	WC159	*C. ferus*	W1

W1-3: wild camel mtDNA haplotypes, **D1-3:** domestic Bactrian camel mtDNA haplotypes.

Table A2. Complete list of all samples considered for phylogenetic analysis

No.	Sample ID	Species inferred from mtDNA haplotype	Haplo-type	Collection place		Data source
1	cDC270	*C. bactrianus*	D3	China	Anji	C
2	cDC271	*C. bactrianus*	D4	China	Anji	C
3	cDC272	*C. bactrianus*	n/a	China	Anji	C
4	cDC273	*C. bactrianus*	D1	China	Anji	C
5	cDC274	*C. bactrianus*	D1	China	Anji	C
6	cDC275	*C. bactrianus*	D8	China	Anji	C
7	cDC276	*C. bactrianus*	D4	China	Anji	C
8	cDC278	*C. bactrianus*	D1	China	Anji	C
9	cDC279	*C. bactrianus*	D1	China	Anji	C
10	cDC280	*C. bactrianus*	D8	China	Anji	C
11	cDC282	*C. bactrianus*	D1	China	Anji	C
12	cDC283	*C. bactrianus*	D1	China	Anji	C
13	cDC285	*C. bactrianus*	n/a	China	Anji	C
14	cDC286	*C. bactrianus*	D8	China	Anji	C
15	cDC287	*C. bactrianus*	n/a	China	Anji	C
16	cDC288	*C. bactrianus*	D4	China	Anji	C
17	cDC289	*C. bactrianus*	n/a	China	Anji	C
18	D36	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
19	D43	*C. bactrianus*	D2	Austria	Herberstein	S
20	D44	*C. bactrianus*	D2	Austria	Herberstein	S
21	D53	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
22	D54	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
23	D56	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
24	D57	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
25	D58	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
26	D60	*C. bactrianus*	D3	Mongolia	Great Gobi A	S
27	D62	*C. bactrianus*	D2	Mongolia	Great Gobi A	S

No.	Sample ID	Species inferred from mtDNA haplotype	Haplo-type	Collection place		Data source
28	D64	*C. bactrianus*	D2	Mongolia	Great Gobi A	S
29	D65	*C. bactrianus*	D2	Mongolia	Great Gobi A	S
30	D66	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
31	D67	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
32	D68	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
33	D69	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
34	D70	*C. bactrianus*	D4	Mongolia	Great Gobi A	S
35	D71	*C. bactrianus*	D2	Mongolia	Great Gobi A	S
36	D72	*C. bactrianus*	D2	Mongolia	Great Gobi A	S
37	D73	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
38	D74	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
39	D75	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
40	D76	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
41	D77	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
42	D80	*C. bactrianus*	D2	Mongolia	Great Gobi A	S
43	D81	*C. bactrianus*	D2	Mongolia	Great Gobi A	S
44	D87	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
45	D92	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
46	D93	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
47	D94	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
48	D95	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
49	D96	*C. bactrianus*	D1	Mongolia	Great Gobi A	S
50	D98	*C. bactrianus*	D4	Mongolia	Great Gobi A	S
51	DC137	*C. bactrianus*	D7	Mongolia	n/a	S
52	DC145	*C. bactrianus*	D7	Mongolia	n/a	S
53	DC156	*C. bactrianus*	D1	Austria	n/a	S
54	DC157	*C. bactrianus*	D2	Mongolia	Great Gobi B	S
55	DC158	*C. bactrianus*	D1	Austria	n/a	S
56	DC164	*C. bactrianus*	D1	n/a	n/a	C
57	DC165	*C. bactrianus*	D1	n/a	n/a	C

No.	Sample ID	Species inferred from mtDNA haplotype	Haplo-type	Collection place		Data source
58	DC166	*C. bactrianus*	D2	n/a	n/a	C
59	DC167	*C. bactrianus*	D1	n/a	n/a	C
60	DC175	*C. bactrianus*	D2	Mongolia	Great Gobi B	C
61	DC176	*C. bactrianus*	D1	Mongolia	Great Gobi B	C
62	DC178	*C. bactrianus*	D1	Mongolia	Great Gobi B	C
63	DC180	*C. bactrianus*	D1	Mongolia	Great Gobi B	C
64	DC186	*C. bactrianus*	D1	Mongolia	Great Gobi B	C
65	DC188	*C. bactrianus*	D3	Mongolia	Great Gobi B	C
66	DC191	*C. bactrianus*	n/a	Mongolia	Great Gobi B	C
67	DC198	*C. bactrianus*	D1	Mongolia	Great Gobi B	C
68	DC199	*C. bactrianus*	D1	Mongolia	Great Gobi B	C
69	DC201	*C. bactrianus*	D1	Mongolia	Great Gobi B	C
70	DC203	*C. bactrianus*	n/a	Mongolia	Great Gobi B	C
71	DC204	*C. bactrianus*	D4	Mongolia	Great Gobi B	C
72	DC205	*C. bactrianus*	D1	Mongolia	Great Gobi B	C
73	DC210	*C. bactrianus*	D1	England	n/a	C
74	DC211	*C. bactrianus*	D4	England	n/a	C
75	DC212	*C. bactrianus*	n/a	England	n/a	C
76	DC214	*C. bactrianus*	D3	Austria	Wien	C
77	DC215	*C. bactrianus*	D1	Austria	Salzburg	C
78	DC244	*C. bactrianus*	D1	Russia	n/a	C
79	DC245	*C. bactrianus*	D1	Russia	n/a	C
80	DC246	*C. bactrianus*	D4	Russia	n/a	C
81	DC247	*C. bactrianus*	D1	Israel	Ramat Gan	C
82	DC248	*C. bactrianus*	D4	Israel	Ramat Gan	C
83	DC250	*C. bactrianus*	D4	Mongolia	West-Mongolia	C
84	DC251	*C. bactrianus*	D4	Mongolia	West-Mongolia	C
85	DC253	*C. bactrianus*	D4	Mongolia	West-Mongolia	C
86	DC254	*C. bactrianus*	D4	Mongolia	West-Mongolia	C
87	DC255	*C. bactrianus*	D4	Hungary	Budapest	C

No.	Sample ID	Species inferred from mtDNA haplotype	Haplo-type	Collection place		Data source
88	DC256	*C. bactrianus*	D4	Hungary	Budapest	C
89	DC257	*C. bactrianus*	D4	Hungary	Budapest	C
90	DC258	*C. bactrianus*	D3	Hungary	Budapest	C
91	DC259	*C. bactrianus*	D4	Hungary	Budapest	C
92	DC260	*C. bactrianus*	D4	Hungary	Budapest	C
93	DC261	*C. bactrianus*	D4	Hungary	Budapest	C
94	DC262	*C. bactrianus*	D4	Kazakhstan	Piervomaiski	C
95	DC263	*C. bactrianus*	D1	Kazakhstan	Piervomaiski	C
96	DC264	*C. bactrianus*	D1	Kazakhstan	Piervomaiski	C
97	DC265	*C. bactrianus*	D1	Kazakhstan	Piervomaiski	C
98	DC266	*C. bactrianus*	D1	Kazakhstan	Piervomaiski	C
99	DC267	*C. bactrianus*	D1	Kazakhstan	Piervomaiski	C
100	DC268	*C. bactrianus*	D1	Kazakhstan	Piervomaiski	C
101	DC290	*C. bactrianus*	D2	Kazakhstan	n/a	C
102	DC291	*C. bactrianus*	D4	Kazakhstan	n/a	C
103	DC293	*C. bactrianus*	D2	Kazakhstan	n/a	C
104	DC295	*C. bactrianus*	D4	Kazakhstan	n/a	C
105	DC296	*C. bactrianus*	D1	Kazakhstan	n/a	C
106	gi:157011955	*C. bactrianus*	D5	n/a	n/a	G
107	gi:157011972	*C. ferus*	W2	n/a	n/a	G
108	gi:223972289	*C. bactrianus*	D2	n/a	n/a	G
109	gi:1566159722	*C. bactrianus*	D5	n/a	n/a	G
110	gi:156615976	*C. ferus*	W2	n/a	n/a	G
111	gi:156615981	*C. bactrianus*	D1	n/a	n/a	G
112	gi:156615983	*C. bactrianus*	D6	n/a	n/a	G
113	gi:156615987	*C. ferus*	W1	n/a	n/a	G
114	gi:156615992	*C. ferus*	W1	n/a	n/a	G
115	H17	*C. ferus*	W1	Mongolia	Great Gobi A	S
116	W1	*C. ferus*	W1	Mongolia	Great Gobi A	S
117	W11	*C. ferus*	W2	Mongolia	Great Gobi A	S

No.	Sample ID	Species inferred from mtDNA haplotype	Haplo-type	Collection place		Data source
118	W13	*C. ferus*	W1	Mongolia	Great Gobi A	S
119	W14	*C. ferus*	W1	Mongolia	Great Gobi A	S
120	W19	*C. ferus*	W2	Mongolia	Great Gobi A	S
121	W20	*C. ferus*	W2	Mongolia	Great Gobi A	S
122	W21	*C. ferus*	W1	Mongolia	Great Gobi A	S
123	W22	*C. ferus*	W1	Mongolia	Great Gobi A	S
124	W4	*C. ferus*	W2	Mongolia	Great Gobi A	S
125	W49	*C. ferus*	W1	Mongolia	Great Gobi A	S
126	W5	*C. ferus*	W1	Mongolia	Great Gobi A	S
127	W50	*C. ferus*	W1	Mongolia	Great Gobi A	S
128	W51	*C. ferus*	W2	Mongolia	Great Gobi A	S
129	W52	*C. ferus*	W2	Mongolia	Great Gobi A	S
130	W6	*C. ferus*	W1	Mongolia	Great Gobi A	S
131	W7	*C. ferus*	W1	Mongolia	Great Gobi A	S
132	WC123	*C. bactrianus*	D3	Mongolia	Great Gobi A	D
133	WC124	*C. ferus*	W1	Mongolia	Great Gobi A	D
134	WC125	*C. ferus*	W2	Mongolia	Great Gobi A	D
135	WC126	*C. ferus*	W2	Mongolia	Great Gobi A	D
136	WC128	*C. ferus*	W3	Mongolia	Great Gobi A	D
137	WC129	*C. ferus*	W1	Mongolia	Great Gobi A	D
138	WC130	*C. ferus*	W2	Mongolia	Great Gobi A	D
139	WC131	*C. ferus*	W2	Mongolia	Great Gobi A	D
140	WC132	*C. ferus*	W1	Mongolia	Great Gobi A	D
141	WC133	*C. bactrianus*	D3	Mongolia	Great Gobi A	D
142	WC134	*C. ferus*	W1	Mongolia	Great Gobi A	D
143	WC135	*C. ferus*	W1	Mongolia	Great Gobi A	D
144	WC136	*C. ferus*	W3	Mongolia	Great Gobi A	D
145	WC137	*C. ferus*	W2	Mongolia	Great Gobi A	D
146	WC139	*C. ferus*	W2	Mongolia	Great Gobi A	D
147	WC142	*C. ferus*	W1	Mongolia	Great Gobi A	D

No.	Sample ID	Species inferred from mtDNA haplotype	Haplo-type	Collection place		Data source
148	WC143	*C. ferus*	W3	Mongolia	Great Gobi A	D
149	WC145	*C. ferus*	W2	Mongolia	Great Gobi A	D
150	WC146	*C. ferus*	W2	Mongolia	Great Gobi A	D
151	WC147	*C. ferus*	W1	Mongolia	Great Gobi A	D
152	WC149	*C. ferus*	W2	Mongolia	Great Gobi A	D
153	WC150	*C. ferus*	W3	Mongolia	Great Gobi A	D
154	WC152	*C. ferus*	W2	Mongolia	Great Gobi A	D
155	WC153	*C. bactrianus*	D1	Mongolia	Great Gobi A	D
156	WC154	*C. bactrianus*	D1	Mongolia	Great Gobi A	D
157	WC155	*C. bactrianus*	D1	Mongolia	Great Gobi A	D
158	WC157	*C. ferus*	W1	Mongolia	Great Gobi A	D
159	WC158	*C. ferus*	W2	Mongolia	Great Gobi A	D
160	WC159	*C. ferus*	W1	Mongolia	Great Gobi A	D
161	WC160	*C. ferus*	W1	Mongolia	Great Gobi A	C
162	WC162	*C. ferus*	W2	Mongolia	Great Gobi A	C
163	WC163	*C. ferus*	W2	Mongolia	Great Gobi A	C
164	WC164	*C. ferus*	W1	Mongolia	Great Gobi A	C
165	WC165	*C. ferus*	W2	Mongolia	Great Gobi A	C
166	WC169	*C. ferus*	W2	Mongolia	Great Gobi A	C
167	WC170	*C. ferus*	W2	Mongolia	Great Gobi A	C
168	WC171	*C. ferus*	W1	Mongolia	Great Gobi A	C

Grey shades: samples used to determine genetic variation in the Mongolian wild (n = 29) and Mongolian domestic (n = 51) camel population; **C:** Charruau, 2012; **D:** present Diploma study; **G:** GenBank; **S:** Silbermayr et al., 2010.

Table A3. Summary of primers used for amplification of the mtDNA fragment (SILBERMAYR et al., 2010)

Primer Name	Annealing temperature	Expected Length	Sequence	mtDNA Position
CB15060_CF-f	57.8 ℃	311 bp	CCTAGCACTTATCCCCATACTG	15060 - 15081
tPRO15371int_CF-r	54.7 ℃		TGAGTCTTAGGGAGAGTGTG	15371 - 15390
CB15279int_CF-f	58.9 ℃	348 bp	AAACCGCATCCTAAAATGAAGA	15279 - 15300
CR15627_CF-r	59.0 ℃		TGTGCTATGCACGAACAAGA	15627 - 15646
tPRO15402_CF-f	56.9 ℃	315 bp	CCAAAGCTGGAATTCTCATT	15402 - 15421
CR15716int_CF+CD-r	58.5 ℃		AGCGGGTTGATGATTTCAC	15716 - 15734
CR15641int_CF-f	58.3 ℃	394 bp	TTTCCAGTCAGTACGCATATC A	15641 - 15662
CR16034_CF-r	58.8 ℃		GGTTGTATGATGCGGGTAAATA	16034 -16055

Table A4. DNA-extraction protocol based on the Nucleospin® Tissue - Standard protocol (MARCHEREY-NAGEL, 2009)

Step	Procedure
Lyse sample (after hair-lysis)	- vortex the samples - add 200µl Buffer B3 - vortex vigorously - incubate at 70 ℃ for 10 min.
Adjust DNA binding conditions	- add 210µl cold* ethanol (96%) - vortex vigorously
Bind DNA	- place one Nucleospin® Tissue Column into a Collection Tube for each sample - apply the sample to the column - centrifuge for 1 min. at 11.000 x g - discard the flow-through
Wash silica membrane	<u>1st wash:</u> - add 500µl Buffer BW - centrifuge for 1 min. at 11.000 x g - discard flow-through <u>2nd wash:</u> - add 600µl Buffer B5 - centrifuge for 1 min. at 11.000 x g - discard flow-through
Dry silica membrane	- centrifuge column for 1 min. at 11.000 x g - residual ethanol removed
Elute highly pure DNA	- place Nucleospin® Tissue column into a 1.5ml tube - add 30µl prewarmed (70 ℃) MilliQ-H2O * - change the 1.5ml tube - add 30µl prewarmed (70 ℃) MilliQ-H2O *

*…Steps adjusted for extraction DNA of non-invasive samples.

Table A5. Single nucleotide sequence variations and amino-acid substitutions between *C. ferus* and *C. bactrianus*

No.	**Position**	**C. ferus**	**C. bactrianus**	**Amino-acid substitution**
1	15159	A	A/G	Thr>Ala
2	15179	G	A/T	Gly
3	15185	A	G	Pro
4	15193	C	C/A	Thr
5	15197	G	G/A	Val>Ile
6	15204	G	A	Iso>Val
7	15217	C	T	Val>Ala
8	15218	A	G	Val>Ala
9	15284	T/C	T	Arg
10	15314	T	C	Nc
11	15465	G	A	Nc
12	15472	T	C	Nc
13	15493	T/indel	indel	Nc
14	15505	G	G/A	Nc
15	15558	A	A/G	Nc
16	15607	T	T/C	Nc
17	15644	T/C	C	Nc
18	15650	G	A	Nc
19	15667	C	A	Nc
20	15668	C	C/A	Nc
21	15693	C	C/T	Nc
22	15710	C	C/A	Nc
23	15723	G	G/T	Nc
24	15780	T	C	Nc
25	15865	G	A	Nc
26	15881	C	A	Nc
27	15882	indel	T	Nc
28	15916	T	C/T	Nc
29	15920	A	A/T	Nc

Numbers 1-29: positions displayed in the median-joining network (s. figure 10); **Nc:** non-coding