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Evolutionary history of African wolf

BACHELOR'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled Evolutionary history of African wolf independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 14. 4. 2023

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Juliana Walterová

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Abstract

This thesis aimed to provide a comprehensive review of taxonomy, geographical range, historical gene flow, population genetics, hybridization, and conflicts of the African wolf (*Canis lupaster*) through literary research of scientific articles.

The African wolf is a unique species genetically distinct from the Golden jackal (*C. aureus*) and the Grey wolf (*C. lupus*). In the literature before 2011, it was considered a subspecies of the Golden jackal thanks to their phenotypic similarity. Speciation started during the Middle Pleistocene when dry and cold glacial periods alternated with more humid interglacial periods. During interglacial periods, Sahara was full of grass, like savannah, and enabled the migration and expansion of many species like the African wolf. Its population differentiated into genetically different Northwestern and Eastern populations between 30 and 16 thousand years ago. The Northwestern population experienced ancient introgression from the Grey wolf and Golden jackal; on the other side of the Sahara is the East population with introgression from the currently endangered Ethiopian wolf (*C. simensis*).

Evidence of the recent hybridization of the African wolf with domestic dogs have recently been discovered in Egypt, Senegal, and Ethiopia. This hybridization is considered as a threat to the species through genetic degradation and pathogen transmission. African wolves however face other threats like habitat degradation through anthropogenic changes of the environment and conflict with humans. Farmers are often persecuting African wolves for killing their livestock and these incidents are not regulated in Africa. Conservation and management of African wolves will be necessary, ideally with the education of farmers on how to protect livestock better against the African wolf.

The African wolf might potentially compete with the endangered Ethiopian wolf because their ecological niches overlap. They both feed on rodents, so competition for sources is possible in the future.

Key words: *Canis lupaster*, hybridization, phylogeny, taxonomy, gene flow

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1. Introduction

The existence of the African wolf (*Canis lupaster*; Fig. 1.) was noted by several authors in 19. century (Ferguson 1981) but remained unrecognised by the general scientific public. It was considered a subspecies of the Golden jackal (*Canis aureus*), although Ferguson (1981) proved the distinctiveness by morphological characters and also using biological, ethological, and geographical evidence. Rueness et al. (2011) finally highlighted this oversight of the African wolf through genetic evidence. After this important genetic discovery, several authors started with more detailed research of genetics, detection of hybridization, population biology and ecology, distribution, etc.

The population status of African wolf is, according to IUCN nowadays, marked as least concerned with decreasing population trends. The current range spreads across Northwest and East Africa (The IUCN 2019). Currently, there are few scientific articles about its range, and we are missing complex data, so data from IUCN might be inaccurate. Well-defined taxonomy and phylogenetic classification are essential for conservation and management and, thus, for the future existence of the African wolf species. In Africa, the number of endangered species is increasing. Without proper research, the African wolf population will be decreasing faster through loss of suitable habitats, fragmentation of habitat, recent hybridization with species from Canidae or poaching.



Fig. 1. The African wolf (*C. lupaster*; vanHoogstraten 2023).

2. Aims of the Thesis

The African wolf (*Canis lupaster*) used to be known as a subspecies of the Golden jackal (*Canis aureus*) until scientists discovered its genetic affinity with the genus *Canis* and probably a hybrid origin of the species. The main goals of the thesis were to study the literature and provide a comprehensive review of the evolutionary history of the African wolf because of its interesting origin and evolution. The comprehensive review concerned taxonomy, population history and genetics, and human-wildlife conflicts. I have done a practical analysis of available nucleotide sequences from NCBI GenBank. This topic is important because up to now, the synthesis of previously published research does not exist, although the African wolf is a common species in Africa and is often poached by humans because of livestock protection.

3. Literature Review

3.1. Taxonomy classification of African wolf

The name and taxonomic classification of the African wolf (*Canis lupaster* Hemprich and Ehrenberg, 1832) is not uniform and it has been developed in recent years. First, it was recognized as the Egyptian jackal (*Canis aureus lupaster* Schwarz, 1926), a subspecies of the Golden jackal (*Canis aureus* Linneaus, 1758) seemingly living only in Egypt. Later, it was differentiated from the Golden jackal and reclassified as a novel species to the Canidae family. In literature, we can find names in Latin like *Canis anthus* Cuvier, 1820 (Koepfli et al. 2015; Eddine et al. 2017; Karssene et al. 2017; Yirga et al. 2017; Karssene et al. 2018) or *Canis mengesi* Noack, 1897 (Krofel et al. 2022) which was translated as African golden wolf (Koepfli et al. 2015; Eddine et al. 2017; Karssene et al. 2017; Yirga et al. 2017; Gopalakrishnan et al. 2018; Karssene et al. 2018; Sarabia et al. 2021). “Golden“ still refers to the past relation with the Golden jackal and because of its phenotype. This name, Mallil et al. (2020) claimed to be misleading; African wolf’s phenotype does not have to be “golden-like“ at all. In this study, I use the simple name African wolf (*Canis lupaster*) as sufficient, like authors from recent publications (Atickem et al. 2017; Viranta et al. 2017, Mallil et al. 2020, etc.).

Differences between the African wolf and the Golden jackal are not easy to spot quickly, as shown in Fig. 2. The African wolf is larger and has longer limbs than Golden jackal (Rueness et al. 2011). There are also differences in cranial metrics and dentice (Viranta et al. 2017). Gaubert et al. (2012) claimed that the African wolf is bigger, has a more heavily built body, ears are bigger and more rounded, the head is wider, and the tail is shorter than the Golden jackal’s. The colour and structure of the coat are also different; the African wolf has darker and longer hair and wider white breast colouring. Machado et al. (2020) observed that the morphology of the African wolf is variable even more than of the Grey wolf (species that lives on three continents).



Fig. 2. Phenotypic variation of the African wolf in Senegal. From A, it is jackal-like, to E, which is very wolf-like. Figure from Gaubert et al. (2012).

Both African wolves and Golden jackals behave differently. Jackals are scavengers feeding on small animals, but African wolves are able to hunt bigger animals like gazelles or livestock (Ferguson et al. 1981). Shepherds in Senegal observed that wolves in packs could hunt bigger prey than jackals, although they preferentially live solitary (Gaubert et al. 2012). African wolves in Ethiopia were mainly active during dawn and morning, and dusk and evening; for the rest of the day, they were resting (Gutema et al. 2022). Scientists speculate that this activity range evolved most likely to avoid competition with bigger carnivores.

Although both African wolves and Golden jackals were recognized as distinct recently, they actually diverged from each other 0.5-1.5 million years ago (Koepfli et al. 2015; Rueness et al. 2015; Krofel et al. 2022).

According to the IUCN (2018), the range of Golden jackals is only in southern Europe, the Middle East, and South Asia. However we know that Golden jackals also live in northern Europe in Germany, the Czech Republic, Poland or Estonia (see the comparison in Fig. 3.; Couto Reis 2015). It is nevertheless strange that the Golden jackal disappeared entirely from big continent like Africa. Some scientists recently wrote about jackals in Africa (Gaubert et al. 2012; Kebede 2017; Temu et al. 2018), but there is no genetic evidence that they are describing the Golden jackal, not the African wolf. Therefore it would be better to do research on Golden jackals in places in Africa.

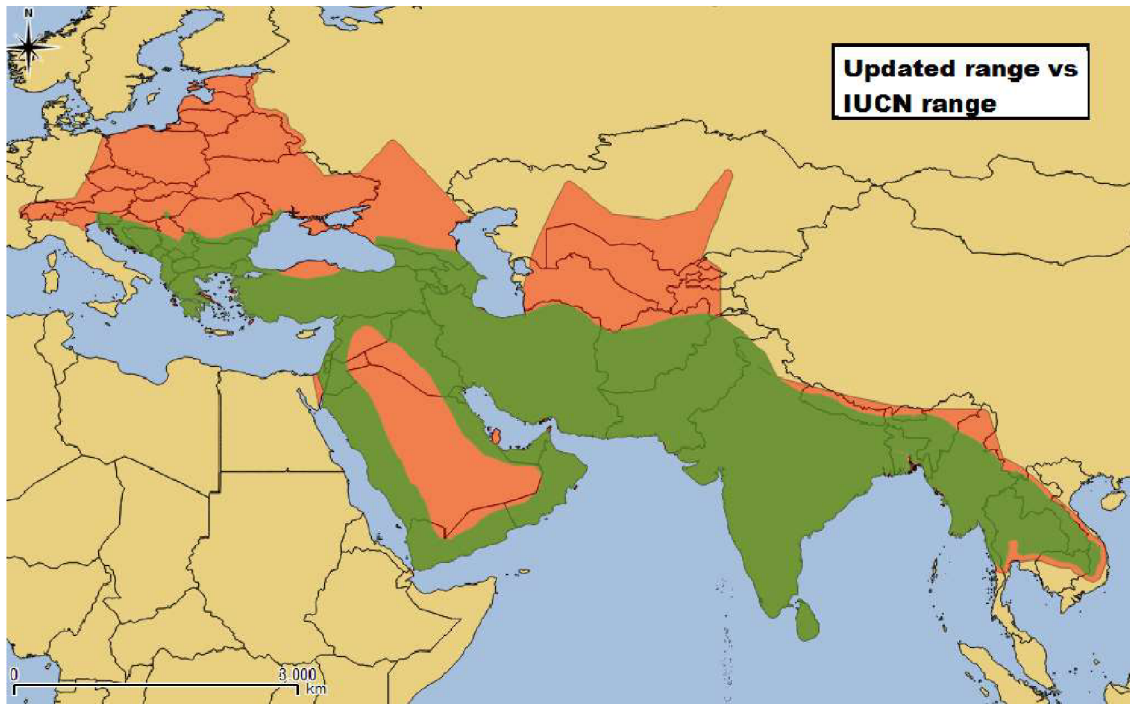


Fig. 3. Geographic range of Eurasian Golden jackal. Green is according to The IUCN Red List of Threatened Species (2018), red is updated global range according to Couto Reis (2015). Figure modified from Couto Reis 2015.

3.2. Distribution of African wolf

According to The IUCN Red List of Threatened Species (2019), the African wolf lives in North, Northwest, and East Africa (see Fig. 4.). It is the original territory of the Golden jackal, from whom the African part was assigned to the African wolf (Gopalakrishnan et al. 2018). But this range is not entirely reliable and is not sufficiently studied by enough scientific research. The bibliography of the IUCN site includes works almost twenty years old or studying only Golden jackal species.



Fig. 4. Distribution of the African wolf according to the IUCN (in orange colour) and according to scientific articles (black stars). Figure modified from The IUCN Red List of Threatened Species (2019).

In 2023, the presence of African wolves was recorded at a few places around the Sahara desert in Africa. It is mostly divided into two geographical locations, the first called the Northwestern clade and the second called the Eastern clade (Gopalakrishnan et al. 2018).

Northwestern populations were sampled in Egypt (Rueness et al. 2011; Gaubert et al. 2012; Viranta et al. 2017; Gopalakrishnan et al. 2018; Sarabia et al. 2021), Tunisia (Karssene et al. 2017; Karssene et al. 2018; Eddine et al. 2020), Algeria (Gaubert et al.

2012; Koepfli et al. 2015; Viranta et al. 2017; Gopalakrishnan et al. 2018; Eddine et al. 2020; Mallil et al. 2020; Sarabia et al. 2021), Senegal (Gaubert et al. 2012; Viranta et al. 2017; Gopalakrishnan et al. 2018; Mallil et al. 2020; Sarabia et al. 2021), Morocco (Koepfli et al. 2015; Viranta et al. 2017; Gopalakrishnan et al. 2018; Sarabia et al. 2021), Western Sahara (Viranta et al. 2017), Mauritania (Koepfli et al. 2015; Viranta et al. 2017) and Mali (Gaubert et al. 2012; Viranta et al. 2017; Mallil et al. 2020).

Eastern populations were sampled in Ethiopia (Rueness et al. 2011; Atickem et al. 2017; Viranta et al. 2017; Yirga et al. 2017; Gopalakrishnan et al. 2018; Mallil et al. 2020; Sarabia et al. 2021; Gutema et al. 2022), South Sudan (Viranta et al. 2017), Kenya (Koepfli et al. 2015; Viranta et al. 2017; Sarabia et al. 2021) and Tanzania (Krofel et al. 2022).

Contrary to the IUCN (2019) report, recent studies do not show any presence of the African wolf in Burkina Faso, Cameroon, Central African Republic, Chad, Djibouti, Eritrea, Guinea, Niger, Nigeria, Somalia, or The United Republic of Congo. Other researches focusing on these localities are needed to confirm the information of the residence of *C. lupaster*.

These two geographical areas are also different in the genetic information of wolf populations living there. Gopalakrishnan et al. (2018) were trying to explain this through gene flow between geographically close canid species. According to them, the Northwestern population admixed with Grey wolves (*C. lupus* Linnaeus, 1758) and Golden jackals. The Eastern population admixed with Ethiopian wolves (*C. simensis* Rüppell, 1840).

Differences between populations do not stop here. There is also some level of genetic difference between Senegalese and Algerian wolves, which might be caused by a long distance of around 4,000 km, as hypothesised by Mallil et al. (2020).

The African wolf has a wide distribution, it lives in many habitats, from green grassland and forest, arid and semi-arid landscapes to areas with a high elevation, and it also can live in zones affected by humans (see references in Sarabia et al. 2021). Yirga et al. (2017) observed a high density of African wolves near anthropogenic areas with available waste compared to bushy areas in Ethiopia. Gutema et al. (2022) recorded using grassland and farmland at night and bushland during the day in Ethiopia. Gaubert et al. (2012) showed a photo of an African wolf occurring in a sandy savannah with shrubs and

in a green agricultural landscape. According to Karssene et al. (2018), African wolves were observed in varied habitats, from desert areas to mountain forests in Tunisia. It is, therefore, evident that this flexible carnivore can survive very well in various habitats.

Thanks to Gutema et al. (2022), there is a lot of information about the ecology of African wolves in protected areas in the Ethiopian Highlands. The home range there varies from 8 km² to 40 km². They appear close to human settlements, unlike Ethiopian wolves, and they reside in anthropogenic areas in grassland. This closeness to humans was explained by using human remains like carcasses from rodents or livestock, and human waste. African wolves are generalist predators (Sarabia et al. 2021), important for the ecosystem because they hunt pest rodents.

African wolves in Ethiopia were found near human settlements (Yirga et al. 2017; Gutema et al. 2022), which the availability of human waste and livestock like sheep and goats as easy prey can explain. Living near human settlements is associated with the consumption of organic and inorganic human waste (plastic bags and aluminium foils were found in faeces, see Belbel et al. 2022). African wolves might prefer to eat human waste before small mammals because of energy savings for other vital functions like reproduction.

The sampling is insufficient and therefore is unclear where all African populations are distributed. More sampling and monitoring will be necessary to better understand the African wolf's distribution, home range, and gene flow.

3.3. Geographical history

According to Sarabia et al. (2021), the speciation of the African wolf started around 1.44 million years ago during the increased aridity in the Sahara. According to Gaubert et al. (2012), the African wolf has occurred on the African continent since the Middle to Late Pleistocene. This information is supported by paleontological records; Geraads (2011) claimed that *C. aureus* (and with him also *C. lupaster*) appeared in Northwestern Africa during the Middle Pleistocene. Koepfli et al. (2015) claimed that, based on their findings, they determined the time of separation from the Grey wolf at 1.3 million years ago and even 1.9 million years ago for separation from the Golden jackal.

Together they agree on the time of speciation around the Middle Pleistocene, which began at 1.25 million years ago and ended at 0.7 million years ago (Clark et al. 2006).

The Pleistocene is characterised by its alternation of glacial and interglacial periods. Glacial periods were very dry and they caused shifts of biomes followed by animals dependent on those biomes. Populations which were isolated in refugia are accumulating new genetic variation through mutation and genetic drift. After some time, new species might evolve over time. The Sahara desert in the Pleistocene was like a savannah full of grass; this is referred to as the Green Sahara periods which lasted from three to six thousand years (Sarabia et al. 2021). African animals could live and migrate through the Sahara during interglacial periods. But when the glacial period came, animals like the African wolf had to migrate back to less arid places like Algeria, Morocco, or Ethiopia. Increased humidity helped its population to expand during interglacial periods, and during glacial periods population sizes decreased, as we can see in Fig. 5. (Sarabia et al. 2021).

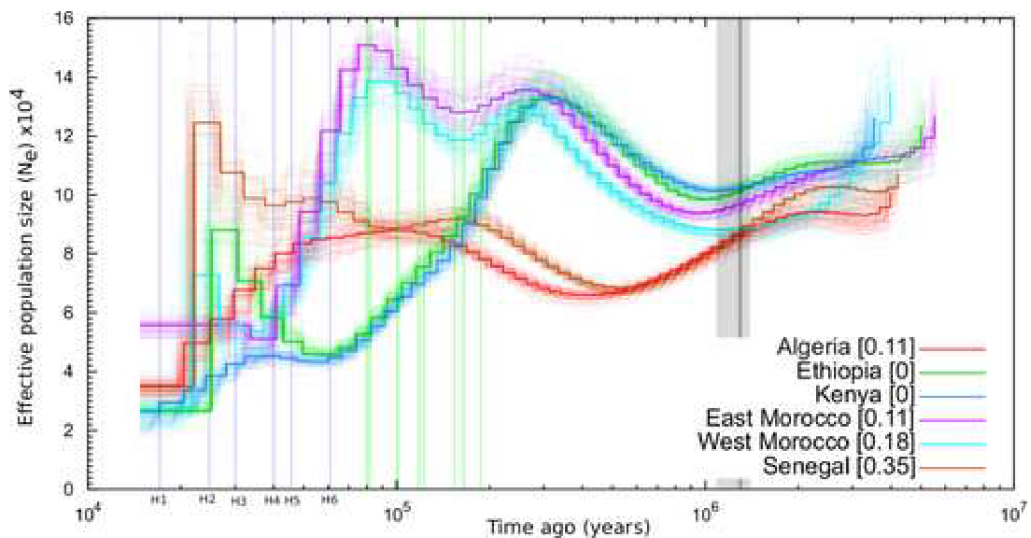


Fig. 5. Development of the effective population size of African wolf populations in history. The grey zone indicates the speciation era. Changes in the effective population size are transforming according to Green Sahara periods (green lines) and Heinrich stadials (H – purple lines). Populations experienced a reduced effective size after Heinrich Event 2 (H2; Sarabia et al. 2021).

Archeological records supported genetic data that suggest a prosperous population during the Pleistocene (Gaubert et al. 2012). Eddine et al. (2020) believed that the population of African wolves experienced an increase in two periods, the first around

50 thousand years ago during the Late Pleistocene and the second between 6,720-3,840 years ago in the Holocene. The last increase should coincide with the end of the wet Holocene Climatic Optimum. It was calculated through demographic analysis of microsatellites as it happened between 1680-960 generations ago (Eddine et al. 2020). Sarabia et al. (2021) used SNP (single nucleotide polymorphism) which supports more reliable results in their research. That is why they disagree with suggestion of Eddine et al. (2020), claiming that the Northwestern population experienced a constant decrease when the Green Sahara periods were rotating with Heinrich stadials (colder glacial periods). That decrease stopped 70 thousand years ago when population size started to increase in the direction from west to east. These population changes could have happened according to climate change during the Late Pleistocene. It is important to add that the influence of climate change did not affect all populations similarly across their range (Sarabia et al. 2021).

We can also notice growth of cattle, goats, or sheep populations bred by humans after the Neolithic revolution. This growth enabled an increase of populations of opportunistic carnivores (Eddine et al. 2020) who did not hesitate to use this opportunity. Because African wolves are medium-sized carnivores, the availability of domestic prey could lead to a demographic growth of the species (Eddine et al. 2020). Population expansion of *C. lupaster* is closely related to homesteads because grazing livestock is available prey for it. In addition, humans were a danger to other carnivores like Spotted hyenas (*Crocuta crocuta* Erxleben, 1777), which were competitors with the African wolf (Sarabia et al. 2021). Eddine et al. (2020) assumed that African wolves lived near human settlements 12 thousand years ago, as nowadays recognized individuals (Gutema et al. 2022).

Sarabia et al. (2021) suggested that during the Green Sahara periods, the African wolf had a wider distribution than today because there are archeological findings in extremely arid habitats where the African wolf is not occurring nowadays. Due to fossil finding, it appears that the African wolf also lived in Saudi Arabia and the Levant (Viranta et al. 2017).

The time of divergence between West and East Moroccan populations was estimated at 21-16 thousand years ago (Sarabia et al. 2021). Sarabia et al. (2021) suggested a few hypotheses for the isolation of the West Moroccan population 18

thousand years ago. One possibility is that this population failed to reconnect with the East One after the last glacial period because they went through local adaptation to changed abiotic conditions. This possibility could be synergistic with another option that the High Atlas Mountains restricted the movement of African wolves, which led to an increased inbreeding coefficient in the West population (Sarabia et al. 2021). More sampling is needed to make a picture of the West Moroccan population and its isolation. The East Moroccan population is genetically close to populations in Senegal and Algeria, which are also geographically close, suggesting gene flow through the high mobility of African wolves migrating through these lands. These three diverged probably less than 3 thousand years ago (Sarabia et al. 2021).

3.4. Population genetics

Populations of African wolves are mainly divided geographically. Not only Northwestern and Eastern, separated by the Sahara desert, but also within one country (see Fig. 6.). For example, in Morocco, the West Moroccan population has a high degree of inbreeding as it seems that it was isolated for 18 thousand years, according to Sarabia et al. (2021). East Moroccan population is surprisingly lowest in the degree of inbreeding compared to the few samples Sarabia et al. (2021) tested.

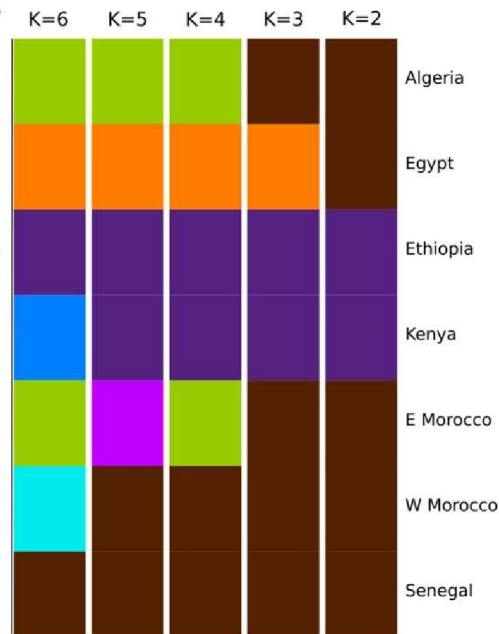


Fig. 6. Genetic clustering between populations of the African wolf according to Sarabia et al. (2021). Orange colour is a sign of historical admixture with domestic dogs. Different colours mean different admixtures.

The Northwestern population is genetically closer to Grey wolves and Golden jackals, which is not surprising due to geographic proximity. The Eastern population is probably admixed with Ethiopian wolves, also geographically close (see Fig. 7.; Gopalakrishnan et al. 2018). These differences are shown in Fig. 6., where the East population has a different colour than the North and Northwest ones. Sarabia et al. (2021) hypothesised that these two populations diverged during dry periods in the Sahara (due to Heinrich stadials) when wolves were isolated in refugia from each other. Populations genetically diverged between 50 and 10.5 thousand years ago with the biggest probability between 30 and 16 thousand years (Sarabia et al. 2021). This divergence might correlate with the last glacial maximum reported in Ethiopian Highlands, 42 to 28 thousand years ago, as Groos et al. (2021) found.

The Egyptian population is genetically somewhere between Northwestern population and the Grey wolf with dog population; it probably had introgressed ancestry (Sarabia et al. 2021). Gopalakrishnan et al. (2018) did interesting research, detecting the possible hybrid origin of the African wolf. It could be a hybrid of Grey wolf and Ethiopian wolf or could have admixed ancestor of these two. At least some relationship between the mentioned species exists, according to their research. This relationship would explain admixture in African wolves.

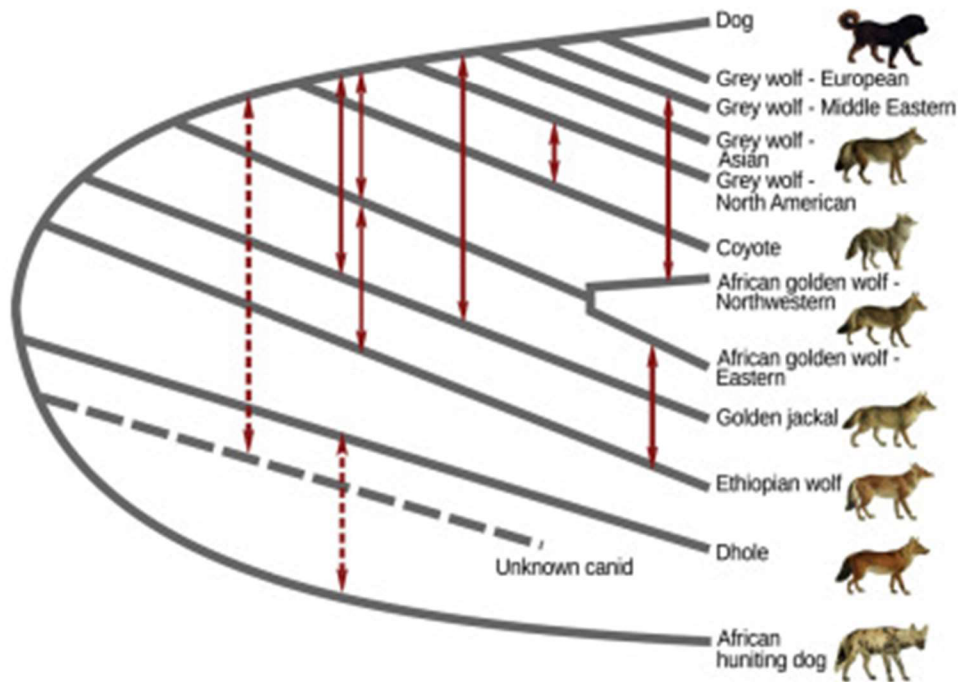


Fig. 7. Phylogenetic tree with gene flow between species (red arrows). Figure is from Gopalakrishnan et al. (2018).

Populations from Algeria and Senegal have the greatest genetic diversity, as Mallil et al. (2020) compared to the African wolf from Ethiopia, African domestic dogs, and Grey wolf. This result is interesting because Viranta et al. (2017) claimed that the Ethiopian population's genetic diversity is higher than that of the Northern one from Algeria, Egypt, and Morocco. Despite this, the above mentioned populations differ from each other. For example, Northwestern wolves are larger in body size than Eastern ones (Viranta et al. 2017). Sarabia et al. (2021) did not rule out the possibility that African wolves diverged to ecotypes according to reaction to climate changes and geographical differentiation. Also, the bottleneck effect is a possibility.

Gaubert et al. (2012) found that the genetic information of the African wolf is unique compared to other Grey wolf lineages. They proved it with the highest level of haplotype and great diversity (Gaubert et al. 2012; Karssene et al. 2018).

Gaubert et al. (2012) found out that there was an ancient lineage with a big female effective population size (80,000 females) in Africa. This ancient lineage is probably the ancestor of the current African wolf and is extinct nowadays. This information could synergize with the research of Rueness et al. (2015) who suggested that the African wolf and the Ethiopian wolf are sister clades with a common ancestor. Recently it was found

that the Himalayan wolf (*C. himalayensis*) has maternal chromosome X after the African wolf and so they have possibly a common ancestry (Werhahn et al. 2017). Rueness et al. (2015) were convinced that the African wolf and Grey wolf also have common ancestors.

The actual population size of the African wolf is not known. Monitoring at least in areas of the previous sampling is necessary and would lead to a great improvement of scientific knowledge.

3.5. Hybridization

When genetically different species reproduce with each other, it is called hybridization or crossbreeding. In small populations, hybridization is an opportunity to avoid inbreeding with relatives. According to Sarabia et al. (2021), the most inbred populations of African wolves are from Egypt, Kenya, and west Morocco.

Among Canid species, hybridization of Grey wolves (*C. lupus*) and domestic dogs (*C. lupus familiaris*) is known across Europe (Randi 2008; Harmoinen et al. 2021; Stronen et al. 2022). It is not surprising to find evidence of the hybridization between the African wolf and African domestic dog (Mallil et al. 2020). It occurs mainly between male dogs and female wolves (Mallil et al. 2020). For example, packs of feral dogs meet with African wolves when searching for food. Also, shepherd's domestic dogs are available for mating with wolves, as seen in Senegal (Mallil et al. 2020). African wolves also have been seen fighting with feral dogs which wanted to mate with a female wolf in degraded landscapes of Senegal (Mallil et al. 2020).

As said, crossbreeding of these distinct species occurs in Senegal, but also in Ethiopia, and there is evidence from Egypt as well. Mallil et al. (2020) discovered the zone of hybridization in northwestern Senegal and central Ethiopia. One female hybrid was identified phenotypically and genetically; the other dog-like individual had African wolf mitochondrial DNA lineage, both from Senegal. In Ethiopia, they identified three African wolves which were assigned to dog lineage. Therefore it is apparent that it is not easy to recognize hybrids solely based on phenotypic characters. Identifying hybrids of the African wolf is complicated by uncertainty about its origin. Although Rueness et al. (2015) claimed that the African wolf should be treated as a unique species and has not a hybrid origin, Gopalakrishnan et al. (2018) suggested that the African wolf is a possible

hybrid of the Ethiopian wolf and the Grey wolf. Ethiopian wolf cross breeds with domestic dogs, so dog genes found in African wolves could be residue inherited from Ethiopian wolves (Krofel et al. 2022). The threshold for recognition of wolf hybrids of the African wolf is not clearly defined and, therefore, difficult.

Except for hybridization between African wolves and dogs, Gaubert et al. (2012) suggested that hybridization may also occur between similarly looking Golden jackals and African wolves in Senegal, although it remains an unconfirmed hypothesis. This hypothesis is based on the observation of two jackal-like-looking individuals genetically assigned to *C. lupaster*. Additionally, in Senegal, a fight was observed between an African wolf and a Golden jackal over a carcass (Gaubert et al. 2012) which may prove that the African wolf and the Golden jackal interact. Viranta et al. (2017) proposed a similar hypothesis of the existence of hybrid zone in eastern Egypt in the Sinai Peninsula. Findings in Israel support this hypothesis about the hybrid zone in Egypt, where live Golden jackals with parts of the African wolf's DNA (Koepfli et al. 2015).

Sarabia et al. (2021) found one individual who they claimed to have Grey wolf or possible domestic dog ancestry. There is also evidence of a Grey wolf + African wolf + Golden jackal hybrid from the Egyptian Sinai Peninsula (Koepfli et al. 2015; Gopalakrishnan et al. 2018), but Koepfli et al. (2015) suggested that this admixture was ancient. The Sinai Peninsula is a place where all these three species can meet, so it is not surprising that this contact could result in hybrids, and we have to ask if there are more of them. It is important to sample individuals also in the distance from Egypt because wolves can reach tens of kilometres per day. For example, Karsene et al. (2018) discovered that African wolves in Tunisia could migrate up to 230 km within three months, which can secure quite high gene flow among populations. These long-distance dispersals might be explained by finding a sexual partner and forming a new pack, seasonal changes in preferred habitat, exploration, or other motivations. Yirga et al. (2017) used vehicles at road transects to record presence of the African wolf, but they were unable to follow individuals to offroad places due to wolves' high mobility.

Last but not least is a suggestion of possible hybridization between the African wolf and the Ethiopian wolf (Mallil et al. 2020). This assumption is evident when these two species are geographically overlapping. There is no evidence yet, but further research will be necessary.

Krofel et al. (2022) recommend a wider sample of African wolves in their geographical presence to fill gaps in knowledge of a number of hybrid individuals and the genetic diversity of all populations.

The history of Canids is complex. Canid species were formed with a high level of gene flow between species and lineages (Gopalakrishnan et al. 2018). Therefore, it is challenging to determine and estimate the recent admixture between the African wolf and other Canid species.

3.6. Types of conflict

3.6.1. Human-wolf conflict

Conflict with humans is complex and must be resolved before the African wolf becomes endangered. Gutema et al. (2022) found two dead African wolves, possibly killed by humans. Rueness et al. (2011) and Gaubert et al. (2012) claimed that farmers kill African wolves (and Golden jackals) because they are a threat to the livestock. Local people even tried to prevent livestock predation; they blocked dens with puppies of the African wolf (Atickem et al. 2017). Karssene et al. (2018) assume that due to the lack of law restrictions for hunting African wolves in Tunisia, individuals might be killed by farmers without persecution. Although there was no observed impact of hunting on genetic diversity, unrestricted hunting might be a problem in the future. Farmers should be better informed about the African wolf population and its important role in the ecosystem. By hunting rodents (or eating carcasses), farmers may find them helpful (Atickem et al. 2017; Yirga et al. 2017; Karssene et al. 2018; Gutema et al. 2019). Atickem et al. (2017) found through a survey that 74.6 % of killed livestock in the Guassa mountains of Ethiopia was killed by African wolves, which caused 78.9 % of economic loss there.

3.6.2. Animal-wolf conflict

The Ethiopian wolf is an endangered species (the IUCN 2011) living in the Ethiopian Highlands. It is primarily a rodent specialist; rodents form 90 % of its diet (Gutema et al. 2019). Because the African wolf's diet also contains rodents (mainly carcasses, but also alive ones), it might be considered as a possible threat to the Ethiopian

wolf. Their niches overlap and there might be competition for resources. The Ethiopian wolf inhabits more intact habitats probably because of the African wolf, who inhabits anthropogenic habitats with rodent prey available. African wolves live in family packs, but some individuals have also been seen alone (Gaubert et al. 2012; Mallil et al. 2020). African wolves usually form larger groups (packs) for more effective hunting and defence of prey against competitors such as Ethiopian wolves, Golden jackals, or Spotted hyenas (Gutema et al. 2022). Gutema et al. (2018) suggested that the Ethiopian wolf could have lived near humans without competition with the more abundant African wolf.

Contacts and breeding with domestic dogs are dangerous because of pathogen transmission (Mallil et al. 2020). The most threatening are rabies, canine distemper, and parvovirus transmitted by domestic dogs and possibly through wild canid species like the Ethiopian wolf (Gutema et al. 2018; Funk 2022).

Hybridization might be another threat to the African wolf. Recently there was evidence of hybridization with domestic dogs (Gopalakrishnan et al. 2018; Mallil et al. 2020; Sarabia et al. 2021). The hybridization could lead to a genetic decrease in wolf genes, threatening the African wolf genome. Hybridization also creates the possibility of forming new individuals that differ morphologically and ecologically from their parents (Machado et al. 2020).

Almost every author writing about African wolves pointed out that finding more relevant information through proper sampling and observation of ecology is necessary for sufficient conservation. Wolf species from around the world are under conservation, the African wolf is the only one that is not. It was caused by the oversight of the existence of this species in Africa, but we have known this long enough to start with management and conservation. According to recent predictions by Karssene et al. (2017), due to global warming, the population of African wolves, at least in Tunisia, will decrease. This

decrease will be caused by a massive reduction of suitable habitat between the 2030s (losing 86 %) and 2080s (losing 97 %); see Fig. 8.

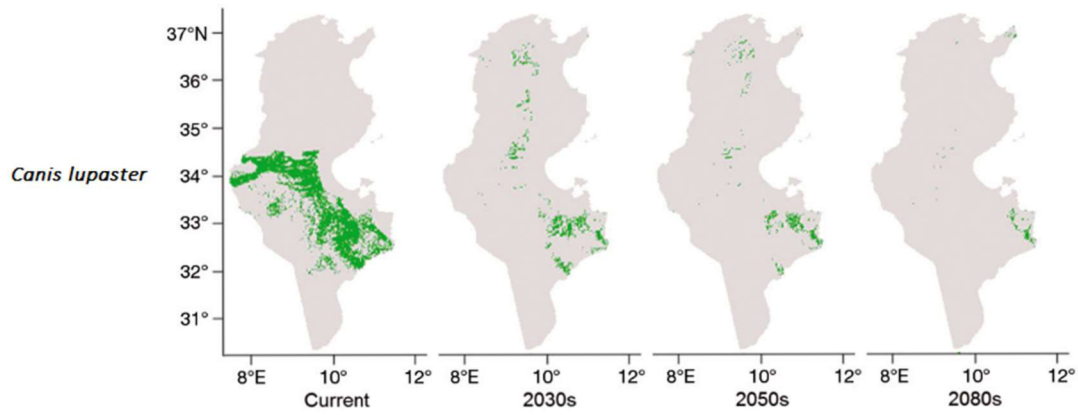


Fig. 8. Habitat suitability map for *Canis lupaster* based on species distribution model in Tunisia. Green and grey are suitable and unsuitable habitats, respectively. Figure modified from Karssene et al. 2017.

4. Methods of data analysis

Data in this bachelor's thesis were obtained from the scientific articles dealing with samples of the African wolf; they are listed in Tab. 1. with the accession numbers. I used nucleotide sequences that have been uploaded in NCBI GenBank. Data are from Tunisia, Morocco, Algeria, Senegal, Mali and Ethiopia. All used sequences are either mitochondrial control region or D-loop.

Tab. 1. Accession numbers for nucleotide sequences from articles used in this study and the locality from which they originate.

Scientific article	Accession numbers	Locality
Rueness et al. 2011	HQ845259.1	Ethiopia
Gaubert et al. 2012	JQ088678.1 - JQ088682.1	Northeastern Algeria
	JQ088683.1	Northern Senegal
	JQ088684.1	Eastern Mali
Urios et al. 2015	KT378607.1	Morocco
Waters et al. 2015	KM670012.1, KM670013.1	Northern Morocco
Karssene et al. 2019	MK392560.1 - MK392572.1	Tunisia
Eddine et al. 2020	MK659615.1 - MK659624.1	Algeria
Mallil et al. 2020	MT108908.1 - MT108910.1, MT108916.1	Northwestern Senegal
	MT108912.1 - MT108915.1, MT108919.1, MT108920.1	Northern Algeria
	MT108911.1, MT108917.1, MT108918.1	Central Ethiopia

Downloaded data was copied into a simple NotePad and loaded into the Geneious program (by Dotmatics) as a FASTA file. In the program, sequences from Tunisia (Karssene et al. 2019) had to be reversed. Data of 47 sequences were aligned and trimmed to the exact size of 230 Nucleotide Sites. Then I exported it into a FASTA file.

I used DnaSP 6 (Rozas et al. 2017) program to see basic information (number, length and mutation of sequences) about the dataset.

I computed pairwise distances in the MEGA11 (Tamura et al. 2021) program to see how different the sequences are to each other.

Staying in MEGA11 (Tamura et al. 2021), I generated a neighbour-joining phylogenetic tree with 500 bootstraps and added colours according to countries belonging in photoshop.

In Geneious (by Dotmatics) I used MrBayes program (Ronquist et al. 2012); for Substitution model I assigned General Time Reversible Model and as Outgroup I assigned access number MT108908.1 because of distant pairwise results. I exported the results into the Newick File and displayed a tree in the FigTree program. Then I also added colours with legend in photoshop.

A FASTA file with trimmed sequences had to be exported into a Phylip file. I worked in TCSv1.23 and created a haplotype network. I saved it as a GML file and put it into online tcsBU - a TCS beautifier (Santos et al. 2016) to add colours.

5. Results

In the DnaSP 6 (Rozas et al. 2017) the total number of haplotypes is 43 (from 47 sequences which means that four sequences were the same) and 42 mutations.

Results of the pairwise distances analysis are shown in Tab. 2. in appendices. Individuals from Ethiopia have low distances, between 0.00454 and 0.02316. Two individuals from Senegal (accession numbers MT108908.1 and MT108909.1; Mallil et al. 2020) have the biggest distances (between 0.06348 and 0.11232) compared to all other data. In the whole dataset is 0.00452 (highlighted with orange colour in Tab. 2.) the smallest number of distance (this distance occurred twenty times) while 0.11232 between Senegalese and Ethiopian individuals (highlighted with blue colour in Tab. 2.) is the biggest distance within the dataset.

The phylogenetic tree (see Fig. 9.) from MEGA11 (Tamura et al. 2021) shows one important branch of Northwestern sequences, another one from Ethiopia (yellow colour) and one distant basal group of two non-classificated individuals (MT108908.1 and MT108909.1).

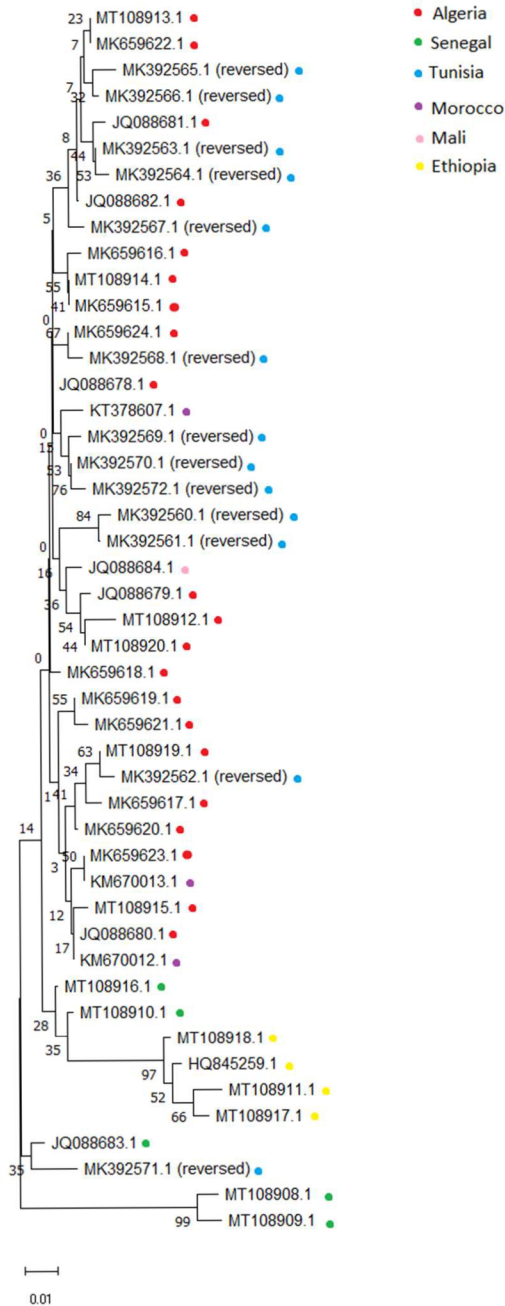


Fig. 9. Phylogenetic tree from MEGA11 (Tamura et al. 2021) with adjusted colours belonging to countries where sequences originate.

The phylogenetic tree (in Fig. 10.) from MrBayes program (Ronquist et al. 2012) displayed in FigTree shows similar results with one main branch of Northwestern sequences from which differ Senegalese individuals (MT108908.1 and MT108909.1). As in the upper case, Ethiopian sequences are separate with some relation with two Senegalese sequences.

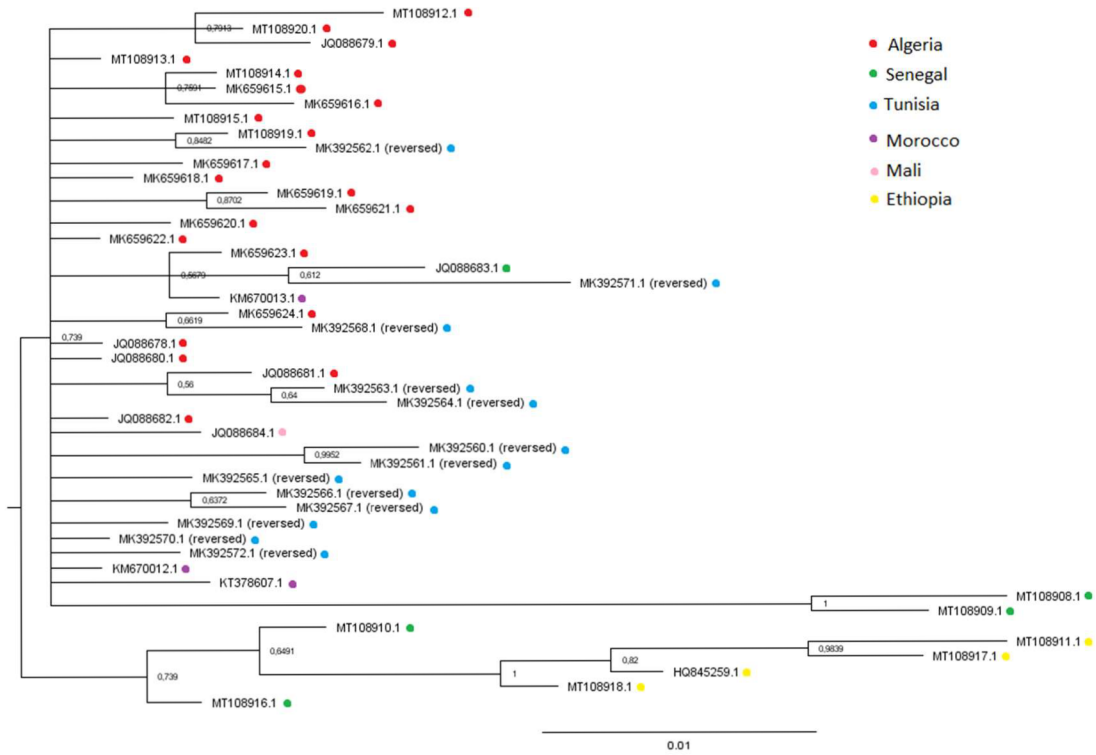


Fig. 10. Phylogenetic tree from MrBayes (Ronquist et al. 2012) with adjusted colours belonging to countries where sequences originate.

Results of the haplotype network are seen in Fig. 11. Sequences of African wolves from Ethiopia (yellow colour) are very distant from the rest places in North and Northwest Africa which corresponds with results of pairwise distances and phylogenetic trees. Two sequences from Senegal (access numbers MT108908.1 and MT108909.1; Mallil et al. 2020) are separated from each other, supporting results of pairwise distance and phylogenetic tree.

Circles of half purple and half red colour are individuals with the same haplotypes from Algeria and Morocco.

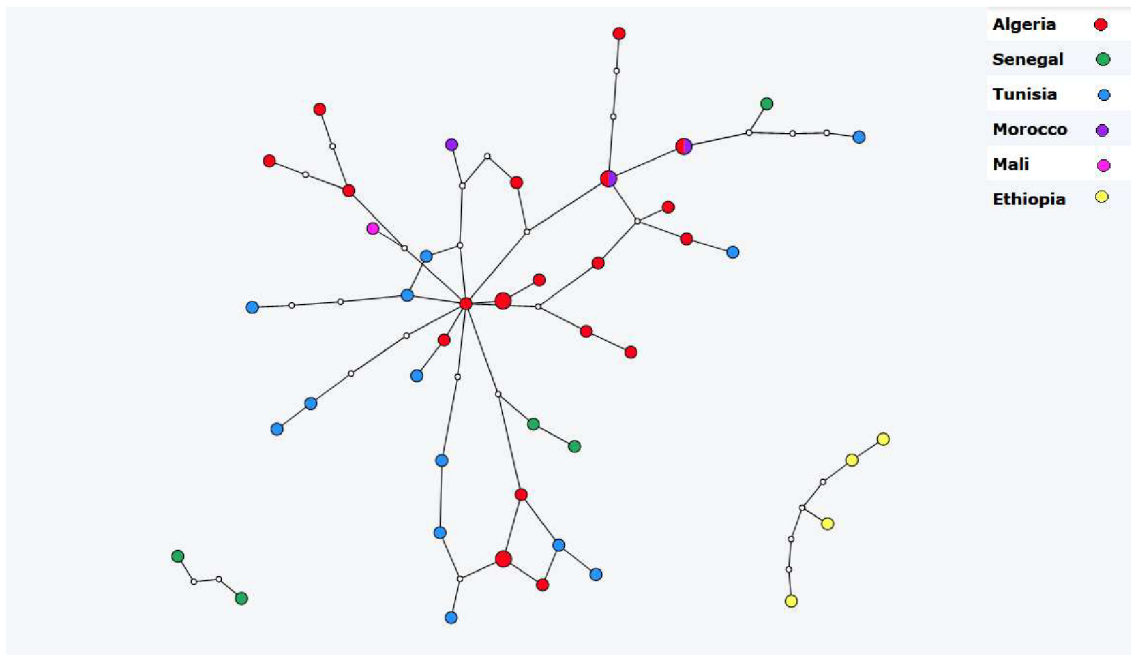


Fig. 11. Haplotype network of nucleotide sequences of the African wolf from NCBI GenBank.

6. Discussion

The pairwise distance of two Senegalese individuals (MT108908.1 and MT108909.1) points to the possibility of belonging to another species, possibly domestic dogs or Grey wolves (Mallil et al. 2020). This result corresponds with the result of Mallil et al. (2020), who assigned them to domestic dogs or Grey wolves (although in GenBank, these data were named *Canis lupaster*). African wolves from Ethiopia have a small distance from each other, which corresponds with the results of phylogenetic trees and the haplotype network. Distances between Ethiopian ones and the rest of the sequences from Northwestern Africa are more significant, clustering them into separate populations, which corresponds with later findings of Gopalakrishnan et al. (2018) and Sarabia et al. (2021). Northwestern sequences from Algeria, Tunisia, Morocco, Mali, and Senegal have small distances from each other, clustering them into a similar cluster, which corresponds with the genetic clustering (Fig. 6.) of Sarabia et al. (2021).

Both phylogenetic trees show a basal group of two Senegalese sequences (MT108908.1 and MT108909.1) and separate the Ethiopian group from the others. Mallil et al. (2020) marked Senegalese sequence number MT108910.1 as a hybrid of the African wolf and domestic dog; it is therefore interesting that Ethiopian sequences are branching from this nucleotide sequence.

The haplotype network supports the results of pairwise distances and phylogenetic trees, separating Ethiopian samples and Senegalese sequences from the biggest group of Northwestern sequences, the same as in Mallil et al. (2020). Two Moroccan sequences from Waters et al. (2015) are the same as two Algerians from Gaubert et al. (2012) and Eddine et al. (2020), as we can see from pairwise distances in Tab. 2.

7. Conclusions

The relatively recent discovery of a “new“ species of the wolf from Canidae is a result of significant improvements in the field of genetic research. It proves that we still have many opportunities to find something surprising, and we have to verify all older discoveries and information.

The African wolf (*C. lupaster*) is a species from the Canidae family, genetically distinct from the Golden jackal, although phenotypically similar. Its range is in North, Northwest, and East Africa. The most studied countries with African wolf populations were Morocco, Algeria, Senegal, and Ethiopia. The IUCN information is not actual, does not reflect recent scientific studies, and for this might be inaccurate. Besides grasslands, the African wolf also occurs near anthropogenic areas resulting in many important interactions like human-wolf conflict (African wolves sometimes kill livestock and, for that, are persecuted by humans), hybridization with domestic dogs, and pathogen transmission.

Populations of the African wolf were differentiated through the Middle Pleistocene when dry glacial periods alternated with humid interglacial periods and created different ecotypes of the African wolf (Sarabia et al. 2021). This explains why geographically distinct African populations are genetically different from each other. The Northwestern population has introgressed genes from the Grey wolf and the Golden jackal; on the other side of the Sahara is the East population with introgressed genes from the Ethiopian wolf (Gopalakrishnan et al. 2018). This introgression was caused by historical hybridization, which is natural and ongoing in all species as part of their evolution. The Moroccan populations are divided by High Atlas Mountain, which caused the isolation of the West Moroccan population for 18 thousand years with a high degree of inbreeding (Sarabia et al. 2020). The East Moroccan population is genetically diverse and closer to the Algerian population.

Recent hybridization with domestic dogs and Grey wolves might cause a threat to the African wolf's genome and uniqueness. There are few pieces of evidence of hybridization in Egypt, Senegal, and Ethiopia, mainly with domestic and feral dogs. Egypt is very close to the Middle East, so there was found a hybrid of African wolf, Grey wolf, and Golden jackal (Gopalakrishnan et al. 2018).

As can be seen from the practical part of this thesis, short mitochondrial data support the results of genomic research. This gives us more space for straightforward research and monitoring of the African wolf populations. Using short mitochondrial sequences gives us the possibility to study more population genetics which is not researched sufficiently in recent publications.

There is still very much to search for about the African wolf. More sampling across its range (estimated by IUCN) is needed not only to find where he lives but also to explain all differences between populations. It would be useful to also estimate Golden jackal's range by sampling to find where can this species meet with the African wolf. Another critical genetic question is how much does the African wolf breed with domestic dogs? We have only a few pieces of evidence of these hybrids, but there are probably more of them in Africa. These missing pieces of information should result in conserving and managing this genetically unique and ecologically essential species.

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Appendices

List of the Appendices:

Tab. 2. Pairwise distances between nucleotide sequences of African wolf individuals. Green are possible dog or Grey wolf individuals. Yellow are sequences from Ethiopia. Orange is smallest distance, blue is the biggest distance. Nucleotide sequences are from GenBank.....In attached Excel file.