CHAPTER ONE

1. Introduction

1.1 Sudan

Sudan is situated in Northern Africa (latitudes 8° and 23°N) with an area 853 km (530 mi) along Red Sea shore. Sudan borders Egypt, Eritrea, Ethiopia, South Sudan, the Central African Republic, Chad, and Libya, covering an area of 1.886.068 km² (728.215 mi²). Sudan is the third largest country in Africa after Algeria and Democratic Republic of the Congo) and the sixteenth largest in the world. The terrain is generally flat plains, broken by several mountain ranges; in the west the Deriba Caldera (3042 m or 9980 ft), located in the Marrah Mountains, is the highest point; in the east are the Red Sea Hills (https://en.wikipedia.org/wiki/Sudan). The amount of rainfall increases towards the south. The central and the northern parts have extremely dry desert areas such as the Nubian Desert to the northeast and the Bayuda Desert to the east. Sudan's rainy season lasts for about three months (July to September) in the north, and up to six months (June to November) in the southern parts. The dry regions are plagued by sandstorms, known as haboob, which can completely block out the sun. In the northern and western semi-desert areas, people rely on the scant rainfall for basic agriculture and many are nomadic, travelling with their herds of sheep and camels. Nearer the River Nile, there are well-irrigated farms growing cash crops. The sunshine duration is very high all over the country, especially in deserts where it could soar to over 4.000 h per year (https://en.wikipedia.org/wiki/Sudan).

Sudan is derived by three permanent rivers: the Blue Nile, the White Nile and the River Nile. The Blue Nile originates from Ethiopia, the White Nile

from Kenya and Uganda, the two rivers meet at Khartoum, the capital of Sudan to form the River Nile which flows northwards through Egypt to the Mediterranean Sea. The Blue Nile's course through Sudan is nearly 800 km (497 mi) long and is joined by the Dinder and Rahad seasonal rivers between Sinnar and Khartoum. The White Nile within Sudan has no significant tributaries. All rivers run from south to north. The River Nile system is a barrier that divides Sudan into two major geographic regions, the western region and eastern region as well as a third region between the Blue Nile and the White Nile.

There are several dams on the Blue Nile and the White Nile. Among these are the Sinnar and Roseires Dams on the Blue Nile, the Jebel Aulia Dam on the White Nile and Marwe dam on the River Nile. There is also Lake Nubia on the Sudanese-Egyptian border. The nation's wildlife is threatened by hunting and habitat destruction. As of 2001, 21 mammalian species and nine bird species are endangered, as well as two species of plants. Endangered species include: the Waldrapp,(*Geronticus eremita*), tora hartebeest (*Alcelaphus buselaphus tora*), slender-horned gazelle (*Gazella leptoceros*), Red-fronted gazelle (*Eudorcas rufifrons*)and hawksbill turtle (*Eretmochelys imbricate*). The Sahara Oryx (*Oryx dammah)*and Addax (*Addaxnasomaculatus*) have become extinct in the wild (https://en.wikipedia.org/wiki/Sudan).

The order of Lagomorpha are found throughout the world either as native or introduced species. Their distribution extends from the equator to 80˚N and from sea level to over 5000 m in the mountains in diverse habitats from desert to tropical forest. Sizes of Lagomorphs range from the small, rodent-like pikas (some less than100 g) to rabbits (one to four kilograms) to the largest hares (in excess of five kilograms). There are about 78 living species,

including 25 pikas, 29 hares and 24 rabbits. The pikas have 26 teeth, rabbits and hares 28 teeth (***Chapman and Flux***, ***1990***). Lagomorphs are almost exclusively herbivorous, with a diet consisting of herbs, as well as fruit, roots, buds, seeds and bark. The only known case of meat- eating as a necessary part of the diet is pika (*Ochotona collaris*) (***Smith***, ***2004***).

The two Lagomorphs' families, Ochotonidae and Leporidae, are easily distinguishable. The Ochotonidae (pikas) have hind legs not much longer than the forelegs; are very small; have rounded ears as wide as they are long; and a skull with no supraorbital bones and a relatively short nasal region. The Leporidae (rabbits and hares) on the other hand, are larger, with hind legs longer than the forelegs; have long ears; and a skull with prominent supraorbital bones and a long nasal region (***Angermann et al., 1990***). Lagomorpha tend to be highly reproductive, especially the leporids, with many species producing large litters each year and young becoming sexually mature at a younger age. Burrowing pikas also tend to have several sequential, large litters or small litters and normally only successful one a year. Lagomorphs are known for their lack of parental care. Some mothers only nurse the young about one time a day, although the milk is highly nutritious (***Smith, 2004***). On the other hand, hares are distinguished from rabbits by giving birth to precocial young fully furred and with the eyes open versus the rabbits that have atricial young born without any fur and with eyes closed (***Smith, 2004***).

Most hares do not dig burrows, instead they rely on camouflage, speed and thick brush cover to escape danger, but cape hare use shallow scrapes in the ground to escape high desert temperatures (***Chapman and Flux, 1990***). Hares appear to be essentially silent, solitary animals; but their behavioral interactions must be complex as they can regulate population density at

levels normally far below the carrying capacity of the environment (Keith and Windberg, 1978). Rabbits live in groups numbering between a single pair and up to 30 individuals (***Leach, 1989***).

The behavior of the European rabbit has been studied more than any other lagomorphs, but it should be remembered that much of this work has been done in Australia and the United Kingdom, where rabbit control is of great importance. These rabbits are now thought to be feral populations derived from escaped domestic stock, which may be atypical in lacking intrinsic population control behavior (***Flux,2001***).

In Africa little information is available about diversity and distribution of Lagomorphs. The situation is more complicated when Sudan, lying in the centre of Africa is considered because of knowledge gap about studies small mammals; while few studies about mammals in general are available in West, East and North African countries bordering Sudan, almost no studies have been conducted in Sudan particularly for Lagomorphs. In Sudan there are no records for the Lagomorphs' species occurrence, distribution, habitat and ecology. This study aims at addressing these parameters.

1.2 Hypothesis

H0 (Null hypothesis): Hare and wild rabbit populations in three regions of Sudan have maintained sufficient gene flow to sustain a genetically admixed population.

H1: Hare and wild rabbit populations are separated by geographical barriers, hindering gene-flow and leading to genetically differentiated populations.

H2: Genetic population differentiation follows an IBD model; the contrast would be that drift is shaping the populations, which is very likely if they are small.

1.3Objectives

Overall objective

1-To determine the morphological characterization of the hares in Sudan.

Specific objective

1- Specific objective wasto determine genetic characterization of the hares in Eastern, Western regions andbetween the Blue and the White Niles region.

CHAPTER TWO
2. Literature Review

2.1 Lagomorpha

The order Lagomorpha is represented by 13 genera and 93 species belonging to three families (Ochotonidae, Leporidae, and Prolagidae) in the world. The family Leporidae (hares, rabbits and jackrabbit) comprises 11 genera and 61 species. The genus *Lepus* L. is represented by 32 species (***Wilson and Reeder, 2005***).

2.1.1Diversity

The family Leporidae, comprising primarily rabbits and hares, includes 54 species from 11 genera. Leporids' biomass ranges from 300 g (1.4 lbs) in pygmy rabbits to 5 kg (11 lbs) in arctic hares. Domestic leporids can be significantly larger, with an average weight of 7 kg. Adult head and body length ranges from 250 to 750 mm. Leporids have long hind limbs and feet, short bushy tales that are sometimes conspicuously marked, and the soles of their hind feet are covered with hair. The toes terminate in long, slightly curved claws (***Angerbjörn, 2011***).The ears, which are also relatively long, are proximally tubular with the lowest point of the external auditory meatus situated well above the skull. Rabbits and hares are often differentiated from Pikas by the length of their tails and ears. Tail length in leporids ranges from 1.5 cm to 12 cm. Rabbits and hares are characterized by their elongated hind limbs and feet and their ears, which can reach 17 cm in antelope jackrabbit; Pikas have short, rounded ears (***Nowak, 1999***).

The European hare (*Lepus europaeus*is) is one of the largest living members of Lagomorpha. Its head and body length can range from 48 cm to 75 cm (19 in to 30 in), and a tail length of 7 to 13 cm (2.8 to 5.1 in). The body mass can range from 2.5 to 7 kg (5.5 to 15.4 lb) (***Burnie and Wilson, 2005***). As

with all leporids, the hare has an elongated ear which, in this species, ranges from 9.4 to 11.0 cm (3.7 to 4.3 in) from the notch. It also has long hind feet, measuring 14 to 16 cm (5.5 to 6.3 in). The fur colour is grizzled yellow-brown on the back; rufous on the shoulders, legs, neck and throat; white on the underside and black on the tail and ear tips. The European hare's fur does not turn completely white in the winter (***Naughton, 2012***) although the sides of the head and base of the ears do develop white areas (***Chapman and Flux, 1991***). Historically, up to 30 subspecies of European hare have been classified, although their status has been variable (***Chapman and Flux, 1991***).

The African savanna hare ***(****Lepus microtis*) is a medium-sized species growing to a length of 41 to 58 cm (16 to 23 in), weighing 1.5 to 3 kilograms (3.3 to 6.6 lb.). The ears have black tips, the dorsal surface of head and body is greyish-brown, the flanks and limbs are reddish-brown and the underparts are white. The general colouring is richer in tone than other hares, especially in mountain regions where the hares are a rather darker shade. The tail is black above and white below. This hare looks very similar to the Cape hare in appearance but can be told apart by its distinctively grooved incisors (***Riegler, 2013***).

Monotypic riverine rabbit is the only species of lagomorpha on the African continent categorized as endangered. Their category is based on the degree of threat to the population, which is exacerbated by its extremely small numbers and habitat specificity (***Duthie et al., 1989***) as well as on its taxonomic uniqueness (***Robinson and Skinner, 1983***). The riverine rabbit superficially resembles the Cape hare *L. capensis*, in external and cranial morphology (***Robinson and Dippenaar, 1987).***Some leporids are extremely social, living in large communal dens, while others are solitary, coming to-

gether in groups or pairs for mating purposes only. The term 'true hares' includes hares and jackrabbit and consists of those species in the genus *Lepus*; all remaining species are referred to as rabbits. While hares are well adapted for running long distances, rabbits run in short bursts and have modified limbs adapted for digging. Hares have long muscle fibers in contrast to the short fibers found in rabbit muscle. Hares are often larger than rabbits, have black tipped ears, and have distinctly different skull morphologies. (***Gould and McKay, 1998 , Schneider, 1990***)

Members of the Leporidae have a nearly worldwide distribution; A Leporid evolutionary relationship, particularly among genera, has proven difficult with conventional phylogenetic approaches. In large part, this is due to convergence in anatomical features, an absence of chromosomal synapomorphies, and the saturation of mitochondrial DNA sequences. Of the early phylogenetic attempts, those based on premolar tooth patterns were the most definitive (***Dawson, 1981***). And the most recent of these incorporated 9 of the 11 leporid genera*Poelagus* and *Bunolagus* were not included.

An analysis of the morphological characters defining *L. capensis* has never been performed, in spite of the compelling need of comparing the nominal subspecies with other *L. capensis* populations and with other species of the genus *Lepus*. The lack of a precise characterization of *L. capensis* is also a result of the absence of holotype material. Moreover, the description of several African hare populations as subspecies of *L. capensis* led to the acceptance of a high morphological variability within this species and to the recognition of *L. capensis* in regions so far away from South Africa as Europe or Asia (***Fernando et al., 2007***). The extent of genetic and morphological diversity suggests that any consideration of population augmentation via translocations must be made cautiously. Although it may

be desirable to manage genetic diversity within areas, a shift of genotypic frequency could be disastrous if haplotypic diversity reflects adaptation (***Shan et al., 2011***).

2.1.2 Geographic Range

Similar to its parent order, Lagomorpha, the family Leporidae has a wide geographic range. Leporids occupy most of the world's land masses with the exception of Southern South America, the West Indies, Madagascar, and most islands southeast of Asia. Although originally absent from South America, Australia, New Zealand, Java, leporids have been introduced to these locations during the last few centuries. The broad geographic range of leporids is largely due to introduction by humans (***Angerbjörn, 2011***).

At least eight of the genera (*Brachylagus*, *Pentalagus*, *Caprolagus*, *Bunolagus*, *Poelagus*, *Romerolagus*, *Oryctolagus*, and *Nesolagus*) have geographically restricted distributions. Apart from *Nesolagus* which includes two species (***Can et al., 2001***), all these genera are monotypic. Of the more widely distributed taxa, *Sylvilagus* contains more than 16 species restricted to North, Central, and South America (***Chapman et al., 1992***; ***Frey et al., 1997***), whereas the African genus *Pronolagus* includes at least four species (***Angermann et al., 1990***; ***Matthee and Robinson, 1996***). The genus *Lepus* (hares and jackrabbit) is characterized by approximately 26 species and is the only taxon with an almost cosmopolitan distribution (***Flux and Angermann, 1990***).

2.1.3 Habitat

Leporids are widely distributed and have adapted to a broad range of habitat types. Their habitats types are open deserts to boreal forests. These habitat types and cursorial ability are tightly linked, and as a result, hares and rabbits have distinct habitat requirements. Hares are most often found in open

habitat where they can use their speed to evade potential predators. They also rely on their well camouflaged pelage to hide from predators among the shrubs and rocks. However, some hare species, such as snowshoe hares (*Lepus americanus*) and Manchurian hares (*lepus mandshuricus*), are well-adapted forest dwellers. While hares are most often found in open habitats, rabbits are confined to habitats with dense cover where they can hide amongst the vegetation or in burrows. Some species of rabbits, such as swamp rabbits and marsh rabbits are excellent swimmers and are considered semi-aquatic. In short, cursorily adapted leporids reside in open habitats, whereas cursorily challenged species reside in closed habitats. (***Angerbjörn, 2011; Hutchins, 2004***).

Color patterns vary between species and among seasons, and range from black to reddish brown to white. Although spots are relatively common in domestic leporids, most wild species have relatively subdued coloration that helps them blend in with their surroundings. The Sumatran rabbit (*Nesolagus netscheri*) is one of two species with stripes. Neither albinism nor melanism are uncommon in leporids, and some species that inhabit higher latitudes have white coats during the winter, which are then molted during spring. Most leporids are counter colored, with dark-colored dorsal pelage and light-colored ventral pelage. Pelage texture can be thick and soft or coarse and woolly (hispid hares) and may become increasingly sparse along the length of the ears.

Leporid skulls are unmistakable; they have an arched profile and are only slightly constricted between the orbits, unlike those of their close relatives, the pikas. They have prominant post- and supraorbital processes and the parietal, occipital and maxillae are fenestrated. In some species, the squamosals are fenestrated as well. They have a moderately robust zygo-

matic arch, a relatively short jugal and tubular external auditory meatuses that are vertically positioned. The dental formula of most leporids is 2/1, 0/0, 3/2, 3/3 = 28. The primary incisors are enlarged, and the secondary are small, peg like, and located immediately posterior to the primaries. The primary incisors resemble those of rodents, except that they are completely encased in enamel. Canines are absent, and a large diastema separates the incisors from the cheek teeth. Their cheek teeth (molars and premolars) have relatively simple cusp morphology, with the occlusal surface being made up of two transverse ridges (bilophodont). The cheek teeth are strongly hypsodont in most species(***Feldhamer et al., 2003***).

2.1.4 Mating Season

Some members of the family Leporidae do not have a specific breeding season while others breed during spring and summer. Female ovulation is induced during copulation, about twelve hours after insemination, and females can come into estrus at various times throughout the year. Many species mate immediately after or just before parturition, as females are able to carry two different litters at once (superfetation).

2.1.5 Reproduction

Most leporid species are polygynandrous. During mating season males and females form small groups in which males compete for access to estrus females and establish a social hierarchy. European Rabbits serve as an exception as they are highly social and have established hierarchies prior to mating season. Males find and attract mates by flagging their tail, involuntary urination, and rubbing against the female prior to copulation. Both sexes have multiple mates and females mate soon after giving birth or while carrying a litter. Gestation typically lasts longer in hares than in rabbits. For example, gestation lasts approximately 55 days in mountain hares and 30 days

in European rabbits. Hares are born in a precocial state, fully furred with their eyes open, and are able to run a few hours after parturition. Rabbits are born in an altricial state and are able to see a few days after parturition. (***Feldhamer et al., 2003; Gould and McKay, 1998; Hutchins, 2004; Nowak, 1999***)

Leporids have high reproductive potential and can produce several litters per breeding season, with several young per litter. Litters usually consist of 2 to 8 young with a maximum of 15 young rabbits (kittens) or hares (leverets) per litter. Resource abundance and quality play a major role in fecundity. For example, Alaskan hares and arctic hares are subjected to prolonged periods of resource scarcity during the winter and have only one litter per year. Black-tailed jackrabbit and antelope jackrabbit live in desert environments and produce several litters a year; however, the litters of these two species are relatively small, containing only 1 to 3 young (***Hutchins, 2004***).

Rabbits are born with no hair and closed eyes but often have full pelage and open eyes within a couple of days after birth. Sexual maturity and weaning can occur at a young age for both groups but varies according to species. Generally, sexual maturation can occur from 3 to 9 months after birth in rabbits and 1 to 2 years after birth for hares which is unusual in mammals, and are able to reproduce before males. Weaning age is also species-specific, but females generally nurse young for at least 3 to 4 weeks, beginning the weaning process about 10 days after parturition (***Hutchins, 2004; Schneider 1990***)**.**

2.1.6 Lifespan

Leporid's face a number of factors that affect their longevity, the most notable being heavy predation from a variety of mammalian, reptilian, and avian predators. In their natural environment, populations of certain species

have been shown to have an average lifespan of less than a year. The oldest recorded age for European hares in the wild was 12.5 years with the maximum age estimated to be between 12 to 13 years (***Feldhamer et al., 2003***)

2.1.7 Behavior

Some leporids are known to dig burrows or occupy those abandoned by other species. Only 4 species of rabbit (European rabbits (*Oryctolagus cuniculus*), pygmy rabbits (*Brachylagus idahoensis*), Amami rabbits (*Pentalagus furnessi*) and Bunyoro rabbits (*Poelagus marjorita*) are known to dig their own burrows, while some hares are known to dig burrows to escape extreme temperatures. For example, black-tailed jackrabbit and cape hares are desert species and dig burrows to escape high temperatures, whereas arctic hares dig burrows in the snow to escape the bitter cold. Many species create forms, depressions in the ground or surrounding vegetation, for rest and protection (***Hutchins, 2004***).

Predation is a constant threat in the lives of leporids and has likely served as significant selective force in their evolution. For example, the musculoskeletal morphology of hares allows for prolonged periods of high speed running, which helps them escape predators. Rabbits, which have shorter legs and more compact musculature than hares, are less efficient runners and elude predators by running into holes and burrows. These markedly different predator avoidance strategies define the rabbit's and hare's differing movement patterns. Hares typically travel long distances and have larger home ranges than rabbits, which are usually restricted to the vicinity of their subterranean safe havens and have relatively smaller home ranges and territories. Leporids are generally solitary and typically only congregate during mating season or as a predator defense mechanism during spring feeding bouts. For example, while arctic hares are solitary for a large portion of the

year, they also form large groups during the spring as a means of reducing per-capita risk of predation. European rabbits have a uniquely complex social system involving large subterranean communities and a highly developed burrow system (***Hutchins, 2004***).

2.1.8 Food Habits

Leporids are obligate herbivores, with diets consisting of grasses, and limited amounts of cruciferous (plants from the Brassicaceae family such as broccoli and brussels sprouts) and composite plants. They are opportunistic feeders and also eat fruits, seeds, roots, buds, and the bark of trees. During periods of high resource abundance, leporids tend to select forage in pre-reproductive and early reproductive stages of development. In general, the leporid diet is deficient in essential vitamins and micro-nutrients. Plant forage is high in fiber and contains cellulose and lignin as well. Mammals do not possess the digestive enzymes needed to breakdown these compounds. To compensate for this, however, the leporid caecum is up to ten times longer than their stomach and contains a diverse microbial community that helps break down cellulose and lignin. In addition, gut flora passing from the cecum into the small intestine are a significant source of protein for leporids, which have a notoriously protein deficient diet. Leporids are also coprophagic, reingesting soft green fecal pellets produced by the cecum. In addition to offsetting their dietary deficiencies, it has been suggested that coprophagy in leporids developed as a predator defense mechanism, allowing them to subsist in the safety of their burrows (***Hutchins, 2004; Whitaker, 1996***)**.**

2.2 Conservation Status

Thirteen species within Leporidae are considered threatened or near-threatened by the International Union for the Conservation of Nature (IUCN), 7 of which are either endangered or critically endangered. Of the 62 species

listed by the IUCN, those threatened with extinction are often the most primitive. As leporid habitat is being destroyed to create room for crops, irrigation, and ranch lands, many species of rabbits and hares are forced to persist on remnant habitat islands that result in significantly decreased genetic diversity and ultimately, genetic inbreeding. Many native species are also vulnerable to increased competition for resources with invasive rabbits, the introduction of new pathogens, and the introduction of new predators. While habitat destruction poses the biggest threat to many native leporids, they are also vulnerable to competition with livestock for food resources, over hunting, and poisoning by farmers. Suggested conservation measures include the eradication of exotic predators, reducing habitat destruction and fragmentation, creating strict hunting regulations and enforcing those already in place, the establishment of habitat reserves, and increasing public awareness about the importance of leporid conservation efforts (***IUCN, 2008***).

2.3 Taxonomy of Hares

Hares belong to the order Lagomorpha. This order was recognized in 1912, when a review by Gridley separated lagomorphs from rodents (order Rodentia), to which they were previously allocated. The distinction is based on some morphological characters, as the presence of a second set of incisors teeth (named peg) behind the upper front incisors in lagomorphs. An elongated rostrum of the skull ***(Flux and Angermann, 1990***) and the presence of a leporine lip are other characteristic anatomical traits of Lagomorpha in comparison with Rodentia. Within Lagomorpha, two families are currently recognized, Ochotonidae and Leporidae. While the former is a monotypic family harbouring the genus *Ochotona* (pikas), the latter contains eleven genera, divided in true rabbits (ten of the eleven genera) and true hares (genus *Lepus).*

The species of the family Leporidae have long hind legs and large movable ears, being adapted to quick movement and flight from danger, as well as large eyes, suited to their crepuscular and nocturnal habits (***Flux and Angermann, 1990***). The genus *Lepus* comprises jackrabbits and hares, with some controversy in the number of species. According to ***Flux*** and ***Angermann*** (***1990***), this monophyletic genus has 29 species, but depending on the authors, the number varies between 18 and more than 30. A more recent survey by ***Wilson and Reeder (2005)*** considers the existence of 33 hare species. The genus *Lepus* is very homogeneous in cytogenetic characteristics, with every species displaying 2n=48 chromosomes and identical G-banded karyotype (***Robinson and Matthee, 2005***). Indeed, the level of variability in cytogenetic markers is generally very low in the Leporidae, suggesting a fast expansion of this family. Within Lagomorpha, the most exclusive characteristic of hares is the fact that their young are born fully furred, with their eyes open and ready to move within minutes (***Corbet, 1983*****).**Compared with rabbits, this feature leads to a difference in behaviour, since rabbits build nests or elaborate warrens in order to protect their young while hares will only use a shallow depression (***Flux and Angermann, 1990***).

Lepus is a widely distributed genus. In fact, hares are the lagomorphs with the most widespread natural distribution, occurring in North and Central America, Europe, Africa and Asia (***Flux and Angermann, 1990***). In general, hares are open country grazers, and so have benefited from habitat changes caused by traditional agriculture. Hare species fit Bergmann's rule, which states that animals tend to be larger in colder regions, in higher latitudes, presumably for reasons of thermoregulation. Another latitude-related characteristic is the fur coloration. The alpine or northern species of hares

change from a darker colour to white in the winter, while the rest of the species have relatively similar "agouti" colorations in various shades of brown on the back and white or pale buff below ***(Flux and Angermann, 1990***).

The genus *Lepus* includes hares and jackrabbits. Due to their exemplary ability of adaptation and some specialized physiological characteristics, members of this genus are spread on all continents except for Antartica (***Chapman and Flux, 2008; Mengoni, 2011; Ristić et al., 2012***). These species have a wide spectrum of phenotypic variations. Additionally, hare furs are valuable commercially and their hairs are used as a disgnostic tool in ecology, wildlife biology, and nature management (***Hausman, 1920; Nowak, 1999; Teerink, 2003***). Brown hare (*Lepus europaeus* Pallas, 1778) the most widespread across the world, is the single hare species distributed in Turkey, the Turkish brown hares (***Kasapidis et al., 2005; Chapman and Flux, 2008; Demirbaş, 2010; Demirbaş et al., 2013***). For instance, ***Chapman and Flux*** (***1990***) suggested 30 subspecies and ***Hoffman and Smith*** (***2005***) found 15 subspecies of *Lepus europaeus*.

2.4 Sexual Dimorphism

Is a phenotypic differentiation between males and females of the same species; this differentiation happens in organisms that reproduce through sexual reproduction, with the prototypical example being for differences in characteristics of reproductive organs. Other possible examples are for secondary sex characteristics, body size, physical strength and morphology, ornamentation, behavior and other bodily traitssuch as ornamentation and breeding behavior found in only one sex imply that sexual selection over an extended period of time leads to sexual

dimorphism (***Dimijian, 2005***). Body mass variation was analyzed separately for males and females; as females are heavier than males the two sexes might differ in their energetic ecology (***García-Berthou, 2001***).

That originated before the dispersal of *L. europaeus* in Western Europe. *L. europaeus* probably originated from an African ancestor and then spread to Europe, perhaps recently and by two different settlements: evidence for the first settlement would be the oldest haplotypes found in three altitude zones in the Apennines. In historical times and in particular during the last century, there has been a massive spread of individuals with different haplotypes from Europe and South America, due to the translocation of hares for hunting purposes. DNA sequences have become the most frequently used taxonomic characters to infer phylogenetic history (***Hillis et al., 1996***) and *mt*DNA is a highly sensitive genetic marker suitable for studies of closely related taxa or populations of a variety of species (***Sunnucks, 2000***).

2.5 The coloration of Lagomorph

2.5.1 Behavior and Physiological Adaptation

Lagomorphs' pelage coloration was matched to habitat type, geographical region, altitude and behavior. First, overall body coloration across lagomorphs tends to match the background as shown for pale and red coloration and perhaps seasonal pelage change. The case for counter shading being a methodof concealment is far less strong. Second, ear tips appear to have a communicative role since they are conspicuous in many different habitats. Third, hypotheses for tail tips having a communicative role, for extremities being dark for physiological reasons, and for Gloger's rule received only partial support (***Chanta et al., 2003***).

Detailed accounts of species natural histories have pointed to the importance of camouflage, communication and physiological processes as evolutionary

causes for coloration patterns in mammals ***(Cott, 1940).***Explored four mechanisms contributing to an animal's concealment: general colour resemblance, variable colour resemblance, obliterative shading, and disruptive coloration. General colour resemblance (background matching) refers to situations in which an animal's coloration generally resembles that of its surroundings (***Cott, 1940; Kiltie, 1989***). Variable colour resemblance occurs when an animal's coloration alters with its changing surroundings. In some mammals, this colour change occurs seasonally. For example, some mammals that live in regions subject to seasonal snowfall(mountain hares, *Lepus timidus*) mould into a white pelage in winter, presumably to blend in with the white environment. Obliterative shading, or 'counter shading' (***Thayer, 1909***), refers to pelage coloration where an animal sports a ventral surface lighter than its dorsum, which is thought to counteract the dark shadows cast upon the animal's lower body by the sun (***Kiltie, 1988***). A possible example in lagomorphs comes from the Sumatran rabbit (*Nesolagus netscheri*) which displays striking dark stripes over its shoulders and across its back (***Surridge et al., 1999***). Coloration may also play a role in communication. For example, some species, such as the arctic hare (*Lepus arcticus),* mould into a white pelage in winter but retain their conspicuous black ear tips. It is possible that these black ear tips are used for signaling since ***Holley (1993)*** has argued that European hares (*Lepus europaeus*) signal to foxes (*Vulpes vulpes*) that they have seen them by standing upright with ears held erect. Similarly, conspicuous white or dark tails may be used to signal to predators or to conspecifics since they are prominent when viewed from behind during flight. ***Poulton (1890)*** suggested that the rabbit's white tail shows conspecifics the way to a burrow. Coloration may also be related to physiological processes. As examples, the presence of dark

coloration on ear tips and tails in many mammals could be related to conditions associated with low temperatures and Gloger's rule states that dark pelages are found in moist, warm habitats, although the underlying mechanism for this association remains unclear (***Gloger, 1833; Huxley***, ***1942***). The subspecies have been distinguished by differences in pelage colouration, body size, external body measurements, and skull and tooth shape (***Suchentrunk et al., 2003***). Unlike most mammals, females are usually larger than males.

2.6 Genetics Characterization

A recent phylogenetic study suggests that brown hare (*Lepus europaeus*) originated in Anatolia (***Mamuris et al., 2010***). Turkish brown hares have high genetical and morphological variations because Anatolia has been a host for hares of north latitudes during the latest Pleistocene and early Holocene, owing to its suitable climatic conditions and biogeographic location (***Kasapidis et al., 2005, Sert et al., 2009; Demirbaş et al., 2012***).

Five species of genus *Lepus* occur naturally in Europe: *L. europaeus*, *L. timidus*, *L. granatensis*, *L. corsicanus*, and *L. castroviejoi*. Of these, the latter two have restricted ranges, *L. castroviejoi* in the Iberian Peninsula and *L. corsicanus* in central and southern Italy.Morphological data show that *L. castroviejoi* and *L. corsicanus* have extensive phenetic similarities, and might be sister taxa, which seems to be supported by a close genetic relationship at the mitochondrial DNA level. This marker also suggests a strong genetic similarity between both *L. castroviejoi* and *L. corsicanus* and *L. timidus*.However, *mtDNA* introgression seems to be a common phenomenon in hares and may confound any phylogeny based solely on this type of marker.***Paulo and Melo-Ferreira (2007)*** confirms a very close relationship between *L. corsicanus* and *L. castroviejoi*, whereas the other

species are phylogenetically clearly separated from each other. *L. corsicanus* and *L. castroviejoi* have a strong genetic similarity, supporting the hypothesis that these species are most likely conspecific.

Currently, all hares from North Africa are considered cape hares (*Lepus capensis L.*, 1758), except for one isolated population of savanna hare (*Lepus victoriaeThomas*, 1823) in north-western Algeria. Some partial mitochondrial (*mt*) cytochrome *b* (cyt *b*) gene sequences suggest that hares from at least some regions in North Africa (Morocco) might represent a species (*Lepus mediterraneus*) different from *L. capensis* (***Pierpaoli et al., 1999; Alves etal., 2003***). Similarly, a restriction fragment length polymorphism (RFLP) analysis of total mtDNA revealed distinct evolutionary divergence between some Moroccon hares (*L. capensisschlumbergeri*) and Spanish brown hares (***Perez-Suarez et al., 1994***). Several molecular studies have been conducted so far to assess the genetic diversity and the phylogenetic relationships of the European hare species (***Thulin et al., 1997; Pierpaoli et al., 1999; Suchentrunk et al., 1999; Alves et al. 2000; Estonba et al. 2006; Ben Slimen et al., 2005).*** However, only ***Alves et al.,*** (***2003***) included all five species simultaneously and analyzed both mitochondrial DNA (*mt*DNA) and a nuclear marker (the transferrin gene). Apart from the latter study, the most comprehensive analyses of the evolutionary relationships among European hare species focused on RFLPs of the total *mt*DNA (***Pérez-Suárez et al., 1994***) and on *mt*DNA control region and cytochrome *b* sequences (***Pierpaoli et al., 1999***). The former, however, did not include *L. corsicanus* and *L.timidus*, and the latter did not include *L. castroviejoi*, but suggested that *L. corsicanus* is clearly different from *L. europaeus* as it was traditionally classified (***Flux and Angermann, 1990***).

On the other hand, analysis based on *mt*DNA by ***Alves et al***.,(***2003***) showed that these two taxa are closely related to *L. timidus* (2.2%-2.7% of divergence), and that the level of differentiation between them is very low when compared with the typical levels among hare species (circa 1.4% *vs.* 9% average between *Lepus* species). Moreover, these authors suggested that this *mt*DNA similarity to *L. timidus* could be due to ancient mitochondrial introgression similar to the one that occurred into the Iberian species (***Melo-Ferreira et al., 2007***).

These *mt*DNA resemblances led ***Wu et al.,*** (***2005***) to suggest that both *L. castroviejoi* and *L. corsicanus* should be considered subspecies of *L. timidus*. However, this work did not consider that the *mt*DNA in hares seems to be the subject of recurrent introgression either due to ongoing or ancient contact and hybridisation (***Thulin et al., 2006; Alves et al., 2006***). Thus, within genus *Lepus*, the analyses based solely on *mt*DNA sequences can be misleading and only data from several unlinked markers should produce reliable estimates of the phylogenetic relationships (***Robinson and Matthee, 2005; Alves et al., 2006; Ben Slimen et al., 2007***).

In ***1999 Pierpaoli et al.***, assessed the genetic distinction of *L. corsicanus*, investigated the genetic variation among populations of the peninsula and Sicily, and reconstructed the phylogenetic relationships between the Italian hare and other species of hares from Europe and Africa. This research, based on mitochondrial DNA (*mt*DNA), has provided the first evidence that *L. corsicanus* is genetically distinct and deeply divergent from the other Eurasian and African.In addition it was shown that Italian and European hares did not share any mitochondrial haplotype, suggesting the absence of interspecific flow past a long separate evolutionary history between the two

species and reproductive isolation. From the study of the Eurasian and African hares we can identify two main groups of haplotypes:

- Group A: includes *L. granatensis, L. corsicanus* and *L. timidus*.
- Group B: includes *L. c. mediterraneus*, *L. habessinicus*, *L. starcki*, *L. europaeus*. These results suggest that the three species belonging to group A, with a common ancestor, would have colonized Europe independent of *L. europaeus* and would have originated for isolation during the Pleistocene glaciations in the southern or northern areas of refuge. A surprising result is the close relationship between the Italian hare and the Mountains hare: times of divergence and bio-geographical structure of the evolution of the genus *Lepus* in Europe indicates that *L. corsicanus* and *L. timidus* are relict species that originated before the dispersal of *L. europaeus* in Western Europe. *L. europaeus* probably originated from an African ancestor and then spread to Europe, perhaps recently and by two different settlements: evidence for the first settlement would be the oldest haplotypes found in three altitude zones in the Apennines. In historical times and in particular during the last century, there has been a massive spread of individuals with different haplotypes from Europe and South America, due to the translocation of hares for hunting purposes. DNA sequences have become the most frequently used taxonomic characters to infer phylogenetic history (***Hillis et al., 1996***) and *mt*DNA is a highly sensitive genetic marker suitable for studies of closely related taxa or populations of a variety of species (***Sunnucks, 2000***).

2.7 Morphological Characterization

Krunoslav et al., (2104) suggested that the variations of morphological features might reflect genetic variations, but considering habitat type, variations of morphological features are caused by species adaptation to climatic conditions and habitat type. Despite these earlier controversies, the

latest taxonomic view accepts five species of genus *Lepus* occurring naturally in Europe: *L. europaeus, L. timidus*, *L. granatensis*, *L. corsicanus*, and *L. castroviejoi*. Of these, the latter two have allopatric and very restricted ranges, the broom hare (*L. castroviejoi*) occurring in the Cantabrian Mountains of the Iberian Peninsula, and the Italian hare (*L. corsicanus*) being present in the Apennines from central and southern Italy, and also in Sicily.

Based on morphological data results correspond to the classification of North African hares into two or more species, such as *Lepus atlanticus* de ***Winton, 1898*** from Morocco and *L. capensis* (with many subspecies) by ***Ellerman and Morrison- Scott*** (***1951***). However, they contradict ***Petter's*** (***1959, 1961, 1972***) concept that all these hares belong to *L. capensis*. The latter author even included brown hares (*Lepuseuropaeus* Pallas, 1778) from Europe and other parts of the western Palearctis (Anatolia etc.) into *L. capensis*. ***Angermann*** (***1965***) too, based on morphological and morphometric data, considered hares from northern Tunisia very similar to brown hares, but was later on (***Angermann, 1983***) this seemed more insecure. The current taxonomic view lists brown hares as a species separate from *L. capensis* (***Flux and Angermann, 1990; Wilson and Reeder, 1993; Nowak, 1999***).

Indeed, recent data show a clear morphometric differentiation between *L. corsicanus* and *L. europaeus* (***Riga et al., 2001***). Nonetheless, in a previous morphological study on the Italian hare, ***Palacios*** (***1996***) showed that despitesome morphological peculiarities, *L. corsicanus* had extensive phenetic similarities to *L. castroviejoi*, and both were clearly different from *L. europaeus*, suggesting that *L. corsicanus* and *L. castroviejoi* might be sister taxa.

2.8 Skulls Characterization of Hares

The term skull has been used to describe the entire skeleton of the head. The skull is both a highly modular and a highly integrated structure. The skull is divided into three primary units, the face, neurocranium and basicranium. The brain case provides protection for the brain and opening for cranial nerve connections, the bone of the face provide a location and protection for the organs of special senses and openings for the digestive and respiratory system. The skull is a mosaic of many bones, mostly paired, but some median and unpaired, that fit closely together to form a single rigid construction (***Reece, 2009; Dyce, 2010***). The shape of the head and skull influence the dynamic of the locomotion and balance. The specific characteristics of a skull often reflect the animal methods of feeding and effect on the muscle of mastication (***Olude and Olopade, 2010***). Skulls differ largely, not only between different species and breed but also between individuals of same breed, age and sex (***Koing and Liebich, 2004***). Craniometric studies of the skull of different animal species continue to be a growing area of applied research, the values obtained from such studies a, apart from being important in osteoarcheological and morphological fields, improve clinical diagnosis and regional anaesthesia of the head and treatment of cranial skeletal disorders (***Shawulu et al., 2011; Yahaya et al., 2011***).Historically, subspecies of hares were classified based on the morphological features of the skull and teeth (***Suchentrunk et al., 2003; Palacios et al., 2008***). Besides morphometric, application of molecular methods over the last years contributed also in elucidating the systematics and distribution of subspecies.

2.9 Relationship between Environmental Factors and Morphological Characterization

Environmental factors are one of the important determinants of postnatal skull ontogeny (***Hall, 1990***) and final size **(*Yom-Tov and Geffen, 2006***). The body and skull sizes of animals are usually considered positively correlated with a decrease in temperature. This is known as Bergmann's rule. Although body mass is the most common reference point for size (***Meiri et al., 2004***), food availability and fasting endurance are the main determinants of body size (***Millar and Hickling, 1990***), and seasonal changes in body mass have been observed in many mammals species. Thus, unlike body mass, the skeleton of mammals is a comparatively stable feature. ***Liao et al.,*** (***2006***) revealed that Bergmann's rule is not universally valid for interpreting animal body size clines, particularly in large mammalian species. On the contrary, ***Yom-Tov,*** (***1967***) stated that Israeli hares showed direct clinal variation from south to north in body and cranial measurements depending on the mean annual temperature and precipitation. ***Sert*** (***2006***) recorded that condylobasal length shows a significant variation in specimens separated by distance (between Europe, Anatolia and South African populations), and it reaches the highest value in the Europe, which has the lowest mean temperature. ***Temizer and Onel*** (***2011***) determined that there was no difference in terms of cranial measurements between Malatya and Elazığ specimens in Anatolia, where the populations are close to each other. ***Mitchell-Jones et al.,*** (**1999**) reported that *Lepuseuropaeus* subspecies in Europe have different coat color types. ***Suchentrunk et al.,*** (***2000***) discussed the effects of ecogenetic factors on coat color and body size in Israeli hares. The authors stated that regional variations in their external

appearances, such as coat coloration, fur texture, body size and ear length, are governed mainly by ecogenetic factors, and Israeli hares have retained a broad phenotypic plasticity in external appearance.

Demirbaş et al., (***2013***) determined that specimens of Turkish hare varied in types of coat color, body weight and hind-foot length depending on geography, body weight and measurements were even observed in different geographical regions. These differences might be based on polymorphism. Moreover, the morphometric analysis confirmed that they were all *Lepus europaeus*, despite any variations in pelage coloration reflecting local adaptation. The hares of Turkey have comparatively diverse seasonal and regional coat color types; however, winter coat colors differ slightly from summer coat color. They presumed that the diversity and admixture observed in the same region and between geographic regions in terms of coat color types in Turkish hares might be a clear signal of different gene flows into Anatolia from neighboring regions (***Demirbaş et al., 2013***). ***Sert et al.,*** (***2005***) suggested that there was little genetic differentiation between the two forms with different coat color (brownish and yellowish ones) in Anatolian hares. On the other hand, ***Demirbas et al.,*** (***2010)*** recorded that the yellowish samples in southeastern Anatolia have a low-level chromosomal difference from the brownish ones. ***Sert et al.,*** (***2005***) pointed out that Anatolian hares have a high genetic diversity. This information may be also confirmed through differences in coat color. Namely, these differences in coat color may reflect different gene pools in Anatolia.

Hares (genus *Lepus*) were a taxonomically notoriously difficult group, mainly due to high degree of morphological variations and the potential of rapid adaptation to the environmental factors. They can live in a variety of terrestrial ecosystems due to their high adaptability (***Flux and Angermann,***

1990).Hares from Turkey are considered as European hares (*Lepus europaeus*) and initial molecular and morphometric analyses (***Sert et al., 2009; Demirbaş et al., 2010, Demirbaş et al., 2013***) found the Turkish hares varied in types of coat color, body weight and hind-foot length, depending on geography, and similar variations in coat coloration, body weight and measurements were even observed in different geographical regions; It is assumed that all these differences might be based on polymorphism. Moreover, the morphometric analysis confirmed that they were all *Lepus europaeus*, despite any variations in pelage coloration reflecting local adaptation.

The leporids express significant variations of morphological features under the influence of environment and diet (***Yom-Tov and Geffen, 2006***). Due to great variations within genera, some authors assume that the phylogenesis and systematics of hares has not been completely clarified (***Chapman and Flux, 1990; Pierpaoli et al., 2003; Ben Slimen et al., 2008***).***Xin*** (***2003***) suggests that analysis of skull development between different animal species exposed to different selection pressure can contribute to understanding of geographical variations of particular populations, as well as life history strategies and evolutionary change.

Mammals' body size is most frequently described by means of body weight and certain linear measurements- usually the body length; other measurements, like sternum length or chest circumference, are less often used (***Szuba et al., 1988; Schmidt-Nielsen, 1994***). In small mammals, apart from body weight and body length, ear height and hind foot length measurements are also used (***Pucek, 1984***). The mentioned parameters are used in systematic, morphological, ontogenetic, and ecological studies;

sometimes also in establishing appropriate relations of allometric character (***Gould, 1966; Szuba et al., 1988; Reiss, 1991***).

Temporal and geographical variation in body size of animals is a common phenomenon, and has been related to many factors (***Yom-Tov and Geffen, 2011***). Among these factors is predation, ambient temperature, fluctuations in various climatic phenomena including climate change, interspecific competition and food availability (***Grant and Grant, 1995; Yom-Tov, 2003; Yom-Tov et al., 2003; Ozgul et al., 2009***). Observed reduction in body size of many species was generally attributed to global climate change (***Gardner et al., 2011; Sheridan and Bickford, 2011***). On the other hand, an increase in body or skull size was attributed to increased food availability, either by human activity or higher primary productivity in northern latitudes (***Yom-Tov and Geffen, 2011***). Recently, ***McNab*** (***2010***) argued that the tendency of mammals to vary in size depends on the abundance, availability and size of resources, and termed this pattern the "resource rule". Among mammals, food availability, especially during the growth period, is a key predictor in determining final body size. Quantity and quality of nutrition during this period affects growth rates and final body size, and these effects on skeletal size carry over into adulthood (***Read and Gaskin, 1990; Ulijaszek et al., 1998; Ohlsson and Smith, 2001; Ho et al., 2010***). Food availability is influenced by both biotic and abiotic factors and fluctuates accordingly in time and space, in turn affecting body size. In Israel, man-made food resources, such as from garbage and agriculture, have increased greatly during the last 60 years.***Yoram and Shlomith*** (***2012***) found that skull size of the red fox increased significantly during the 20th century, possibly due to improved food availability from man-made resources such as agricultural produce and garbage. No temporal trend in body size was detected for the

jackal and hare.***Scholander*** (***1955***) argued that heat dissipation and conservation are not as efficient as other mechanisms such as vascular control and fur insulation. Others have suggested that body size is better correlated with basal metabolic rate, cost of transport, dominance in a community, success in mating, size and type of food, and competition (***Calder, 1984; Schmidt-Nielsen, 1984; Dayan et al., 1989***). ***James (1970)*** suggested that body size variation is related to a combination of climatic factors, mainly moisture and temperature, and that small body size is associated with hot and humid conditions and larger size with cooler and drier conditions. ***Roedenbeck and Voser*** (***2008***) found that edge habitat, especially in conjunction to forest, is the most important habitat factor for hares, and lack of shelter possibly limit juvenile survival in some habitats (***Smith et al., 2004; Jennings et al., 2006***).

CHAPTER THREE

3. Material and Methods

3.1 Base of the Study

This study was conducted in three ecologically isolated regions. These are the western region separated by the White Nile and the River Nile, the eastern region separated by the Blue Nile and the River Nile and the region lying between the Blue and the White Niles as shown in (Fig. 3.1).

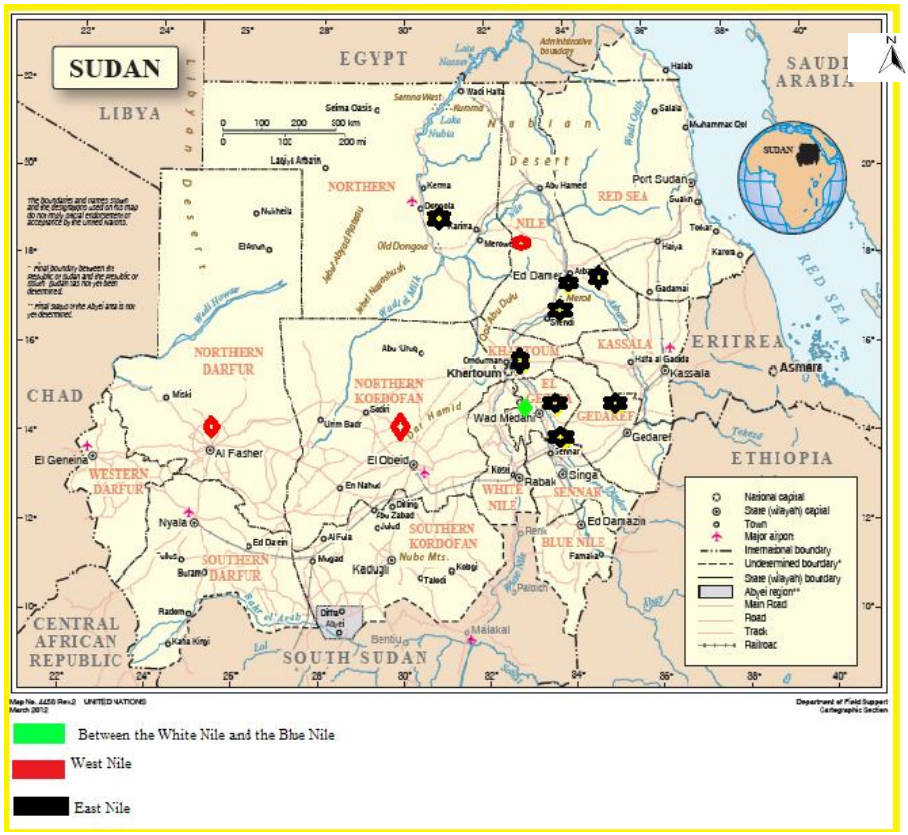


Figure 3.1: The signs show the three geographic regions lying west of the Nile, between the White and the Blue Niles and East of the Nile.

(Soures : https://en.wikipedia.org/wiki/Sudan.)

3.2 Study Area

Climate Sudan is divided to seven zonesaccording to Sudan Meteorological Authority (http://www.ersad.gov.sd/seasonalar)as shown in (Fig 3.2). Rise in temperature in all parts of the Sudan in the period from March to July, is rated42 °C at day time and 23°C at night. Low temperatures in the period from November to February are up to 30°C at noon and 16 °C at night, especially in the North as shown in (Appendix 1). Rainfall rate ranges between 75 to 300 mm in the central regions, 400 to 800 mm in the Southern and Alooasit regions, and 800 to 1500 mm in the tropics as shown in (Appendix 2). The most important soil types for farming in Sudan are dominated by expanding clay. Such soils cover most of central Sudan and the Eastern plains. They are calcareous and moderately well drained, but generally contain little nitrogen. The Eastern plains lying North, Southeast and East of the Gadaref town and the central plains from Gezira to Rosaries on the Blue Nile, are the best example of soils: loam, deep and generally well drained. The soils of the Northern agricultural zones along the flood plain of the Nile are Loam, stratified and calcareous (***Osman, 2005***). ***Harrisonand Jackson*** (***1958***) classified the vegetation cover of the study area into three major vegetation zones: semi desert vegetation cover in the north followed by low woodland savannah in the central part of the state and high woodland savannah in the far south.

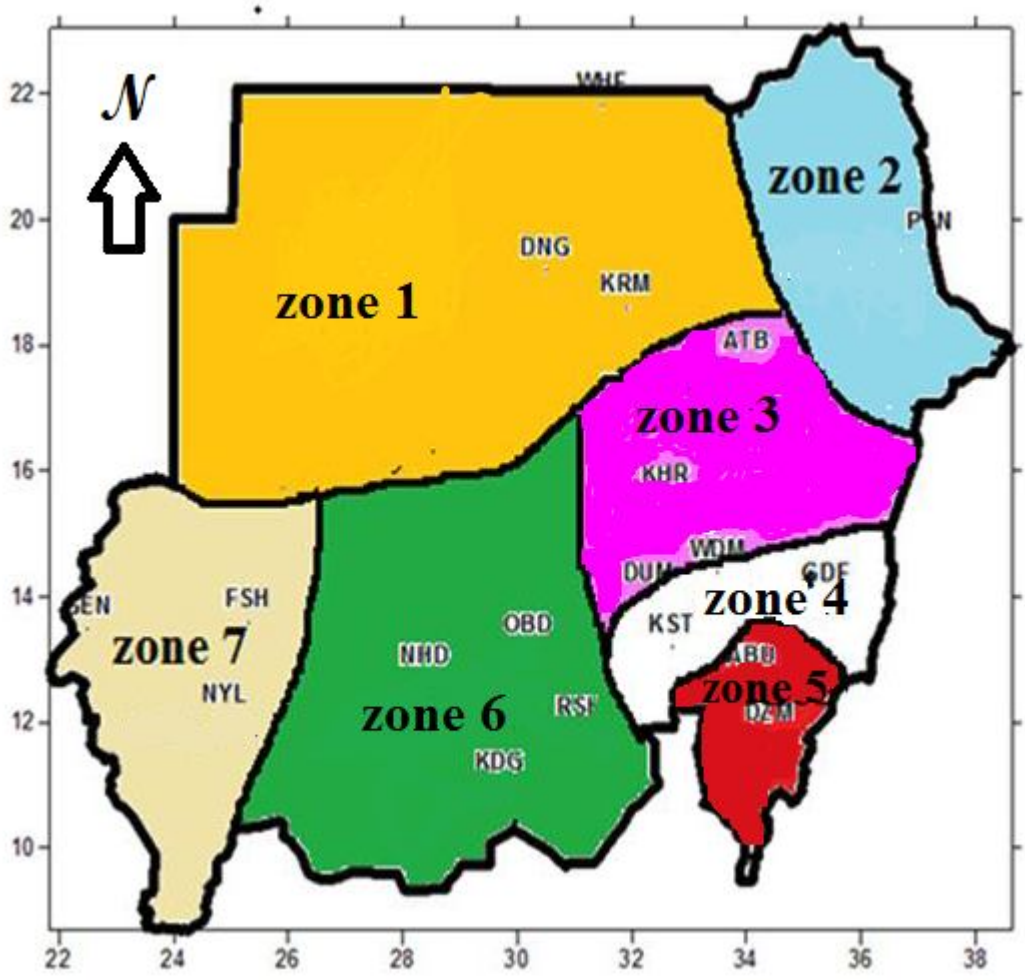


Figure (3.2): Climatic zones in Sudan where samples were collected. Sours. : http://www.ersad.gov.sd/seasonalar (see Appendices1 and 2 for rain fall and temperature data)

3.2.1Eastern Region

3.2.1.1Dongla Area

Is located at Northern state of Sudan (zone 1). It is Lay between latitudes 16-22° North and longitudes 32-35° east. It is characterized by a desert climate with very low and irregular rainfall, very hot summers and cool winters. With the short rainy season and the highest average the temperature apart from the winter months (***El Belattagey, 2000***).The vegetation caver includes

Acacia ehrenbergiana (Salm), *Moura crassifolia (*Sarah*)* and*Acaciatortilis*(Samar)

3.2.1.2Aldamer Area

It is located at River Nile State (zone 1)between Latitudes 16-22°N, and Longitudes 32-35°S. The annual average of maximum temperature is 42.6 C° while the maximum temperature is 47C° (May-June) and the annual average of minimum temperature is 29C° while its minimum temperature is 8C° (January-February). *Acacia ehrenbergiana* (Salm), *Moura crassifolia (*Sarah*)* and *Acaciatortilis*(Samar), *Capparis decidua* (Tondub), *Acacia seyal* (Taleh), *Prosopis juliflora* **(**Mesquite**)** the area covered Alfalfa and Potato.

3.2.1.3 GadarifArea

Is located in the eastern part of Sudan (zone 4)between 33-37° E and Longitudes 12-16° N. The state is bordered to the east by Ethiopia and Eritrea. The mean temperature is 29°C, the mean maximum is 37°C and the mean minimum is 21°C. The annual rainfall in the area ranges between less than 300mm in the North to more than 800mm in the South.The dominant soil in the study area is dark, heavy, deep clay belonging to the vertisol ***(Sulieman and Elagib, 2012***). The vegetation includes, Acacia *mellifera* (Kitir); *Acacia oerfota* (Laout); *Acacia Senegal* (Hashab).

3.2.1.4 Wad-Ais Area

The area is located near Singatownat Sinnar State (Zone 4). It is located in an area (12.7653° N, 33.6176° E). The State lies within the low rainfall Savanna zone, where the average annual rainfall is between 400-600 mm, while the Southern part lies in the high rainfall Savanna zone, with the average annual rainfall of about 800mm. The mean temperature ranges from 35°C to 40°C in summer and from 20°C to 25°C in winter (***Abdelaziz, 2010***).The vegetation is a complex mixture of grasses, herbs and woody

species.*Acacia oerfota*(Laout);*Acacia mellifera* (Kitir)*Capparis decidua*(Tondub);*Acacia nilotica* (Sonut); *Ziziphus spina-chiristi* (Sedir), Damblab (*Aristida mutabilis*) and *Trianthma pentndra*(Rabaa). Also the area included the residue of many crops (sorghum, onion and watermelons).

3.2.1.5 Algayle Area

Is located at Northern of Khartoum state (Zone 3)the coordinates (14°49'48"N 33°14'40"E) with an annual average rainfall of 160 mm. Temperatures peak at the end of the dry season. During the warmest months (May and June), the temperature can reach 48 °C contributing to average highs of 41°C (***Metz, 1992***). The plant includes; *Acacia mellifera* (Kitir) and *Acacia oerfota* (Laout), vegetablesand onion.

3.2.2 Western Region

3.2.2.1Aldamer Area

It is located at River Nile State (Zone 1)between Latitudes 16-22°N, and Longitudes 32-35°S. The annual average of maximum temperature is 42.6 C° while the maximum temperature is 47C° (May-June) and the annual average of minimum temperature is 29C° while its minimum temperature is 8C° (January-February). In general, this season is characterized by being short and warm in Sudan. However, the River Nile State has relatively cold and long winters. *Acacia tortilis*(Samar),*Capparis decidua*(Tondub),*Lpomoea cardofana (*Tabar)and thecrops are including wheat, legumes and vegetables.

3.2.2.2Rahad Abu-Dakana

It is located at Northern Kordofan State (Zone 6)is located 12° 42' 56" N, 31° 31' 29" E lies in the arid and semi-arid zones the region in rainfall which generally vary from 150-450 mm/year. The region is bordering the desert zone, there is a persistent threat associated with shifting

sand *Acacia mellifera* (Kitir)*Acacia seyal*(Taleh) and*Acacia Senegal*(Hashab).

3.2.2.3 Alfasher Area

Alfasher situatedat North Darfur state (zone 7) at latitude 13°37'N and longitude 25°22' E. The average maximum monthly temperatures vary from 32°C in Dec. and Jan. to 40°C in May and June while the average minimum monthly temperatures lie between 10 and 20°C. The average Rainfallannual precipitation in North Darfur State ranges from zero in the north to some 600 mm in the south, the rainy season in the state extends from June to September every year (***Institute of Environmental Studies(I.E.S.)1988***).The plants included; Acacia Senegal (Hashab); *Acacia mellifera* (Kitir);*Balanites aegyptica* (Heglig), *Cucumis melo* (Humaidh).

3.2.3 Between the White and the Blue Niles Region

3.2.3.1 GanibArea

It is located at the Gezira states (Zone 3)and lies between the Blue Nile and the White Nile in the east-central region of the country Coordinates14°49'48"N 33°14'40"E. the soil type the heavy dark clay. Dominant vegetation are; *Acacia mellifera* (Kitir); *Acacia oerfota* (Laotu);*Capparis decidua*(Tondub);*Acacia nilotica* (Sonut); *Ziziphus spina-chiristi* (Sedir).*Dinebra retroflexa* (Um mamlaiha), *Cyperus rotundus* (Seida), *Cajanus Cajan* (pea) and*Sorghum vulgar*(AbouSabeen) Cassia *senna* (Sana Maka). Also the area closed to Blue River include the residue of many crops (onion, Watermelons and vegetables).

3.3 Samples' Collection

Ninety sex of hares were collected from three geographical regions from Sudan (Table 3.1) after permission from the Wildlife Conservation General Administration (WCGA) the hares were shot by local hunters during the

period 2012 to 2015. Specimens collected for the present purpose were weighed and measured in the field, or immediately after they were brought to the laboratory of Fisheries in College of Animal Production Science and Technology (SUST).Tissues samples for DNA extraction were cut from liver, kidney, heart or ear of individual hares either immediately in the field or when the specimens were brought to the laboratory. The tissue was transferred to an “Eppendorf” test tube containing 80% ethanol, and then stored in Fridge until Carried to Liebniz Institute for Zoo and Wildlife Research (IZW) in Berlin.

Table 3.1: Sample size collected from three geographic regions in Sudan..

Geographic region	Description	Females	Males
West of the Nile	Embraces the area west of the White Nile, and the River Nile.	9	8
East of the Nile	Embraces the area east of the, Blue Nile and the River Nile.	29	30
Between the two rivers	Embraces the area lying between the White Nile and the Blue Nile.	14	6

3.4 Lab Work

3.4.1 Morphometric

Tail length, ear length, total length, hind foot length, Length of font leg, Length of back leg, distance between ears, distance between eyes, Neck length, Height and Back length were measuredusing a tape according to ***Anthony andRobert*** (***1979***) and the weights were recorded from 96 fresh(immediately after collected) animals during field work and weighed by

digital balance to the nearest 0.05 mm (***Nagorsen, 1985; Harrison and Bates, 1991***).

3.4.2 Craniometric

Twenty measurements were taken from each skull (96 samples) using a digital Vernierto the nearest of 0.01 mm, following ***Palacios*** (***1996***). These measurements included, anterior nasal width (ANW); external nasal length (ENL); foramen incisivum length (FIL); foramina incisive width (FIW); facial tubercle length (FTL); internal nasal length (INL); lower cheek tooth-row length (LCTRL); upper cheek tooth-row length (UCTRL); mandible height (MH); mandible length (ML); palatal length (PL); posterior nasal width (PNW); post palatal width (PPW); posterior zigomatic width (PZW); rostral width (RW); smallest frontal width (SFW); tympanic bulla length (TBL); tympanic bulla width (TBW); total length (TL); and width between facial tubercles (WFT) (see Fig 3.3).

3.4.3 Dental Shape Examination

All the skulls of hares collected during the field work were examined after boiling for one hour and then all meat and muscles were removed from the bones by scalpel to examination the labial groove of the first upper incisorof the all skullsusing the Dissection Microscope(***Suchentrunk et al., 1991***).

3.4.4 Species Identification

According to the present collection, different morphological typesseen in plates (4.1, 4.2, 4.3,4.4 and 4.5) were identified from external morphological features. To diagnostic features for *L. capencies* were assessed of the ear, surface of the fur and upper and lowersidesand also tail sides (***Flux and Angermann, 1990***).

3.5 Molecular Analyses

3.5.1 Extraction of DNA

Total DNA was extracted from tissues (liver, kidney, heart and ear) of 94 animals, using standard extraction method, the all -tissue DNA Kit described by ***Gen-Ial***(***2007***). A piece of tissue sample was taken from each individual and put in 2 ml tube. Then 550µl lyse (500µl Lyse1 and 50 µl Lyse2) and 10µl Enzyme (proteinase k) were added, the material was completely covered by Lyse1 and Lyse2. The mixture was incubated overnight at 65°C. After that 375 µl Lyse 3(Ca 75 volume% of the Lysate) were added. Then the mixture was kept in freezer at (-15˚to-22˚C) for 5 minutes then centrifuged for 10 minutes at 13.525 rpm. Then 640 µl (0.8vol %) isopropanol was added, mixed carefully by inversion and carefully transferred 800µl from supernatant into new 2ml tube. The supernatant was incubated overnight at 4°C. The tubes were centrifuged again for 15 min at 13.525 rpm. Then the supernatant was completely removed and washed with 150 µl cold 70% ethanol and centrifuge for 5 minutes at 13.525 rpm to allow DNA pellet to dry at room temperate. Then the DNA pellet was dissolved in 100 µl ddH2O. The quantity of DNA was checked through spectrophotometer.

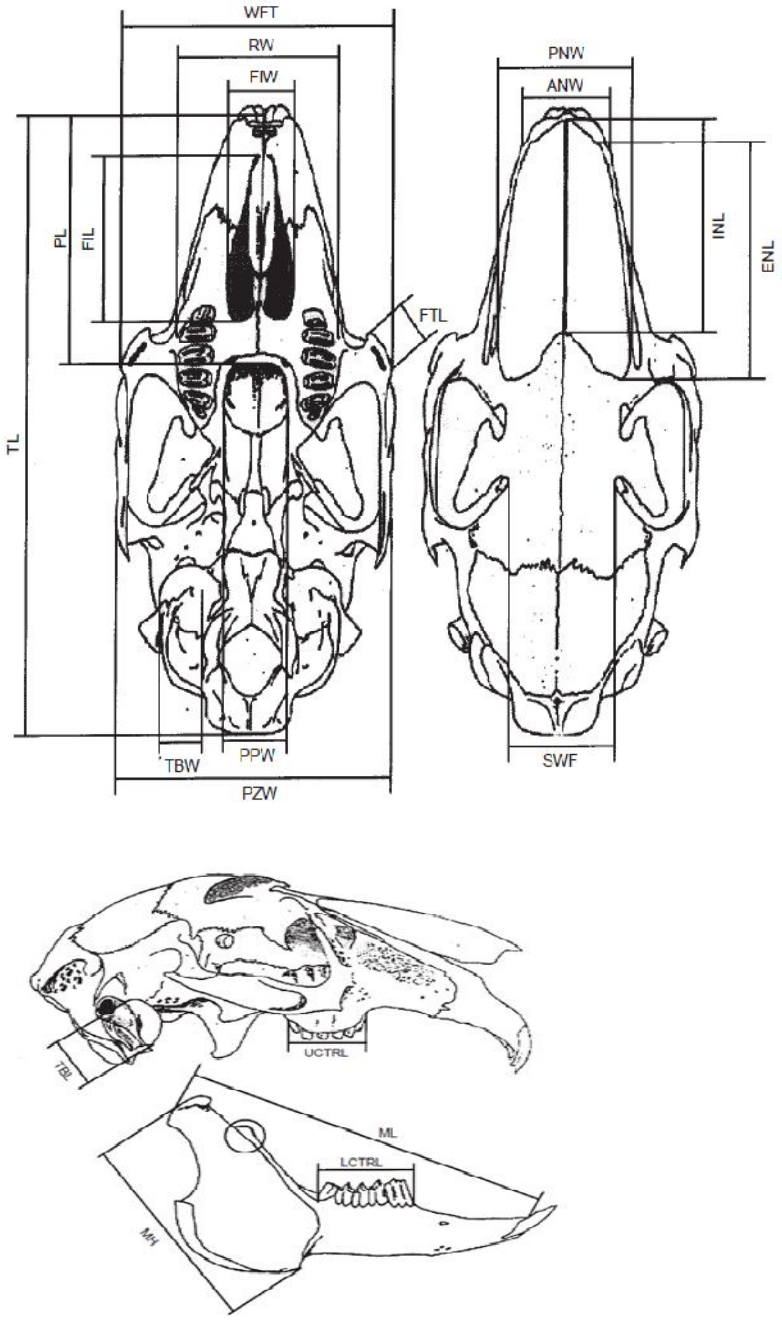


Figure 3.3: Characters measured on the skull and the mandible of hares (*Lepus spp*.). See text for explanation of abbreviations (***Riga et al., 2001***).

3.5.2 Sequences of Cytochrome b (Cyt b) and D-loop

From 94 specimens Cytochrome *b* (Cyt *b*) gene and control region (*D*-Loop) were amplified, using primers CB1L 5′CCATCCAACATCTCAGCATGATGAAA3′ and CB2H 5′ CCCTCAGAATGATATTTGTCCTCA3′ (***Kocher et al., 1989***) and LeCTR1L 5′ATCCAAGTAACTTGTCACTTG3′ and LeCTR2L 5′GGGCGATCTTAGGGTTATGG 3′ designed by ***Fickel et al.,*** (***1999***), respectively.

The PCR reaction following, the Promega manufacturers for master mix comprised the following: 1× PCR buffer, 2mM $MgCl_2$, 0.4µM dNTP-Mix, 1.25µM GoTaq DNA Polymerase and for each primer 0.5µM and 150µg template DNA. PCR amplification conditions were followed as: 95°C initial start for 2 Minutes, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 50°C for 30 sec and extension at 72°C for 30 sec, a final extension at 72°C for 7 minutes. To see the positive results of PCR products, 5µl PCR products and 1µl loading dye in 1.5% Agarose gel were loaded and run at Gel photograph (Mupid-one) for 18 minutes with 135V and binding DNA with an UV (Gel Red). PCR products were purified, using 5µl of a mixture included Exonuclease 0.1µl, Termosensitive 0.25µl and 4.65µl H_2O, using the following thermo cycling program: (37°C x 15 minutes → 80°C x 15 minutes) →4°C.

The PCR Sequencing was carried out by Big Dye^R Terminator v1.1 cycle sequencing ***kits protocol*** (***2007***) for 1µl of PCR product with 6µl master mixes, the mixer included, 1µl primer, 1µlBig Dye terminator, 1.5µl the buffer and 1.5µl H_2O,using the following thermo cycling program: (95°C x 10sec → 50°C x 5sec → 60°C x 1.5 minutes) for 18 cycles → 4°C.

The Big Dye^{R} XTerminator^{TM}purification Kit protocol (2010) were added to purified PCR sequences to finish the reaction with 35µl, the master mix included 29µl (SAM) solution Buffer and 6µl Big Dye and vortexes for all plats for 2 seconds.

The sequencing was carried out on an ABI (3130XlGenetic Analyzer Fig 3.4), using Big Dye version 1.1 (Applied Biosystems) to run capillary electrophoresis system after putting all plates with PCR sequence products to obtain and to recognize the sequences results. Then sequences data were checked carefully, queried by BLAST searches versus Gen-Bank to confirm homology.

Figure 3.4: The sequencing process allows to read sequences of DNA by theuse of automated sequencers (3130Xl ABI Genetic Analyzer).

3.5.3 Microsatellites Amplification

PCR products were analyzed, using the ***GIAGEN Multiplex kit*** (**2011**). Nine microsatellites were chosen for this study (Table 3.2), based on their known used Mix (1) (Sat8, D7UTR, Sol33), Mix2 (OCELAMB, Sol28, Sol3) and Mix3 (Sol8, Sol44, Sol3) table (3.3). 85µl 2xQiagen, Primer Mix17µl (BioTez), 42. 5µl and 1. 5µl PCR products. Sequences were analyzed using Gene Mapper analysis date collection v3.0, and genotyper1.1software. All PCR sequences started with decreasing the annealing temperature by 2°C, the cycling conditions were optimized for each multiplex, starting from the following general PCR program: 95°C x 5'→ (94°C x 30''→63 °Cx1' 30''→72°C x 30") → (94°C x 30'' →61°C x 1' 30'' →72°C x 30'') → (94°C x 30''→61° Cx1' 30''→72°C x 30'') → (94°C x 30''→59° Cx1' 30''→72°C x 30'') for one cycles→ (95°C x 30''→57° Cx1' 30''→72°C x 30'') 31 cycles→(95°C x 30''→52° C x 1' 30''→ closed 72°C x 30') for 4 cycles→.

All sequences were analyzed using Gene Mapper analysis, date collection v3.0, and genotyper1.1software.To Analysis phylogenetic 94 sequences were aligned to converted Cyt *b* (297bp) and D-loop (413bp) used Bio Edit v0.6. To test Phenotypes analyzed by MEGA to construct a neighbor joining tree based on Tamura-3 and 1000 bootstraps (TN92) HKY+G+I], $\alpha = 0.21$ distances and evaluated the robustness of the tree topology by bootstrap robustness of the tree topology by bootstrap support values .

Table 3. 2: Microsatellite loci used for genotyping of hares.

Locus	Size of Primer (bp)	Sequence (5'-3')	Reference
Sat08	20 22	F: CAGACCCGGCAGTTGCAGAG R: GGGAGAGAGGGATGGAGGTATG	Mougel *et al.,* (1997)
Sol03	24 24	F: TACCGAGCACCAGATATTAGTTAC R: GTTGCCTGTGTTTTGGAGTTCTTA	Rico et al. (1994)
Sol08	24 25	F: GGATTGGGCCCTTTGCTCACACTT R: ATCGCAGCCARATCTGAGAGAACTC	Rico *et al.,* (1994)
Sol28	23 25	F: ATTGCGGCCCTGGGGAATGAACC R: TTGGGGGGATATCTTCAATTTCAGA	Rico *et al.,* (1994)
Sol33	20 24	F: GAAGGCTCTGAGATCTAGAT R: GGGCCAATAGGTACTGATCCATGT	Surridge, (1997)
Sol44	22 22	F: GGCCCTAGTCTGACTCTGATTG R: GGTGGGGCGGCGGGTCTGAAAC	Surridge, (1997)
D7UTR	23 21	F: ACACCTGGGGAATAAACAACAAG R: GAGGGAGGCAGAGGGATAAGA	Korstannje *et al.,* (2003)
OCELAMB	22 24	F: AGTCACATTTGGCATTTCGTGA R: TCCTTTGAATTTAGGATCCACAGC	VanHearingen *et al.,* (1997)
OCLS1B	24 24	F: ACTGCTATATCAAAGGCATGACCC R: TCAGGTATTTGGAAAGTGAATCCC	VanHearingen *et al.,* (1997)

Table 3.3: Multiplex dye used for genotyping of hares.

Locus	Multiplex	Dye	References
Sat8	1	6FAM	Rico *et al.,(*1994)
D7UTR			Korstannje, (2003)
Sol33			Surridge,(1997)
OCELAMB,	2	HEX	VanHearingen, (1997)
Sol28			Rico *et al.,* (1994)
Sol3			Rico *et al.,* (1994)
Sol8	3	6FAM	Rico *et al.,*(1994)
Sol44			Surridge, (1997)
Sol3			Rico *et al.,* (1994)

3.5.4 Microsatellites Analysis

Microsatellite analysis consists in clearly separates 2 alleles present for six fragments in automated capillary sequencers the electrophoresis does not require the gel preparation because they could automatically inject it in a series of capillaries through which fragment migration takes place (mechanism of operation of an automated sequencer was described in the previous paragraph). When the labelled DNA fragment passes a pre-set location the fluorescent dye is picked up by a laser and the emission of fluorescence is detected and measured by the all sequences were analyzed using Gene Mapper analysis data collection v3.0, and genotyper1.1software. To see the different alleles in an image file and in an electropherogram in which the molecular weights of the alleles is precisely determined by the use of internal standards (Fig.3.5).

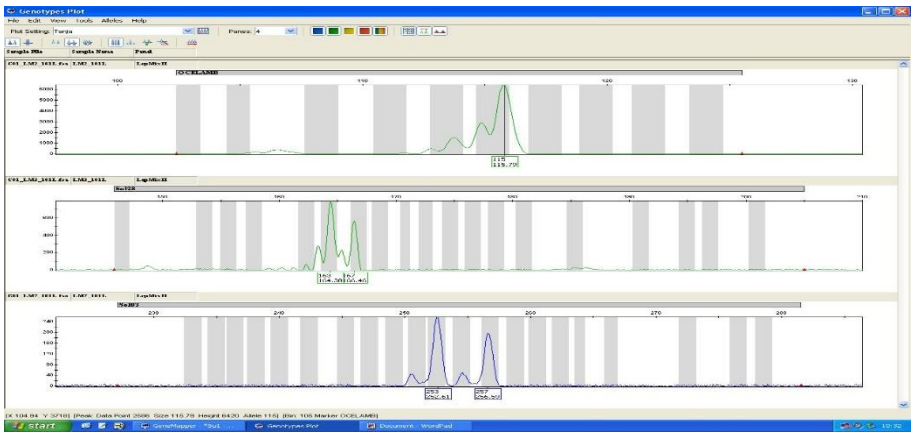


Figure 3.5: Gene Mapper analysis to see the different alleles in image.

3.5.5 Population Genetic

Allele frequencies, mean number of alleles (A), observed (Ho), expected (He) heterozygosity, as well as F*is* values (indicating a deficit of heterozygous genotypes) were calculated separately for each locus and for each sampling region, i.e., for samples from (1) East of the Nile, (2) between the two rivers and (3) West of the Nile with GENETIX 4. 05.02 (***Belhhir, 2004***). Numbers of alleles shared by all three regions, and numbers of "private alleles", i.e., those that occurred only in one of the three regions, were counted from the GENETIX output table.

Microsatellite loci were tested for deviation from Hardy-Weinberg equilibrium (HWE) using the Markov chain method implemented in GENEPOP version 4.5.1 (***Raymond and Rousset, 1995***) and the default parameter settings of 1000 demomorizations, 100 batches, and 1000 iterations per batch. The same program was also used to test for genotypic linkage disequilibrium (non-independence of loci) separately for each region, applying the following Markov chain parameters: 10000 dememorisation, 20 batches and 50000 iterations per batch. In those test runs

and in all further test series significance levels ($\alpha = 0.05$) were adjusted using strict Bonferroni correction for multiple comparisons (***Rice, 1989***).

The GENETIX software was used to calculate pairwise Cavalli-Sforza Edwards chord (CSE) distances (absolute genetic distances) between the three regions and to run a factorial correspondence analysis (FCA, ten factors) based on individual composite genotypes, to infer potential spatial differentiation between the three regions. The ARLEQUIN 3.11. software (by L. Excoffier 1998-2007: Computational and Molecular Population Genetics Lab CMPG, Zool. Inst., University of Berne, Switzerland) was used to run an Analysis of Molecular Variance (AMOVA) to test for significant partitioning of relative genetic variation into the three sampling regions were East,Between the two rivers and West. ***Weir and Cockerham´s (1984)*** estimators of *Fst* values of relative genetic differentiation between all pairs of sampling regions were calculated with the Fstat program 2.9.3.2/Feb. 2002 (J. Goudet, Inst. of Ecology, Univ. Lausanne, Switzerland; Windows NT 6.1. (Build 7601: Service Pack 1) and significance was determined by permutation tests (60). The Fstat program was also used to calculate mean locus-specific(allelic richness), based on a rarefaction approach to account for different sample sizes. Region-specific allelic richness (AR) was calculated as arithmetic mean over all locus-specific values, respectively, and a Kruskal-Wallis test as well as a Friedmann test was used to test for significant variation of AR across the three regions (by using the SPSS program). Whereas the Kruskal-Wallis test was based on untied locus-specific allelic richness values, the Friedman test used tied locus-specific values, which allows for inference on whether or not there is a systematic change of locus-specific values across the regions.

The Geneclass 2.0, 11/2003 software (***Piry et al., 2004***) was used to assign each individual hare to one of the three sample regions using a Bayesian statistical approach (***Paetkau et al., 1995***) based on obtained individual composite genotypes and simulating 10000 individuals according to the algorithm of ***Paetkau et al.,*** (***2004***). A high proportion of individuals being assigned to the regions where they were collected indicated onthe one hand a significant geographical differentiation and that the applied molecular marker system is capable of detecting the underlying geographical structure.
Finally, the geographical genetic structure and admixture of composite genotypes of individuals was inferred from a STRUCTURE analysis (***Pritchard et al., 2000; Falush et al., 2003***) using a Bayesian statistical approach, i.e. a likelihood analysis to assess the most likely number of population groupings (clusters) compatible with the observed genotypic distribution. The likelihood when assuming different numbers of genetic clusters (k) was calculated under the following specifications: admixture model, correlated allele frequencies among regions, burn-in 150.000, MCMC 500.000, 1–10 k and 10 iterations for each k. Mean, maximum, minimum, and standard deviation of ln [Pr(XIk)] were calculated for each k, and delta K for each k, based on the second order rate of change of the likelihood function with respect to k (***Evano et al., 2005***) was calculated by using the on-line Structure Harvester Platform. In a second approach, STRUCTURE runs were repeated with the same specifications as above but with using the region information as prior, and for 1-4 k and 8 iterations per k.

3.6StatisticsAanalysis

SPSS 20.0 statistical package was used for analyses of morphometric and Craniometric, whereas means were separated by least significant difference (LSD)

CHAPTER FOUR
4. Results
4.1 Morphometric

Parameters for body measurements were body weight (kg), total length (cm), tail length (cm), hind foot length (cm), ear length (cm), length of fore leg (cm), length of hind leg (cm), distance between ears (cm), distance between eyes (cm), neck length (cm), height (cm) and back length (cm) (Table 4.1).

Tail length, height and the distance between eyes were different among the three geographic regions. The tail length was longer ($P < 0.0001$) in the Western geographic region followed by the region between the two Rivers. Hares in the Eastern Region were taller ($P < 0.04$), followed by those between the two Rivers. The distance between eyes was wider ($P < 0.0001$) for hares between the two Rivers followed those on the Western region, and finally the distance between ears was slightly wider ($P < 0.084$) in hares between the two Rivers followed by hares in the Western region.

Table 4.1: Morphometricof hares collected from three geographical regions in Sudan during 2012 -2015.

Geographical region	Parameters											
	Body weight (kg)	Total length (cm)	Ear length (cm)	Tail length (cm)	Hind foot (cm)	Length of fore leg (cm)	Length of hind leg (cm)	Distance between ears (cm)	Distance between eyes (cm)	Neck length (cm)	Height (cm)	Back length (cm)
East (n=59)	1.42±0.3	43.13±6.4	10.75±1.4	7.37± 1.5[c]	10.02±1.2	22.55±3.4	27.63±3.8	3.25±0.9	2.87±0.5[c]	4.15±1.3	16.91±3.2[a]	26.84±3.8
West (n=19)	1.57±0.4	42.71±4.4	10.59± 1.0	9.12±0. 9[a]	9.85± 0.5	21.53±2.2	26.94±2.0	3.00±0.7	3.33±0.8[b]	3.91±0.6	14.18±2.9[b]	28.29±1.9
Between Blue and White Niles [(n=18)]	1.34±0.3	41.81±4.1	10.00± 0.7	8.18±0.8[b]	10.28 ± 0.6	22.95±1.9	28.60±2.5	3.58±0.6	4.25±0.7[a]	4.63±0.8	15.53±2.4 [ab]	27.65±2.7
95% confidence	1.36-1.5	41.46-43.61	10.41-10.92	7.56-8.14	9.84-10.25	21.85-23.05	27.03-28.39	3.11-3.43	3.11-3.43	3.98-4.44	15.5-16.77	26.59-27.94
P-values	0.129	0.884	0.724	0.000	0.432	0.324	0.313	0.084	0.000	0.293	0.004	0.242

[a,b,c] means within the same column followed by different superscript are at significantly($p < 0.05$)

4.1.1 Sexual Dimorphism

Paired t-test (Table 4.1 and Table 4.2) revealed that sex morphometric weresimilar (P ranges from < 0.171 to P < 0.995) except the live weight and neck length: females were heavier (P < 0.003) than males with longer necks (P < 0.034).

Table 4.2: Sex morphometricof hares collected from three geographical regions in Sudan during 2012 - 2015.

Sex	**Parameters**											
	Body weight(Kg).	Total length (cm)	Ear length (cm)	Tail length(cm)	Hind foot (cm)	Length of fore leg (cm)	Length of hind leg (cm)	Distance between ears (cm)	Distance between eyes (cm)	Neck length (cm)	Height (cm)	Back length (cm)
Male$^{(n=43)}$	1.36±0.3^{b}	42.22±5.1	10.57±1.8	7.81± 1.6	10.08±1.1	22.68±2.8	27.78±3.6	3.25±0.7	3.18±0.7	4.46±1.3	16.11±3.4	27.11±3.4
Female$^{(n=51)}$	1.49±0.4^{a}	42.81±5.4	10.75± 1.3	7.89±1.3	10.01 ± 0.9	22.26±3.1	27.64±3.1	3.29±0.9	3.45±0.8	4.00±0.9	16.15±3.0	27.39±3.3
P-value	0.003	0.637	0.290	0.256	0.269	0.373	0.695	0.356	0.171	0.034	0.414	0.995

Correlation matrix of hares' morphometric (Table 4.3) indicated three types of correlations. First, there were fair, positive correlations with most of the variables, the strongest being between the lengths of the fore leg and lengths of hind leg ($r = 0.828$, $P < 0.01$), foot length and total length ($r = 0.815$, $P < 0.01$), length of hind leg and length of hind foot ($r = 0.801$, $P < 0.01$). Second, there were weak, positive correlations between the following variables: Body weight (BW) and distance between eyes ($r = 0.096$, $P > 0.05$), BW and height ($r = 0.009$, $p > 0.05$), total length (TL) and distance been eyes ($r = 0.194$, $P > 0.05$), ear length (EL) and tail length ($r = 0.195$, $P > 0.05$), EL and distance between eyes ($r = 0.194$, $P > 0.05$), tail length (TL) and Body weight (r 0.146, $P > 0.05$), TL and EL ($r = 0.195$, $P > 0.05$), TL and distance between ears ($r = 0.12$, $P > 0.05$), distance between ears (DBE) and BW ($r = 0.096$, $P > 0.05$), DBE and TL ($r = 0.12$, $P > 0.05$), DBE and TL ($r = 0.12$, $P > 0.05$), DB eyes and TL ($r = 0.149$, $P > 0.05$, DB eyes and EL ($r = 0.04$, $P > 0.05$), NL and EL ($r = 0.119$, $P > 0.05$), height and BW ($r = 0.009$, $P > 0.05$) Third, variables that indicated weak, negatively correlation were Height and TL ($r = - 0.142$, $P > 0.05$), Height and distance between eyes ($r = - 0.132$, $P > 0.05$), Height and NL ($r = - 0.074$).

It is worth mentioning that the morphometric of hind foot, fore leg, hind leg and the back are all strongly and positively correlated with morphometric of the remaining variables.

Table 4.3: Morphometric correlation matric of hares collected from three geographic regions* in Sudan 2012 - 2015.

	Body weight(cm)	Total length(cm)	Ear length(cm)	Tail length(cm)	Hind foot (cm)	Length of fore leg(cm)	Length of hind leg(cm)	Distances between ears(cm)	Distances between eyes(cm)	Neck length(cm)	Height(cm)	Back length(cm)
Body weight (cm)	1	0.706**	0.322**	0.146	0.553**	0.467**	0.469**	0.096	0.219*	0.315**	0.009	0.528**
Total Length (cm)	0.706**	1	0.692**	0.428**	0.815**	0.678**	0.738**	0.375**	0.194	0.399**	0.338**	0.735**
Ear length(cm)	0.322**	0.692**	1	0.195	0.684**	0.623**	0.600**	0.260*	0.04	0.119	0.626**	0.623**
Tail length (cm).	0.146	0.428**	0.195	1	0.434**	0.292**	0.348**	0.12	0.429**	0.308**	-0.142[a]	0.379**
Hind foot (cm)	0.553**	0.815**	0.684**	0.434**	1	0.763**	0.801**	0.355**	0.281**	0.519**	0.385**	0.692**
Length of foreleg (cm).	0.467**	0.678**	0.623**	0.292**	0.763**	1	0.828**	0.222**	0.202*	0.560**	0.377**	0.739**
Length of hind leg (cm).	0.469**	0.738**	0.600**	0.348**	0.801**	0.828**	1	0.449**	0.316**	0.637**	0.372**	0.762**
Distance between ears(cm)	0.096	0.375**	0.260*	0.12	0.355**	0.222**	0.449**	1	0.238*	0.283**	0.303**	0.343**
Distance between eyes (cm)	0.219**	0.194	0.04	0.429**	0.281**	0.202*	0.316**	0.238*	1	0.285**	-0.132[a]	0.310**
Neck length (cm)	0.315**	0.399**	0.119	0.308**	0.519**	0.560**	0.637**	0.283**	0.285**	1	-0.074[a]	0.353**
Height (cm)	0.009	0.338**	0.626**	- 0.142[a]	0.385**	0.377**	0.372**	0.303**	-0.132[a]	-0.074[a]	1	0.401**
Back length (cm)	0.528**	0.735**	0.623**	0.379**	0.692**	0.739**	0.762**	0.343**	0.310**	0.353**	0.401**	1

*The geographic regions were: West of the Nile, East of the Nile and between the two Rivers (White Nile and the Blue Nile).
Shaded areas correlations were insignificant (P > 0.05); [a] Insignificant negative correlations (P > 0.05).

4.2 Craniometric

Craniometric of the hares collected from the three geographic regions are shown in (Table 4.4) anterior nasal width (ANW) was wider ($P < 0.001$) for the Western region, followed by the region between the two rivers. The external nasal length (ENL) was longer ($P < 0.03$) for hares in Western region followed by that of the Eastern region. The internal nasal length (INL) was slightly longer ($P < 0.076$) in hares in the Western region followed by that in hares in the Eastern region. The Lower cheek-tooth row length (LCTRL) was longer for Western region followed by that of hares Between the Two Rivers. The upper cheek-tooth row length (UCTRL) was longer ($P < 0.027$) for hares in Western region but about equal in the remaining two regions. The Mandible height was larger ($P < 0.045$) for hares in the Western region followed by that of hares in the Eastern region. These findings suggest that hares in the Western region are distinct from those in the Eastern and thosebetween the two rivers region, whereas; no difference was apparent between hares in Eastern Region and those occurring between the two rivers.

The Craniometricindicated three groups: in the first group, means were higher in the Western region for (ENL ($P < 0.023$), ANW ($P < 0.001$), PNW ($P < 0.01$), LCTRL ($P < 0.007$), MH ($P < 0.04$), and TBL ($P < 0.001$), followed by the Eastern region and lastly the Region between the two rivers; in the second group, UCTRL mean is higher ($P < 0.02$) in the Western region but about equal in the Eastern region and the Region between the two rivers; and in the third group, TBW mean was higher ($P < 0.0001$) in the Eastern region, followed by the Western region and lastly the region between the two Rivers. There was no variation in measurement for smallest frontal width (SFW) ($P < 0.751$), internal nasal length (INL) ($P < 0.076$), mandible length (ML) (0.14), posterior zigomatic width (PZW) ($P < 0.678$), post palatal width (PPW) ($P < 0.431$), total length (TL) ($P < 0.124$), palatal length (PL) ($P <0.256$),

Foramen incisivum length (FIL) (P < 0.390), facial tubercle length (FTL) (P < 0.350), FIW (P < 0.823), rostral width (RW) (P < 0.88), and width between facial tubercle (WFT) (P < 0.094) (Table 4.4) among the groups in the three geographic. Most Craniometricwere passively correlated (Table 4.5).

Craniometric correlation matrix is presented in Table (4.5) the Craniometric of all bones are more or less strongly and positively correlated (P < 0.05) except the smallest frontal width (SFW). The measurement of this bones was weakly and oftentimes negatively correlated with measurements of many bones: SFW showed weak correlations (P > 0.05) with the 14 bones; among these, its correlations were positive with ENL, NL, ANW, PNW, LUCTL, PZW, negative with the remaining 8 bones.

Table 4.4: Craniometric of hares collected from the Western, Eastern and between the two Rivers Regions in Sudan, 2013 – 2015.

Region	**Parameters**									
	ANW (mm)	ENL (mm)	FIW (mm)	FTL (mm)	INL (mm)	FIL (mm)	LCTRL (mm)	UCTRL (mm)	MH (mm)	ML (mm)
East (n=59)	11.43±1.1[a]	31.77±3.5[a]	8.71±0.9	8.46±0.9	28.64±3.4	19.65±2.2	13.69±1.2[b]	13.62±1.3[b]	36.35±3.6[a]	57.17±5.0
West (n=19)	11.70±0.9[a]	31.87±2.7[a]	8.74±0.9	8.38±0.6	29.04±2.5	19.73±2.2	14.62±1.0[a]	14.47±1.0[a]	37.10±2.6[a]	58.41±4.2
Between the two Rivers (n =18)	10.59±0.5[b]	29.46±3.4[b]	8.57±0.8	8.11±0.9	26.76±3.9	18.78±2.2	14.10±1.1[a]	13.69±1.1[ab]	34.46±2.8[b]	55.31±4.4
95%Confidence	11.12-11.53	30.66-32.05	8.51-8.87	8.20-8.55	27.68-29.06	19.06-19.94	13.73-14.21	13.55-14.05	35.45-36.83	56.09-58.04
Significance level	0.001	0.032	0.823	0.350	0.076	0.290	0.007	0.027	0.045	0.140

ANW: anterior nasal width. FIL: foramen incisivum length.
ENL: external nasal length. LCTRL: lower cheek-tooth row length.
FIW: foramina incisive width. UCTRL: upper cheek-tooth row length.
INL: internal nasal length. MH: mandible height.
FTL: facial tubercle length. ML: mandible length.

[a,b,] means within the same column followed by different superscript are at significantly($p < 0.05$)

Table 4.4: continued

Region	Parameters							
	PNW (mm)	PPW (mm)	RW (mm)	SFW (mm)	TBL (mm)	TBW (mm)	PL (mm)	PZW (mm
East(n=59)	16.92±2.1[a]	7.02±0.8	21.51±1.4	12.33±0.8	13.17±1.2[a]	8.52±0.9[a]	31. 17±3.2	37.6
West(n=19)	17.12±1.8[a]	6.96±0.6	21.67±1.1	12.31±0.9	13.50±0.7[a]	8.43±0.6[a]	31.38±3.0	38.1
Between Blue and White Niles (n=19)	15.51±1.7[b]	6.77±0.6	21.61±0.9	12.31±0.9	12.22±0.9[b]	6.77±1.3[b]	29.86±3.1	37.4
95%Confidence	16.29-17.11	6.82-7.11	21.30-21.82	12.10-12.45	12.83-13.29	7.59-8.13	30.32-31.61	37.2 38.1
P-values	0.019	0.431	0.88	0.751	0.001	0.000	0.256	0.67

PNW: posterior nasal width.
PPW: post palatal width.
RW: rostral width.
SFW: smallest frontal width
WFT: width between facial tubercles.
TBW: tympanic bulla width.
PL: palatal length.
PZW: posterior zigomatic width
TL: Total length.
TBL: tympanic bulla.

[a,b], means within the same column followed by different superscript are at significantly ($p < 0.05$).

Table 4.5: Craniometric correlation matrix of hares collected from three geographic regions* in S

	SFW	ENL	NL	ANW	PNW	ML	LUCTL	MH	UCTL	TBL	PZW	PPW	TBW	PL	FIL
SFW	1	0.001	0.029	0.015	0.078	-0.061[a]	0.052	-0.061[a]	-0.059[a]	-0.132[a]	0.127	-0.112[a]	-0.094[a]	-0.029[a]	-0.007[a]
ENL	0.001	1	0.922	0.779	0.832	0.874	0.703	0.854	0.720	0.592	0.781	0.703	0.568	0.908	0.895
NL	0.029	0.922	1	0.756	0.825	0.837	0.652	0.818	0.655	0.551	0.768	0.672	0.521	0.868	0.876
ANW	0.015	0.779	0.756	1	0.784	0.734	0.545	0.788	0.609	0.527	0.684	0.587	0.570	0.788	0.770
PNW	0.078	0.832	0.825	0.784	1	0.765	0.604	0.782	0.669	0.539	0.736	0.633	0.538	0.810	0.841
ML	-0.061[a]	0.874	0.837	0.734	0.765	1	0.821	0.912	0.811	0.581	0.79	0.679	0.480	0.892	0.885
LUCTL	0.052	0.703	0.652	0.545	0.604	0.821	1	0.728	0.846	0.404	0.778	0.606	0.222	0.718	0.731
MH	-0.061[a]	0.854	0.818	0.788	0.782	0.912	0.728	1	0.786	0.610	0.763	0.627	0.537	0.882	0.867
UCTL	-0.059[a]	0.720	0.655	0.609	0.669	0.811	0.846	0.786	1	0.474	0.807	0.630	0.357	0.761	0.783
TBL	-0.132[a]	0.592	0.551	0.527	0.539	0.581	0.404	0.610	0.474	1	0.480	0.425	0.470	0.544	0.555
PZW	0.127	0.781	0.768	0.684	0.736	0.790	0.778	0.763	0.807	0.480	1	0.635	0.413	0.796	0.811
PPW	-0.112[a]	0.703	0.672	0.587	0.633	0.679	0.606	0.627	0.630	0.425	0.635	1	0.377	0.697	0.727
TBW	-0.094	0.568	0.521	0.570	0.538	0.480	0.222	0.537	0.357	0.470	0.414	0.377	1	0.463	0.476
PL	-0.029[a]	0.908	0.868	0.788	0.810	0.892	0.718	0.882	0.761	0.544	0.796	0.697	0.463	1	0.954
FIL	-0.007[a]	0.895	0.876	0.770	0.841	0.585	0.731	0.867	0.783	0.555	0.811	0.727	0.476	0.954	1
FTL	-0.008[a]	0.649	0.644	0.604	0.579	0.660	0.520	0.700	0.588	0.536	0.623	0.439	0.443	0.682	0.654
FIW	0.102	0.710	0.673	0.645	0.714	0.699	0.644	0.670	0.672	0.443	0.731	0.610	0.342	0.718	0.777
RW	0.024	0.737	0.691	0.670	0.666	0.811	0.743	0.830	0.804	0.419	0.812	0.599	0.341	0.808	0.823
WFT	0.032	0.781	0.758	0.763	0.749	0.852	0.742	0.876	0.832	0.508	0.802	0.599	0.460	0.847	0.852

Shaded areas indicate weak correlations; [a] negatively correlated.

4.2.1 Sex Craniometric

Sex Craniometric are presented in table (4.6)apparently, there were no differences (P ranges from < 0.09 to < 0.868) among all bone measurements except the Tympanic bulla width (TBW) which was wider (P < 0.013) for males than females.

Table 4.6: Sex Craniometric of hares collected from three geographic regions* in Sudan, 2013 – 2015.

Sex	**Parameters**									
	ANW (mm)	ENL (mm)	FIW (mm)	FTL (mm)	INL (mm)	FIL (mm)	LCTRL (mm)	UCTRL (mm)	MH (mm)	ML (mm)
Male	11.29±1.0	31.48±3.5	8.64±0.9	8.4±0.8	28.36±3.4	19.49±2.0	14.06±1.3	13.88±1.3	36.17±3.4	57.47±4.80
Female	11.33±2.0	31.14±3.4	8.71±0.9	8.35±0.9	28.28±3.5	19.40±2.3	13.87±1.1	13.71±1.3	36.01±3.5	56.62±4.86
P-values	0.728	0.686	0.760	0.096	0.785	0.176	0.692	0.349	0.513	0.313

ANW: anterior nasal width. FIL: foramen incisivum length.

ENL: external nasal length. LCTRL: lower cheek-tooth row length.

FIW: foramina incisive width. UCTRL: upper cheek-tooth row length.

INL: internal nasal length. MH: mandible height.

FTL: facial tubercle length. ML: mandible length.

*The geographic regions were: West of the Nile, East of the Nile and between the White Nile and the Blue Nile.

Table 4.6: continued:

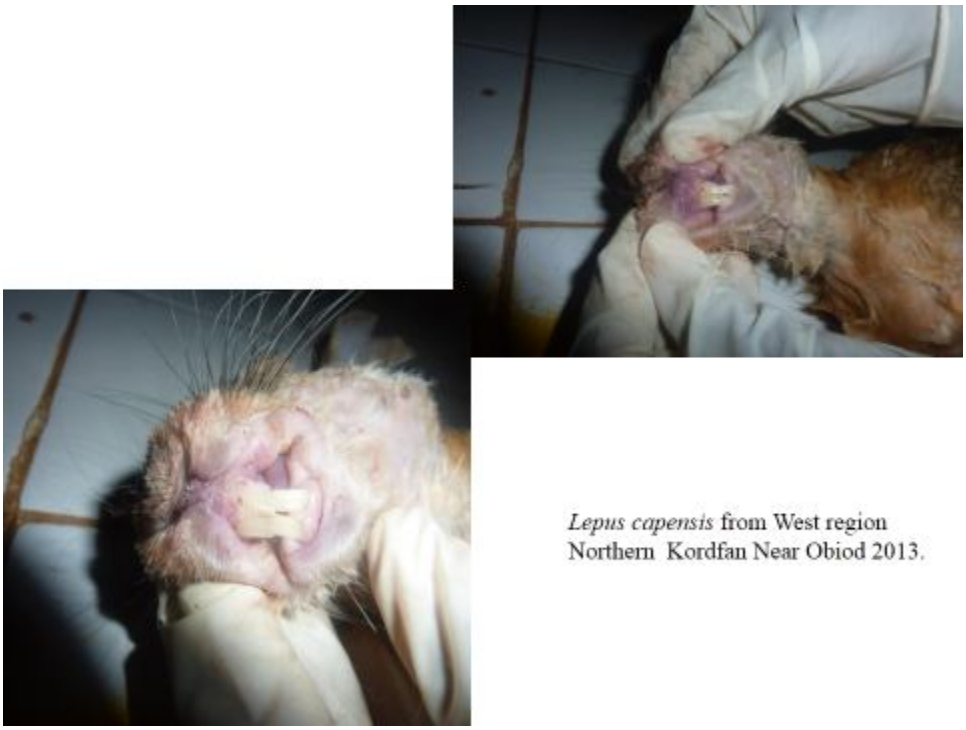
Sex	**Parameters**							
	PNW (mm)	RW (mm)	PPW (mm)	SFW (mm)	TBW (mm)	TBL (mm)	PL (mm)	PZW (mm
Male	16.74±2.0	21.47±1.3	7.07±0.8	12.07±0.8	8.0±1.1	13.14±1.1	30. 98±3.1	37.65±2.8
Female	16.58±2.0	21.62±1.3	6.9±0.7	12.47±0.9	7.72±1.5	12.95±1.2	30.83±3.3	37.63±2.0
P-values	0.810	0.795	0.868	0.739	0.013	0.361	0.156	0.09

PNW: posterior nasal width. TBW: tympanic bulla width.
PPW: post palatal width. PL: palatal length.
RW: rostral width. PZW: posterior zigomatic width
SFW: smallest frontal width TL: Total length.
WFT: width between facial tubercles. TBL: tympanic bulla.
*The geographic regions were: West of the Nile, East of the Nile and between the White Nile and the Blue Nile.

4.5The Dental and External Phenotype of Specimens

4.5.1 Dental Shape

The microscopicexamination of the labial groove of the first upper incisorof all skulls showed simple pattern, typical for *Lepus capensis*; the fold was quite deep or in several cases it was shallow. In most cases this fold was fillet with cement (plate 4.1).



Lepus capensis from West region
Northern Kordfan Near Obiod 2013.

Plate 4.1: Dental characterization of hare.

4.5.2 The Phenotype

The fur colour was grey, brownish–yellow to brownish; ears were blackish in the outside at the tip and underneath were slightly darker and the tail is blackish on the upper side similar to the *Lepus habessinicus*. The nep colour was light brownish, sometime the back was relatively quiet dark. The lateral transient from the dorsal grayish-brownish to whitish, ventral was not sharp and seem to be pulley brownish

at transient zoneas shownin plates (4.2, 4.3, 4.4 and 4.5). All these results, namely, dental analysis and external phenotype as well as the skull shape indicated that all specimens belong to *Lepus capensis* in a wide sense.

Phenotype of *L. capensis* collected from from,River Nile State, Photo taken during field trips of this study from Sudan,

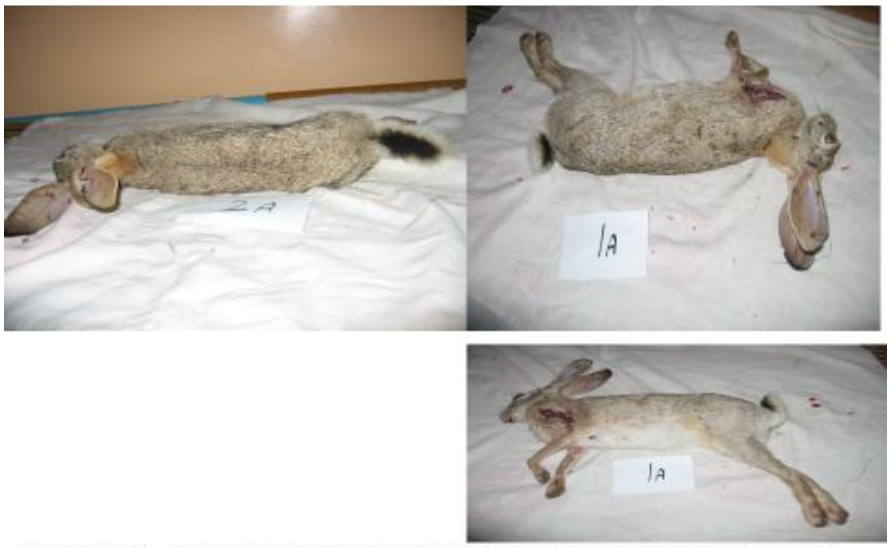
Plate 4.2: Samples from West region.

Phenotype of*Lepus capensis* collected fromSinnar State near. Photo taken during field trips of this study from Sudan

Plate 4.3: Samples from Eastern region.

Hare (*Lepus capensis)* from East region,River Nile State, Close to Damer, Sudan 2014

Plate 4.4: Samples from Easternregion.



Phenotype of *L. capensis* collected from Gaziera State near Ganib. Photo taken during field trips of this study from Sudan,

Plate 4.5: Samples from Region Between two rivers

4.6. Molecular Genetics

4.6.1 Cyt (*b*) Sequences of Samples and D-loop

The median joining network in (Fig. 4.1)is not meant to present a complete phylogenetic model for these hare species from Africa, because not all available scrub hare sequences and no cape hare sequences from Africa except from Sudan are included. The preliminary alignment of all respective sequences on GenBank was not successful for all available African sequences. This needs further exploration. Moreover, for a comprehensive phylogenetic analysis of the Sudanese hares, all available and reliable sequences of other *Lepus* species should be included. Currently, there is only one cyt b sequence of the African savanna hare, *L. victoriae*, available from GenBank, but there are a few more *L. saxatilis* sequences available. However, until today there is no clarity about the phylogenetic relationship between *L.saxatilis* and *L. victoriae* they may be conspecific or two good species. In South Africa, the scrub hare has originally been described only from the SW of the country, and unknown sample locations (as is the case for most sequences on GenBank) may be either from the range of *L. saxatilis* or *L. victoriae*. Due to all these reasons, the median joining network includes only few selected haplotypes with reliable regional assignment. In the network, each open circle represents a cyt b haplotype, circle size is proportional to the number of hares harboring the respective haplotype, black continuous lines between haplotypes indicate the modelled evolutionary trajectories between haplotypes and numbers along lines indicate substitutions (numbers of point mutations) if more than one; if there is only one substitution between two haplotypes no number is given. The dashed circle encompasses all Sudanese haplotypes found presently.All mtDNA-lineages of the Sudanese hares are phylogenetically closely related (Fig. 4.2) without major phylogenetic gaps. This

parallels the population genetic results based on the microsatellite allele frequencies indicating only shallow gene pool differentiation.

4.6.2 Variation of Population Genetics

As shown in table (Table 4.7)a total of 89 alleles were identified at the nine loci studied, 84 in the East region, 38 in the sample from the region between the two rivers and 72 from West region. Of all alleles 32 (38.1%) were shared by all three regions and 16 (19.05%) were private allelesthat occurred in only one region.Most of private alleles (12) were occurred in the East region; the region between the two rivers harboured one, and the West region three private alleles. All but one locus (Sol33) harbored at least one private allele. Frequencies of all private alleles were generally low, with a mean of 4.504% and ranging from 0.81% to 16.67%.According to the Genepop software results there were no significant deviations from linkeage equilibrium, but there was an overall highly significant deviation of genotype frequencies from Hardy-Weinberg expectations. The latter result indicated a significant substructuring of the overall genotypic data set.The following indices of genetic variability are given for each locus and regional sample in (Table 4.7) total number of alleles detected (A_N), most frequent allele (A_F) and respective allele frequency (F), mean observed heterozygosity (H_o), expected heterozyogsity corrected for small sample sizes ($H_{e\ n.b.}$). For each region the mean number of alleles per locus (A), the number of “private alleles” (A_P), the mean allelic richness (AR) as calculated by a rarefaction approach to account for sample sizes using Fstat (see Material and Methods), and the F_{is} value is also given.

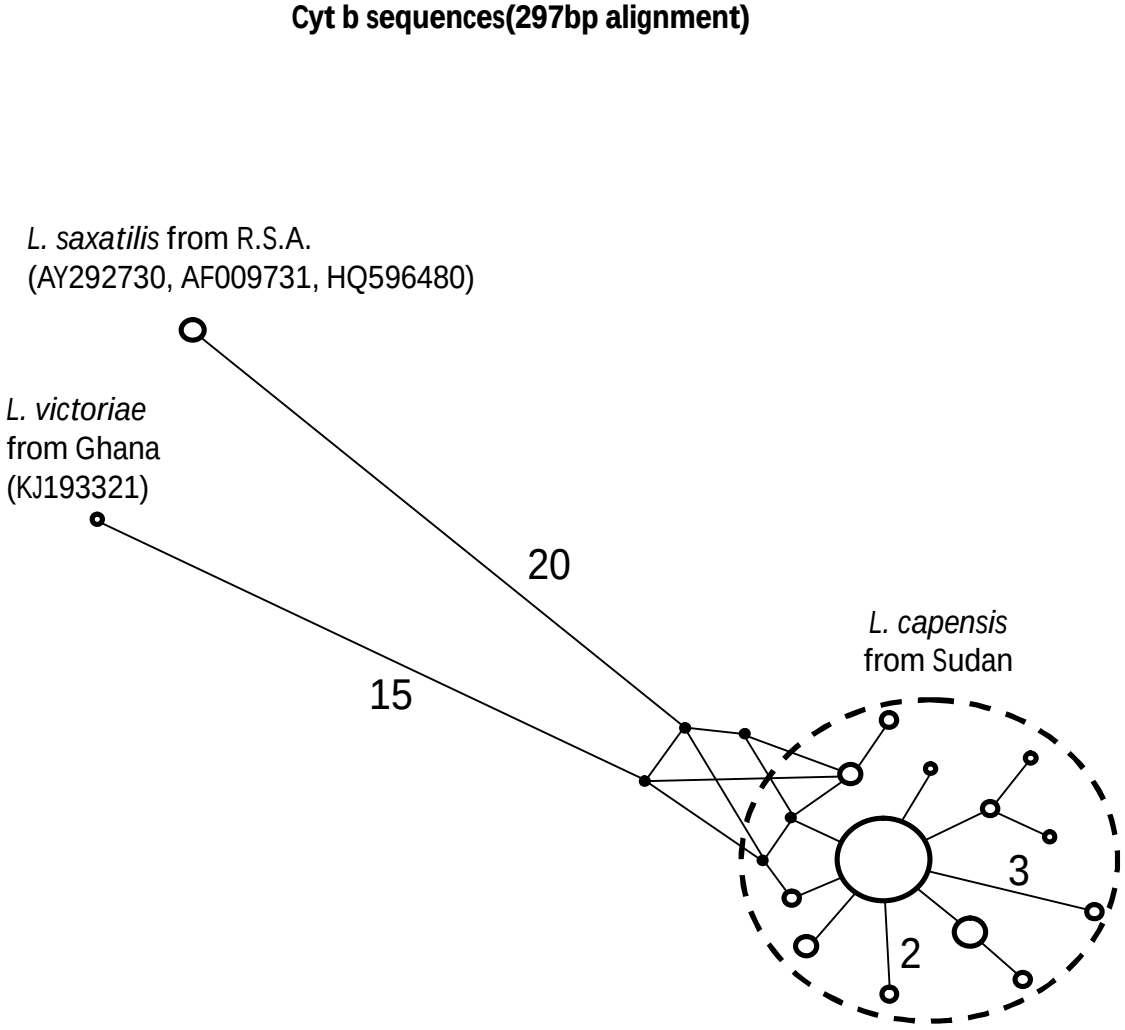


Figure 4.1: Median Joining Network (Bandelt *et al.*, 1999) of cyt b sequences (297bp) of all currently sequenced hare samples from Sudan, including three sequences of scrub hares (*L. saxatilis*) from the Republic of South Africa and one sequence of the African savanna hare (*L. victoriae*) from Ghana, as example to demonstrate the evolutionary mtDNA differentiation between Sudanese cape hares and the other two currently acknowledged *Lepus* species of which sequences were available from GenBank.

Figure 4.2: Neighbor-joining (NJ) phylogenetic tree computed by *Mega* using toalign 94 sequences were converted Cyt *b* (297bp) and D-loop (413bp) of hares from Sudan.

The F_{is} value indicates whether there is a heterozygote deficit or surplus at the population level (significant deviation from zero is indicated by an asterisk; significance at a nominal alpha = 0.05, after strict Bonferroni corrections for multiple testing). A significant deviation of F_{is} from zero means that there is a significant deviation from the Hardy-Weinberg equilibrium in the region/population.

Kruskal-Wallis and Friedmann tests were used to carry outwhether allelic richness varies significantly locus-by-locus across populations(Table 4.7 and Fig. 4.3). Allelic richness was similarly high in the regions East and West, but significantly lower in richness in the region between the two rivers (P< 0.016, Kruskal-Wallis test andP< 0.011, Friedmann test) (Appendixes3 and 4).

Based on all nine microsatellite loci, there was a shallow but significant genetic differentiation between all three regions currently considered (East, between the two rivers and West); values of absolute (CSE distances) and relative (F_{ST} values) genetic differentiation between these regions are given in table (4.8). This shallow significant genetic differentiation was confirmed by the AMOVA (analysis of molecular variance) that revealed that 6.76% (p<0.0001) of the overall relative genetic variability was due to partitioning into the three discriminated regions, whereas 8.81% (p<0.0001) were due to partitioning among individuals within regions, and the biggest portion of 84.43% (p<0.0001) were due to partitioning within individuals (Appendix 5).

Table 4.7: Overview of allelic variability of cape hares from the three regions East, between the two rivers and West. The total number of alleles found at each locus is given with the locus name. Separately for each region, the following locus-specific values are given: AN – total number of alleles, R – length range of alleles, AF – length of the most frequent allele, F – frequency estimate of the most frequent allele, He n.b. – expected heterozygosity (corrected for small sample sizes), Ho – observed heterozygosity, AR – allelic richness, AP – number of "private alleles". Further, for each region the mean number of alleles per locus (A) and the FIS value are given (significant departure from zero is indicated by an asterisk; 95% confidence intervals are given in parentheses).

Region		**Loci**									**Mean (A)**	F_{IS}
		D7UTR 12	Sat 8 10	Sol 33 5	OCELAMB 7	Sol 03 16	Sol 28 8	OCLS1B 12	Sol 08 7	Sol 44 12		
East	A_N	11	10	5	7	15	7	11	7	11	9.33	
	R	121-143	89-109	212-	109-121	233-	155-	152-172	125-137	138-160		
	A_F	133	93	222	117	279	171	164/166	127	150		0.096*
AR=6.5	F	0.23	0.52	214	0.40	253	161	0.19	0.45	0.19		(0.041 - 0.133)
A_P=12	$H_{e\ n.b.}$	0.88	0.67	0.66	0.76	0.29	0.60	0.87	0.73	0.89	0.754	
	H_o	0.88	0.66	0.52	0.79	0.86	0.60	0.76	0.67	0.89	0.682	
				0.38		0.65	0.46					
Between Blue and White Nile	A_N	6	5	4	3	8	3	4	1	4	4.22	
	R	119-143	89-105	212-	115-119	233-	155-	152-166	127-127	150-156		
	A_F	135	89	222	117	279	163	158	127	150		0.214*
AR=3.9	F	0.50	0.46	214	0.69	245	161	0.58	1.0	0.55		(0.002 - 0.337)
A_P=1	$H_{e\ n.b.}$	0.71	0.63	0.77	0.46	0.31	0.58	0.59	0.0	0.60	0.533	
	H_o	0.54	0.84	0.41	0.46	0.84	0.56	0.54	0.0	0.50	0,423	
				0.0		0.85	0.08					
West.	A_N	9	8	4	6	11	7	10	6	11	8.00	
	R	125-141	98-107	212-	109-119	233-	157-	152-174	125-135	132-160		
	A_F	131/133/1	93	220	115	277	175	162	127	142		0.186*
AR=6.9	F	35	0.52	220	0,29	253	161	0.24	0.34	0.23		(0.058 - 0.250)
A_P=3	$H_{e\ n.b.}$	0.18	0.70	0.47	0.80	0.22	0.44	0.86	0.77	0.91	0.801	
	H_o	0.88	0.53	0.66	0.74	0.89	0.74	0.84	0.58	0.73	0.656	
		0.89		0.42		0.72	0.44					

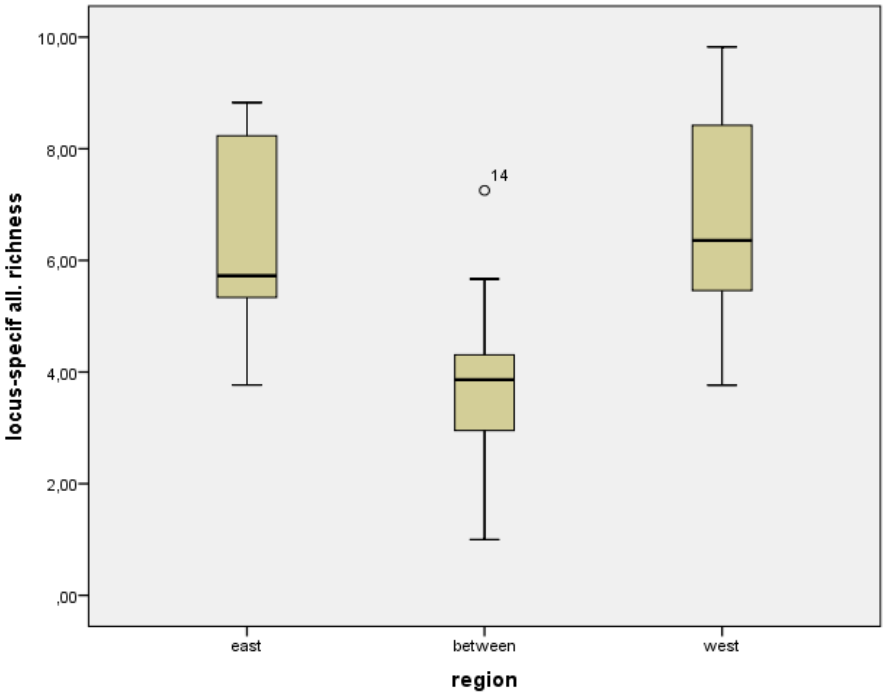


Figure 4.3: Thedistribution of the nine locus-specific allelic richnessvalues forthe three regions.

Table 4. 8: Genetic differentiation of cape hares from the three regions (East (E), between the two rivers (B), and West (W) studied in Sudan, as based on allele frequencies at the nine microsatellite loci and as calculated as relative (F_{ST} values) and absolute (Cavalli-Sforza-Edwards chord distances -CSEd) genetic differentiation. Pairwise F_{ST} values are above the diagonal and pairwise CSEd values are below the diagonal. Significance (i.e., values higher than zero) are marked by asterisks.

Region			
	E	B	W
E	-	0.112*	0.024*
B	0.132*	-	0.134*
W	0.046*	0.147*	-

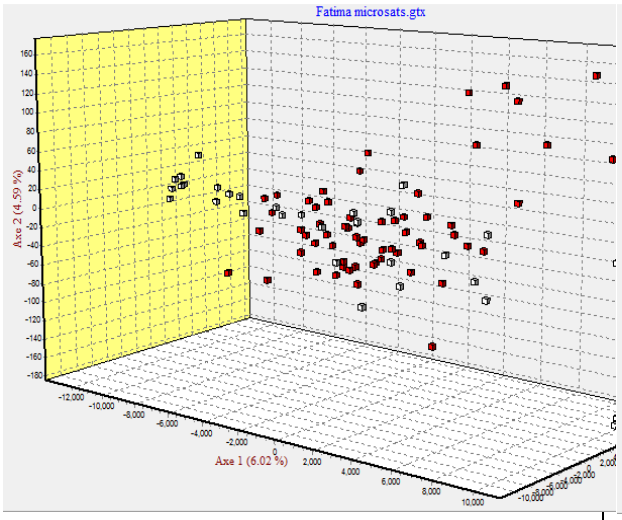
The overall shallow genetic divergence pattern between the three regions was also evident by the FCA (factorial correspondence analysis); though it revealed some little divergence of individual scores (but no significant gaps) of individual scores along the first three factorial correspondence axes (Fig.4.4) that conveyed only 14.85% of the total allelic variation.

Moreover, the first ten factors covered only 38.28% of the total allelic variability harbored by all studied hares, which accords to the interpretation of a shallow pattern of genetic differentiation.

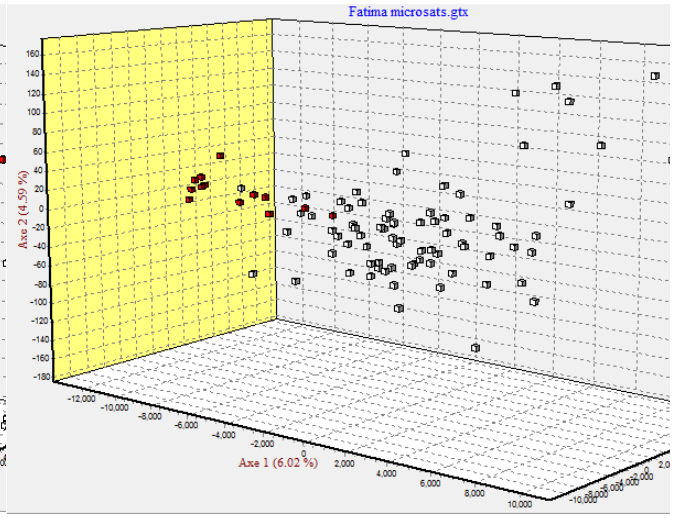
Nevertheless, the overall likelihood to assign each hare to the region where it was collected based on its composite genotypes was unexpectedly high: using the GENECLASS software (see Material and Methods), only eleven (11.6%) out of hares used for microsatellite analysis could not be assigned “correctly” to the region where they were collected. Whereas all hares from West regionwere assigned to their collection region, 84.6% of the hares from between the two rivers region were assigned to this region, and 85.7% of the hares from East regionwere assigned to the region where they originated from.

Finally, the Bayesian STRUCTURE analysis indicated that there were two or three most likely genetic clusters (groupings/populations) that were underlying the composite data set; those two to three clusters coincided more or less nicely with the three geographical regions (Fig.4.5).

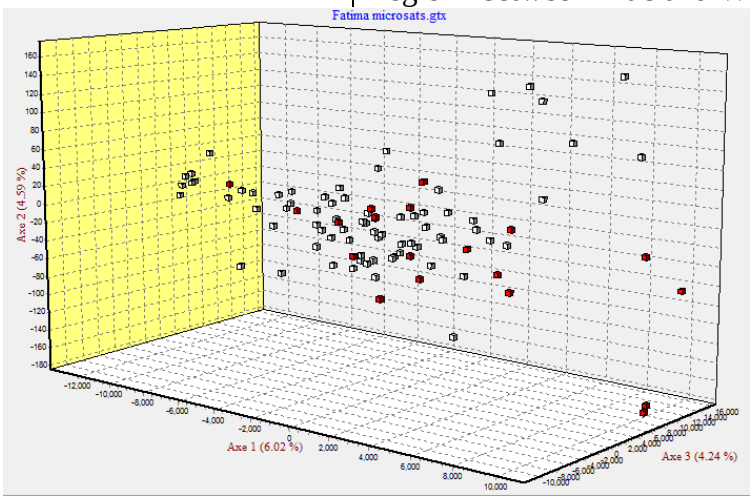
Whereas the admixture model runs with no prior regional information for the individuals resulted in a most likely number of two genetic clusters (supported by the probability statistics and the delta K statistics according to Evanno), the runs with prior region information for each individual resulted in most likely three genetic clusters (K = 3) underlying the overall data set. The latter result reflected very well the results as obtained by the other population genetic statistics of differentiation.



Region East



Region "between Blue and White Nile



Region West

Figure 4.4: Factorial correspondence analysis for genetic divergence of individual between the three regions.

Moreover, the results of the STRUCTURE analysis indicated relatively little genetic admixture on the individual level (Fig. 4.5).

Three-dimensional plots (Fig.4.6) of individual scores for the first three factors from the Factorial Correspondence Analysis (FCA) based on allele frequencies at all nine studied microsatellite loci. Each of the three graphics shows the total individual scores scattered across the three-dimensional space (as defined by the three axes), and the score positions of respective individuals from the three regions are highlighted in red (first plot is for the East region; second plot for the region between the two rivers, and third plot for the West region.

Moreover, the first ten factors covered only 38.28% of the total allelic variability harbored by all studied hares, which accords to the interpretation of a shallow pattern of genetic differentiation. Nevertheless, the overall likelihood to assign each hare to the region where it was collected based on its composite genotypes was astonishingly high: using the GENECLASS software (see Material and Methods), only eleven (11.6%) out of hares used for microsatellite analysis could not be assigned “correctly” to the region where they were collected. Whereas all hares from West regionwere assigned to their collection region, 84.6% of the hares from between the two rivers region were assigned to this region, and 85.7% of the hares from East regionwere assigned to the region where they originated from.

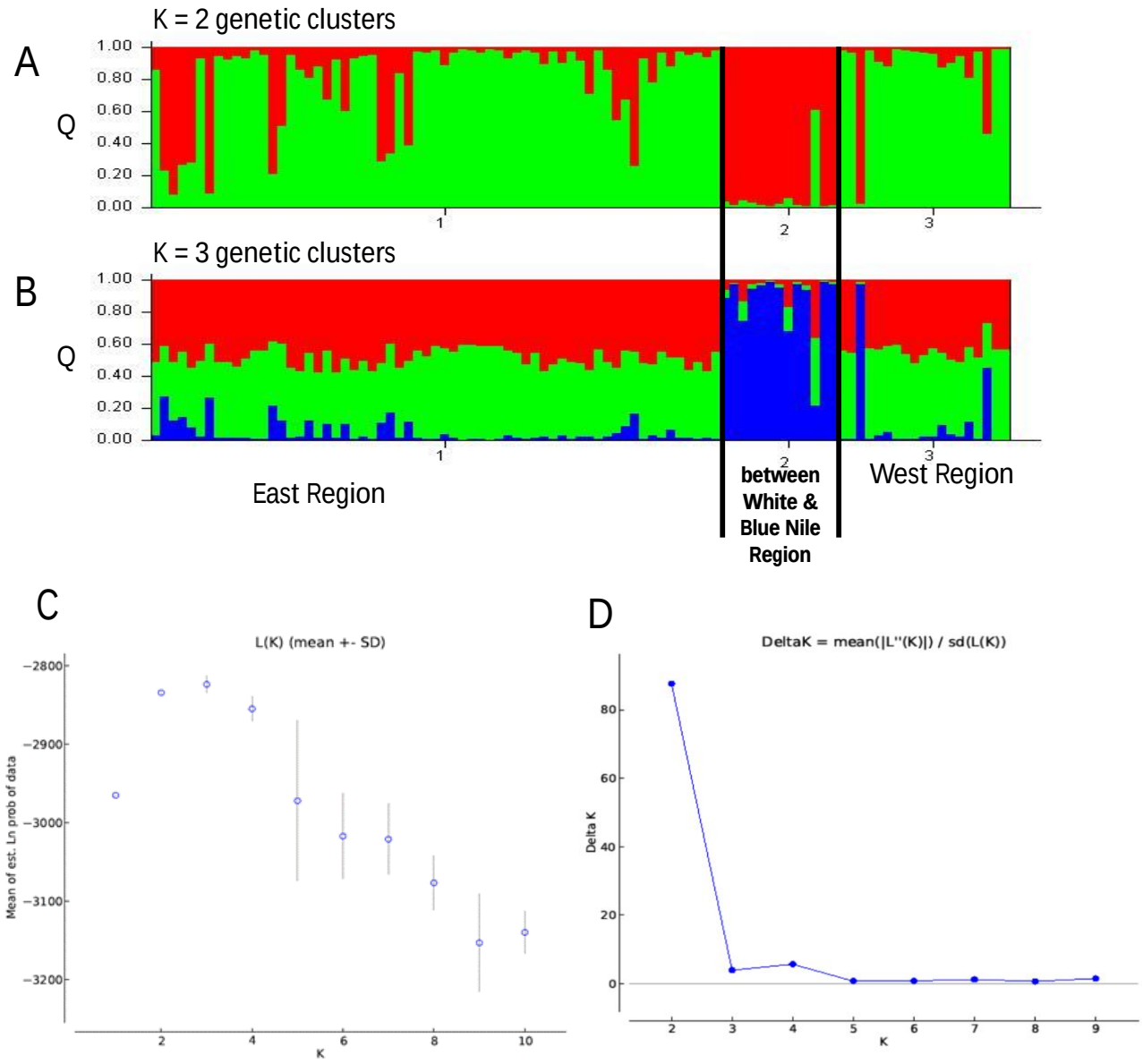


Figure 4.5: Genetic STRUCTURE results for Sudanese cape hares. Upper charts A and B depict the genetic admixture of individuals and structure across the three regions, when assuming the presence of two (A) and three (B) genetic clusters (K). The Q value indicates the percentage of anIndividual´s genetic composition belonging to a certain cluster. According to the Likelihood values (C) for the data as obtained from 10 iterations per K and from Evanno´s delta K statistics (D), two genetic clusters are likely better describing the overall microsatellite variation than three genetic clusters. This is also indicated by chart B showing that three clusters (colours) do notadd any further meaningful information for understanding the geographic distribution of genetic

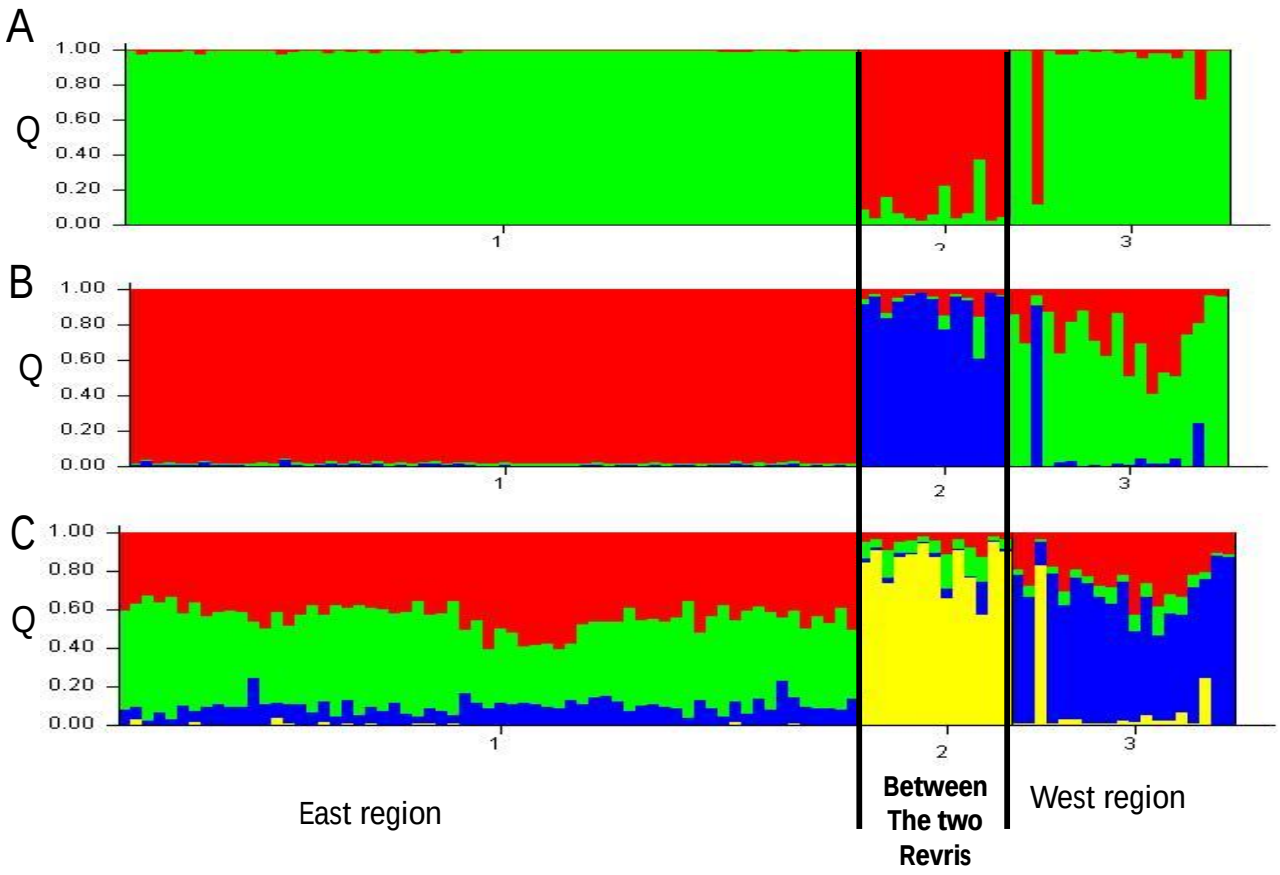


Figure 4.6: Genetic STRUCTURE results for Sudanese cape hares, when using the a priori information of the region, where each individual was collected in the model runs ("with region as prior"). A: two genetic clusters are assumed, B: three assumed clusters, C: four clusters. This additional regional sampling information in the model runs results in most likely three genetic clusters being present in the data set (green, red, blue in chart B). Assuming one more genetic cluster (chart C) does not meaningfully increase the information on geographic distribution of genetic clusters and on individual genetic admixture. Assuming three genetic clusters (B) indicates that all three regions are genetically differentiated from each other. However, according to the likelihood statistics, the most likely number of clusters in the STRUCTURE runs with a priori information on sampling region of each individual was nevertheless two clusters. But as additionalInformation on geographical genetic structuring is increased when assuming three clusters, thisnumber of clusters can be maintained. This also corresponds to the results of absolute (pairwiseCSE genetic distances) and relative (pairwise F_{st} values) genetic differentiation.

CHAPTER FIVE

5. Dissection

5.1Morphometrics

The research findings revealed that there are considerable variations in tail length, height and distance between eyes among hares collected from East of the Nile, West of the Nile and between the Blue Nile and the White Nile.Differences in these morphometrics implied that different species occur in the three geographic regions. However, the conclusion drawn from phenotypic and dental analysis that all specimens collected from the three geographic regions belong to one species, *Lepus capensis,* comply with findings of many researcher worldwide***Yom-Tov and Geffen, 2006Freckelton et al., 2003; Meiri, and Dayan, 2003Demirbaş et al., 2013***. Geographical variation in body size of both homeotherms and ectotherms is a very common phenomenon (***Freckelton et al., 2003; Meiri, and Dayan, 2003***) and has been related to many factors, including inter- and intraspecific competition, food availability and reaction to ambient temperature in accordance with Bergmann's rule which states that within a broadly distributed taxonomic clade, populations and species of larger size are found in colder environments, and species of smaller size are found in warmer regions(***Yom-Tov and Geffen, 2006***). ***Burnett*** (***1983***) work reveals that moisture has a statistically more significant influence on wing and skull size of the bat *Eptesicus fuscus* than temperature, and attributed this correlation to the need to conserve water in hot and dry environments. Research in Turkish hares indicates variations in body weight and hind foot length, depending on geographic regions (***Demirbaş et al., 2013***). ***James*** (***1970***) suggests that body size variation is related to a combination of climatic factors, mainly moisture and temperature, and that small body size is associated with hot and humid conditions and larger size with cooler and drier conditions. In

Australia, body size variation in five species of mammals is better correlated with moisture index and precipitation than is temperature, leading to the conclusion that food supply is the main factor determining body size (***Yom-Tov and Nix, 1986***). Studies on the geographical variation of *L. capensis* (***Yom-Tov, 1967***), *A. cahirinus* (***Nevo, 1989***) and *S. ehrenbergi* (***Nevo et al., 1986***) in Israel and Sinai, show that in these species northern animals are larger than southern ones, and that body size is negatively correlated with temperature variables and positively so with plant cover (reflecting productivity or food resources) and water-related parameters such as the number of rainy days, annual rainfall and relative humidity. Comparing body weights of brown hares living in Poland to representatives of this species from Western Europe (***Mace et al., 1981; Voss et al., 1990***), fully grown up individuals from Poland are about twice as heavy, reflecting the adaptation of the species to different environments in these regions. Our findings that there are variations in body size agreed with the findings many researches (***Krunoslav et al., 2014***;***Demirbaş et al., 2013;Riga et al., 2001)***. In fact, morphological variation may determine individual performance and may be driven by ecological and behavioural adaptations (***Garland and Carter, 1994; Garland and Losos, 1994; Harris and Steudel, 1997***). Due to great variations within genera, some authors assume that the phylogenesis and systematics of hares has not been completely clarified (***Pierpaoli et al., 2003; Ben Slimen et al., 2008***).It is difficult, however, to decide which factor influences the variation in body morphometrics and to assume the relative effect of each factor.

Results of the present study revealed that morphometric procedure is useful for determining hare's sex, as females are always heavier than males with longer necks. These findings have never been reported by (***Riga et al., 2001; Cervantes and Lorenzo, 1997***) have found no sexual dimorphism in body size. ***Piotr et***

al.,(***2004***) have attempted morphometric comparisons between males and females with no positive results.

Morphometric correlation matrix (Table 4.3) has some implications statistical models could be developed to predict easily measured morphs from those that are difficult to measure. For example, the length of the back could be predicted from the length of the fore foot, Total length from foot length and so forth.

5.2 Craniometric

Like morphometric, cranometric also varied among the three geographic regions (Table 4.4).The specific characteristics of a skull often reflect the animal methods of feeding and effect on the muscle of mastication (***Olude et al., 2010***). Skulls differ largely, not only between different species and breed but also between individuals of same breed and sex (***Koing and Liebich, 2004***). Historically, subspecies of hares were classified on the basis of the morphological features of the skull and teeth (***Suchentrunk et al., 2003; Palacios et al., 2008***). ***Xin*** (***2003***) suggests that analysis of skull development between different animal species exposed to different selection pressures can contribute to the understanding of geographical variations of particular populations, as well as life history strategies and evolutionary change. On the other hand, an increase in skull size is attributed to increased food availability, either by human activity or higher primary productivity (***Yom-Tov and Geffen, 2011***).

Like morphometric, craniometric correlation matrix (Table 4.5) is useful for developing predictive statistical models. For example, ENL could be usefully predicted from NL, Pl from ENL, and so forth. That SFW relationship with the cranial bones is weak, and either positive or negative suggests that it is useless to be included in developing predictive models. Its negative correlations with MH, UCTL, TBL, PPW, TBW, PL and FIL need further investigations.

5.3 Sex Craniometric

One important finding is that craniometric is similar for males and females except Tympanic bulla width (TBW) which is wider for males; this finding is useful for determining sexual dimorphism in hares. This contradicts the findings by ***Krunoslav et al.,*** (***2014***) in the island of Vir and in continental north-west Croatia, that no variations in craniometric, based on sex, is apparent although significant variations among geographical regions are evident.

5.4 PopulationGenetics

By and large,the allelic diversity found presently in the overall sample of Sudanese cape hares is compatible to that found in other cape hares (*L. capensis* senu stricto and *L. capensis* s.l.) from South (***Kryger, 2002; Suchentrunk et al., 2009***) and North Africa as well as in brown hares (*L. europaeus*) from different locations in Europe (***Fickel et al., 2005; Ben Slimen et al., 2008***). The level of allelic diversity is similar in the regions East and West of the Nile but significantly lower in the population studied in the region between the two rivers. In this latter population allelic diversity amounts to roughly half of that found in the hares regions East and West and between the two rivers. This is indicated by all population genetic parameters indicative of allelic diversity. The reason for the lower genetic diversity in this region is not clear. But the relatively small sample size for this population might have contributed to the low diversity, as many alleles that may occur at relatively low frequencies would not have been samples by the few individuals. Nevertheless, the “allelic richness“ value, which accounts for unbalanced (also small) sample sizes in the comparison with the values for the other two regions is significantly reduced for the population from between the two rivers. This latter finding may suggest some underlying biological cause for the low allelic diversity there. A potential effect of frequent presence of null alleles (i.e., alleles that are

missed due to technical reasons, such as amplification failures in the PCR runs) cannot be excluded, particularly when considering the absence of any heterozygous genotypes at two loci – Sol33, Sol08, and the very low frequency of heterozygotes at the Sol28 locus). However, a likelihood test for the presence of null alleles in this population could not be carried out, due to the small sample size.

A potential biological reason for the low genetic diversity of the population between the Blue and White Nile could be one or repeated genetic bottlenecks in the recent past, i.e., very low population density for short time or extending over many generations, possibly due to flooding, bush fires, too strong natural predation pressure, overharvesting by humans, parasitic or other infectious diseases etc. A clear answer would necessitate an intensive ecological, pathological, parasitological etc. study of the population. Also, the current results do not allow to generalize the finding of low genetic diversity in the local population where the samples have been collected for the whole region between the two rivers. An investigation of clearly more samples in the whole region between the two rivers in genetic, parasitologic, phathologic etc. terms may shed light on the underlying biological causes that could have led to the low genetic diversity in this area.

In addition to the relatively low genetic diversity found in the population sample from between the Blue and White Nile, this population is also clearly genetically different from the hares roaming the region East and West region. The cape hares from the latter two regions are also differing to some extent in their overall genetic make-up; overall, genetic differentiation is significant, but at a low level, compatible to what can be expected between diverse cape hare populations in South and North Africa (***Kryger, 2002, Ben Slimen et al., 2008, Suchentrunk et al., 2009***) or in brown hares from central Europe (***Fickel et al., 2005***). A recent investigation on genetic differentiation of Indian Hares (*L. nigricollis dayanus*) from east and west of the River Indus in Pakistan did not show any significant

genetic divergence (***Ali et al., 2016***). In general, low genetic differentiation among populations across large geographic areas in diverse hare species, indicating relatively high gene flow suggests large "effective population sizes" (i.e., population sizes that are genetically/evolutionarily relevant), and this may be associated with long speciation times in many species of the genus. Further microsatellite analyses combining Sudanese cape hares and cape hares from North and South Africa should help to understand gene flow patterns and levels of genetic differentiation across large ranges and should help to prove or disprove conspecificity of all hares in Africa currently considered *Lepus capensis* sensu lato. They should also help inferring the systematic position of *L. habessinicus* form Ethiopia that is currently considered a separate species (***Gebremariam, 2016***) and help to understand evolutionary genetics of the cape hares of Africa.

The currently studied *cyt b* sequences of Sudanese cape hares are on the one hand all closely related and on the other hand clearly differentiated evolutionarily from the haplotype of the three scrub hares (*L. saxatilis*) from the Republic of South Africa (that have identical sequences of the 297bp alignment) and the haplotype of the single African Savanna hare (*L. victoriae*) from Ghana. The evolutionary position of the Sudanese cape hares within *L. capensis* s.l. from Sudan needs further comprehensive molecular analysis and should include reliable sequences with known sampling locality. The comparison of *cyt b* sequences obtained in the course of the present thesis with all reliable *cyt b* sequences of cape hares (*L. capensis* s.l.) and other *Lepus* species available from GenBank and several tree-building approaches (neighbor joining, maximum likelihood, and Bayesian inference – not shown in details in the current thesis) suggest close phylogenetic relationships to haplotypes of North African cape hares, hares considered *L. europaeus* (brown hares) from the Near East, and also to Asian forms considered *L. capensis s. l.* Whether or not the taxon "*Lepus capensis* sensu lato" represents a

single species, a complex of closely related species (perhaps indicating incipient speciation), or even a group of partially not very closely related species remains to be investigated by a combination of phylogenetic, phylogeographic, and population genetic analyses, accompanied by analyses of external phenotypes and skull morphometric. Importantly, for all individuals in such a study, unambiguous information on collection locations must be available. Currently, quite a fair number of *cyt b* sequences of "*L. capensis*" are available from GenBank for such comparisons, but seemingly the taxonomic information associated with many is not always clear (e.g., sometime "*L. capensis*" is used for brown hares that are currently considered *L. europaeus*), and in many cases no clear information on sampling locations or morphological/phenotypical characteristics is available. Therefore, currently it is too premature to work out comprehensive phylogenetic relationships based on *cyt b* sequences (this may rather add to tentative confusion that already exists for this taxon). The systematic relationship between African cape hares and European brown hares (*L. europaeus*) from the Near East needs further study, but so far the conspecificity hypothesis of ***Petter*** (***1959***) cannot be refuted as shown by a first microsatellite study by ***Ben Slimen et al.,*** (***2008***). In any case, the currently analyzed*cyt b* sequences of Sudanese hares fit nicely to sequences of African *L. capensis* s.l. and are also closely related to sequences of what is currently considered*L. europaeus* from the Near East. Should both currently acknowledged species turn out in the future to be indeed conspecific, the correct species name for the currently studied Sudanese hares will be maintained *L. capensis*, provided African *L. capensis* is not partitioned into different species, due to future molecular results.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

In this study, analyses of morphometric and craniometric have indicated considerable variations of these measurements among the three geographic regions from which hares were collected. The implication is that different species of hares might have occurred in the geographic regions. Further investigation on morphological, phenotypic and dental features; however, confirm that the specimens belong to *Lepus capensis*. This supports the abundant literature that environmental factors in different geographic regions influence morphometric and craniometric features. Both morphometric and craniometric measures are useful for determining sexual dimorphism, based on the findings that females are always heavier than males, with longer necks; and that tympanic bulla width is always wider in males compared with females. Correlation matrices of morphometric parameter and Craniometric could be used to develop statistical models to predict a particular easily measurable variable from the one variable that is difficult to measure.

Low genetic diversity found in the population sample from between the two rivers this population is also clearly genetically different from the hares roaming the East of the Nile and West of the Nile. The study identified 89 alleles in nine loci and 32 were found in three regions while the private alleles were 16 that occurred in only one region.

Recommendations

There are some gaps in knowledge that need to be addressed in the future to reinforce the findings of this study. These are:

1. Collection of metrological data and assessment of habitat condition are essential in determining variations in environmental factors in the geographic regions from which hares are collected.
2. Basic information about food quality and quantity in the three geographic regions is essential to assess variations in hares' morphometric and craniometric.
3. Long-term Studies of reproductive biology, ecology and behavior of hares relative to food quantity and quality in the three regions are needed to determine how these variables affect hares' population dynamics.
4. Future research is needed about trans boundary hares in order to determine the gene flow between the countries.
5. Further analyses of *mt*DNA cytochrome b gene with additional specimens of hares from Sudan are needed to clarify the taxonomic status.

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Appendix (1): Mean of temperature (c°) in (6) climatic zones of Sudan.

Year	Obied (zone 5)		Aldamer (zone 1)		Alfashir (zone 7)		Khartoum (zone 3)		Sinnar (zone 4)		Gadarif (zone 4)		Wadmadeni (zone 3)		Dongola (zone 1)	
	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
1961	33.5	19.0	36.3	21.8	33.7	16.0	36.0	21.8	36.9	20.4	35.9	21.1	36.3	19.4	35.1	18.0
1962	34.1	19.4	37.6	22.6	34.9	17.0	37.2	22.7	37.0	20.7	36.3	21.4	36.7	19.9	37.0	19.6
1963	34.6	20.0	38.2	23.2	34.8	17.2	37.7	23.2	36.8	21.0	36.3	21.4	33.8	18.7	37.9	17.7
1964	33.6	19.1	36.5	21.7	34.1	16.5	36.3	21.9	35.9	19.6	35.5	20.7	35.9	19.8	36.0	17.9
1965	34.3	19.3	37.3	22.2	34.7	16.3	37.4	22.5	36.6	20.2	36.5	21.1	33.2	19.9	36.7	18.6
1966	34.7	20.3	38.0	22.9	34.7	17.5	37.9	23.4	36.9	21.0	36.5	21.5	37.1	20.5	36.9	18.9
1967	33.9	19.5	36.7	21.9	34.1	16.2	36.4	21.9	36.1	19.9	35.6	20.8	35.9	19.6	35.4	18.3
1968	34.4	19.7	37.1	22.2	34.4	16.3	36.9	22.0	36.2	20.0	36.0	21.0	36.4	19.5	36.4	17.9
1969	35.3	21.1	38.0	22.9	35.2	17.4	38.1	23.2	37.8	21.3	37.2	21.5	37.5	20.6	37.4	18.5
1970	34.6	20.2	37.5	22.2	34.8	17.3	37.3	22.7	36.9	20.9	36.3	21.5	36.7	20.3	36.3	17.2
1971	34.3	19.8	37.2	21.6	34.7	16.9	36.8	20.9	36.4	19.8	35.8	21.0	30.7	19.8	36.5	17.5
1972	34.8	20.2	37.5	22.1	35.4	18.1	37.4	22.7	36.8	20.9	36.4	21.4	37.0	20.4	36.5	17.5
1973	35.5	21.0	38.2	22.5	35.6	18.2	37.8	23.0	37.2	21.1	36.8	22.0	37.6	21.2	36.9	17.9
1974	34.1	20.0	37.2	22.0	34.2	17.2	36.6	22.4	36.5	20.3	35.7	20.9	36.5	20.0	36.3	17.5
1975	34.4	20.4	37.1	21.8	34.4	17.6	36.8	22.6	36.7	20.3	36.0	21.1	36.3	20.3	36.2	17.5
1976	35.2	20.7	37.3	22.7	35.2	18.5	37.2	22.9	36.9	20.8	36.5	20.8	37.0	20.6	36.8	18.4
1977	34.2	19.8	36.3	21.6	34.0	17.3	36.2	22.1	36.6	20.4	36.4	20.7	36.5	19.3	35.7	17.1
1978	34.7	20.3	37.1	22.2	34.5	17.9	37.0	22.3	36.6	19.5	36.5	21.1	36.6	19.8	36.6	17.9
1979	35.3	20.9	37.8	22.9	35.3	18.4	37.5	23.0	36.7	19.7	36.9	21.3	37.2	20.9	37.4	19.0

Appendix (1) continued

1980	35.4	20.7	37.9	22.9	35.0	17.9	37.5	22.9	36.9	20.4	36.8	21.3	37.4	21.0
1981	34.8	20.4	37.5	22.8	34.6	17.5	37.0	22.6	36.8	20.0	36.6	20.1	37.0	20.8
1982	34.9	20.3	36.8	22.1	34.6	17.8	36.8	22.2	36.7	19.4	36.5	20.0	36.9	20.5
1983	34.5	20.2	36.8	21.9	34.6	17.8	36.9	22.7	36.9	19.5	36.7	21.3	33.7	20.3
1984	35.7	21.3	37.9	22.5	35.1	17.0	37.7	23.5	38.0	20.2	38.0	22.0	34.9	21.6
1985	34.6	21.2	37.7	22.7	34.5	18.4	37.0	21.5	36.9	20.0	36.6	21.5	36.6	21.9
1986	34.7	20.6	37.3	21.7	34.8	16.2	36.8	22.6	36.9	19.5	36.8	21.1	37.8	20.4
1987	35.1	21.1	37.9	22.7	35.0	17.5	37.5	23.2	37.7	20.2	37.1	22.0	37.8	20.9
1988	34.3	20.8	37.3	22.5	34.6	15.3	36.9	23.3	36.7	19.6	36.9	21.6	37.2	21.7
1990	35.6	19.1	38.1	22.7	35.0	17.2	38.0	24.1	38.6	20.7	38.1	22.0	38.4	21.1
1991	35.0	20.3	38.2	21.5	34.7	15.5	37.3	23.8	36.8	18.9	37.3	22.0	30.9	20.6
1992	34.0	17.8	37.1	21.1	33.7	17.8	36.0	22.4	35.7	18.0	36.7	21.1	36.5	19.2
1993	34.7	19.6	38.4	22.7	34.6	19.0	30.8	23.7	36.7	18.4	37.1	21.7	37.3	20.1
1994	34.3	18.0	37.9	21.4	33.9	17.8	36.2	22.7	36.6	17.8	36.7	21.7	36.8	19.9
1995	34.7	18.7	38.4	22.3	34.8	18.8	37.0	22.7	37.1	17.5	37.1	21.5	37.2	19.8
1996	35.3	19.2	38.6	22.1	35.1	19.0	37.2	23.0	36.9	18.8	36.9	21.6	37.1	20.0
1997	34.6	19.6	38.0	22.6	34.8	19.2	37.0	22.8	37.0	20.2	36.5	21.8	37.2	20.4
1998	34.9	19.4	38.3	22.4	34.9	19.0	37.4	23.6	37.2	20.3	36.8	22.1	37.0	19.8
1999	34.7	19.6	39.1	22.9	34.9	18.8	37.6	23.3	36.9	20.7	36.8	21.9	37.4	19.7
2000	34.4	20.7	38.5	22.7	34.8	18.4	37.5	21.8	36.7	20.4	36.8	21.8	37.6	21.2
2001	34.1	20.9	38.6	23.0	35.2	19.1	37.4	22.9	37.0	20.6	37.2	22.2	37.5	21.0
2002	35.2	21.3	38.6	22.3	35.1	19.5	37.5	23.1	37.5	20.3	37.8	22.2	-48.9	20.9
2003	35.0	21.5	38.5	21.8	35.3	19.7	37.2	23.6	37.4	20.4	37.4	22.2	37.7	21.2
2004	35.2	21.2	38.7	22.3	34.4	19.1	37.9	23.5	37.7	20.4	37.3	22.2	38.1	21.2

Appendix (1) continued

2005	35.3	21.5	39.0	22.7	35.0	19.4	37.9	23.8	37.7	20.7	37.8	22.4	38.0	21.4
2006	34.6	21.0	38.6	22.2	34.5	20.0	37.4	21.3	37.0	20.1	37.0	21.9	37.4	21.1
2007	34.6	20.6	38.6	22.8	34.6	19.1	37.4	23.3	36.5	20.1	37.0	22.3	36.9	20.8
2008	33.6	20.3	38.9	22.6	34.7	18.5	38.2	23.8	36.7	20.3	37.3	22.3	38.0	20.9
2009	35.7	21.7	34.2	23.5	35.5	20.1	38.1	24.2	37.7	20.6	38.4	22.9	38.2	21.1
2010	35.6	21.8	35.0	24.3	35.6	20.0	38.3	24.3	37.3	21.2	37.8	23.1	38.5	21.1
2011	34.9	20.7	33.4	23.1	34.8	18.8	37.6	21.8	37.3	19.7	37.7	22.4	38.3	20.6
2012	34.6	20.6	38.8	23.5	34.7	19.0	38.1	21.9	37.5	18.1	37.4	22.2	37.9	21.0
2013	37.6	21.3	38.6	23.4	35.1	19.3	37.7	24.6	37.4	19.6	37.8	22.8	38.2	21.4
2014	34.8	21.3	38.0	23.6	34.9	19.8	36.7	23.8	36.8	20.9	36.8	22.7	37.1	21.6
2015	34.9	21.2	39.3	24.8	36.0	20.2	38.1	25.0	38.0	21.5	38.3	23.6	39.1	22.1

Appendix (2): Mean of rain fall in (7) climatic zones of Sudan.

The Zones						
One	Two	Three	four	five	Six	Seven
6	5	153	410	558	422	311

Appendix (3): Indicates significant differences of allelic diversity among the three regions by Kruskal-Wallis-Test.

Regions	**Number of alleles**	**Ranges**
East	9	16.50
Between	9	7.56
West	9	17.94
Significant	27	0.011

Appendix (4): Indicates significant differences of allelic diversity among the three regions by Friedman-Test.

Regions	Number of alleles	Ranges
East	9	2.33
Between	9	1.22
West	9	2.44
Significant	27	0.016

Appendix (5): Analysis of Molecular Variance (AMOVA) results of the overall genetic variability among population in the regions.

Source of variation.	Degree of freedom	Sum of square	Variance components	Percentage variation of Variance	p-values
Among populations	2	27.93	0.22	6.76%	0.0001
among individuals within populations	92	308.99	0.29	8.81%	0.0001
within individuals	95	264.00	2.78	84.43%	0.0001
Total	189	600.916	3.29		