

1.2 Mitochondrial DNA Variation among Dogs of Mongolian, Tuvian and Altaic Nomads

Daria Sanna¹, Ilenia Azzena^{1,2}, Piero Cossu², Fabio Scarpa¹, Massimo Scandura², Marco Apollonio², Francesca Lugli³, Paolo Francalacci⁴, Paolo Mereu¹, Marco Casu²

¹Dipartimento di Scienze Biomediche, University of Sassari, Viale San Pietro 43/b, 07100 Sassari, Italy, darsanna@uniss.it, fscarpa@uniss.it, pmereu@uniss.it

²Dipartimento di Medicina Veterinaria, University of Sassari, Via Vienna 2, 07100 Sassari, Italy, iazzena@uniss.it, picossu@uniss.it, scandura@uniss.it, marcoapo@uniss.it, marcasu@uniss.it

³Associazione Italiana di Etnoarcheologia, Via Principe Umberto 41, 00185 Roma, Italy, luglifrance@gmail.com

⁴Dipartimento di Scienze della Vita e dell'Ambiente, University of Cagliari, Via Ing. Tommaso Fiorelli 1, 09126 Cagliari, Italy, paolo.francalacci@unica.it

Corresponding author: Daria Sanna darsanna@uniss.it

Abstract

Dogs originated from the domestication of Eurasian grey wolves. From a genetic viewpoint, they can be grouped into two main clusters: the first is represented by several breeds obtained by artificial selection, whereas the second is of dogs that adapted to a human commensal lifestyle. Here we have provided a molecular survey aimed to infer on the genetic variability of dogs from nomadic camps in Mongolia, and the Republics of Tuva and Altai belonging to the Russian Federation. The results provided evidence of typical marks of expanding populations with multiple origins. Such a scenario could be the result of genetic exchanges among dogs from different camps, that were likely mediated by nomads.

Keywords: *Canis familiaris*, mtDNA, control region, genetic variability, Mongolia, Siberia.

1 Introduction

The dog (*Canis familiaris*) was the first domesticated species, which likely originated in the upper Paleolithic from the domestication of the Eurasian grey wolf (*Canis lupus*) (i.a. Shannon *et al.* 2015). From a genetic viewpoint, domestic dogs can be ranked into two main, highly divergent, groups: the first is represented by a large variety of pure breeds obtained by means of human-mediated artificial selection; the second encompasses large and strongly diversified groups of free-ranging animals adapted to a human commensal lifestyle (the so-called village dogs). Genetic data collected worldwide support a single geographical origin for domesticated dogs. In this context, the supposed first centre of domestication is located in Central Asia, as suggested by the highest levels of genetic variation that are generally reported in populations from this region.

Mitochondrial DNA (mtDNA) molecular markers were extensively used to infer on the phylogenetic relationships among canine populations distributed throughout the world. The mtDNA is a separate genome located inside cytoplasmic organelles (the mitochondria) in all eukaryotic cells (Anderson *et al.* 1981). It is a small circular molecule, which is present in multiple copies per cell and is inherited maternally. Savolainen *et al.* (1997) described the occurrence of two highly informative, hypervariable regions (HVS-I

and HVS-II) in the canine mtDNA. Pang *et al.* (2009) used these mtDNA regions to analyse 1,543 dogs spread across the Old World, evidencing the presence of six phylogenetic mitochondrial haplogroups (i.e. groups of similar sequences that share a common ancestor), named as clades A-F. Clades A, B and C occur at high frequencies in every canine population, suggesting the hypothesis of a possible common origin of these groups from a single domestication event. Conversely, the clades D, E and F showed a limited geographical dispersal and low frequencies of distribution.

The dogs of nomadic populations that live in areas near to the first centre of wolf domestication are generally poorly influenced by foreign gene flows and might show peculiar genetic traits that deserve to be investigated (Irion *et al.* 2005; Boyko *et al.* 2009; Pedersen *et al.* 2013; Shannon *et al.* 2015). In nomad camps, dogs are fundamental to protect livestock against wolves and predators. Therefore, nomads usually pick up dogs when they are puppies, preferring the bravest cubs with the strongest physical structures and peculiar morphological features, such as specific coat colours (Lugli 2016).

Current Mongolian and Siberian pastoralism can be considered the result of a multi-millenary process which started in prehistoric times. Current nomadism has had to face modernity and its market and social

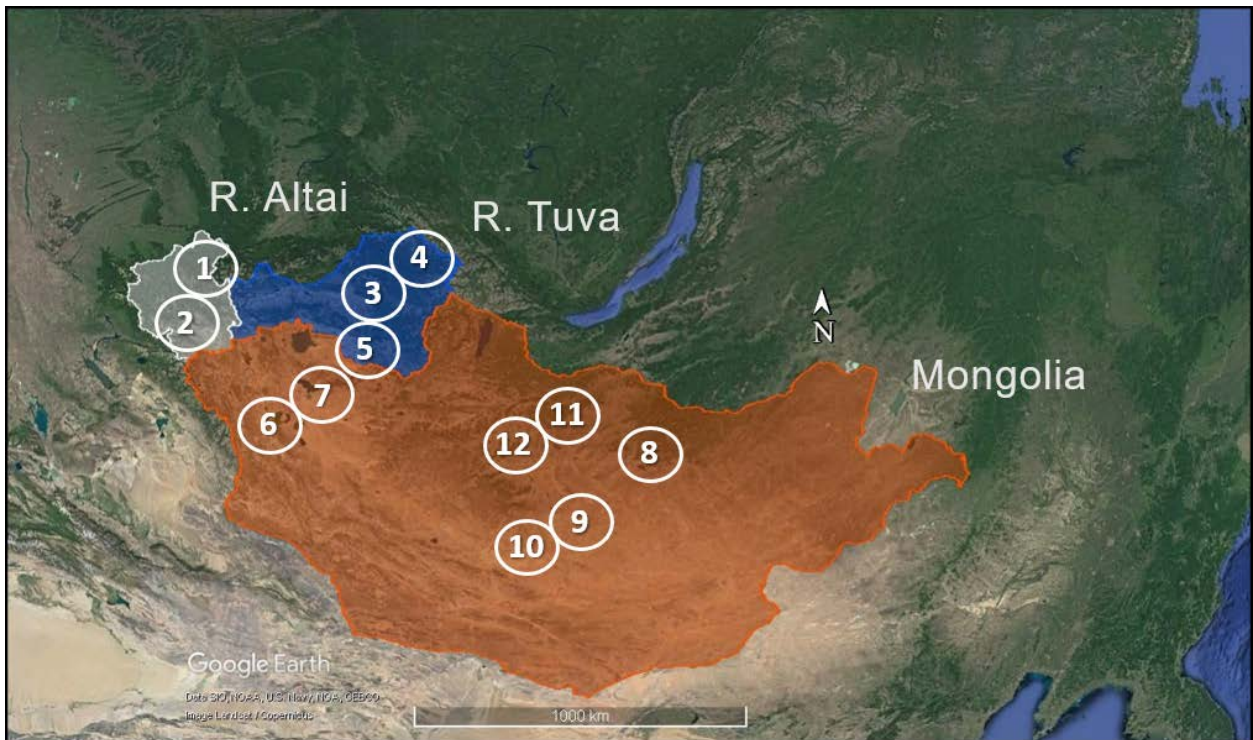


Figure 1. Sampling map showing the countries and the areas where dog hair samples were collected for DNA extraction. Arabic numbers inside white circles indicate the sites where sampling was performed. Republic of Altai:(1) Kurmach Baygol and (2) Kulada. Republic of Tuva: (3) Kyzyl, (4) Systig-Hem, Ador Kezhik, Toora Hem, (5) Erzin region. Mongolia: (6) Khovd aimag, (7) Zavkhan aimag, (8) Ulan Bator, (9) Delgerkhantai (Dundgovi aimag), (10) Övörkhantai aimag, (11) Mogod, Ulziit, Tal Bulag, Tsagaan Khust (Bulgan aimag), (12) Jargalant, Erdenet Mandal, UndurUlaan, BayyanUul (Arkhangai aimag).

models which push young people to abandon their traditional lifestyle.

In such a context, here we have provided a preliminary molecular survey, performed by means of the mitochondrial hypervariable region I (HVS-I) marker, aimed to infer for the first time on the genetic variability and the phylogeographic relationships among village dogs from nomadic camps of rural areas of Mongolia, and the Republics of Tuva and Altai (the Russian Federation).

2 Material and methods

In 2005 the Italian Association for Ethnoarchaeology (AIE) with the sponsorship of the Ministry of Foreign Affairs and International Cooperation – Italy (MFA) started the research project ‘The camps of Mongolian nomads: an ethnoarchaeological perspective’, which aimed to study the various crucial points of steppe pastoralism in different Mongolian ecosystems.¹

¹ The project was conducted in collaboration with Tserenkhand (2002–2006) (National University of Ulaanbaatar; Academy of Sciences) and Dulam Sedenjav (after 2007) (National University of Ulaanbaatar). The missions were conducted by F. Lugli and G. Capitini and students and graduated of the National University. The research was conducted in various regions to register differences and variabilities.

This project also included research on dogs, which started in Mongolia in 2012. Consequently, the Italian Association for Ethnoarchaeology (AIE) with the sponsorship of Ministry of Foreign Affairs and International Cooperation – Italy MFA, started the mission ‘Siberian nomads and their dogs’ in 2013, which was conducted in the Republics of Tuva (2013, 2017), and Altai (2014, 2016) (the Russian Federation), and in the Kemerovo region (2015) in collaboration with the Novosibirsk State Conservatory, the Institute of Philology (SB RAS, Novosibirsk), the Institute for Humanities and Kyzyl College of Arts (RT), and the Institute of Altaistics (RA).²

The Mongolian and Siberian projects aimed to study and document the relationship between nomads, hunters and dogs in various socio-historical contexts and different ecosystems. Villages and camps of various regions were visited in order to document traditional situations. Hair samples from dogs owned by the families that were studied and interviewed were taken during the missions both in Mongolia and in Siberia.

² The project was conducted in Tuva (2013, 2017), Republic of Altai (2014, 2016), and Kemerovo region (2015) by F. Lugli and G. Sychenko (see Lugli and Sychenko in this volume) in collaboration with Novosibirsk State Conservatory, Institute of Philology (SB RAS, Novosibirsk), Institute for Humanities and Kyzyl College of Arts (RT), Institute of Altaistics (RA).

Table 1. Estimates of genetic diversity obtained for the mitochondrial HVS-I fragment of dog populations analysed here. n: sample size; S: number of polymorphic sites; H: number of haplotypes; h: haplotype diversity; and π : nucleotide diversity.

Country	Sampling date	n	S	H	\bar{h}	π
Mongolia	Total	33	16	14	0.888	0.01328
	Nov. 2013	14	15	9	0.923	0.01279
	Oct. 2014	14	9	6	0.813	0.01137
Republic of Tuva	Oct. 2013	23	18	12	0.917	0.01094
Republic of Altai	Total	23	11	11	0.806	0.01118
	Oct. 2016	15	8	6	0.648	0.00832
	Jul. 2014	8	10	7	0.964	0.01252
Total		79	24	24	0.908	0.01276



Figure 2. Some of the individuals, from Mongolia, the Republic of Altai and the Republic of Tuva, whose hairs were collected to perform non-invasive DNA extractions in the present study (Photos by F. Lugli).

There was usually a preference to take the samples in traditional and isolated contexts. A few samples were also taken from a dog farm in Ulaanbaatar in Mongolia in order to analyse the Mongol Bankhar mastiffs.

The molecular analysis of a 348 base pairs-long HVS-I mitochondrial fragment was performed on 79 dogs

from seven sites in Mongolia (33 individuals), and five sites from two states of the Russian Federation, being Republic of Tuva (23 individuals from two sites) and Republic of Altai (23 individuals from three sites) (Figure 1 and Table 1 for details). The individuals analysed included not only non-breed dogs, but also representatives of three canine breeds (Laika, Mongol

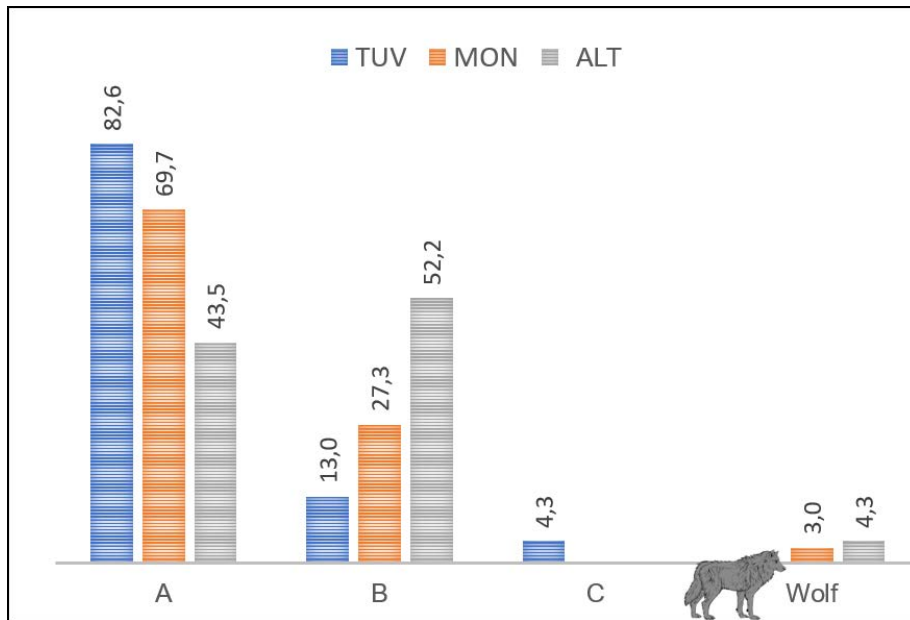


Figure 3. Distribution frequencies of the canine mitochondrial HVS-I haplogroups found for the dogs analysed here. MON: Mongolia; ALT: Republic of Altai; TUV: Republic of Tuva.

Bankhar, and Volkodav) (see Figure 2). Hair samples were collected from individuals with the help of their owners during 2013, 2014, and 2016. Genomic DNA was extracted from hairs by means of the InstaGene™ Matrix (Bio-Rad) according to the manufacturer's protocol. Sample quality and DNA concentration were determined via spectrophotometry using a ND-8000 (NanoDrop Technologies, Thermo Fisher Scientific Inc., Wilmington, DE). PCR was carried out using modified mtDNA HVS-I universal primers (Kocher *et al.* 1989) and sequencing was performed by an external service (Macrogen, The Netherlands). Newly generated sequences were aligned using the BioEdit 7.2.5. software package (Hall, 1999). The genetic variation was assessed estimating the number of polymorphic sites, the number of haplotypes (H), the haplotype diversity (h), and the nucleotide diversity (π) using the software package DnaSP 6.12 (Librado *et al.* Rozas, 2009). Genetic relationships among haplotypes were investigated by a Median-Joining network using the software package Network 10.2.0.0 (www.fluxus-engineering.com).

3 Results and discussion

High levels of genetic variability, resulting in a total of 24 haplotypes (*i.e.* nucleotide sequences corresponding to the same DNA fragment that differ among each other in some informative nucleotide sites), were found at each sampling location (see Table 1 for details). The lowest levels of genetic variation were found in the camps from the North of the Republic of Altai (Turochaksky district). In the present study, the two main worldwide distributed canine mitochondrial haplogroups (A, B) showed distributions of frequencies (Figure 3) that are



Figure 4. Images of the Laika-like individuals whose mitochondrial HVS-I sequence was also found in Siberian wolves. (a) a 3-year-old male from Mongolia; (b) a 1-year-old female from the Republic of Altai (Photos by F. Lugli).

consistent with those generally retrieved for almost all dog populations from the Old World (Savolainen *et al.* 2002; Ardalan *et al.* 2011; and references therein), with the haplogroup A showing the highest frequencies. Dogs from the Republic of Altai were the only exception as they showed the highest frequencies of distribution for the haplogroup B, in accordance with a similar trend already reported for Southwest Asian dogs (Ardalan *et al.* 2011). We hypothesise this latter finding may be a consequence of genetic drift mediated by human artificial selection. This evolutionary force may have acted on the Altaic population with repeated introductions of dogs carrying less common haplogroups just by chance. The haplogroup C was found only in dogs from the Republic of Tuva, and the genetic drift may be invoked in this case as well. As briefly outlined above, genetic drift is a stochastic evolutionary force whose strength depends on the population size. When a new population originates from a very small number of individuals (the so-called founders), genetic drift may trigger the loss of genetic variation changing the frequencies of haplotypes. As a consequence, the distribution of haplogroups in the new established populations may diverge from those reported for the original population.

It is noteworthy that a mitochondrial lineage, that is present in Siberian wolves, was found in two morphologically Laika-like individuals, one from Mongolia (a 3-year old male from the district of Bulgan) and one from the Republic of Altai (a 1-year-old female from the district of Kurmach Baygol) (Figure 4). Such a finding may be the result of past accidental domestications of wolf females or cubs in nomad camps. Indeed, although nomads usually prefer to select dogs directly from their canine families, they do not always follow this choice criterion and puppies can also be rescued from stray mothers or lost adults can be adopted (Lugli 2016).

However, it should be taken into account that the uncommon mitochondrial lineage found in these two individuals may also belong to the mitochondrial canine clade D, whose distribution is restricted to North Europe, Siberia, Southwest Asia and the Mediterranean Sea (Angleby and Savolainen 2005; Pang *et al.* 2009). Some sub-haplogroups of this clade are the products of a dog-wolf cross-breeding, rather than of independent domestication of wolves (Ardalan *et al.* 2011).

The network analysis (see Figure 5a and its legend for more details) evidenced that many sequences were exclusively found in single individuals, probably due to very recent multiple introductions of new dogs. The occurrence of star-like configurations in the plot suggests the lack of genetic divergence among areas, along with the occurrence of many founder effects.

Indeed, here the network star-like configurations are represented by a common central haplotype, usually shared among individuals from many regions, that is surrounded by many lesser-frequent (and private to single individuals) haplotypes differing by a few mutations. The most common haplotypes in the network likely correspond to sequences belonging to the first dogs introduced in the nomads' camps, which had the opportunity to breed extensively. Such findings are consistent with the general trend of genetic homogeneity worldwide reported for dog populations (*i.a.* Pang *et al.* 2009).

A less frequent and highly divergent haplotype (Figure 5b) was found exclusively among dogs from a Mongol Bankhar breeding farm in Mongolia: we hypothesised the occurrence of a mtDNA matrilinear relationship among all individuals born in the farm, which likely descend from a group of related females.

The network analysis also evidenced that two Volkodav dogs from the Republic of Tuva show private-owned haplotypes (not shared with the other breeds). This finding suggests that the genetic divergence reported for these dogs is likely consistent with the different history and geographic origin of their breed.

4 Conclusions

The study of the genetic makeup of village dogs and central Asian local breeds represents an important step to depict the complex evolutionary history of these animals (Shannon *et al.* 2015).

In such a context, we have reported the first and preliminary molecular inference on dogs from the mountains of Mongolia and from the Russian Republics of Altai and Tuva. The results pointed out high levels of genetic divergence at each sampling site, and a lack of geographic differentiation among regions. Our findings reflect the typical marks of expanding populations with multiple origins. We hypothesise that such a scenario could be the result of repeated genetic exchanges among dogs from different nomads' camps, which were likely mediated by human activities. Indeed, Mongolian nomads usually move to villages to pick their dogs (Lugli 2016). Accordingly, the haplotype distribution frequencies and the founder effects evidenced by the network analysis, further account for the signature of artificial selection, which drastically skewed the genetic diversity within village dogs and local breeds such as the Mongol Bankhar mastiff. Within this framework, it should also be considered that the mitochondrial genetic variability reported for village dogs from nomadic camps may be sex-biased because of the maternal inheritance of the mitochondrial molecular marker here used. In the present study,

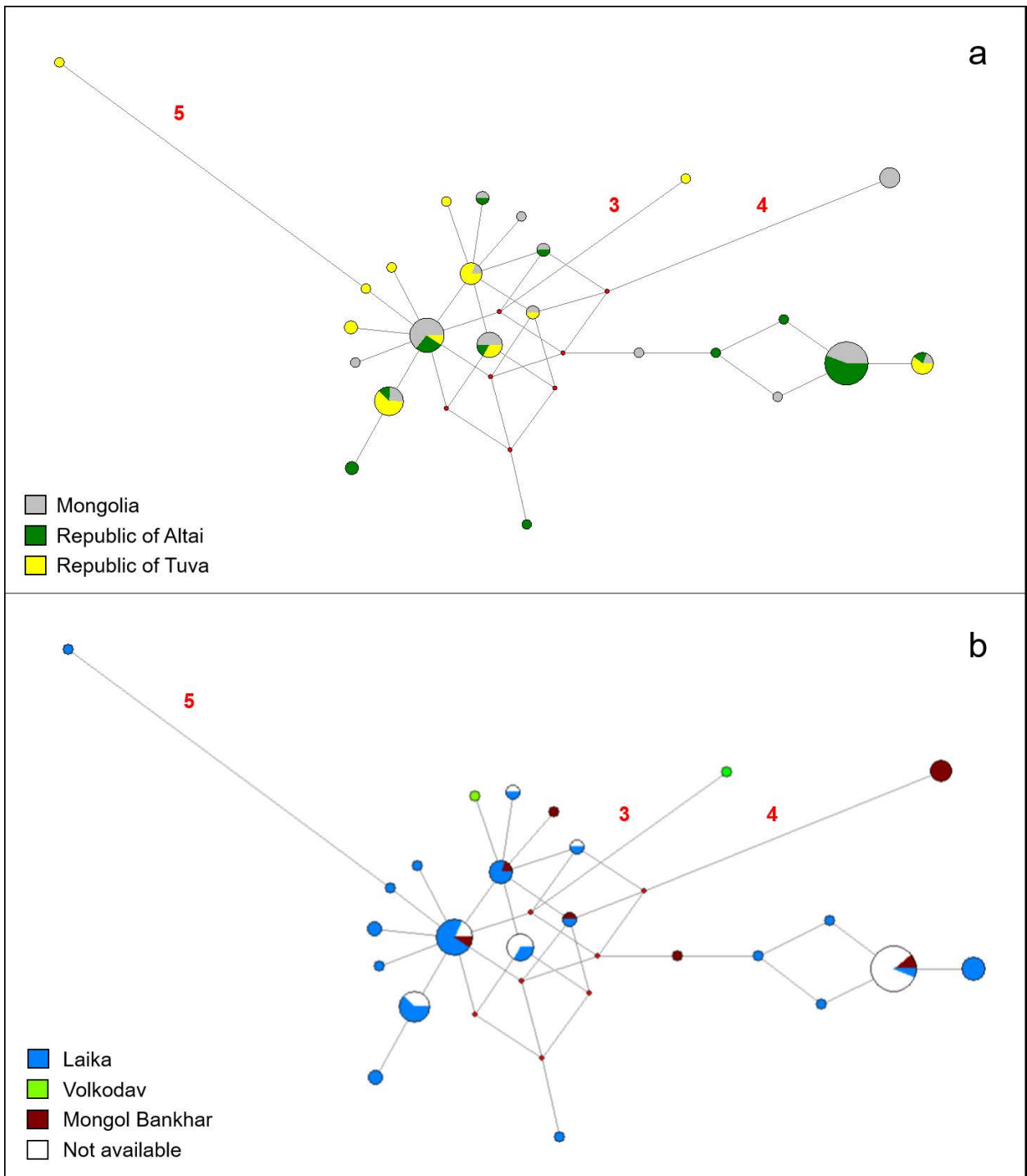


Figure 5. Median-Joining networks showing the phylogenetic relationships occurring among the mitochondrial haplotypes found in the present study. Haplotypes are represented by the circular spots on the graphic; the diameter of spots is proportional to the number of individuals that share the haplotype; the length of branches in the graphic are proportional to the number of nucleotide mutations occurring between the two haplotypes at the edges of the branch. The spots are coloured according to the characteristics of the individuals that share the haplotype. Little red spots in the graphic represent the median vectors that are crucial sequences likely existing in nature but not found among the specimens collected for the present study. In the present graphic all haplotypes diverge from each other for a single mutation except for the cases where Arabic red numbers on network branches indicate the occurrence of a higher number of point mutations. (a) the spots on the network are coloured according to the geographic origin of dogs; (b) the spots on the network are coloured according to the breed of dogs. Please note that it was not possible to identify the breed for all individuals.

the general trend of genetic homogeneity evidenced among areas may be the result of the sex-based choice criterion that nomads apply to pick their dogs. For example, 99% of Mongolian nomads' families decide to only have male dogs because they consider females too difficult to manage during their oestrus cycle (see Lugli 2016 for more details). As a consequence, the common ancestors of the dogs considered in the present study could be represented by a reduced number of females that were likely used as breeders. This choice might have decreased the effective population size (*i.e.* the number of mating individuals that contribute to the genetic pool of the next generation) and increased the genetic homogeneity evidenced by the mitochondrial DNA, which is matrilineally transmitted to the offspring.

The main mitochondrial haplogroups found in the present study among dog populations suggest a recent origin, common to other European canine populations.

In the future, the analysis of a larger number of individuals from further Mongolian, Altaic and Tuvian sites, will shed further light on the evolutionary processes that might have shaped the genetic patterns of dog populations living in these Asiatic regions.

References

- Anderson, S., A.T. Bankler, B.G. Barrell, M.H.L. de Bruijn, A.R. Coulson, J. Drouin, I.C. Eperon, D.P. Nierlich, B.A. Roe, F. Sanger, P.H. Schreier, A.J.H. Smith, R. Staden and I.G. Young 1981. Sequence and organization of the human mitochondrial genome *Nature* 290: 457–464.
- Angleby, H. and P. Savolainen 2005. Forensic informativity of domestic dog mtDNA control region sequences. *Forensic Science International* 154(2–3): 99–110.
- Ardalan, A., C.F. Kluetsch, A.B. Zhang, M. Erdogan, M. Uhlén, M. Houshmand, C. Tepeli, S.Z. Miraei Ashtiani and P. Savolainen 2011. Comprehensive study of mtDNA among Southwest Asian dogs contradicts independent domestication of wolf, but implies dog-wolf hybridization. *Ecology and Evolution* 1(3): 373–385.
- Boyko, A.R., R.H. Boyko, C.M. Boyko, H.G. Parker, M. Castelano, L. Corey, J.D. Degenhardt, A. Auton, M. Hedimbi, R. Kityo, E.A. Ostrander, J. Schoenebeck, R.J. Todhunter, P. Jones, and C.D. Bustamante 2009. Complex population structure in African village dogs and its implications for inferring dog domestication history. *Proceedings of the National Academy of Sciences* 106(33): 13903–13908.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Irion, D.N., A.L. Schaffer, S. Grant, A. N. Wilton and N.C. Pedersen, 2005. Genetic variation analysis of the Bali street dog using microsatellites. *BMC Genetics* 6: 6.
- Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Pääbo, F. Villablanca and A.C. Wilson 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the USA* 86: 6196 – 6200.
- Librado, P. and J. Rozas 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451–1452.
- Lugli, F. 2016. Mongolian nomads and their dogs, in S. Biagetti and F. Lugli (eds) *The Intangible Elements of Culture in Ethnoarchaeological Research*: 125–139. Springer: Cambridge.
- Pang, J-F., C. Kluetsch, X-J. Zou, A-B., Zhang, L.Y. Luo, H. Angleby, A. Ardalan, A., C. Ekström, A. Sköllermo, J. Lundeberg, S. Matsumura, T. Leitner, Y.-P. Zhang and P. Savolainen 2009. mtDNA data indicate a single origin for dogs south of Yangtze River, less than 16,300 years ago, from numerous wolves. *Molecular Biology and Evolution* 26(12): 2849–2864.
- Pedersen, N., H. Liu, G. Theilen and B. Sacks 2013. The effects of dog breed development on genetic diversity and the relative influences of performance and conformation breeding. *Journal of Animal Breeding and Genetics* 130(3): 236–248.
- Savolainen, P., B. Rosen, A. Holmberg, T. Leitner, M. Uhlen and J. Lundeberg 1997. Sequence analysis of domestic dog mitochondrial DNA for forensic use. *Journal of Forensic Science* 42(4): 593–600.
- Savolainen, P., Y.P. Zhang, J. Luo, J. Lundeberg and T. Leitner 2002. Genetic evidence for an East Asian origin of domestic dogs. *Science* 298(5598): 1610–1613.
- Shannon. L.M., R.H. Boyko, M. Castelano, E. Corey, J.J. Hayward, C. McLean, M.E. White, M. Abi Said, B.A. Anita, N.I. Bondjengo, J. Calero, A. Galov, M. Hedimbi, B. Imam, R. Khalap, D. Lally, A. Masta, K.C. Oliveira, L. Pérez, J. Randall, N.M. Tam, F.J. Trujillo-Cornejo, C. Valeriano, N.B. Sutter, R.J. Todhunter, C.D. Bustamante and A.R. Boyko 2015. Dog genetic diversity reveals Central Asian origin. *Proceedings of the National Academy of Sciences* 112.44: 13639–13644.