TYPOLOGY, GENETIC DIVERSITY AND GENOME WIDE SCAN FOR SIGNATURES OF SELECTION OF PRODUCTIVITY TRAITS IN INDIGENOUS GOATS IN THE DEMOCRATIC REPUBLIC OF CONGO

BY

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Declaration

I declare that the work documented in this thesis is my own composition. This work has never been submitted by anyone to any other institution. Specific contributions by others are acknowledged.

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Preface

This thesis has been presented under the supervision of Professor JOSEPH OWINO JUNGA, Doctor JOEL WINYO OCHIENG in the Department of Animal production (University of Nairobi), and Dr. CHRISTIAN KEAMBOU TIAMBO of the Centre for Tropical Livestock Genetics and Health (CTLGH) – ILRI, Nairobi. This work has been structured into seven chapiters consisting of general introduction, literature review, general materiel and methods, and manuscripts published or to be submitted in peer reviewed journals. In the chapter one which is the general introduction, the purpose of the study was justified. In chapter two, a literature review has been presented on several aspects of goat domestication, genetic diversity and population structure of goats (molecular techniques used and parameters to analyze). Genome wide scan for the signatures of selection and genome wide association studies for phenotypes economic traits have been reviewed. In chapter three, the study area and data collection method were described. Chapter four, five, six and seven have been prepared in manuscript paper format. Chapter four, present the results on the phenotypic traits, the reproductive and milk production performances of goats (Journal of Dairy, Veterinary and Animal Research). Typology, production management and critical traits for selection of indigenous goats (*Capra hircus*) in three agro-ecological zones in the Democratic Republic of Congo was prepared as chapter five (journal of applied animal research). In chapter six, a scientific paper titled "Haplotype analysis of the mitochondrial DNA *d-loop* region reveals maternal origin and historical dynamics among the indigenous goat populations in east and west of the Democratic Republic of Congo" was prepared to be submitted to the journal (Journal of evolution and ecology). "Genome-wide scan for signatures of selection in indigenous goats from the east and west of the Democratic Republic of Congo" was presented as chapter seven to be submitted to the journal. The last part of this study contains conclusions and recommendations of the research findings.

Dedication

To my lovely grandfather; RAMAZANI BAENYI who loved me and always wanted me to succeed. A special gratitude to my loving wife, TANTINE MASTAKI JULIENNE for the daily encouragement. My son, KUMBAALA BAENYI DJIBRIL and my daughter ASIFIWE BAENYI ESTHER DJENNY for their love, prayers, support and patience during the time of my absence.

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I am truly thankful

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List of abbreviations

◦ C : Degree Celsius

µl : Microliter

AEZs : Agroecological zones

AGIN : African Goat Improvement Network

AMOVA : Analysis of Molecular Variance

AnGR : Animal genetic ressources

BecA : Biosciences eastern and central Africa

BSP : Bayesian Skyline Plot

DA : Discriminant Analysis

DAPC : Discriminant Analysis for Principal Components

DAVID : Database for Annotation, Visualization, and Integrated Discovery

DNA : Deoxyribonucleic Acid

DnaSP : DNA Sequence Polymorphism

DRC : Democratic Republic of Congo

D-loop : Displacement loop

EDTA : Ethylene Diamine Tetra Acetic Acid

FAO : Food and Agriculture Organization

F_{IS} : Inbreeding coefficient

F_{ST} : F-Statistics

GIS : Geographic Information System

GO : Gene ontology

GPC : Genetic Power Calculator

GPS : Geographic Power Calculator

GWAS : Genome wide association studies

H_E : Expected heterozygosity

H_O : Observed heterozygosity

HVI : Hypervariable I region

HWE : Hardy Weinberg Equilibrium

ILRI : International Livestock Research Institute

Kb : Kilobase

LD : Linkage Disequilibrium

MAF : Minor allele frequencies

MAS : Marker Assisted Selection

MEGA : Molecular Evolutionary Genetics Analysis

MJ : Median-joining network

mtDNA : Mitochondrial DNA

NCBI : National Center for Biotechnology Information

Ne : Effective population size

NJ : Neighbor joining tree

ng : Nanogram

PCA : Principal Components Analysis

PCR : Polymerase Chain Reaction

RAPD : Random Amplified Polymorphism DNA

RFLP : Restriction Fragment Length Polymorphism

SNP : Single Nucleotide Polymorphism

tRNA : Transfer ribonucleic acid

UoN : University of Nairobi

QC : Quality control

QTL : Quantitative Traits Locus

XP-EHH : Cross-population extended haplotype homozygosity

ABSTRACT

Genetic characterization and assessment of signatures of selection in domestic animals adapted to local environments provide a large amount of knowledge and give a clear perspective on the population structure that assists in the decision-making of future breeding programs. This study aimed at characterizing the phenotypic and genetic diversity, the typology and production management, and carrying out a genome-wide assessment of signatures of selection on candidate genes of productivity traits (prolificacy, disease resistance and adaptability) in three Congolese indigenous goat populations (small goat, Kasai goat, and dwarf goat) across three agro-ecological zones (AEZs) in the Democratic Republic of Congo (DRC). The three AEZ regions: Kinshasa (in the stratified savannah plateau), South Kivu (in the high altitude volcanic mountains) and Tshopo (in the equatorial forest), were selected based on their accessibility and the socio-economic importance of goats. Using a structured questionnaire, information on production management, the economic benefit of keeping goats, and reproductive performance (focused on litter size) was collected from 202 farms from where phenotypic measurements were recorded for 320 adult females. From each of the 320 sample animals, five to ten (5-10) ml of total blood was collected for DNA analysis. Total genomic DNA (gDNA) was used for mtDNA *d-loop* region sequencing and genotyping carried out using the Goat 60K SNP chip panel.

The results showed that the oldest animals were 3.34 ±1.48 years old found in South Kivu with 3.85±1.71 number of kidding. Based on the hierarchical clustering on principal components analysis, the studied goat populations were clustered into three clusters well distinguished by double and triple kidding. Prolific goats were mostly represented by goats from South Kivu mostly found in cluster two (48.33%) and three (37.22%). 82.69% of goats from Tshopo were clustered into cluster one characterized by goats with low reproductive performances (less double and triple kidding frequencies). The Canonical Discriminate

Analysis (CDA) revealed that the body measurements, particularly the body length were an important variable both to discriminate and to classify goats from the three geographical regions. The Mahalanobis's distances revealed that indigenous goats from Kinshasa and South Kivu were not genetically distant while the largest genetic distance was observed between goats from Kinshasa and Tshopo or South Kivu and Tshopo (F-stat, $p< 0.001$). No improvement techniques in feeding, disease control, and reproduction management were applied in goat breeding in DRC. However, goats were considered as a source of income and saving method in smallholder farmer's households. The adaptability of goat to the region, disease resistance, and prolificacy were the farmer-preferred traits in the selection of goats.

The analysis of 339 mitochondrial DNA displacement loop (mtDNA d-loop) sequences with a total length of 1,169 base pairs (bp) revealed a total of 568 segregating sites, resulting in 192 haplotypes. Only 9 of the 192 haplotypes were shared between the Congolese goats and goats from Kenya, Ethiopia, Algeria (1 haplotype), Zimbabwe (1 haplotype), Pakistan (1 haplotype), Cameroon (3 haplotypes), and Mozambique (3 haplotypes). Average haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (K) were 0.987, 0.015 and 14.74, respectively. The analyses of molecular variance (AMOVA) revealed that 5.88 % of Congolese goat populations' variation was observed among the population and 94.12% within the population. A weak genetic differentiation was revealed between the three Congolese indigenous goat populations based on the population pairwise analysis (F_{ST}). Neighbor-joining (NJ) and Median Joining (MJ) analyses revealed both the three studied Congolese indigenous goat populations to belong to the haplogroup A with one maternal origin as revealed by the mismatch distribution analysis. Negative and significant (p-value < 0.05) values for *F*u's *F*s (-20.418) and Tajima's (-2.189) tests showed that the three Congolese indigenous goat populations have expended in history. The Approximate Bayesian Computation (ABC) analyses indicated that the Congolese goats operated from the northern

Africa before reach Central Africa, 354 generations in later times (~1,062years ago). Specific putative signatures of selection were identified in the three Congolese indigenous goat populations using the cross-population expended haplotype homozygosity (XP-EHH) statistical method applied across Congolese and other (Cameroon and Keffa in Ethiopia) goat populations. Positive genomic regions were enriched and annotated using the *Capra hircus* Genome Data Viewer Assembly ARS1 (GCF_001704415.1) and the web-based tool, Database for Annotation, Visualization, and Integrated Discovery (DAVID) software, respectively. Ten out of forty three positive genomic regions were enriched with genes involved in signalling pathways associated directly or indirectly with body size (DEPTOR, MAGEL2), behaviour and nervous systems (DCDC2, PANK3, ITSN1, COL6A3, ENPP2), prolificacy (CMK4), disease control such as a decrease in salmonella proliferation (EIF3J) and hair colour measurement, hair measurement and hair colour (PADI2).

The results of this study suggest a high phenotypic and genetic diversity in Congolese indigenous goat populations clustered into three clusters well distinguished by the reproductive performances (double and triple kidding). Adaptability in the region, disease resistance and prolificacy were the major farmer-preferred traits for selecting goats to raise. A weak genetic differentiation and a single maternal origin were observed for the studied goats that underwent an expansion in the history. Novel insights into the genetic and physiological architecture of goat's adaptation and reproduction were revealed by the gene ontology (GO) enrichment of the positive selection regions. However, similar to the relatively poor annotation of the caprine genome, information provided by the GO analysis is limited. Since most of these candidate genes have been previously reported to be under positive selection for several traits in other species; further research should be conducted on the candidate genes reported in this study to clarify their implication and association with the reported and

unreported traits in goats in DRC. These results could have implications for managing improvement strategies and long-term conservation of Congolese indigenous goats.

Key words: Adaptability, candidate genes, indigenous goat, genetic diversity, prolificacy, DR Congo

CHAPTER ONE: GENERAL INTRODUCTION

1.1. Background information

Goats are observed to be among the first animals to be domesticated and largely distributed in the world (MacHugh and Bradley, 1997), with a major role in the production of milk, meat, fiber, and hides (Shrestha and Fahmy, 2005). Due to their multiple uses and their adaptability, goats are most appreciated by farmers and consumers since they contribute significantly to household income (Chentouf *et al.*, 2011). Therefore, lack of sufficient information on the reproduction performances as well as the genetic diversity is affecting goats' productivity in their environment of production (Monteiro *et al*., 2017; Assan, 2020).

In the long term, genetic diversity positively affects species and population sustainability (Sjöqvist *et al*., 2016, Hendricks *et al.,* 2020). Hence, it needs to be assessed to be able to provide selection for desired species diversity (Mahmoudi *et al*., 2011). Genetic variability and diversity are regarded as the pillars of animal and crop diversification (Govindaraj *et al.,* 2015). Each living organism can develop specific genetic traits based on specific natural ecosystems, environmental and socio-economic conditions (Gregory, 2009). This variability, developed by each living organism in particular conditions, is essential for genetic conservation (Lacy, 1997).

Different techniques including morphological and biochemical traits, geographical origin, and molecular markers (microsatellites, RFLP, AFLP, RAPD, mtDNA, Y-chromosome, SNP) have been used for animal genetic diversity studies (Filippo *et al*., 2015).

The displacement loop (*d-loop*) region of the mtDNA has been widely used and has proved its efficiency in the genetic diversity, population structure, and population dynamics assessment in domestic animals (Liu *et al*., 2009; Tarekegn *et al*., 2018 and 2019). Providing many advantages including both genetic diversity and candidate genes linked with economic traits

assessment, signatures of selection analysis using the genome-wide single nucleotide polymorphisms (SNPs) technique has been widely used in genome exploration in livestock in general and in goat breeds (Brito *et al*., 2017, Tarekegn *et al*., 2018, 2019; Onzima *et al*., 2019).

Both artificial and natural selection were reported to be more apparent respectively in exotic and local breeds, leading to high productivity and adaptation in various environments. However, these traits (high productivity and adaptation) could be attributed to different signatures within certain genomic regions which might be imparted by both artificial and natural selection (Fu-ping *et al*., 2016). The genome-wide scan based on the Illumina caprine SNP50 Genotyping Bead Chip array technology has facilitated the detection of positive signatures of selection in goats (Onzima, 2019; Chokoe *et al*., 2020; Tilahum *et al*., 2020). An understanding of agnostic evaluation of the association between genetic variants (positive signatures of selection) and important phenotype economic traits (prolificacy, disease resistance, drought tolerance, milk production, etc.) is required to elucidate if these traits are based on the genetic or environmental factors (Witte, 2014; Tam *et al*., 2019). The genetic variants across the genome of different species including human and domestic animals have been reported to be tested based on the genome-wide association studies (GWAS) to identify genotype-phenotype association (Tam *et al*., 2019, Tilahum *et al*., 2020). In previous studies, the GWAS have revealed the association between candidate genes with important phenotype economic traits in goats, for instance, prolificacy (Islam *et al.*, 2020; Wang *et al*., 2021), disease resistance (Silva *et al*., 2018), body morphological traits (Nazari-Ghadikolaei *et al*., 2018; Rahmatalla *et al*., 2018), milk quality and production (Mucha *et al*., 2018), growth (Grigoletto *et al*., 2019), and adaptability (Tilahum *et al*., 20120).

1.2. Problem startement

In the Democratic Republic of Congo (DR Congo) the goat represents the second important domestic animal after the chicken (FAOSTAT, 2018) and contributes 72% to rural household income, which represents 75% of the Congolese population (Wasso *et al*., 2018). It has been demonstrated that the productivity of indigenous goats is low (Gasigwa *et al*., 2017; Baenyi *et al*., 2018). Factors such as lack of sufficient information on the genetic structure and networks of goat production and the absence of an established goat genetic improvement program in DRC could be attributed to the observed low productivity in these goat populations (Wasso *et al*., 2018; Baenyi *et al*., 2021).

This study therefore aimed to characterize the phenotypic and genetic diversity, the typology and production management, and to determine the signatures of selection on candidate genes associated with traits of economic interest (prolificacy and adaptability) in indigenous goat populations in the DRC. Such work would potentially provide a scientific basis for enhancing indigenous goat productivity and livelihood improvement in DRC.

1.3. Justification of the study

To facilitate increased goat productivity, characterization of the phenotypic and genetic diversity, production management, and assessment of signatures of selection on genes linked with traits of economic interest such as adaptability and prolificacy in indigenous goat populations in the AEZs of the DRC are important. This knowledge will provide information on the genetic potential of goats that has the potential to contribute to the development of election tools (maker-assisted selection) for the improvement of goat breeding programs in the DRC.

1.4. Research objectives

1.4.1. Overall objective

Generally, this study aimed at investigating the phenotypic and genetic diversity, the typology and production management, and genome wide assessment of signatures of selection on genes associated with survival and productivity in indigenous goat populations in the DRC.

1.4.2. Specific objectives:

1. To characterize the typology, the production system, and the management of indigenous goat populations in east and west of DRC;
2. To characterize the genetic diversity, the population structure, and dynamics of indigenous goat populations in east and west of DRC;
3. To determine the genome-wide scan for the detection of signatures of selection on candidate genes linked with traits of adaptability and prolificacy in indigenous goat populations in east and west of DRC.

1.5. Null Hypotheses

1. There is no genetic diversity among and between indigenous goat populations in different agro ecological zones of the DRC;
2. Congolese indigenous goat populations are not genetically differentiated
3. There are no signatures of selection on candidate genes linked to survival (disease resistance, adaptability) and productivity (prolificacy) in Congolese indigenous goat populations.

CHAPTER TWO: LITERATURE REVIEW

2.1. Introduction

Goats play an important role in the food production systems and contribute significantly to household incomes in many developing countries in the world (Shrestha and Fahmy, 2005; Chentouf *et al*., 2011). Therefore, in most of these countries, goats are raised in traditional methods without proper knowledge on their genetic diversity (Deldar-Tajangookeh *et al*., 2009). The molecular investigation of the genetic diversity presently considered as one of the FAO priorities for breed characterization has been proposed as a valuable complement and sometimes proxy to phenotypic diversity of local breeds. Genetic variability is more important and it seems to positively affect the conservation sustainability of populations in long term (Kahilainen *et al*., 2014). Livestock conservation and improvement programs require proper knowledge on the genetic diversity, the production systems, and the population structure that include different genetic sources of diversity within and among breeds (Mahmoudi *et al*., 2009). In goat species, litter size that indicate the prolificacy of an animal is appreciated at the second kidding and it is considered as a crucial and complex economic trait within the goat industry (Zhang *et al*., 2012; Marhous *et al*., 2013). Palai *et al*. (2013) have demonstrated that the number of kids per parity was under the control of various genes and different biological and physiological factors that cannot be increased with traditional improvement methods (selection) due to the low heritability of the reproductive performances. Therefore, genomic selection and marker-assisted selection would be useful in the conservation and improvement programs of animal genetic resources.

This chapter provided an overview of the origin, the domestication, the genetic diversity, the population structure, and the methods of detecting signatures of selection on candidate genes linked to traits of economic interest in indigenous goat populations.

2.2. The origin and domestication of goat

The domestication of the goat was an integral part of the rise of agriculture and the adoption of agricultural practices throughout much of the world (Fernández *et al.,* 2006; Luikart *et al*., 2006). Around 11,000 years ago, goat is believed to be the first wild herbivores to be domesticated in the Near East at the beginning of the revolutionary transition from hunter-gatherer to agriculture-based societies (Zeder, 2012). From that period, goats have contributed to vital cultural, religious, and socio-economic roles in the civilizations of humans worldwide. The vast expansion of the ability to detect the context, locations, and timing of initial domestication of goats, and the ability to trace the migratory trajectories used by humans to worldwide spread goat were facilitated with genetics and archeology studies (Zeder, 2008; 2012).

Based on different studies, divergences were observed in the origin and the domestication centers of the goat. A potential origin of the goat was suggested in the Balkans or Carpathian Mountain regions of Romania when comparing the geographic distribution of goat Y-chromosome lineages and mtDNA lineages. Divergent mtDNA lineages (lineage C) were mainly found in Switzerland and Slovenia, while a distinctive Y-chromosome lineage was only found in the Carpathian Mountains (Fernández *et al.,* 2006; Pereira *et al*., 2010). Therefore, doubt was cast on these three regions suggested as centers of *Capra hircus* domestication due to sample size limitations. In addition, the hypothesis of domestication started by Luikart *et al*. (2001) for lineage C, and the putative domestication centers as demonstrated by Naderi *et al*. (2008) are contradictory to these suggested goat domestication centers making questions on the previous premises of the domestication of goat in general.

Levant and Mehrgarh have been respectively suggested by Horwitz *et al*. (1999) and Sultana *et al*. (2003) as two ancient centers for the domestication of goats. However, the DNA analysis of the ancient goat in the Inner Mongolia region was genetically closed to modern

Chinese goat which led to the assumption that China could also be a goat domestication center, specifically for sub-haplogroups B1 and B2. The analysis of the hypervariable (HVI) region of the displacement loop (*d-loop*) region of the mitochondrial DNA of the ancient goat, revealed that the central Zagros played a major role in the domestication of goats (Mazdarani *et al*., 2014). Moreover, the first Neolithic migration waves could accompany the presence of lineage A and C in Southeast and Central Europe (Colli *et al*., 2015).

Divergences are still observed on the origin and the goat domestication centers. However, the goats that have been firstly domesticated for meat production might be one of the oldest domesticated animals and probably the first ruminant to be domesticated after wolf domestication (MacHugh and Bradley, 2011).

2.3. Genetic diversity assessment

2.3.1. Definition and importance of genetic diversity

Genetic variability and diversity are regarded as the pillars of animal and crop diversification (Govindaraj *et al*., 2015). Each living organism can develop specific genetic traits based on specific natural ecosystems, environmental and socio-economic conditions. (Gregory, 2009). This variability, developed by each living organism in particular conditions, is essential for genetic conservation (Lacy, 1997). Collectively, these particular traits constitute species diversity of the Earth (Hailu *et al*., 2015).

The knowledge of the genetic variability and process that underlie livestock's origins and maintenance have crucial importance to provide critical insights into the structure and dynamics of livestock populations (Caliskan, 2012). Genetic variability is required for any population to evolve and cope with climate change, new diseases, and pest epidemics (Woodruff, 2001; Caliskan, 2012; Govindaraj *et al*., 2015) . The opportunity to trace the population history, and to link them with their ancestors is facilitated by genetic diversity which can be assessed at the morphological, biochemical, and molecular levels (Pérez *et al*.,

2019). The observed diversity requires being especially utilized, improved, and conserved. Strategies for the improvement and the conservation of animal populations are focused on a proper genetic characterization coupled with phenotypic characterization (Halima, 2007).

Animal genetic resources (AnGR) are a capital component of the biological foundation for food security in the world (FAO, 2012). A number estimated to hundreds of millions of poor people living in rural areas keep animals and frequently depend on them to provide many different services and products (FAO, 2011). The genetic characterization of livestock is considered as the basic step when considering solid management and conservation of a specific population (Caliskan, 2012; FAO, 2017). A good understanding of the genetic variation within and among breeds is important for the improvement of traits of economic interest (Govindaraj *et al*., 2015). Hence, the coming breed improvement depends on the existing genetic diversity in the population of parents (Xu *et al*., 2017).

2.3.2. Phenotypic characterization

Phenotyping and genotyping are both essential to identify and to classify both animal or crop genetic resources (Donelli *et al*., 2013). Phenotypic characterization of animal genetic resources is the process of identifying distinct breed populations and describing their external and production characteristics in a given environment and under given management, taking into account the social and economic factors that affect them (Karnuah *et al*., 2018) . In 2007, The Global Plan of Action for Animal Genetic Resources was adopted and was considered as the first internationally aggred framework for the management of biodiversity in the livestock sector (FAO, 2012). The guidelines on phenotypic characterization of animal address strategic priority area 1 of the Global Plan of Action-Characterization, inventory and monitoring of trends and associated risks that adce on how to conduct a well-targeted and cost-effective phenotypic characterization study that contributes to the improvement of animal genetic

resources management in the context of country-level implementation of the Global Plan of Action for Animal Genetic Resources (FAO, 2012). The phenotypic characterization strategiy focus on the evaluation of the quantitative phenotypic traits based on the body measurements (e.g. total body lengh, heigh at witchers, heigh at sterns, body weigh,…) and the qualitative phenotypic traits focused on the presence or absence (e.g. horn, beard, tassels, etc.), the body or hair coat colors and the shape of some organs (Lauvergne *et al*., 1993; FAO, 2012). Inventory of species and breeds, their population sizes, geographic distribution and possibly their genetic diversity is generally undertaken as a first step in any national programme for the management of animal genetic resources for food and agriculture (Tixier-Boichard *et al*., 2008). Phenotypic characterization has been applied to many domestic animal species worldwide and has provided necessary information in several animal breeding and conservation programs (Hall and Bradly, 1995).

2.3.3. Molecular tools/ markers for animal genetic diversity studies

Phenotypic traits, biochemical and molecular markers are largely used to characterize animal genetic resources (Msoffe *et al*., 2004; Joshi *et al*.; 2013). However, the information given by phenotypic and biochemical markers has been revealed very little and is limited to give more information on the variability of a particular population (Filippo *et al*., 2015). The advances in DNA polymorphism techniques and subsequent data analysis in the last two decades have greatly increased the ability to understand the genetic relationship among species at the molecular level. The DNA markers have now been used for molecular genetic characterization and genetic diversity studies in numerous species. The most widely used are microsatellites probing, restriction fragment length polymorphism of nuclear DNA, random amplified polymorphism DNA (RAPD), microsatellites PCR amplification, amplified fragment length polymorphism (AFLP), mitochondrial DNA, and Single Nucleotide

Polymorphisms (SNPs) (Jeffreys and Morton, 1987; Litt and Luty, 1989; Loftus *et al*., 1994). SNPs and microsatellites markers were the most emerged DNA markers (Yadav *et al*., 2017). Advantages offered by the next-generation molecular tools development have facilitated the investigation of both the genetic diversity and the signatures of selection at the genome level either for important production or adaptive traits in goat breeds (Mrode *et al*., 2018).

2.3.3.1. Mitochondrial DNA (mtDNA) analysis for goat genetic diversity assessment

DNA sequencing can directly assay the differences of mt DNA sequences among populations; these differences can also indirectly be assayed through restriction site analysis of the DNA (Loftus *et al.,* 1994; Bradley *et al*., 1996; Kikkawa *et al*., 1997). Universal or specific primers allowing amplification of sequences as much as several thousand base pairs lengths are used to directly sequence mtDNA sequences which can rapidly be analyzed to assess the genetic diversity, the relationship of recently diverged populations in which different genes are involved at different rates.

The *d-loop* region, called in some cases the control region, is defined as the most variable and non-coding region of the mtDNA and has been considered as one of the most amplified regions for the assessment of genetic diversity, population relationship, population structure, and population dynamics of animal species (Wilson *et al*., 1985). Boore (1999) characterized for the first time the mechanism of mtDNA replication in cultured mouse cells. It has been demonstrated that the replication of the H-strand starts at the fixed point (oriH) in the *d-loop* region, then replicated DNA displaces the non-replicated single strand from the *d-loop*. After the H-strand replication fork passed through, the replication of the L-strand initiates at oriL within a tRNA cluster far from the *d-loop*. The replication of the complementary strands of mtDNA is asynchronous. The *d-loop* region is a non-coding and controlling region for transcription and replication lies between two tRNA genes on mtDNA which his length is approximately 1kb in vertebrates, excluding tandem and direct repeated sequences, which are

frequently found in various species. The phylogenetic relationships among breeds and species are established through the analysis of the mtDNA *d-loop* which is a highly useful molecular tool used in population relationships studies (Zhao *et al.,* 2011). The analysis of the mtDNA *d-loop* region has been largely used to assess the genetic diversity in a large number of livestock including sheep (Agaviezor *et al.,* 2012), cattle (Ilie *et al*., 2015), chicken (Hoque *et al.,* 2013), goat (Naderi *et al*.., 2007; Kul and Ertugrul, 2011, Tarekegn *et al*., 2018, 2019) in different production systems worldwide.

Parameters linked to the genetic diversity, the population structure, and the population dynamics can be assessed through the analysis of the mtDNA *d-loop* (Liu *et al*., 2006; Gerbault *et al*., 2012; Seyedabadi *et al.,* 2016; Periasamy *et al*., 2017; Tarekegn *et al*., 2018).

2.3.3.1.1. Genetic diversity and population differentiation based on mt DNA *d-loop* region analysis

Complete sequences of the mtDNA *d-loop* region were analyzed for the assessment of genetic diversity and differentiation in goat populations in developing as well as in developed countries. The understanding of the genetic differentiation between populations is of interest to population genetics since it reflects the number of alleles exchanged between populations which influence the genetic composition of individuals within populations (Balloux and Lugon-Moulin, 2002). Due to the huge global population size in contemporary domestic goats, weaker intercontinental population structuring has been observed in domestic goats than other livestock as revealed by DNA markers studies (Luikart *et al*., 2001). Six monophyletic mtDNA haplogroups including haplogroup A, haplogroup B, haplogroup C, haplogroup D, haplogroup F, and haplogroup G were revealed through mtDNA *d-loop* sequences analysis in the Mahabali goat population. Within these haplogroups, haplogroup A was found to represent more than 90% of the studied goat populations worldwide (Naderi *et*

al., 2008; Mohammadi *et al.*, 2018). That scenario could not have changed dramatically in the expanding goat population since domestication could have dispersed more successfully, more often and more extensively than other domestic animals (Fernandez *et al*., 2006; Naderi *et al*., 2008). Intercontinental variation for the mtDNA in the hypervariable region (HVI) have been revealed to be lower (10%) in domestic goat than estimates of 54% to 80% in cattle. Polymorphic sites, haplotypes, haplotype diversity, nucleotide diversity, Analysis of Molecular Variance (AMOVA), Neighbor-Joining (NJ) tree, and Median Joining network (MJ) are the most considered analyses for the genetic diversity and differentiation evaluation based on the mtDNA marker. Haplotype diversity and nucleotide diversity are useful indices for assessing population polymorphism and genetic differentiation within and between populations (Liu *et al*., 2006; Liu *et al*., 2007; Kang *et al*., 2011; Harlistyo *et al*., 2014; Naqvi *et al*., 2016; Silva *et al*., 2016; Ahmed *et al*., 2017; Tarekegn *et al*., 2018; Kivila *et al*., 2018; Bwihangane *et al*., 2018).

2.3.3.1.2. Genetic relationships and population structure evaluation based on the analysis of the mtDNA *d-loop* region

The population genetic structure is defined as the study of the genetic variation that occurred in space and in time within a population (Janes and Batista, 2016). The combined effects of evolutionary processes such as natural selection, mutation, genetic drift, recombination, demographic history drive the population structure of the population (Gao *et al*., 2017). Traditionally, the assessment of population genetic structure gives information on the dispersal of species and population boundaries. For any organism, the genetic structure is shaped by different factors which vary significantly over space (Janes and Batista, 2016). A good understanding of the genetic relationships and the natural selection that occurred in a population require the analysis of the population structure (Luo *et al*., 2019). In addition, it

facilitates subsequent association mapping studies which the presence can lead to false-positive associations between markers and traits (Eltaher *et al.,* 2018). The population genetic structure analysis is considered as the first step to conduct genome-wide association studies (GWAS) for the identification of the true existing association between traits and markers by underlying genes which control these traits. F-statistics measured by levels of heterozygosity into components of within and between population is considered as the commonest used method of summarizing structure within genetic variability (Wright, 1965). Structure and principal component analysis (PCA) results give also a good understanding of the population relationships. The analysis of mtDNA *d-loop* variation has been widely used to assess the genetic relationships among and between several goat breeds adapted in various environments (Liu *et al*., 2006; Liu *et al*., 2007; Kang *et al*., 2011; Harlistyo *et al*., 2014; Naqvi *et al*., 2016; Silva *et al*., 2016; Ahmed *et al*., 2017; Tarekegn *et al*., 2018; Kivila *et al*., 2018; Bwihangane *et al*., 2018).

2.3.3.1.3. Population dynamic evaluation based on the analysis of the mtDNA *d-loop* region

Useful insights into different evolutionary processes are provided through the reconstructing of population demographic history by evaluating correlations between paleoclimatic and demographic events (Ho *et al*., 2011). This analysis allows testing the elements driving past population dynamics, to trace the transmission and the expansion of viruses (Kitchen *et al*., 2008). Since mt DNA is the more informative among DNA markers in tracking human evolutionary history, it has been one of most use in studying population dynamics in goats (Tarekegn *et al*., 2019).

2.3.3.2. Genetic diversity assessment based on the SNPs analysis

The high polymorphism of microsatellite markers had made them more informative and extensively used in the studies of genetic diversity (Sunnucks, 2000). However, according to a wide hypervariable markers number in goats, the genotyping and scoring were labor-intensive. With the emergency of molecular biology technology, 50 to 60,000 SNP chips were designed for goats based on their low coast, automatic allele calling, and robustness, and quickly replaced microsatellites in genetic diversity assessment as well as in paternity testing. Additionally, the goat genome investigation at high resolution was facilitated with the density of the SNPs chip panels. This offered an advantage of assessing the genetic diversity, the population structure, and the signatures of selection on genes associated to traits of economic interest in goat populations. SNPs markers have also allowed reconstructing the demographic history and detecting recent and historical admixture of known and unknown goat populations (Onzima *et al.,* 2019, Tarekegn *et al.,* 2019, Chokoe *et al.,* 2020).

SNPs markers represent modifications or variations in a DNA sequence that occur when a single nucleotide in the sequence differs at least one percent from the common sequence constitution of the population (Brito *et al.*, 2017). Once SNPs occur inside the gene, they modify it in creating different alleles or variants of that gene. SNPs have become principal markers for genetic diversity assessment and whole-genome studies. Used for the genotyping of different goat populations, SNPs markers have shown a high polymorphism and have revealed considerable variations within goat breeds. As the important raw materials for breeding are represented by genetic diversity and since it has practical implications for implementations of genomic selection, the investigation on genetic diversity levels based on the SNPs markers is relevant (Chokoe *et al.,* 2020). Whole-genome sequencing of six goat breeds that included Creole, Saanen, Boer, Savanna, Alpine, and Katjang has revealed 53,347SNPs in a Goat50K SNP panel which has been widely used to assess the genetic

diversity, the population structure, and the signatures of selection on genes linked to traits of economic interest in different goat populations (Tosser-Klopp *et al.,* 2014).

2.3.3.2.1. Parameters for the genetic diversity and population structure analyses based on SNPs markers

2.3.3.2.1.1. Genetic diversity and Linkage Disequilibrium (LD)

The expected heterozygosity is considered as a common factor used for the quantification of the diversity in a population. It is considered as the likelihood that 2 alleles chosen in a population by chance are different (Toro and Caballero, 2005). A higher value of the expected heterozygosity ($H_E > 0$) indicates higher genetic variability while a lower value ($H_E=0$) implies inbreeding and high selection pressure within a population (Mburu and Hanotte, 2005). The Analysis of Molecular Variance (AMOVA) can also be used to further partition the genetic variability or differentiation within and among populations (With AMOVA value = 0, the overall population is not differentiated with its subpopulation; if it is equivalent to 1, then populations are differentiated). The quantification of the genetic diversity within populations can also be made by the measurement of the Linkage Disequilibrium (LD) which is considered as a non-random relationship of alleles at two or more loci that depend on some demographic factors such as genetic drift, migration, and selection in populations (Slatkin, 2008). The LD is measured based on 2 statistical parameters that included the squared association (r^2) between alleles at two loci and D', both extend from zero to one. A zero value means no disequilibrium and the one value means complete disequilibrium. In biallelic markers, D' equals one if one or more of the four probable haplotypes is not present; It is inferior to one if the four probable haplotypes are available (Pritchard and Przeworski, 2001). Complete disequilibrium is considered for r^2 if only 2 out of the 4 haplotypes are present within a population, while D' statistic requires large data (Khatkar *et al*., 2008).

2.3.3.2.1.2. Inbreeding and effective population size

Inbreeding is the level of related animals. Based on the calculation of the inbreeding coefficient (F_{IS}), it is used to estimate the genetic diversity in populations. Keller and Waller (2002) have demonstrated that: a $F_{IS} > 0$ means loss of genetic diversity occurred through more inbreeding than expected at random; a $F_{IS} < 0$ means less inbreeding than expected at random. The effective population size (Ne) is used to assess the loss of genetic diversity (genetic erosion). Also, it assists in explaining the evolution of a population.

2.3.3.2.1.3. Population structure

The effectiveness of the strategies for the management and the conservation of animal genetic resources requires the knowledge of the population structure that can be obtained by the analysis of the population differentiation and population dynamics (Patterson *et al*., 2006). The Principal Component Analysis (PCA) and the clustering model analysis such as ADMIXTURE and STRUCTURE are the most common approaches to illustrate the population structure (Alexander *et al*., 2009; Pritchard *et al*., 2000). The PCA analysis has facilitated the detection of the gene pool introgression in the population and the historical events that happened and that shaped the population during the domestication and breeds formation.

The allele frequency information is exploited by The STRUCTURE analysis to distinguish parental populations, to estimate admixed individuals, and to detect and allocate individuals to their populations (Pritchard *et al*., 2001). This technique uses a model-based clustering method that employs a Markov Chain to estimate the posterior distribution of each individual's admixture co-efficient. The average of this distribution signifies several individual genes that are acquired from one of the assumed parental populations (Pritchard *et al*., 2001). The identification and the description of clusters of genetically related individuals

have been facilitated through the designee of the discriminant analysis of Principal Components method analysis (DAPC) (Jombart *et al*., 2010). DAPC approach was revealed to perform better than STRUCTURE at assessing subdivisions in population; It uses sequential K means and model selection for genetic clusters inferring (Jombart *et al*., 2010). A Summary of some genetic diversity indices for some goat populations in the Sub-Saharan African region is provided as illustrations (**Table 2.1**).

Table 2. 1:Genetic diversity of some Sub-Saharan African indigenous goats based on the analysis of the SNPs markers

Population	N	Ho	H_E	F_{IS}	Country	Reference
Agew, Ambo, Afar, Gumuz, Gondar and Keffa	336	0.35-0.38	0.37-0.38	0.00-0.34	Ethiopia	Tarekegn *et al*. (2016)
Boer, Kalahari red, Savanna, Xhosa, Zulu and Tankwa	216	0.35-0.41	0.33-0.40	0.03-0.40	South Africa	Mdaladla *et al.* (2016)
Binga, Matopo, Tsholotsho, Chipenge and Shurugwi	246	0.60-0.64	0.60-0.63	-0.01-0.03	Zimbabwe	Zvinorova (2017)
Nubian, Desert, Taggar and Nilotic	95	0.39-0.39	0.39-0.40	-0.01-0.01	Sudan	Rahmatalla *et al.* (2017)
Boer, Karamojong, Kigezi, Mubende and Small East Africa	144	0.34-0.38	0.38-0.41	-----	Uganda	Onzima *et al*. (2018)
North west Highland, Forest goat Central Highland and Djallonke	324	0.33-0.46	0.35-0.50	-----	Cameroon	Tarekegn *et al*. (2019)
Free State, Gauteng, Limpopo, North West	117	0.39-0.50	0.42-0.51	-----	South Africa	Chokoe *et al.* (2020)

N = total number of samples, Ho = Observed homozygosity, H_E = Expected homozygosity, F_{IS}= inbreeding coefficient

2.4. Genome-wide scan for the detection of signatures of selection

The potential of the signatures of selection detection is mentioned to elucidate the identities of genes and mutations linked with traits of economic interest in livestock (Brito *et al*., 2017).

The SNP chips were developed for several livestock species (Fan *et al*., 2010). Genome-wide scan for the detection of signatures of selection was firstly performed in humans (Harris and Meyer, 2006; Oleksyk *et al*., 2010), before being performed in domestic animals such as cattle (Gautier *et al.,* 2009; Stella *et al*., 2010), chicken (Rubin *et al*., 2010; Groenen *et al.,* 2011), sheep (Ramos *et al*., 2009), and goats (Brito *et al*., 2017; Tilahum *et al*., 2020) through the analysis SNPs markers. Statistics approaches using for the detection of signatures of selection are summarized in **Table 2.2**.

Table 2. 2: Statistics approaches for the detection of signatures of selection (Weigand and Leese, 2018)

Method	Type of data	Number of populations	Genomic position	Ancestral state	Recombination map
Linkage disequilibrium-based methods					
LRH	Haplotypes	One	Yes	No	No
iSH	Haplotypes	One	Yes	Yes	Yes
XP-EHH	Haplotypes	Two	Yes	No	Yes
Rsb	Genotypes or Haplotypes	Two	Yes	No	No
H12	Genotypes	One	Yes	No	No
(t) statistic	Haplotypes	One	Yes	No	No
HapFLK	Genotypes	Multiple	Yes	No	No
Site frequency spectrum-based methods (SFS)					
Tajima's D	Genotypes or allele frequencies	One	Yes	No	No
Fu and Li's tests	Genotypes or allele frequencies	One	Yes	Optional	No
Fay and Wu's H	Genotypes or alleles frequencies	One	Yes	Yes	No
CLR	Allele frequencies	One	Yes	Optional	No
XP-CLR	Allele frequencies	Two	Yes	No	Yes
Pool-HMM	Pooled sequence data with quality score	One	Yes	Optional	No
Population differentiation-based method					
FDist	Allele frequencies	Multiple	No	No	No
BayeScan	Allele frequencies	Multiple	No	No	No
FLK	Allele frequencies	Multiple	No	Optional	No

CLR: composite likelihood ratio test; FDist: function distribution; FLK: extended Lewontin and Krakauer test; HapFLK: haplotypes differences between population; H12, iHS: integrated haplotype score; LRH: long-range haplotype test; Pool-HMM, Rsb, XP-CLR: cross-population composite likelihood ratio test; XP-EHH: cross-population extended haplotype homozygosity.

2.5. Genome-wide association studies and identification of candidate genes affecting phenotype

One of the most important and time-consuming steps leading to good and reliable results when doing GWAS is the quality control (QC) check (Corvin *et al*., 2009). The QC is the step that is focused to remove SNPs and subjects with unreliable data and assessing biases that might lead to spurious results (Corvin *et al*., 2009). Any of the following reasons may lead to the removal of an individual SNPs: excessive disagreement among duplicated samples, imprecise mapping to the genome, low minor allele frequency (< 1%), excessive missing genotypes on subjects (> 5%), observed genotype frequencies deviate markedly from the Hardy-Weinberg equilibrium ($p< 0.000001$) (Corvin *et al*., 2009). The logistic regression model is the widely used model for GWAS analysis if using SNPs and subjects that have passed QC. In this model, the case-control status is considered as the dependent variable, while a single SNP is considered as the predictor. Covariates such as sex, indicators of ancestry, or age may be included in the logistic regression model (optional). The SNP is coded as 0, 1, or 2 representing the copies numbers of one of the two alleles for an additive test with 1 df. The success of GWAS requires both multiple test correction and statistical power which are inseparable issues for it. Statistical evidence for GWAS must be held to a high standard when testing large numbers of SNPs to avoid false-positive associations risks. To well perform GWAS test statistics, all samples in the analysis are assumed to be unrelated and selected from a uniform, and random-mating population. Any departure from this assumption can cause unexpected results, especially in large study cohorts (Scherer and Christensen, 2016). Many studies based on the GWAS have been conducted in goat species around the world for different traits including body morphological traits (Zonaed *et al*., 2020), milk production, and quality (Mucha *et al*., 2018; Scholtens *et al*., 2020; Tilahum *et al*., 2020), reproduction traits (Islam *et al*., 2020), disease resistance (Silva *et al*., 2018). The table

2.3 shows some candidate genes found to affect some major economic traits such as prolificacy, disease and immune response, body morphological traits, milk production and quality, adaptability and drought tolerance, growth and body size in different goat breeds.

Table 2. 3: Some candidate genes associated with major economic traits in different goat breeds

Gene	Economic traits	Authors (sources)
AA-NAT, BMP15, BMPR1B, BMP4, CART, CSNS1, FSHβ, FSHR, GDF9, GH, GnRHR, GPR54, IGF1, INHbb, KDM6A, KTLG, KiSS-1, LHβ, PRLR, SETDB2, KHDRBS2, WNT10B, SETDB2, PPP3CA, FOXL2, MTNR1A, AMEL, SRY	Physiological mechanism linked with prolificacy/ litter size	Supakorn *et al*., (2009), An et al., (2013), Li *et al*., (2011), Lai *et al*., (2016), Mekuriaw *et al.*, (2018), Islam *et al*., (2020), Zonaed *et al*., (2020)
PROM1, FGFBP1, LIMCH1ADAMTS3, SUCLG1, MHC, ABCC4, PRAME, CD163L1, KIR3DL1, TRIM56, CFH, TRIM5, MHCI,	Disease resistance and immune response	Supakorn *et al*., (2009), Silva et al., (2018), Zonaed *et al*., (2020)
CNTNAP5, ASIP, ITCH, AHCY, RALY, KIT, PDGFRA, POU1F1, MREG, DUOX1, ADGRV1, PMEL, TRPM1, DCT, TYRP1, ELOVL3, ASIP, KAP, MC1R	Body morphological traits	Supakorn *et al*., (2009), Peng et al., (2015), Martin et al., (2016) Nazari-Ghadikolaei *et al*., (2018), Zonaed *et al*., (2020)
DGAT1, STAT5A, CSN3*B, YBOX2, LEP, LEPR, IGF1, GHR, PRLR, AGPAT6, CSN1S1, CSN2, CSN1S2, CSN3	Milk production and quality	Kiplagat *et al*., (2009), Talouam *et al*., (2020), Zonaed *et al*., (2020)
CDK2, SOCS2, NOXA1, ENPEP, CDK2, SOCS2, EPAS1	Adaptability and drought tolerance	Zonaed *et al*., (2020)
LCORL, IGF1, GH, IGF1, POUIF1, MSTN, BMP15, LDB2, TBX15, DGCR8, CDC25A, RDH16, MYADM,	Growth, body size	Supakorn *et al*., (2009), Alakilli *et al*., (2012), Naicy *et al*., (2017), Saif *et al.*, (2020), Zonaed *et al*., (2020)

CHAPTER THREE: PHENOTYPIC TRAITS, REPRODUCTIVE AND MILK PRODUCTION PERFORMANCES OF INDIGENOUS GOATS OF SOUTH KIVU IN THE DEMOCRATIC REPUBLIC OF CONGO

3.1. Abstract

Quantification of the phenotypic variations of indigenous goats in South Kivu and their relationship with farmer preferered economic traits could provide ways for the conservation and the breeding options for goat improvement. This study quantified the variation and the association of questionnaire-based phenotypic characteristics with reproductive performances in indigenous goat populations of South Kivu. Six reproductive traits, three lactation parameters, and fourteen morphometric traits were analyzed following a general linear model. The shape of the horn, the shape of the tail, and the eye color explained the variabilities among goat populations. The length and the thickness of the tail positively correlated ($p < 0.01$) with the number of kidding per year, with the age of doe at the first service, and with the number of kidding. The lactation length was correlated ($r= 0.33$) ($P < 0.05$) with the estimated age of kids at weaning. The ages of the does highly correlated ($r = 0.80$) ($P < 0.05$) with the number of kidding. The results suggest that the length and the thickness of the tail can act as phenotypic markers for goat selection. However, further research based on the genome-wide association studies is required to confirm and verify these findings and to elucidate either they are genetically based or are from environmental influence.

Keywords: DR Congo, female goat, milking potential, phenotypic traits, prolific

3.2. Introduction

Goat farming is practiced worldwide with products having a good image (Gooki *et al*., 2019; Khorshidi-Jalali *et al*., 2019). The number of goats has increased globally, both in countries with high and low-income resources (Robinson *et al*., 2011). In developing countries, goats play important nutritional, socio-economic, and cultural roles in rural households (Bettencourt *et al*., 2015; Onzima *et al*., 2017). To more significantly contribute to farmers' household socio-economy, the goat should present more productive traits in terms of economic farm traits, including adaptability, disease resistance, prolificacy, milk production, drought tolerance, and other traits associated with their productivity. However, the improvement of productivity requires the integration of technologies, good farming practices, adapted breeds, and good management of the production environment (Thornton *et al*., 2010).

In the Democratic Republic of Congo, goat breeding is favorable for the development of the livestock sector (Lafleur *et al*., 2018). In the South Kivu region, livestock is an integral part of the region's mixed farming systems. Farmers' focus on small livestock kept accumulating household reserves that were strongly invested in children's education (Maass *et al*., 2012). Additionally, goats and chickens are the most accessible by farmers in the different environments of the region (FAOSTAT, 2018). Due to its adaptation to the production system, goat breeding contributes to 40% of rural household income (Wasso *et al*., 2018; Baenyi *et al*., 2020).

Therefore, livestock numbers per household that remain low could affect goat's productivity and reflect the poverty because of violent conflict in the region (Maass *et al*., 2012). To alleviate the problem and increase the size of goat herds in South Kivu, breeding goats were distributed to 150 households through a food security project funded by the United Nations Office for the Coordination of Humanitarian Affairs (OCHA) in 2014 (Nachigera *et al*., 2017). Production system based on free-range grazing, the introduction of exotic breeds as

well as the proximity of South Kivu with neighboring countries to DRC could affect the genetic diversity of goat populations impacting on their productive and reproductive performances (Gasigwa *et al*., 2018; Wasso *et al*., 2018). The assessment of goat variability, the estimation of the relationship between phenotypic variability, productive parameters including milk production and reproductive performances would be necessary for increasing goat's productivity and developing efficient selection programs (Montaldo *et al*., 2002; Weppert et *al*., 2004; Torres-Vázquez *et al*., 2009; Montaldo *et al.*, 2010).

This study focused on an understanding of the phenotypic variability and its possible association with reproduction and lactation parameters in indigenous goat breeds in South Kivu in the DRC.

3.3. Materials and methods

This study was conducted in the South Kivu region located in the eastern part of the DRC. The region is characterized by a tropical rainforest along the Congo River and two rainy seasons, occurring from March to May and from September to December, followed by two short dry seasons stated by June to August and from January to February. Average temperatures vary between 24 and 25°C with limited variability throughout the year. South Kivu shares limits on the North with North Kivu, with the Kivu Lake on the northeast, Maniema on the west, Katanga on the South, Burundi, Rwanda, and Tanzania countries on the east. South Kivu is located at 3.0167°S, 28.2667°E with 1531m of altitude above sea level. The average rainfall is about 1,500 mm with more than 50% of the total land used for animal grazing. According to the Koppen Geiger Climate classification, South Kivu's climate is classified as tropical wet and dries (AW). Livestock and agriculture are some of the major pillars contributing to the economy of rural households in the region. Goat populations are distributed in all the sub-agro-ecological zones of the region (**Table 3.1**).

Table 3. 1:Geographical description of the study area

Region	zone	Latitude (South)	Longitude (East)	Average annual tº C	Average Altitude (meter)	Ecology of the area
South Kivu	Mwenga	3 º03’	28 º26’	26	1650	Highland and forest area
	Kabare	2 º30’	28 º30’	22.6	2225	Cold mountain climate
	Walungu	2 º38’	28 º40’	18.6	1765	Cold mountain climate
	Uvira	4°20’	29 º30’	26	833	Humid and semi-arid tropical type

3.3.1. Assessment of reproductive and milk production

Data were collected using a purposive stratified sampling method in the goat farms. All the selected farms were characterized by the same production system which is the free-grazing system. Farm’s structure, farmers’ experience in goats breeding (more than 10 years), phenotypic variability mostly observed by body hair coat color were considered as the inclusion criteria for the selection of farms. Morphological traits, reproductive traits (prolificacy and some lactation parameters) were recorded on one adult doe (with more than one kidding) that was randomly selected in each farm to avoid the effect of consanguineous due to the small flock size varying between 5 to 13 animals. The selected farms were distributed in 4 of 8 territories of the region including Uvira (n = 49), Walungu (n = 31), Kabare (n = 28) and Mwenga (n = 40). In order to avoid the distortion of data due to pregnancy, efforts were made to ensure that the animals selected were not pregnant. Information on 6 reproductive parameters including age at the first service (AFS), number of kidding (NK), number of kids born at the last kidding (NKBLK), number of kidding per year (NKY), and number of kids weaned at the last kidding (NKWLK) (during the investigation for this study) and the age of kids at weaning (AKW) were recorded for the analysis. The estimation of milk production performance was based on the length of lactation, and the use

of milk-produced parameters (Lôbo *et al*., 2017). Milk yield was not quantified and considered as a studied parameter since goats were not milked in South Kivu.

Live body weight, height at withers, total body length, head profile, presence or absence of the shape and horn, presence or absence of beard, orientation of ear, presence or absence of tassels, body hair coat color type, body hair coat color pattern, hair shape and light, the shape of the tail and body conformation were recorded for the phenotypic traits. The shape of the tail and the body conformation were appreciated by palpation according to the body condition scores evaluating method (McKenzie-Jakes *et al*., 2007). The shape of the tail was considered as thin when the tail bone was quite pronounced; moderate or medium when it was less prominent and little fat covering the tail head area or when the tail born area felled spongy to the touch. The tail was considered as fat when fat was observable and palpable over the head the tail. The length of the tail was measured from the base of the tail to the pinpoint of the tail without hair. It varied between 9 and 16 cm. Accordingly, tail with 9 cm of length, was considered as short; between 9 and 12 cm, it was considered as medium, and over 12 cm as long. The body conformation of the goat was considered as normal conformation when the spine and the ribs were individually identified by palpation, but feel rounded rather than sharp (some fat is over the ribs) or when the goat had a good overall appearance (fat is over the ribs, hips, and tail bone areas and feels spongy to the touch). The body conformation was considered as vigorous when the fat was palpable over the ribs and tail head area or when the animal appeared fleshy and obviously carries a considerable amount of fat (very spongy fat covers ribs and tail head areas). According to the farming system (free grazing system) and the effect of feeding on measurements, all measurements were taken in the morning between 6 and 8 am before the animals were released for grazing. For the collection of these phenotypic traits, coat color patters developed by Lauvergne *et al*. (2013) were applied while body measurements were collected following the method described by Mahmud *et al*. (2014)

and Bouchel *et al*. (2017) and based on the recommendations of FAO (2012) for the phenotypic characterization of animal genetic resources. Bodyweight measurements were done using suspending balance having 50 kg capacity with 0.2 kg precision; metric tape and measuring sticks were used for the measurements on all animals. These morphological traits were considered according to their possible association with litter size and milk production performances (Lefebvre *et al*., 1976; Shongjia *et al*., 1992; Odubote *et al*., 1994).

3.3.2. Data analysis

Qualitative as well as quantitative data were subjected to the descriptive analysis using the Xlstat 2019.1.2 software (Vidal *et al*., 2020). A general linear model was used for analyzing data. The fixed effects in the model included locations or territories, phenotypic traits like the shape of the tail, ear orientation, shape of the horn, and eye color. Mean percentages and Chi-Square simulated p-values were calculated for qualitative parameters, while the mean and the standard deviation were determined for quantitative variables. Analysis of variance was performed to estimate the reproductive performances based on morphometric traits (Atoui *et al*., 2018). Pearson correlation coefficient (r) values were calculated to assess the relationship between body measurement traits, reproductive and milk production performances.

3.4. Results

3.4.1. Phenotypic traits identified in indigenous goats

Based on the phenotypic appearance, it was found that the most variable ($p < 0.01$) phenotypic traits were the horn shape, ear orientation, and the shape of the tail. However, the body hair coat color; mostly grew (33.78%), the eye color mostly represented by the umber color (83.11%) varied with the goat's geographic location or territory (Table 3.2). The observed variations were based on the geographic location since the production system was the same and characterized by the free-grazing system in all the regions.

Table 3. 2: Morphological characters of indigenous goat breeds from South Kivu

Parameters	Kabare (%)	Mwenga(%)	Uvira (%)	Walungu (%)	Mean (%)	(Khi²)
Coat color type						
white	3.57	7.50	4.08	6.45	5.41	0.004*
black	21.43	22.50	24.49	16.13	21.62	
grey	28.57	22.50	40.82	41.94	33.78	
Grey with black spots	17.86	5.00	14.29	25.81	14.86	
Black with white spots	28.57	32.50	8.16	6.45	18.24	
Grey with white spots	0.00	10.00	8.16	3.23	6.08	
Eye color						
umber	82.14	70.00	83.67	100.00	83.11	< 0.001
blue	17.86	15.00	16.33	0.00	12.84	
brown	0.00	15.00	0.00	0.00	4.05	
Beard presence						
no	85.71	87.50	87.76	87.10	87.16	0.184
yes	14.29	12.50	12.24	12.90	12.84	
Tassels						
no	7.14	2.50	14.29	0.00	6.76	0.038*
yes	92.86	97.50	85.71	100.00	93.24	
Horn shape						
absent	3.57	15.00	4.08	0.00	6.08	< 0.001
shot	0.00	7.50	0.00	0.00	2.03	
curved	82.14	72.50	89.80	100.00	85.81	
spiral	7.14	5.00	6.12	0.00	4.73	
cut	7.14	0.00	0.00	0.00	1.35	
Ear orientation						
erect	67.86	47.50	61.22	93.55	65.54	< 0.001
pendulous	0.00	47.50	0.00	0.00	12.84	
horizontal	32.14	5.00	38.78	6.45	21.62	
Body hair coat colour pattern						
plain	92.86	77.50	87.76	100.00	88.44	0.004**
patchy	7.14	22.50	12.24	0.00	11.56	
Shining of body hair						
yes	10.71	37.50	38.78	41.94	33.78	0.041*
no	89.29	62.50	61.22	58.06	66.22	
Shape of the tail						
Short and thin	28.57	22.50	10.20	6.67	16.33	< 0.001
Short and medium	28.57	30.00	0.00	93.33	32.65	
Short and fat	42.86	25.00	83.67	0.00	42.86	
Long and thin	0.00	2.50	2.04	0.00	1.36	
Long and medium	0.00	7.50	0.00	0.00	2.04	
Long and fat	0.00	12.50	4.08	0.00	4.76	
Body conformation						
normal	10.71	7.50	16.33	0.00	9.46	0.041*
vigorous	89.29	92.50	83.67	100.00	90.54	

significant at 0.05 level, significant at 0.01 level

3.4.2. Vatiations in morphometric and reproductive performances detected in goats

The highest body length and height at withers were recorded on goats from Uvira 60.78 ± 3.49 cm and 54.84 ± 3.09 cm, respectively while goats from Kabare shown the highest body weight 36.86 ± 5.54 kg. Live body weight, total body length, height at withers, were statistically different ($P < 0.01$) among goats from different locations or territories. The variation ($P < 0.01$) in the age for the first service, the age of kids at weaning, and the number of kidding per year was observed in goat populations with the geographic location. The highest (10.82 ± 2.79 months) age at the first service was observed in goats from Mwenga and the lowest in goats from Kabare (6.75 ± 0.61months). Particularly, goats used for the first service with the age below ten months, specifically between 7 to 8 months, registered a high number of kidding (3.9± 1.76) in Walungu and in Uvira (3.86± 1.99) with high number of kids born at the last kidding and with the high number of kidding per year except in Uvira territory (**Table 3.3**).

Table 3. 3: Variations in morphometric and reproduction performances detected in goats

	Kabare Mean ± SD	Mwenga Mean ± SD	Uvira Mean ± SD	Walungu Mean ±SD	Mean±SD	p-value
Morphometric parameters	Body length (cm)					
	59.29 ± 6.13^{ab}	59.15±3.27 ^{ab}	60.78 ± 3.49 ^{b}	58.00 ± 3.92 ^{a}	59.32 ± 4.23	0.032*
	Height at withers (cm)					
	54.32 ± 4.51 ^{ab}	53.60 ± 2.7^{ab}	54.84 ± 3.09 ^{b}	52.61 ± 3.54 ^{a}	53.93± 3.47	0.035*
	Body weight (kg)					
	36.86 ± 5.54 ^{b}	28.10± 3.36 ^{a}	29.77 ± 3.51 ^{a}	29.99 ± 6.52 ^{a}	30.70 ± 5.55	< 0.0001
Reproduction parameters	AFS (months)					
	6.75 ± 0.61^{a}	10.82±2.79^{c}	8.10±0.98^{b}	7.87±0.61^{ab}	8.54±2.17	< 0.0001
	NK					
	3.48± 1.64^{a}	3.25± 1.97^{a}	3.86± 1.99^{a}	3.9 ± 1.76^{a}	3.66 ± 1.87	0.437
	NKBLK					
	2.32 ± 0.62^{a}	2.10 ± 0.62^{a}	2.33 ± 0.47^{a}	2.4 ± 0.4^{a}	2.29 ± 0.55	0.183
	NKWLK					
	2.04 ± 0.73	1.92 ± 0.60^{a}	2.31 ± 0.46^{ab}	2.40 ± 0.49^{b}	2.19 ± 0.58	0.03*
	AKW (months)					
	5.36 ± 1.27^{c}	4.28 ± 0.71^{b}	4.00 ± 0.76^{b}	3.11 ± 0.28^{a}	4.12 ± 1.09	<0.0001
	NKY					
	1.38 ± 0.48^{ab}	1.26 ± 0.25^{a}	1.22 ± 0.38^{a}	1.75 ± 0.41^{b}	1.39 ± 0.43	<0.0001

significant at 0.05 level; a,b,c = letters showing the statistically significant differences among variables with a < b < c,*; NK*= Number of kidding; AFS= Age of doe at the first service; NKBLK= Number of kids born at the last kidding; NKWLK= Number of kids weaned at the last kidding; AKW= Age of kids at weaning; NKY= Nomber of kidding per year

3.4.3. Correlation between reproduction and considered lactation parameters

It is observed (Table 3.4) that the estimated age of the does (r = -0.038), the estimated age of the doe at the first kidding (r = -0.05), the number of kidding (r = -0.10), and the number of kidding per year (r = -0.17) were not significant and were negatively correlated with the lactation length, whereas the age of kids at weaning was positively correlated (r = 0.33; p < 0.01) with the lactation length. The age of does was positively correlated (r = 0.24, 0.80 and 0.39; p < 0.05) with the age of doe at the first kidding, the number of kidding, and the number of kids born at the last kidding, respectively.

Table 3. 4: Coefficient of correlation between reproduction and lactation parameters

Variables	AD	AFK	NK	NKBLK	NKWLK	AKW	NKY	LL	KB	QMP
AD	**1**	0.24**	0.80 **	0.39 *	0.24 ^{ns}	-0.02 ^{ns}	0,01 **	-0.04 ^{ns}	-0.16 *	-0.09 ^{ns}
AFK		**1**	0.034**	-0.26 *	-0.24 ^{ns}	-0.03 ^{ns}	-0.08 ^{ns}	0.05 ^{ns}	-0.05 ^{ns}	-0.11 ^{ns}
NK			**1**	0.43 ^{ns}	0.3 ^{ns}	-0.07 **	0.22 **	-0.10 ^{ns}	-0.007 ^{ns}	-0.004 ^{ns}
NKBLK				**1**	0.79 **	-0.25 ^{ns}	0.09 ^{ns}	-0.04 ^{ns}	- 0.05 ^{ns}	-0.08 ^{ns}
NKWLK					**1**	-0.35 **	-0.008 *	-0.09 ^{ns}	0.04 ^{ns}	-0.052 ^{ns}
AKW						**1**	-0.28 **	0.33 **	-0.19 ^{ns}	-0.02 ^{ns}
NKY							**1**	-0.17 ^{ns}	0.12 ^{ns}	0.099 ^{ns}
LL								**1**	-0.07 ^{ns}	-0.090^{ns}
KB									**1**	0.20*
QMP										**1**

ns, non-significant (P > 0.05); significant at 0.05 level; significant at 0.01 level
AD = age of Doe, AFK = Age of Doe at the First Kidding, NK = Number of Kidding, NKBLK = Number of Kids Born at the Last Kidding, NKWLK = Number of Kids Weaned at the Last Kidding, AKW= Age of Kids at Weaning, NKY = Number of Kids per Year, LL = Length of Lactation, KB = Kids Breastfed, QMP = Quantity of Milk Produced

3.4.4. Reproductive performances variations estimated based on morphological traits

It is shown in Table 3.5 that the age at the first service, the number of kidding, and the number of kidding per year varied with the shape of the tail. The number of kidding positively correlated with the short and fat tail ($p < 0.05$) (Figure 3.1a). The age at the first service

positively correlated (P < 0.01) with the long and thin tail (Figure 3.1b). Additionally, the age at the first service positively correlated with the long and medium tail (p < 0.01) (Figure 3.1c). The long and fat tail (Figure 3.1d) did not affect the variation of the considered reproductive traits. The number of kidding per year varied ($p < 0.05$) with a short and fat tail. Positive variation was also observed for the age of kids at weaning ($p < 0.01$) with the erect horn.

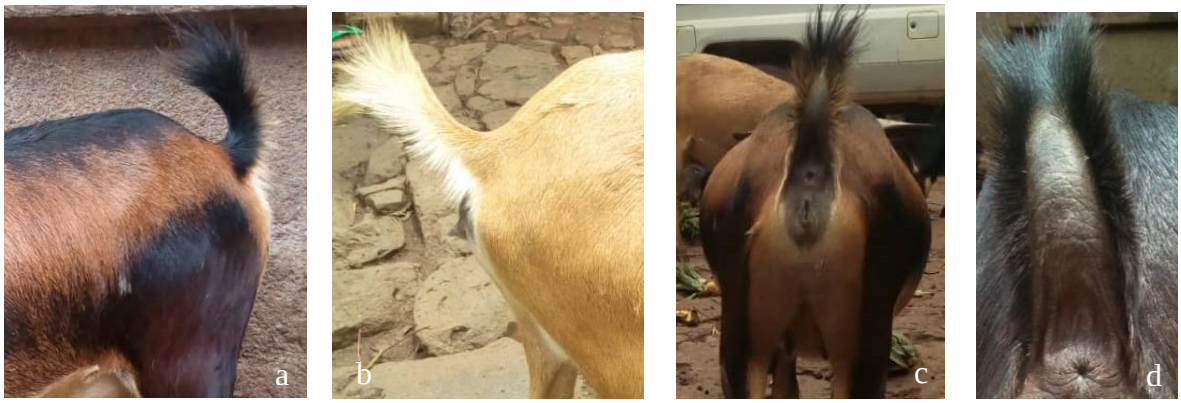


Figure 3. 1: a. Short and fat tail b. Long and thin tail c. Long and medium tail d. Long and fat tail

Table 3. 5: Estimation of reproductive performances based on morphological traits: ANOVA

Morphological traits		Reproductive performances														
		AFS			NK			NKWLK			AKW			NKY		
Shape of tail	Variables	value	SE	Pr	value	SE	Pr	value	SE	Pr	value	SE	Pr	value	SE	Pr
	1	-1.16	0.79	0.14	0.38	0.82	0.65	0.22	0.27	0.41	-0.82	0.47	0.084	-0.023	0.18	0.90
	2	-1.23	0.74	0.10	0.91	0.77	0.24	0.22	0.26	0.32	-1.33	0.44	0.003	0.38	0.17	0.03
	3	-1.50	0.75	0.05	1.55	0.77	0,05	0.2	0.26	0.45	-0.55	0.44	0.25	-0.033	0.17	0.85
	4	14.33	1.85	< 0,0001	0.33	1.93	0.86	-1.00	0.64	0.12	1.00	1.10	0.37	0.25	0.42	0.56
	5	-0.67	1.22	0.58	3.667	1.26	0.004	0.000	0.42	1.000	-1.33	0.72	0.07	0.42	0.28	0.14
	6	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00
Ear orientation	1	0.307	0.48	< 0,0001	-0.49	0.42	0.24	0.17	0.13	0.21	-0.58	0.24	0.02	0.042	0.1	0.67
	2	2.01	0.64	0.52	0.014	0.56	0.98	0.08	0.18	0.65	-0.42	0.32	0.2	-0.05	0.13	0.71
	3	0.000	0.00	0.002	0.00	0.00	0.08	0.000	0.00	0.000	0.00	0.00	0.000	0.00	0.00	
Shape of horn	1	6.29	1.56	< 0.0001	-0.43	1.50	-0.77	-0.14	0.47	0.76	-3.21	0.82	0.000	-0.071	0.35	0.84
	2	5.67	1.77	0.002	-1.67	1.71	0.33	0.33	0.54	0.54	-3.50	0.93	0.000	-0.17	0.40	0.68
	3	2.29	1.38	0.10	-0.26	1.34	0.84	0.23	0.42	0.58	-3.43	0.73	< 0,0001	-0.11	0.31	0.72
	4	1.83	1.59	0.25	0.17	1.53	0.91	0.000	0.48	1.00	-3.33	0.83	0.000	-0.25	0.36	0.49
	5	0.000	0.00	0.001	0.000	0.00	0.002	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.00
Eye color	1	-4.8	0.82	< 0.0001	-0.34	0.78	0.66	0.40	0.25	0.10	-0.42	0.46	0.36	0.06	0.18	0.74
	2	-3.97	0.95	< 0,0001	0.07	0.90	0.94	0.23	0.28	0.41	-0.07	0.53	0.90	0.000	0.21	1.00
	3	0.000	0.000	< 0.0001	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000

*(Significant at 0.05 level***).** Legend**:** *AFS = Age of Doe at the First Kidding, NK = Number of Observed Kidding, NKWLK = Number of Kids Weaned at the Last Kidding, AKW = Age of Kids at Weaning, NKY = Number of Kids per Year,*

The shape of the tail: 1: shot and thin; 2: shot and moderate; 3: shot and fat**;** 4: long and thin**;** 5: long and moderate**;** 6: long and fat**. Ear** orientation: 1: erect**;** 2: pendulous**;** 3: horizontal**. The shape of horn**: 1: absent**;** 2: scurs; 3: curved**;** 4: spiral**;** 5: cut**.** Eye color 1: umber**;** 2: blue; 3: Brown

3.4. Discussion

The characterization of livestock provides large knowledge and gives a clear perspective on the population structure assisting in the decision-making of future breeding programs. In this study, it was found that the dominant hair coat color type for the local goats in South Kivu was grey (33.78%) followed by black (21.62%). A different observation was made by Hansen *et al*. (2012) showing that the most frequent coat color for Ethiopian indigenous goats was white with spots (20.7%) and that black coats with or without spots or patches were less frequent than brown or white ones. Therefore, it is believed that black-colored animals, including goats, have superior adaptation to seasonal cold weather or cold nights as the dark pigment allows them to warm up earlier than goats with other coat colors (Robertshaw *et al*., 2006). The white coloration could be an advantage in an intense radiant environment due to its reflectance property as reported by Hansen *et al*. (1990). As reported by Odubote *et al.* (1994), coat color influences radiant heat loss affecting on its turn the body weight and other productive adaptability factors in livestock species in the tropical environment. Light coat colors such as white, grey, and fawn have an impact on radiant heat loss (Baenyi *et al*., 2020). The relative proportion of light color observed in the goat populations in South Kivu is an indication that the small goats breed of the region were not thermal stressed and their productivity could not be affected (Baenyi *et al*., 2020).

In this study, the majority of goats were not bearded (87.16%); this result lines with the findings of Odubote *et al.* (1994) and Adedeji *et al.* (2006) who have shown that majority of indigenous goat populations in Nigeria were not bearded (82.6%) despite the numerous benefits associated with the presence of beard in goats reared under hot and humid environments. Generally, among the most important benefits of the beard are the thermoregulatory functions and its association with reproductive traits such as higher prolificacy, higher milk yield, higher litter size, fertility index, and conception rate (Osinowo

et al., 1988; Yakubu *et al*., 2010). Being characterized by the mountain climate, temperature variation in South Kivu could not affect the adaptability of goats. Thus, the reproductive performances could be explained by other phenotypic traits than the presence of beards.

The body length and height at withers of the small goats in this study were higher than previous findings for indigenous goat breeds in southern Nigeria as reported by Fajemilelin *et al.* (2008) and by Hagan *et al.* (2012) in Ghana. The observed difference can be explained by the difference of the geographic location, the climate characteristics and the phenotypic traits of indigenous goats in the western part of Africa mostly closed to dwarf goats characterized by their short body size. As reported by Cam *et al.* (2010), morphometric measurements and how they relate to each other can describe roughly an animal's production status and breed characteristics. According to Devendra *et al.* (1982), goats are classified as large when they weigh between 20-60 kg and with a height at withers above 65 cm. On this basis, the local goats observed in this present study could be classified as small-sized breeds (Wilson, 1991).

As reported by Hagan *et al.* (2012), linear body measurements reflect breed characteristics and the management conditions under which the animals are kept. Thus, the observed homogeneity based on the considered linear body measurement on South Kivu's small goats could be attributable to similarities in feed resource availability base (in terms of quantity and quality), grazing field, and management conditions to which the animals were subjected (Cam *et al.* 2010). On the other hand, the observed homogeneity in the goats in South Kivu could mean that there is no effect of gene interaction from the crossbreed between goats in South Kivu and exotic breeds or goat exchange and gene flow from neighboring countries.

Results of this study showed that the age of the doe was positively correlated with the age at the first service ($r = 0.24$; $p < 0.01$), number of kidding per year (0.80; $p < 0.01$), and number of kids born at the last kidding ($r = 0.39$; $p < 0.01$). At the same time, it has been observed that most of these reproductive performances (number of kidding per year, age of doe at the

first service, and number of kidding at the third kidding) were in positive association with both shapes of tail and horn (little literature for further discussion). It has been reported that higher age, heavier body weight at breeding, higher parity order was associated with the chance of triplet and quadruplet births on prolific Black Bengal goats and on meat type goats (Haldar *et al*., 2014; Pan *et al*., 2015). In this study, the high number of kidding in a lifetime, the high number of kids per year, and the high number of kids born at the last kidding were observed in goats used earlier for reproduction as was the case for goats from Uvira and Walungu.

Results of this study have shown that the lactation length significantly varied ($p < 0.01$) with the locations (territories) and was correlated ($r= 0.033$) with the age of kids at weaning. Gökdal *et al*. (2017) have shown that the lactation milk yield, daily milk yield, and lactation length of the does were not significantly different between the weaning groups' ages. Additionally, it has been reported by Zumbo *et al.* (2007) that the higher daily milk yield was high for terziparous goats compare to uniparous goats. This finding is not in accordance with the finding in this study, from where most of the goats were approximately at the fourth (3.66 ± 1.87) kidding with approximately three kids per parity but with less production of milk. Diet, environmental conditions, breed, litter size, parity, and season of kidding might affect the significant variation observed between the average lactation length of different breeds (Mourad *et al*., 2001; Güler *et al*., 2007. Findings in this study revealed significant effects of the geographic location, the age of kids at weaning on the lactation length variation. The highest age of kids at weaning observed in Kabare corresponded with the longest lactation length, whereas the lowest age of kids at weaning which was observed in Walungu corresponded with the shortest lactation length. Further researchers should be considered to investigate and to more explain the correlation between the shape and the length of the tail with some reproductive traits.

3.5. Conclusion

The shape of the horn and the tail, the body coat color, ear orientation, and eye color explained the phenotypic variability (heterogeneity) among goat populations in South Kivu. Reproductive traits like number of kidding per year, age of kids at weaning, and age of doe at the first service and the the lactation length were positively correlated with the length and the thickness of the tail. These findings suggest that these traits can act as phenotypic markers that can be used in the selection of reproductive traits especially the number of kidding per year and the lactation length. Further research based on molecular characterization through genome-wide association studies is required to confirm the association between the shape of the tail, the shape of the horn, the ear orientation with the number of kidding per year, and the lactation length. This could be useful and considered as a phenotypic marker for the selection and improvement of goats in South Kivu.

CHAPTER FOUR : TYPOLOGY, PRODUCTION MANAGEMENT AND FARMER PREFERRED TRAITS FOR SELECTION OF INDIGENOUS GOATS (*Capra hircus*) IN THREE AGRO-ECOLOGICAL ZONES IN THE DEMOCRATIC REPUBLIC OF CONGO

4.1. Abstract

Data on the characterization of animal genetic resources are essential in the development of breeding and conservation schemes to ensure their sustainable use. The present study aimed at assessing the phenotypic traits, reproductive performances, production management, and smallholder farmer-preferred traits in the selection of indigenous goats in three agro-ecological zones in the Democratic Republic of Congo (DRC). Based on a structured survey, baseline data were recorded on 320 adult and unrelated goats from 202 goat farms. Only female goats (does) were considered in this study (justified in chapter 5). It has been observed that the oldest doe in farms was 3.34 ±1.48 years old and found in South Kivu with 3.85±1.71 number of kidding. Hirarchical clustering on principal components revealed three clusters in the studied goats well distinguished by the double and triple kidding. Prolific goats were mostly clustered into the cluster two and three represented by goats from South Kivu while 82.69% of goats from Tshopo were clustered into cluster one characterized by low reproductive performances. The Canonical Discriminant Analysis (CDA) revealed that the body length was an important variable both to discriminate and to classify goats from the three geographical regions. The Mahalanobis's distances revealed that indigenous goats Kinshasa and South Kivu were not distanced (ns) while the largest distance was observed between goats from Kinshasa and Tshopo or South Kivu and Tshopo (F-stat, $p< 0.001$). No innovations or good management practices were applied in goat breeding. However, goats

were considered as a source of income and saving method in smallholder farmer's households. Adaptability to the region, disease resistance, and prolificacy were the important traits considered by farmers in selecting goats. These results give the first step in the decision-making towards goat improvement in the DRC.

Keywords: Characterization, Twinning, Environmental adaptation, Goat management, DRC

4.2. Introduction

The domestication of goats is thought to have begun as early as 10,000 years before Christ in Southeast Asia as revealed by the study of the mitochondrial genes (Nomura *et al*., 2013). From the domestication centers, goats are dispersed throughout the world, including developing countries, where they play important socio-economic, nutritional, and cultural roles in the rural community (Onzima *et al*., 2017; Monau *et al*., 2020). In a different production environment, especially in difficult conditions, reproductive performances in goats are one of the indicators of their adaptation. In does, the reproductive efficiency is determined by different processes including the length of the breeding season, the overall rate, the age at puberty, the age at the first service, the age at the first kidding, the litter size, and the weight of kids at birth (Moaeen-ud-Din *et al*., 2008, Zang *et al*., 2008). Besides the genetic aspect, these factors are directly or indirectly influenced by the environmental conditions where goats are raised (Zang *et al*., 2008).

The Democratic Republic of Congo (DRC) is located in the central Africa region and characterized by three agroecological zones (AEZs) including the alluvial basin or the humid zone (in the northeast and the central part), the savannah, or the subhumid zone (in the central, the western and the south-east part) and the high-altitude volcanic mountains or the highland zone (in the eastern part) (FAO, 2005). The indigenous goat populations are spread throughout all AEZs of the country and are estimated to be 4,065,709 heads (FAOSTAT, 2018). Goats represent the second domesticated livestock species after chicken, representing between 30-60% of total livestock units kept for meat production and commercial transactions, and contributing up to 72% of households' income in rural areas at the country level. Several studies have been conducted to assess the productivity and population dynamic of local goats by estimating the number of kids born by a doe in five years (Sabimana *et al*., 2018), the socioeconomic impacts (Wasso *et al*., 2018), and the physiological adaptation and

heat tolerance of goats (Baenyi *et al*., 2020) in different AEZs in the country. In contrast, little is known on the genetic diversity, the production systems, and the farmer-preferred traits for the selection of indigenous goats in DRC.

To facilitate improved management and conservation of goats, the assessment of the genetic diversity and the management of the production systems is important (Mekuriaw *et al*., 2016). Several tools, including phenotype and molecular markers (Gama *et al*., 2011) have been developed to characterize animal genetic resources as the basis for the genetic diversity studies of animal species (Lanari *et al*., 2003). However, animal characterization begins with the knowledge of variations in the morphological traits (Delgado, 2001) followed by characterization at the molecular levels. A combination of the phenotypic and molecular information yields clues about the association and the genetic potentials of livestock populations to further design or consider the molecular tools (Molecular Assisted Selection) for use in breeding programs.

This study aimed at determining the typology, the production management, and the farmers-preferred traits in the selection of indigenous goat populations in three agro-ecological zones in DRC.

4.3. Materials and methods

4.3.1. Study area

This study was carried out in DR Congo in three provinces including South Kivu, Tshopo, and Kinshasa selected based on the statistic distribution of indigenous goat populations and the characteristics of the climate representing all the AEZs of the country (**Figure 4.1**, **Table 4.1**). Generally in the study area, most smallholder farmers do not receive any animal husbandry training (Akilimali *et al*. 2018). The average size of goats per farm increased from 2015 (4.2 ± 3.02) to 2017 (6.26 ± 3.49) (case of South Kivu province). However, disease,

sales, donation, poor nutrition, and theft are the major factors decreasing goat numbers (Wasso *et al*., 2018). Despite the difficulties smallholder farmers experience, goat farming allows them to meet their needs such as schooling their children, health care, fertilization of farms, farm rental, and nutrition.

Three basic components of the African Goat Improvement Network (AGIN) sampling protocol including geographical information system (longitude, latitude, and elevation), physical characteristics, and photo characterization were recorded for the standardized phenotyping (Session TRR 2011; Huson *et al*., 2012). The geographic information (GI) was collected at each sampling site using a global positioning system (GPS) tool, to provide accurate details on the region where sampling was done as well as coordinates to direct subsequent collection of climatology data

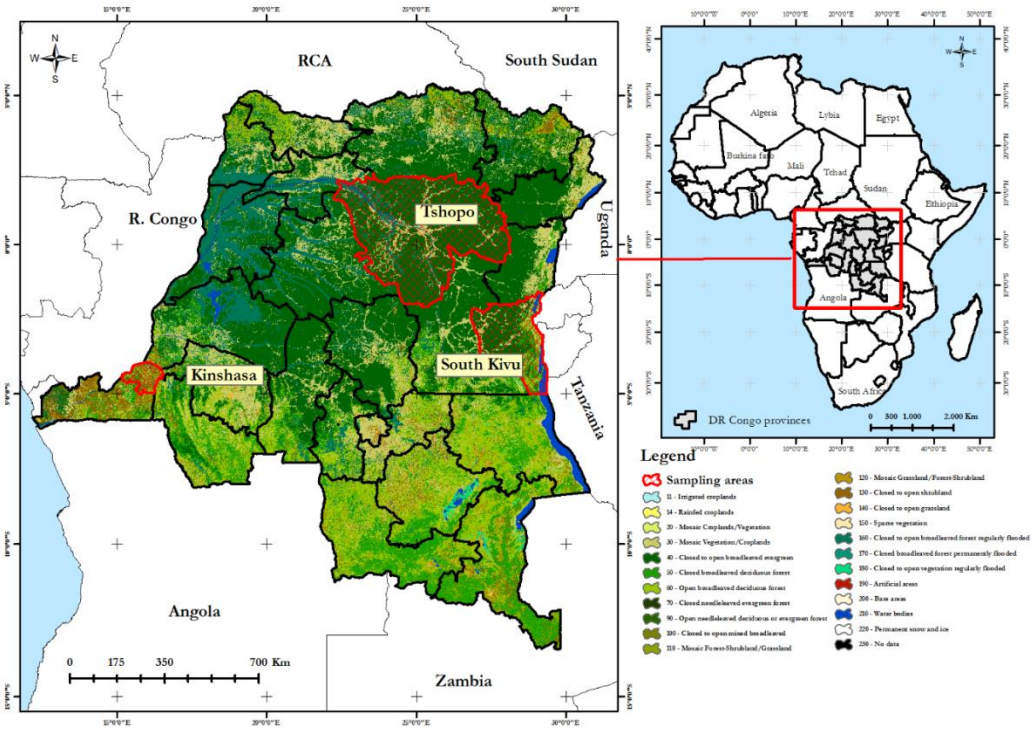


Figure 4. 1: Sampling areas in the different agro-ecological zones in the Democratic Republic of Congo Source: GIS Lab UEA, 2021

Table 4. 1: Summary of agro ecological zones the sampling areas

Province	zone	Latitude	Longitude (East)	Altitude (meter)	Average temperature in Celsius degree	Ago-ecology
South Kivu	Fizi	-4.1082	29.0931	790	24	High altitude Volcanic Mountain (highland zone)
	Kabare	-2.21633	28.82783	1579	19.5	
	Walungu	-2.62683	28.66821	1780	21	
	Uvira	-3.23464	29.16653	833	26	
Kinshasa	Maluku	-4.097385	15.529493	350	25.6	The savannah (sub-humid zone)
	Masina	-4.31113	15.302732	427	25.6	
	Nsele	-4.353662	15.22842	316	25	
Tshopo	Makiso	0.512158	25.193142	412	25	Alluvial basin Equatorial Forest (Humid zone)
	Buta	0.705575	25.227631	402	26.5	
	Ubundu	0.413975	25.218069	397	25.5	

4.3.2. Evaluation of phenotypic traits for Congolese indigenous goat populations

4.3.2.1. Sample size calculation

Sample size is one of the critical factors affecting the accuracy of the estimation of population genetic diversity parameters (Bashalkhanov *et al*., 2009). Since small sample sizes lead to significant errors in determining the allelic richness which is one of the most important and commonly used estimators of genetic diversity in populations, the sample size calculation in this study was done according to the Genetic Power Calculator (GPC) and the African Goat Improvement Network (AGIN) sampling protocol for the standardized phenotyping (Session TRR 2011, Huson *et al.,* 2014). The AGIN sampling protocol was completed by the results of Toro *et al*. (2011) who demonstrated that in small farm animal population, the acceptable level for effective population size for the genetic variation studies can be considered from different angles leading to a conclusion that it should be at least 50 to

100. Accordingly, in this study, purposive sampling method was used to select a total of 320 adult female goats from three goat populations (Kinshasa, n= 120, South Kivu, n= 120 and Tshopo, n=80) were randomly selected within each strata in 202 goat farms. Basic information of the production systems (feeding management, health care management, reproduction management, goat management according to the seasons, and the socio-economic benefits of keeping goat) (**Appendix I**) were collected at the farms level. The selection of farms was done based on some fixed inclusion criteria related to the experience of the farmer in kipping goat (more than 5years), the structure of the farm (more than 10 goats in the farm), the accessibility in the farm, and the phenotypic variability observed on goats in the farm. Phenotypic traits based on measurement and visual observations were recorded on the 320 female goats (Only female goats were concerned to allow the sequencing m of tDNA d-loop region; chapiter 5).

4.3.2.2. Morphological traits

Four quantitative parameters including body weight (kg), body length (cm), height at the stern (cm), height at withers (cm), and eight qualitative parameters including the color of the body hair coat type, facial profile, ear orientation, presence or absence of beard and tassel, horn shape, the color pattern of the body hair coat and its shine were respectively measured and observed on each animal. Measurements were taken early in the morning using a measuring tape and weighing scale on a total of 340 adults and unrelated does (South Kivu, n=120; Kinshasa n=120; and Tshopo n=80) distributed in 202 farms (South Kivu, n=62; Kinshasa, n=60, Tshopo, n=80). For the collection of these phenotypic traits, coat color patters developed by Lauvergne *et al*. (2013) were applied while body measurements were collected following the method described by Mahmud *et al*. (2014) and Bouchel *et al*. (2017) and based on the recommendations of FAO (2012) for the phenotypic characterization of animal genetic resources. Bodyweight measurements were done using suspending balance having 50

kg capacity with 0.2 kg precision; metric tape and measuring sticks were used for the measurements on all animals.

4.3.2.3. Reproductive performances

Reproductive performances mainly focused on the kidding history (number of kidding, number of double and triple kidding, number of kids weaned, litter size at first, second, and last kidding during this study's period) (**Appendix I**) were recorded on the same number of measured animals for the phenotypic data recording (Onzima *et al*., 2018; Bhattarai *et al.*, 2019). Farmers selected these animals and provided these informations.

4.3.2.4. Production management and benefits of kipping goats

Information on feeding management, health care management, reproduction management, goat management according to the seasons, and the socio-economic benefits of keeping the goat was recorded on each farm in a questionnaire form (**Appendix I**). Farmer-preferred traits in the selection of goats as suggested by Onzima *et al*. (2018) were recorded from each farmer in the study area (**Appendix I**).

4.3.3. Data analyses

The collected qualitative parameters related to morphological traits, production management data, benefits of keeping goats , and traits assisting in goat selection by farmers were submitted for the descriptive analysis (Trochim *et al*., 2001). The Chi-Square statistic and Fisher's exact test were used for testing relationships between qualitative parameters and provinces while the analysis of variance (ANOVA) was used to statistically compare morpho-biometric traits and reproductive performances between regions and clusters. The suitability of the dataset for clustering analysis on principal components was tested statistically using Bartlett's test of sphericity to minimize the danger of interpreting factor analytic results which can be attributed entirely to chance. The obtained p-value < 0.001,

indicated that hierarchical clustering on principal components (HCPC) was very likely to be useful. Then, clusters were obtained using HCPC on body measurement and reproductive performances. Canonical Discriminant Analysis (CDA) was implemented with the Candisc package version 0.8-0 computing canonical scores and vectors (Friendly and Fox, 2017). The total variation explained by each canonical variable (Can) and coefficient was calculated. The scores for each Can and individual were plotted in the canonical space. The first two Can (Can 1 and Can 2) were considered in building a graph. Differences between the three regions were obtained by F-test ($p<0.05$) over Mahalanobi's distances expressing the distance between the centroids of each group through the HDMD package version 1.1 (McFerrin, 2013). Statistical analyses were performed using R Statistical Software version 4.03 (R Core Team, 2017). Relative frequencies, mean and standard deviation of various characterization parameters were summarized in tables and figures.

4.4. Results

4.4.1. Vatiations in morphological traits

The descriptive analysis of morphological characters of indigenous goats in the three AEZs revealed that the dominant goat's hair coat color type was black (31.26%) followed by grey (23.40%) (**Table 4.2**). The frequency of the gray body coat color was higher in South Kivu (33.63%) than in Kinshasa (19.29%) and Tshopo (17.30%). Other dominant phenotypic characters observed in the three AEZs were straight facial head (100%), absence of bear (88.8%), curved horn shape (91.56%), absence of tassels (94.53%), the plain color pattern of the body hair coat (92.10%). Ear orientation was mostly horizontal in Tshopo (73.07%) and Kinshasa (97.36%) but erected in South Kivu (45.90%). The presence of light hair was observed on goats from South Kivu (62.27%) and Kinshasa (75.43%) while absent on goats in Tshopo (63.46%). There were statistically significant relationships between all qualitative morphological traits and sampling regions ($p \leq 0.0001$) (Table 4.2).

Table 4. 2: Morphological traits of indigenous goat breeds from three agro-ecological zones in DRC

Parameters		Location				
		South Kivu	Tshopo	Kinshasa	Mean	P-value
		Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	
Body hair coat color type	Black	34.09	30.76	28.94	31.26	< 0.0001
	Black and grew	0.00	1.92	0.00		
	Black and white	2.27	15.38	8.77		
	Black, white and grew	0.00	0.00	0.87		
	Black and white head	0.00	0.00	0.87		
	Black with white spots	12.27	0.00	1.75		
	Brown	0.00	1.92	6.91		
	Brown and black	0.00	0.00	3.50		
	Brown and white	0.00	0.00	3.50		
	Brown, black and white	0.00	0.00	1.75		
	Brown and black legs	0.00	0.00	0.87		
	Grew	33.63	17.30	19.29	23.40	
	Grew and black	0.00	3.84	9.64		
	Grew and white	0.00	11.53	4.38		
	Grew, white and black	0.00	0.00	0.87		
	Grew with black spots	10.00	0.00	0.87		
	Grew with white spots	4.09	0.00	0.87		
	Mixed	0.00	3.84	4.38		
	White	3.63	9.61	0.87		
	White and black	0.00	1.92	0.87		
	White and grew	0.00	1.92	0.00		
Head (facial) profile	Straight	100.00	100.00	100.00		ns
Beard	Absence	89.09	88.46	88.86	88.8	0.677
	Presence	10.90	11.53	11.14		
Tassels	Absence	94.09	90.38	99.12	94.53	0.035
	Presence	5.90	9.61	0.87		
Horn shape	Absent	4.09	9.61	1.75		< 0,0001
	curved	90.45	90.38	93.86	91.56	
	shot	1.36	0.00	4.38		
	spiral	3.18	0.00	0.00		
Ear orientation	Erect	45.90	26.92	2.63	25.16	< 0,0001
	horizontal	45.45	73.07	97.36	71.96	
	Pendulous	8.63	0.00	0.00		
Body hair coat color pattern	plain	91.32	96.15	92.10	93.19	< 0,0001
	patchy	8.67	3.84	7.89		
Hair light	not	37.72	63.46	24.56	41.91	< 0,0001
	light	62.27	36.53	75.43	58.07	

4.4.2. Analysis of variance for quantitative traits (morphological) and reproductive performances

Significant statistical differences ($p \leq 0.0001$) were observed among goats for all measured morpho-biometric traits and reproductive performances according to the regions. The highest body length (61.95 ± 5.52 cm) and body weight were recorded on goats from South Kivu (**Figure 4.2**C) and Kinshasa (**Figure 4.2**B). The highest height at withers (55.25 ± 4.13 cm) and stern (52.50 ± 3.75 cm) were recorded on goats from Kinshasa province followed by goats from South Kivu. The goats from Tshopo province had the lowest values for all observed morpho biometric traits compared to goats from Kinshasa and South Kivu (**Figure 4.2**A, **Table 4.3**). The oldest does in the farms were in goats from South Kivu (3.34 ±1.48 years old) which registered the highest number of kidding (3.85 ± 1.71) with a higher number of twins (2.01 ± 1.1) and triplets (0.66 ± 0.90). In general, goats from South Kivu were characterized by the highest reproductive performances compared to goats from Kinshasa and Tshopo, which had the lowest reproductive performances. The average number of kids born per kidding increased significantly ($p < 0.0001$) from the first parity to the last parity in all three regions.

Table 4. 3: Morpho biometric traits and reproductive performances of indigenous goat breeds from 3 agro-ecological zones in DR Congo

Variables	South Kivu	Tshopo	Kinshasa	
	Morpho biometric traits			
	Mean ± SD	Mean ± SD	Mean ± SD	P-value
Body length (cm)	61.95 ± 5.52 ^{b}	51.50 ±2.04 ^{a}	61.72 ± 4.37 ^{b}	< 0.0001
Height at withers (cm)	53.55 ±5.19 ^{b}	47.69±1.76 ^{a}	55.25 ± 4.13 ^{c}	
Height at stern (cm)	50.71 ±4.86 ^{b}	46.68±1.87 ^{a}	52.50 ± 3.75 ^{c}	
Body weight (cm)	32.75 ±6.04 ^{b}	30.34±5.09^{a}	33.23 ± 4.04 ^{b}	
Reproductive performances				
Estimated age (year)	3.34 ±1.48 ^{b}	2.05±0.73 ^{a}	2.56 ± 1.12 ^{a}	< 0.0001
Number of kidding	3.85 ± 1.71^{b}	1.96 ± 0.94^{a}	2.52 ± 1.21^{a}	< 0.0001
Double kidding	2.01 ± 1.1^{c}	0.88 ± 0.80^{a}	1.47 ±1.05^{b}	
Triple kidding	0.66 ± 0.90^{b}	0.04 ± 0.19^{a}	0.32 ± 0.74^{a}	
Number of kids weaned	2.92 ± 1.61^{c}	1.36 ± 1.06^{b}	2.23 ± 1.73^{a}	
Litter size at the last kidding	1.93 ± 0.00^{b}	1.31 ± 0.12^{a}	1.80 ± 0.00^{b}	
Litter size at the second kidding	1.59 ± 0.07^{b}	1.06 ± 0.14^{a}	1.32 ± 0.09^{ab}	
Litter size at the first kidding	1.47 ± 0.77 ^{c}	0.84 ± 0.82 ^{a}	1.12 ± 0.88 ^{b}	

cm= centimeter; a, b and c = letters showing the statistically significant differences among variables with a < b < c, SD= standard deviation

Figure 4. 2: Goats of DR Congo. A goat from the alluvial basin, equatorial forest in Tshopo province, B goats from the savannah plateau, Kinshasa province, and C goat from the high-altitude volcanic mountains, South Kivu province.

4.4.3. Typology of goat populations from three agro-ecological zones in DR Congo

The HCPC analysis based on the morpho-biometric and reproductive traits grouped indigenous goat populations from South Kivu, Tshopo, and Kinshasa into three clusters (**Figure 4.3**). The principal component analysis (**Figure 4.3**), considering the origins of goats, indicated that the two axes showed up to 66% of the variability observed in the studied goat populations. The first axis which retained 51.2% of the total inertia, was represented by most of the considered reproductive performances and body weight. The second axis which retained 14.8% of the total inertia, was mostly represented by the considered morpho-biometric traits, including the body length, the height at withers, and the height at the stern. The reproductive performances differentiated better the goats in the studied populations. There was a slight difference between goats from different provinces in terms of reproductive performances (all groups have very close centers of gravity as shown in **Figure 4.3**).

Based on the morpho-biometric traits including live body weight, body length, height at withers, and height at the stern, the cluster two and three were characterized by large and heavy animals compared to cluster one (**Table 4.4**). The analysis of morpho-biometrics traits by region showed that while goats from the three different regions were represented in the three clusters, there was a high representation of goats from Kinshasa in cluster one (42.98%)

and cluster two (35.96%) whereas cluster three included mostly goats from South Kivu (37.22%) (**Figure 4.4** and **Table 4.5**). Therefore, most of the indigenous goats from Tshopo clustered into cluster one (82.69%) with only a few animals segregated in clusters two (17.31%) and none in cluster three (0%) (**Figure 4.4** and **Table 4.5**). Based on reproductive performances including the number of double and triple kidding at three levels of kidding (**Table 4.5**), the results for the clusters revealed that goat populations from Tshopo were more represented in cluster one (**Figure 4.4** and **Table 4.5**) characterized by a low number of kids born at the three stages of kidding, and a low number of double and triple kidding (**Figure 4.4** and **Table 4.5**). Therefore, from the same data (**Table 4.4**), clusters two and three were characterized by a high number of kids born at the three stages of kidding, a high number of double and triple kidding, and were more represented by goats from South Kivu 48.33% (cluster two) and 37.22% (cluster three) (**Figure 4.4** and **Table 4.5**). Although the body measurements did not explain well the three clusters of goats, nevertheless they were factors discriminating the goats in the three geographical regions (**Figure 4.5**). The CDA revealed that canonical variables presented the highest weights for body length and wither height showing that body length is important both to discriminate and to classify goats from different regions. 100% of the total variation was explained by the first two canonical variables with 73.61% and 26.39% respectively (**Table 4.6**). The pairwise Mahalanobis' distances and probability of a significant (F-test) effect of contrasts between indigenous goats from the three regions revealed that the smallest distances were observed between goats from Kinshasa and South Kivu with a non-significant probability (P= 0.0937). The largest distances were observed between goats from Kinshasa and Tshopo or South Kivu and Tshopo (**Table 4.7**).

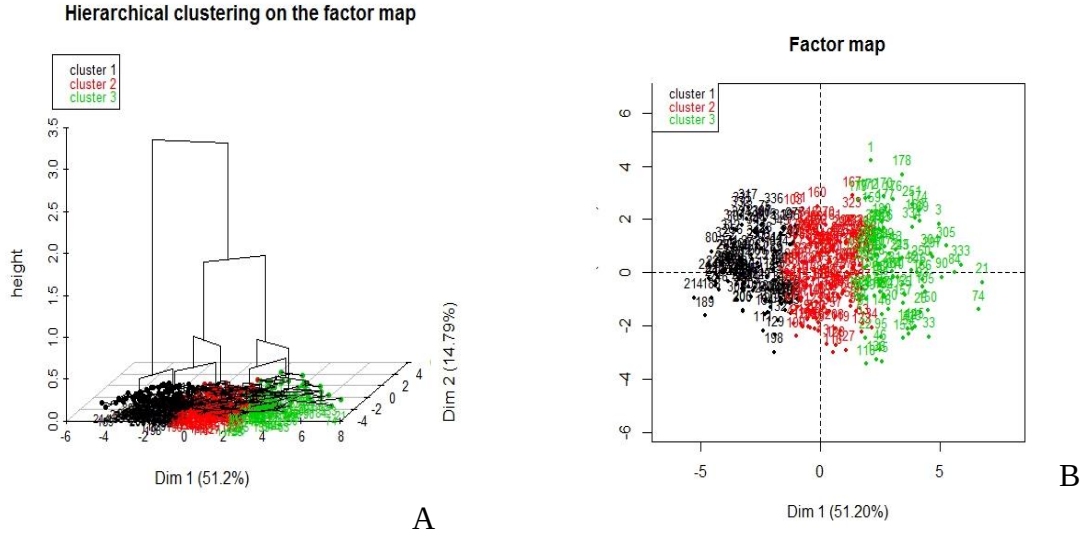


Figure 4. 3: Hierarchical clustering (A) and factor map clustering (B) of Congolese indigenous goat populations based on body measurement and reproductive performances in three AEZs in DR Congo. Cluster 1 in black and cluster 3 in green (with large and heavy goats)

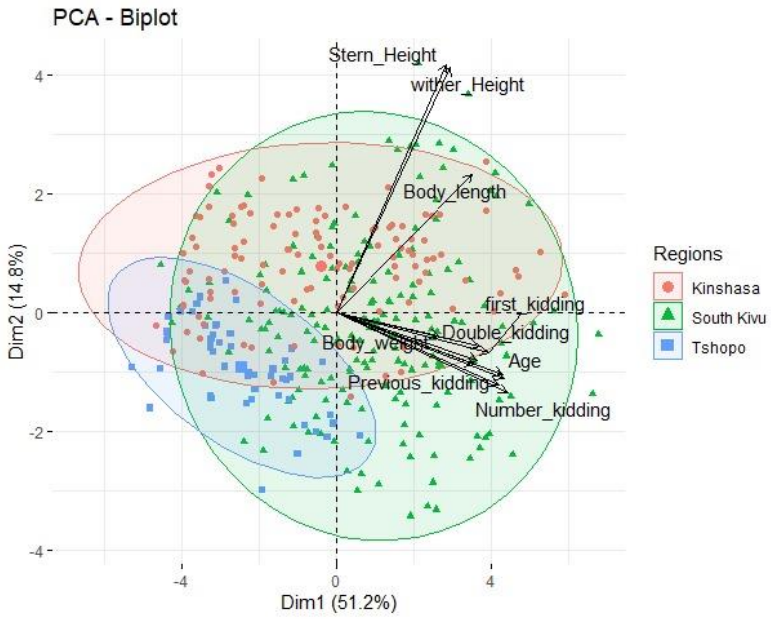


Figure 4. 4: Principal component analysis of Congolese goat populations based on body measurement and reproductive performances in three ago ecological zones. This figure shows the repartition of the three indigenous goat populations in the three identified cluster

Table 4. 4: Description of clusters based on body measurement and reproductive performances

Clustering-based to body measurement					
Class	Body length	Height at withers	Height at stern	Bodyweight	Estimated age
1	64.438	57.528	54.596	38.581	3.653
2	56.306	50.782	48.482	28.977	2.048
3	66.469	46.625	44.906	38.156	3.156
Clustering based on reproductive performances					
Class	Double kidding	Triple kidding	Last kidding	Second kidding	First kidding
1	0.983	0.0011	1.430	0.972	0.492
2	2.022	1.613	2.753	2.581	1.849
3	2.757	0.095	2.041	1.405	1.973

Table 4. 5: Proportion of animals from different regions in each cluster

Region	Clusters		
	1	2	3
Kinshasa	42.98	35.96	21.05
South Kivu	14.44	48.33	37.22
Tshopo	82.69	17.31	0

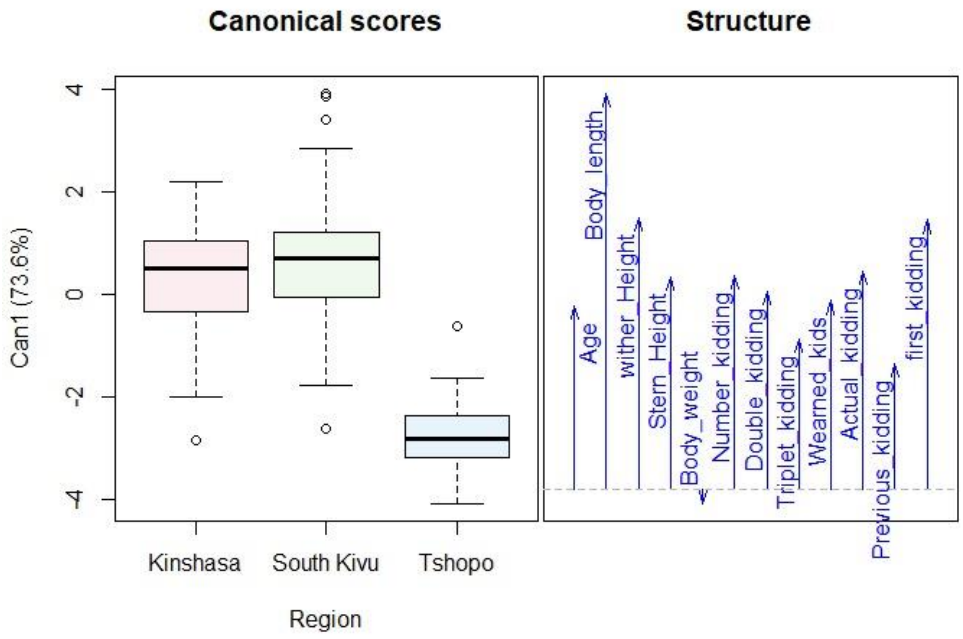


Figure 4. 5: Canonical scores of goats per region and canonical structure coefficients of variables

Table 4. 6: Total-sample standardized canonical coefficients and total variation explained by each canonical variable

	Canonical variables	
Traits	Can1	Can2
Age	0.124	0.269
Body length	0.978	0.321
wither Height	1.866	-0.189
Stern Height	-1.972	0.657
Body weight	-0.543	-0.560
Number of kidding	0.102	-0.893
double kidding	0.081	0.014
triplet kidding	-0.047	-0.088
earned kids	-0.122	0.220
Actual kidding	0.183	0.049
Previous kidding	-0.234	0.383
First kidding	0.253	-0.607
squared canonical correlations	0.577	0.328
Eigen value	1.363	0.488
Total variation explained (%)	73.613	26.387

Table 4. 7: Pairwise Mahalanobis' distances and probability values for the contrasts between local goats from different regions in the DRC.

Regions	Kinshasa	South Kivu	Tshopo
Kinshasa	0.00	4.73	14.58
South Kivu	ns	0.00	14.48
Tshopo	***	***	0.00

The squared Mahalanobis' distances are above the diagonal line. The probability values for the contrasts by the F-test (***= P<0.001 and ns= non-significant) are below the diagonal line

4.4.4. Production management and benefits of keeping goats

The results in **Table 4.8** showed that free grazing was the common method used by farmers to feed goats. This practice was observed more in Tshopo (100%) than in South Kivu (89.87%) and Kinshasa (54.90%) where the feeding system was supplemented by kitchen residues. In all the three regions studied, no technical input was applied in the management of the production system neither for the goat health care nor for the reproduction or during the seasons' variation. Keeping indigenous goats was considered by farmers as a source of income in South Kivu (98.73%) and Tshopo (90.27%) while in Kinshasa it was mostly considered as a saving method (60.78%). For that purpose, only live adult goats were fully marketed (100%), mostly in local markets in Tshopo (98.61%) and South Kivu (81.013%) and urban markets in Kinshasa (88.23%). The price of goats was significantly ($p < 0.0001$) higher (USD 58.42 ± 10) in Kinshasa than in South Kivu (USD 52.05 ±11.79) and in Tshopo (USD 46.63±9.68). The price variation could be justified by the fact that Kinshasa is the capital city of the country and has almost one-fifth of the estimated population of DR Congo of 90 million while Tshopo is in the remote forest area, leading to higher demand in Kinshasa compared to Tshopo. Additionally, in Kinshasa and South Kivu goats play an important role in cultural ceremonies than in Tshopo where that habit is less important.

Table 4. 8: Production management and benefits of keeping goats

Variables		Location			
		South Kivu	Tshopo	Kinshasa	
	Intensity	Frequency (%)	Frequency (%)	Frequency (%)	P-value
Feeding management	Grazing	89.873	100	54.902	< 0.0001
	Grazing, cassava leaves	5.063	0.00	11.765	
	Raving, kitchen residues, and cassava leaves	2.532	0.00	3.922	
	Grazing and kitchen residues	2.532	0.00	29.412	
Health management	Deworming	3.898	2.778	11.765	< 0.0001
	nothing	92.405	97.222	76.471	
	Vaccination	2.532	0.00	9.804	
	Veterinarian support	1.266	0.00	1.961	
Reproduction management	Yes	8.861	1.389	7.843	< 0.0001
	No	91.157	98.612	92.157	
Goat management	Nothing	100	100	100	
Socio economic benefits of keeping goat	Social status	1.266	0.00	1.961	< 0.0001
	Source of income	98.735	90.278	37.491	
	Savings	0.00	9.723	60.783	
Market orientation	Fully marketed	100	100	100	ns
Targeted market	Local market	81.013	98.612	11.764	< 0.0001
	Urban market	18.987	1.389	88.236	
Last product marketed	In live adult goat	67.089	77.778	100	< 0.0001
	Kids	15.190	9.722	0.00	
	Only male kids	17.722	12.500	0.00	
Price at the market (USD)	In live adult goat	52.05 ±11.79 [b]	46.63±9.68 [a]	58.42 ± 10 [c]	< 0.0001

4.4.5. Description of important phenotypic traits assisting the selection of goats raised by farmers

The essential traits that smallholder farmers from the three AEZs prefer in selecting indigenous goats to be raised included the adaptability in the region, the resistance to disease,

the prolificacy, and the ability of doe to breastfeed and to protect her kids up to the weaning age. The frequency of these traits varied according to the region (**Table 4.9**). In Kinshasa, 38.592% of the farmers preferred adaptability and resistance to disease traits, while 30.7% of farmers in the same region also preferred prolificacy. In South Kivu, adaptability to the region (45.55%) and prolificacy, and both adaptability in the region and disease resistance (27.727%) were preferred by farmers. In Tshopo, farmers mostly considered the ability to protect kids, and resistance to disease (19.23%), only resistance to disease (15.384%), and both adaptability and resistance to disease (13.461%).

Table 4. 9: Description of important phenotypic traits assisting the choice of doe raised by farmers

Important traits	Location			
	South Kivu	Tshopo	Kinshasa	P-value
	Frequency (%)	Frequency (%)	Frequency (%)	
Body conformation	0.00	1.923	1.754	< 0.0001
Weight	0.455	1.923	0.00	
Adaptability in the region	45.455	1.923	5.263	
Adaptability and resistance to disease	15	13.461	38.592	
None	0.00	1.923	3.509	
Ability in protecting kids	0.00	3.846	4.386	
Ability in protecting kids and resistance to disease	0.00	19.23	0.00	
Prolificacy	0.455	7.692	0.87	
Prolificacy and adaptability	6.364	5.773	0.87	
Prolificacy, adaptability and resistance to disease	27.727	3.846	30.70	
Prolificacy, ability in protecting kids and resistance to disease	0.00	7.692	11.40	
Resistance to disease	0.455	15.384	0.00	
Prolificacy and resistance to disease	4.091	9.615	0.87	
Prolificacy and ability in protecting kids	0.00	5.769	1.754	

4.5. Discussion

Various approaches have been developed to characterize domesticated animals. The phenotypic characterization is considered the first step to better understand the typology and production performances of animal genetic resources in different production environments.

4.5.1. Morphological traits

In this study, black color followed by gray color, both in a plain or uniform pattern was found to dominate the goat body hair coat color in the three AEZs of DR Congo studied. These findings corroborate with those in Ethiopian goat populations (Amesha, 2001; Halima *et al*., 2012; Gebreyowhens and Rohatash, 2017) with the variations in the morphological traits and the predominance of black coat color. This variability in the morphological traits can explain

the absence of any selection through structured selective breeding (Halima *et al.*, 2012) in the studied indigenous goat populations. Black-colored goats are believed to have a superior adaptation to seasonal cold weather or cold nights as the dark pigment helps them to warm up earlier than goats with other coat colors (Robertshaw, 2006). This affirmation does not align with the reality in Kinshasa and Tshopo, two of the hottest regions in DR Congo. However, the choice of black goats can be justified by its positive association with the reproduction performance since prolificacy was mentioned as one of the preferred traits in selecting goats by farmers (Ebozoje *et al*., 1998). Variations within and between goat ecotypes were also observed in the horn shape, ear orientation, and the presence or absence of beard and tassels. In this study, most of goat populations were characterized by the curved horn (91.56%), horizontal ear in Tshopo and Kinshasa while erect in South Kivu. The presence of horns has been considered as an advantage for the drainage of blood through the cavernous sinus as a control mechanism for thermal homeostasis and better reproductive performances (Al-Ghalban *et al.,* 2004; Kridli *et al*., 2005; (Robertshaw 2006). Our results for the absence of bear (88.8%) and tassels (94.53%) on the studied goats is different from the findings in indigenous Twana goat in Botswana where 95% of goats were respectively bearded and horned (Monau *et al*., 2018). Horned and bearded goats were also observed in other goat populations (Manzi *et al*., 2011; Halima *et al*., 2012).

Average weight and linear body measurements of adult does vary significantly ($p < 0.001$) among the goat populations. The goat ecotype populations from Kinshasa were significantly ($p < 0.001$) heavier and larger with 61.72 ± 4.37 cm, 55.25 ± 4.13 cm, 52.50 ± 3.75 cm, 33.23 ± 4.04 kg, respectively, for the body length, height at withers, height at stern and body weight than goat ecotype populations from Tshopo with 51.50 ± 2.04 cm, 47.69 ± 1.76 cm, 46.68 ± 1.87 cm and 30.34 ± 5.09 kg, respectively for the same traits. Gebreyowhens and Rohatash (2017) obtained 28.9 ± 0.35 kg, 60.0 ± 0.40 cm, and 59.3 ± 0.31 cm, respectively, for the

body weight, body length, and height at withers in indigenous Maefur goats from the north of Ethiopia. These findings corroborate the results from this study in terms of body weight and height at withers, respectively, in goats from Tshopo and Kinshasa. The body weight of 31.8 kg equal to the body weight of indigenous goats for this study (30.34 ± 5.09 kg) was observed in indigenous goats in the highland zones in the Sub-Saharan region. Peters and Horst (1981) suggested that body size is a suitable criterion for the classification of goats since it gives clues to potential performance. Accordingly, Devendra and Burns (1983) classified tropical goats into three groups; large (>65 cm at withers), small (51 to 65 cm at withers) and dwarf (<50 cm at withers). Based on this classification, indigenous goats from this study were classified into the small (53.55 ± 5.19 cm for goats from South Kivu; 55.25 ± 4.13 cm for goats from Kinshasa) and into the dwarf (47.69 ± 1.76 cm for goats from Tshopo province). The Canonical Discriminant Analysis (CDA) revealed that canonical variables presented the highest weights for body length and wither height showing that body length is important both to discriminate and to classify goats from different regions. Similar results were obtained in two indigenous goats in Nigeria showing that the body measurements were important factors discriminating the two-goat populations. The rump height followed by the body length were the most discriminate variables between the two goat populations (Yakubu et al. 2010).

4.5.2. Reproductive performances

The oldest does in this study were found in South Kivu (3.34 ±1.48 years old) with 3.85 ± 1.71 kidding. The average litter size increased with the number of kidding. Average litter size of 1.7 kids at the first kidding and 2.4 kids at the sixth kidding was observed in Creole goats (Alexander et al. 1999). Chowdhury et al. (2002) observed average litter sizes of 1.29; 1.71; 1.87; and 2.17 respectively at the 1^{st}, 2^{nd}, 3^{rd}, and the 4^{th} parity in black Bengal goat under semi-intensive management. Adeeb Khanum *et al.* (2006) observed an average litter size of

1.8 ± 0.8 in female dwarf goats under traditional conditions at NIAB Farm in Pakistan at the second kidding. However, in this study, litter size of 2 kids per parity was observed at the third kidding in the indigenous goat in Kinshasa (1.80 ± 0.00) and South Kivu (1.93 ± 0.00). In contrast, Sabimana *et al*. (2018) registered a litter size of 2 kids at the second kidding and 3 kids at the sixth kidding with the probability of 6.8% of the total number of plain females in goats raised in Banza Ngungu district in the Savanah and sub-humid AEZ in Kongo Central Province in the DRC. At the third kidding, litter size was 1.93 for indigenous goats' populations in South Kivu considered as the largest litter size in the three AEZs studied. The number of double kidding was superior to triple kidding in all the three provinces. Đuričić *et al*. (2012) found the same results in Boer goats in a moderate climate zone in Croatia, where the number of double kidding was superior to the triple kidding. These differences could be justified by the influence of genetic potential, environment, and management (Pamo *et al*., 2007)

4.5.3. Production management and benefits of keeping goats

Indigenous goats also play an important socio-economic role under the stressful environment in which they produce (Edea *et al*., 2012). Across studied production systems, indigenous goat ecotypes had significantly contributed to the farmers' households. Income generation and savings methods were considered as the overriding goal of keeping goats. Farmers used the income derived from goats keeping for children's education, health, and other basic needs of farmers' households (Wasso *et al*., 2018). This was realized mainly through the conservation and the sale of live animals both in local and urban markets. This finding concurs with other studies in developing countries that underscore the importance of livestock in generating income for the farmers (Kugonza *et al*., 2011; Okeno *et al*., 2011; Wendimu *et al*., 2018). The productivity of domestic animals depends on production management, including the production system, the breeds, and the management of the

environment of production (Philip *et al*., 2010). However, no management under the local production system was reported in keeping goats in the three AEZs of this study. Goats were fed through free grazing on natural pastures without any supplementary or complementary feed; However, in Kinshasa, goats sometimes benefited from residues from the kitchen during the night by sharing the same home with people. This finding corroborates the report by Wendimu *et al*. (2018) on the goat production system in the Assosa zone in Ethiopia. The feeding system by free grazing constitutes the source of diseases to animals, causing some crucial losses of the high reproductive performance (Lamy *et al*., 2012; Agoussu *et al*., 2017). However, Garcia *et al*. (2012) shown that, if managed adequately, free grazing can be a useful tool for environmental conservation. Although indiscriminate crossbreeding negatively affects the genetic potential of goats by causing genetic erosion, reducing their adaptative values (loss of genetic diversity), and reducing their opportunities for their efficient utilization (Hanssen *et al*., 2012); mating of goats in the area of this study was uncontrolled. Similar results were observed by Mamabolo and Webb (2005) in the production system of goats in Southern Africa.

4.5.4. Important traits in a selection of goat

The involvement of farmers in assessing breeding objectives and designing programs is crucial for the success of livestock improvement programs (Dossa *et al*., 2009; Mueller *et al*., 2015). To rank animals in a rather objective and accurate manner, farmers focus on some preferences based on weightings or indices becoming a powerful tool for them (Onzima *et al*., 2017). This participatory approach of farmers is important and has been used in some developing countries to provide information for developing breeding objectives for Nile tilapia (Omasaki *et al*., 2016), goats in Ethiopia (Gebreyesus *et al*., 2013), Maasai sheep (Zonabend Konig *et al*., 2015) and dairy goat in Kenya (Bett *et al*., 2009). Selection, whether based on individual, family, or pedigree, is dependent upon the economically important traits

e.g., meat, milk, disease resistance, drought tolerance, etc. (Bhattarai *et al*., 2019). Goats in DR Congo have been mostly selected for adaptation in the production environment, disease resistance, prolificacy, and the ability of the doe in protecting her kids. Similar findings were reported in the study on the production system and participatory identification of breeding objective traits for indigenous goat breeds in Uganda (Onzima *et al*., 2017). With the West African dwarf goats, disease resistance was similarly considered essential in selecting goats (Ogah, 2016). The findings from this study were in line with those from previous studies in Somalia (Marshall *et al*., 2016**)** and Ethiopia (Gizaw *et al*., 2010). It is important to highlight that the understanding and the inclusion of the adaptation traits in the breeding program are very critical for developing sustainable improvement programs for indigenous goats (Monau *et al*., 2020). However, selection for adaptation traits may be more complicated unless if they are positively associated with the production performances of animals (Onzima *et al*., 2017). In this perspective, farmers from the three AEZs studied included the prolificacy performance essentially considering the number of kids per parity and the ability of the doe to breastfeed (enough milk) and to protect her kids as production traits in the selection of does to keep. Farmers could mention the adaptation traits in these regions according to the limited inputs (agro-inputs, access to professional veterinary services, capacity building in goats farming) characterizing the production systems (Wasso *et al*., 2018). Indigenous goat breeds are superior to exotic breeds in terms of survival and economic performance in the tropical environment (Ayalew *et al*., 2003 and Onzima *et al*., 2014). These indigenous goats can also valorize low-quality feed resources, disease resistance, and environmental stress compared to exotic breeds (Philipsson *et al*., 2006; Kugonza *et al*., 2012), thus the adoption by the farmers of these characteristics in their selection traits.

4.6. Conclusion

Goats from three AEZs of DR Congo were characterized by the black coat colour and curved horn. They clustered into three groups well distinguished by the reproductive performances that included the number of double (twins) and triple kidding. Clusters two and three, characterized by goats with high number of double and triple kidding, were represented mainly by goats from South Kivu and Kinshasa, while cluster one was characterized by a low number of double and triple kidding included goats from Tshopo. The CDA revealed that canonical variables presented the highest weights for body length and wither height, showing that body length is important to discriminate and classify goats from the three different regions. Goats were considered as a source of income and savings method contributing to farmer's household income. However, no technical inputs were supplied in the management (in feeding, reproduction and disease control) of goats by smallholder farmers in the 3 AEZs. The physical adaptation in the regions, the disease resistance and the prolificacy were the most farmer-preferred traits in selecting goats. These results indicate the first step in the decision-making towards goat improvement in DRC. Molecular characterization by genotyping and genomics association analyses should be considered to elucidate whether the observed phenotypic differences, are genetically based and/or environmentally influenced

CHAPTER FIVE: HAPLOTYPE ANALYSIS OF THE MITOCHONDRIAL DNA *d-loop* REGION REVEALS THE MATERNAL ORIGIN AND HISTORICAL DYNAMICS AMONG THE INDIGENOUS GOAT POPULATIONS IN EAST AND WEST OF THE DEMOCRATIC REPUBLIC OF CONGO

5.1. Abstract

This study aimed at assessing haplotype diversity and population dynamics of three Congolese indigenous goat populations that included Kasai goat (KG), small goat (SG), and dwarf goat (DG) of the Democratic Republic of Congo (DRC). The 1,169 bp *d-loop* region of mitochondrial DNA (mtDNA) was sequenced for 339 Congolese indigenous goats. The total length of sequences was used to generate the haplotypes and evaluate their diversities, whereas the hypervariable region (HVI, 453 bp) was analyzed to define the maternal variation, the demographic dynamic, and history. Approximate Bayesian Computation (ABC) simulations were modeled to investigate the demographic history of the three Congolese indigenous goat populations. A total of 192 haplotypes were generated by 568 segregating sites obtained after the analysis of the 1,169 bp of the mtDNA *d-loop* region of these 339 Congolese goat sequences. Phylogenetic analyses using reference haplotypes from the six globally defined goat mtDNA haplogroups showed that all the three Congolese indigenous goat populations studied clustered into the dominant haplogroup A, as revealed by the Neighbor-joining (NJ) tree and median-joining (MJ) network. Nine haplotypes were shared between the studied goats and goat populations from Pakistan (1 haplotype), Kenya, Ethiopia and Algeria (1 haplotype), Zimbabwe (1 haplotype), Cameroon (3 haplotypes), and Mozambique (3 haplotypes). The population pairwise analysis (F_{ST}) indicated a weak differentiation between the Congolese indigenous goat populations. Negative and significant (p-value < 0.05) values for *F*u's *F*s (-20.418) and Tajima's (-2.189) tests showed the

expansion in the history of the three Congolese indigenous goat populations. The Approximate Bayesian Analyses indicated that the Congolese goats operated from the northern African goats after 354 generations in later times (~1,062years ago). These results suggest a weak differentiation and a single maternal origin for the studied goats. This information will contribute to the improvement of the management strategies and long-term conservation of indigenous goats in DRC.

KEYWORDS

Genetic diversity, haplogroup, mismatch distribution pattern, population expansion, DRC

5.2. Introduction

In most developing countries, agriculture and particularly livestock farming constitutes an important source of income in rural households (Herrero *et al*., 2013). In that respect, goats, one of the first domesticated animals, provide meat and milk as a major source of income for smallholder farmers (Naderi *et al*., 2008; Aziz et al., 2010, Skapetas *et al*., 2016).

Estimated to 4,065,709 heads, indigenous goat populations in DRC are grouped into three local breeds locally called "chèvre *moyenne du Congo" or* small goat (SG) of Congo*, "chèvre du Kasai"* or Kasai goat (KG) and "*chèvre de Bandundu*" or dwarf goat (DG) of Congo (Lafleur *et al*., 2018; FAOSTAT, 2018; accessed March, 2021). These goats are spread throughout all the agro-ecological zones (AEZs) of the country where they are kept by farmers (FAOSTAT, 2018; accessed March 2021). In South Kivu, goats contribute up to 40% to farmers' household income (Wasso *et al*., 2018). As in eastern Africa, goats in DRC are raised in marginal areas, where crops production is not possible, in different production systems with the predominance of an extensive system that is characterized by low breeding inputs (Muigai *et al*., 2017).

Due to uncontrolled livestock movements across borders, exotic goat breeds would have been introduced and crossbred with the three Congolese indigenous goat populations. Such a practice increases the risk of the disappearance of resilient and adapted local breeds. To mitigate this risk, the locally adapted goat breeds in DRC need to be characterized, conserved, and utilized sustainably. Characterization of local breeds provides large knowledge and gives a clear perspective on the population structure that will assist in the decision-making of future breeding programs (Groeneveld *et al*., 2010; Yang *et al*., 2016). Because animal mitochondrial DNA (mtDNA) evolves faster than a nuclear genetic marker, it represents a good informative region for the study of phylogenetic and evolutionary biology (Ladoukakis and Zouros, 2017). It also permits the faster examination of the relatedness of populations and has become important in biogeographic and anthropologic studies (Lehman *et al*., 2006). The mtDNA polymorphism,

especially the displacement loop (*d-loop*) region, is one of the important tools that have been used to better understand the genetic diversity, the population structure, and the population dynamics in different animal species including goats (Phyu *et al*., 2017; Tarekegn *et al*., 2018 and 2019), sheep (Agiviezor *et al*., 2012), and chickens (Liu *et al*., 2009).

In general, six mtDNA haplogroups (A, B (B1 and B2), C, D, F, and G) were identified and found distributed in different geographic areas in the world. The haplogroup A was shown to have a large geographic distribution (Pereira *et al*., 2005) and was more reported in a large part of African regions (Luikart *et al*., 2001). In addition, haplogroups B and G were also reported in some African countries; with haplogroup B particularly found limited in the South part of Africa, especially in South Africa and Namibia while the haplogroup G was reported in Egypt (Naderi *et al*., 2007), Kenya (Kibegwa *et al*., 2016), Ethiopia (Tarekegn *et al*., 2018), Sudan and Somalia (Al-Araimi *et al.*, 2017). A previous study on indigenous goat from *Peste des Petits Ruminants* outbreak zones in South Kivu province of DRC based on mtDNA *d-loop* variation revealed the presence of two haplogroups A (commonest) and B within the goat population in South Kivu (Bwihangane *et al.*, 2018). However, the result from this study was limited to goats from *Peste des Petits Ruminants* outbreak zones in the South Kivu region and could not reveal more information on the genetic diversity of indigenous goat breeds in the whole country and did not mention the goat populations dynamics and history. Therefore, this study aimed at describing the haplotype diversity, the population structure, and the demographic dynamics and history of three indigenous goat breeds in three AEZs of DRC based on the mitochondrial DNA *d-loop* region.

5.3. Materials and Methods

5.3.1. Sampling and DNA extraction

Sampling was conducted in collaboration with the Ministry of Agricultural, Livestock, and Fisheries of DRC through the representative inspections in each sampling region that included Kinshasa, Tshopo, and South Kivu (**Figure 4.1**).

Blood samples were collected from jugular-vein into sterile tubes containing Ethylenediamine tetra acid (EDTA) as an anticoagulant using a sterile vacutainer syringe from 340 adult and female goats from which physical observation, body measurement, and reproductive performances were recorded (section 4.3.2.1 Sample size calculation, chapter 4). The collected blood samples were stored at -20 °C till the extraction of DNA. Farms were selected based on some inclusion criteria related to the experience of the farmer in kipping goat (more than 5years), the structure of the farm (more than 10 goats in the farm), the accessibility in the farm, and the phenotypic variability observed on goats in the farm.

A total of 339 blood samples representing three Congolese indigenous goat populations (Kasai goat, n=108; dwarf goat, n=114 and small goat, n=117) were sampled from farmer's flocks from the three AEZs of DRC and used for the study. To avoid blood sampling on closely related goats, one to two goats chosen by the farmers based on their genealogical information were sampled on each farm. The socio-economic factors of goats (Wasso *et al.*, 2018), the environmental characteristics (the high land volcanic mountain), and the proximity with neighbor countries: Tanzania, Rwanda, and Burundi facilitating animal exchanges that can lead to an uncontrolled intercross breeding between goat populations were the major reasons for chosen South Kivu. Tshopo was chosen based on its geographic location (the equatorial forest region) which could affect goat management and productivity, while Kinshasa (capital city) was chosen based on the environmental characteristic (high temperature), the commercial transaction with surrounding

regions including Bandundu, Kasai central, Congo Central, and the productivity history of goats (Sabimana *et al.,* 2017).

Genomic DNA was extracted from blood samples using the QIAamp® DNA Mini kit (Qiagen) according to the manufacturer's protocol. DNA quality control (QC) was done using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, USA) and DNA integrity was checked on 1% agarose gel electrophoresis (Tarekegn *et al.,* 2018). (Appendix II).

5.3.2. PCR amplification and sequencing of the *d-loop* region

The 1,169 bp of the mtDNA *d-loop* region was amplified using designed primers (F: 5'-ACCAGAAAAGGAGAATAGCC-3'; R: 5'–GGTACACTCATCTAGGCATT-3') using a three steps PCR after the protocol optimization. PCR reactions were carried out in 25 µl reaction volumes composed of Phusion master mix (2x concentrated solution which included Taq DNA polymerase (0.05 U/µl), reaction buffer, 4 mM $MgCl_2$ and 0.4 mM of each dNTP), 0.2 µM of each primer (F and R), 2% of Dimethyl Sulfoxide (DMSO) and 40 ng of template DNA. The three steps PCR involved an initial denaturation at 98°C for 30 seconds following by 35 cycles of amplification (denaturation at 98°C for 10 seconds, annealing at 61°C for 30 seconds, and extension at 72°C for 30 seconds) and completed by the final extension step at 72°C for 7 minutes. The PCR products were purified using the QIAquick® PCR purification kit (Qiagen) following the manufacture's protocol. The reverse primer (R: 5' – GGTACACTCATCTAGGCATT -3') and a pair of GDLS2 primers (GDLS-2F: 5'-ACCTAAAATCGCCCACTC-3'; GDLS-2 R: 5' TGATCTAGTGGACGGGATAC-3) were respectively used as external and internal primers to sequence the purified PCR products (Tarekegn *et al*., 2018).

5.3.3. Data analyses

Default values and parameters inherent in algorithms and software were used for all analyses undertaken in this study. Only deviations from the default were mentioned. Before the analyses, all the chromatograms were visualized using CLC Genomics workbench v8.0 software. MEGA v6.4 software was used for the multiple sequence alignments with the ClustalW algorithm (Tamura *et al.,* 2013). The variable sites were scored against the *Capra hircus* reference sequence (GenBank accession number: GU223571: direct submission). In total, 339 sequences were generated from where haplotypes were generated with DnaSP v5 (Rozas *et al*., 2003). Genetic diversity parameters that include the number of haplotypes (N), haplotype diversity (Hd), nucleotide diversity (π), and mean of nucleotide differences between haplotypes (K) and their standard deviations (SD) were analyzed for each goat population and across all populations using DnaSP v5 (Rozas *et al*., 2003; Librado *et al*., 2009).

A phylogenetic tree was constructed using haplotypes generated in Congolese indigenous goat and 22 reference haplotypes representing the six haplogroups (A, B, C, D, G, and F) defined based on the variation in the first HVI with 481 bp of length size (Luikart *et al*., 2001; Naderi *et al*., 2007, 2008) corresponding to positions 15,709 – 16,190 bp of the *Capra hircus* mtDNA reference sequence (Gene Bank accession number GU295658) with the neighbor-joining (NJ) algorithm implemented in MEGA v6.4 with the level of confidence associated with each bifurcation evaluated with 1,000 bootstrap replications. To visualize Congolese indigenous goats in the context of the regional and global caprine diversity and obtain further insights into genetic relationships between the haplotypes, a total of 336 published sequences and 22 reference sequences of domestic goats representing the six globally defined mtDNA *d-loop* haplogroups (Naderi *et al*., 2007) were retrieved from the GenBank and included in the NJ tree and MJ network analyses using Network v4.6 software (Bandelt *et al*., 1999). The added sequences were from 13 African countries: Cameroon (central Africa), Kenya, Ethiopia (East Africa), Egypt,

Algeria, Libya, Tunisia and Morocco (North Africa), Senegal, Nigeria (West Africa), Namibia, Zimbabwe, and Mozambique (southern Africa). In addition, sequences from 10 Asian countries (India, Iraq, Saudi Arabia, Pakistan, China, Laos, Iran, Mongolia, Jordan, Azerbaijan) and 6 European countries (Turkey, Austria, France, Italy, Switzerland, and Spain) were included in the analysis (**Appendix III**).

The genetic variation among Congolese indigenous goat populations was evaluated through the analysis of molecular variance (AMOVA) following 1,000 permutations in Arlequin v3.5.2 (Excoffier and Lischer, 2010). Pair-wise genetic differentiations (F_{ST}) (Reynolds *et al.,* 1983) were estimated between the three Congolese and with the mentioned non-Congolese goat populations grouped according to the geographical regions using Arlequin v3.5.2 software (Excoffier and Lischer, 2010) with the number of permutations for significance estimated at 100 at the significance level of 0.05.

We inferred population demographic history and dynamics from haplotype mismatch distribution patterns (Rogers and Harpending, 1992) and the expected distributions plus their 95% confidence intervals for the three Congolese indigenous goat populations. Departures of the observed sum of squares differences (SSD) from the simulated model of expansion were tested with the chi-square test of goodness of fit statistic and Harpending's raggedness index 'r' (Harpending, 1994) following 1,000 coalescent simulations. Analysis of mismatch distribution patterns was augmented with two coalescent-based estimators of neutrality; Fu's FS (Fu, 1997) and Tajima's D (Tajima, 1989) statistics. The significance of these two statistics was tested with 1,000 coalescent simulations in Arlequin v3.5.2.

To further investigate the demographic history of the three Congolese indigenous goat populations and understand their route of expansion, the Approximate Bayesian Computation (ABC) approach implemented in DIYABC v 2.1.0 (Cornuet *et al*., 2014) was used. HVI region sequences for the Congolese indigenous goat populations and reference sequences grouped based on geographical

regions as follow: north Africa (Morocco, Algeria, Libya and Egypt), east Africa (Ethiopia and Kenya), west Africa (Senegal, Nigeria) and Cameroon, and southern of Africa (Mozambique, Zimbabwe and Namibia) were considered for the ABC analysis. All the scenarios corresponded to a classic invasion history from an ancestral population assuming an initial size reduction (noted: Ne2b for population 2 and Ne3b for population 3, Ne4b for population 4 and Ne5b for population 5) when a new population is created from its ancestral population since the invasive population generally starts with a few immigrants (Cornuet *et al*., 2014). Accordingly, four demographic scenarios were tested (**Figure 5.1**) assuming that Congolese indigenous goat populations were descended from north Africa (scenario 1); east Africa (scenario 2), west Africa and Cameroon (scenario 3), and southern Africa (scenario 4). The reference tables were built by one million pseudo-observed data sets per scenario using uniform prior distributions of the effective population size (Ne) (considering Ne1>Ne2>Ne3>Ne4>Ne5), and time parameters (t) (considering t5>t4>t3>t2>t1) with defaults mutation settings according to Tamura and Nei (1993). Between two successive events affecting a population, we assume a discrete change in effective population size and that the population diverged in time by using "VarNe" and "merge" keywords, respectively in designing the scenarios. Tajima's D (Tajima, 1989) was used as one sample summary statistics, while the F_{ST} (Hudson *et al*., 1992) was used as two summary statistics (Jackson *et al*., 2015). Pre-evaluation of each scenario was performed by the Principal Component Analysis (PCA) within DIYABC v 2.1.0. To identify the best scenario, posterior probabilities across the 500 simulated pseudo-observed data sets were compared using DIYABC's logistic regression on the 1% of the simulated datasets (**Appendix IV**). For the best models, posterior distributions of the parameters were estimated with a logit-transformed linear regression on the 1% simulated datasets closest to the observed data. Scenario confidence was evaluated by comparing observed and simulated summary statistics. Finally, the goodness-of-fit of the posterior parameters for the best performing scenarios was tested via the model checking option with

default settings (Montana *et al*., 2017). The skewness of the posterior distribution graph, the mode value which represented the highest posterior probabilities of the posterior distribution for the scenarios was used to determine time of arrival of goat populations in DRC.

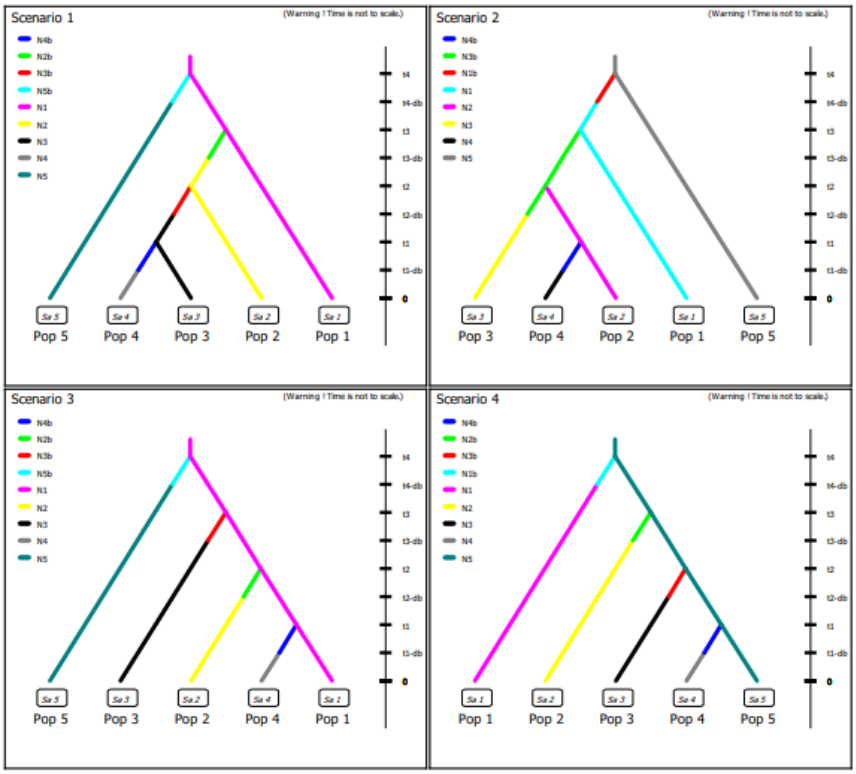


Figure 5. 1: Regional representation of goat population to infer the historical gene flow towards DRC examined using Approximate Bayesian Computation implemented in DIYABC

5.4. Results

5.4.1. mtDNA *d-loop* sequence variation and genetic diversity

From the 339 generated sequences of the mtDNA *d-loop* region representing the three Congolese indigenous goat populations spanning the entire 1,169 bp, a total of 192 haplotypes defined by 568 segregating sites were detected from the *d-loop* region of these sequences aligned against the caprine reference (Accession number GU223571). Out of the 192 haplotypes, 23 were shared between the Congolese indigenous goat populations (seven haplotypes shared between the three Congolese indigenous goat populations, eight shared between KG and SG, three between KG and

DG, and five between DG and SG. Furthermore, nine haplotypes were shared between the three Congolese indigenous goat populations and the goat populations in Africa. These include one haplotype (mostly represented by a small goat) shared with goat populations from Ethiopia, Kenya, and Algeria, three haplotypes mostly represented by DG shared with goat populations from Cameroon, three and one haplotypes mostly represented by KG were respectively shared with goat populations from Mozambique and Zimbabwe. Outside Africa, one haplotype was shared with Pakistan goat.

The three Congolese indigenous goat populations showed high levels of genetic diversity (average haplotype diversity 0.994 ± 0.03 for SG, 0.994 ± 0.003 for KG and 0.973 ± 0.007 for DG). The average nucleotide diversity was higher for DG ($\pi = 0.018 \pm 0.004$) than for SG ($\pi = 0.013 \pm 0.002$) and KG ($\pi = 0.012 \pm 0.022$) (**Table 5.1**).

Table 5. 1: mtDNA d-loop sequence variation and genetic diversity

Population	N	S	H	Hd± SD	π ±SD	K
KG	108	187	91	0.994± 0.003	0.012±0.022	13.899
SGC	117	200	96	0.994 ± 0.03	0.013 ±0.002	14.806
DG	114	495	60	0.973± 0.007	0.018 ± 0.004	18.104
All population	339	568	192	0.987± 0.002	0.015 ± 0.003	14.740

N= Number of populations; S= number of segregating sites; H= number of haplotypes detected; Hd= haplotype diversity; SD= standard deviation; π = nucleotide diversity; K= average number of nucleotide differences. KG= Kasai goats (Kinshasa); SGC= small goats (South Kivu); DG= dwarf goats (Tshopo).

5.4.2. Population phylogenetic and relationship analysis

The first hypervariable (HVI, 453bp) sequences both for Congolese indigenous goat populations and the reference sequences representing the six-domestic goat haplogroups were aligned using the ClustalW algorithm implemented in MEGA v6.4 and considered for the NJ tree analysis. All the three Congolese indigenous goat populations (100%) were clustered into mtDNA lineage A

(**Figure 5.2**). A MJ (as described in the data analysis section) constructed to provide a wider resolution of the phylogenetic relationship between Congolese indigenous goat populations with those of others regions (**Figure 5.3**), supported the result obtained by the NJ tree analysis.

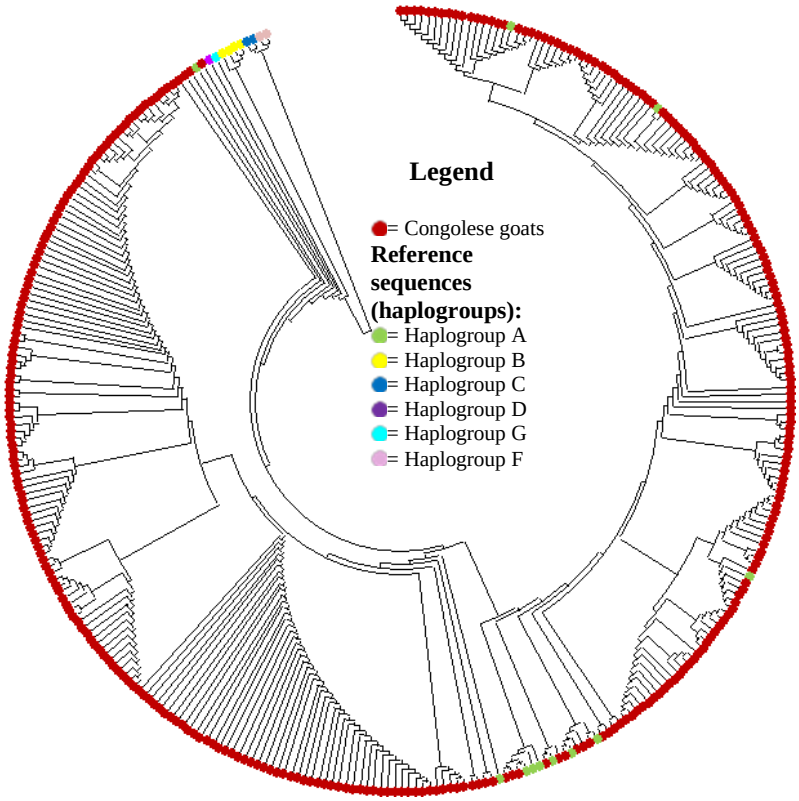


Figure 5. 2: Neighbor-joining tree constructed using the HVI region of the mtDNA d-loop of 3 Congolese indigenous goat populations including reference haplotypes representing six globally defined haplogroups (A, B, C, D, G, and F) observed in goats (Naderi *et al.*,2007).

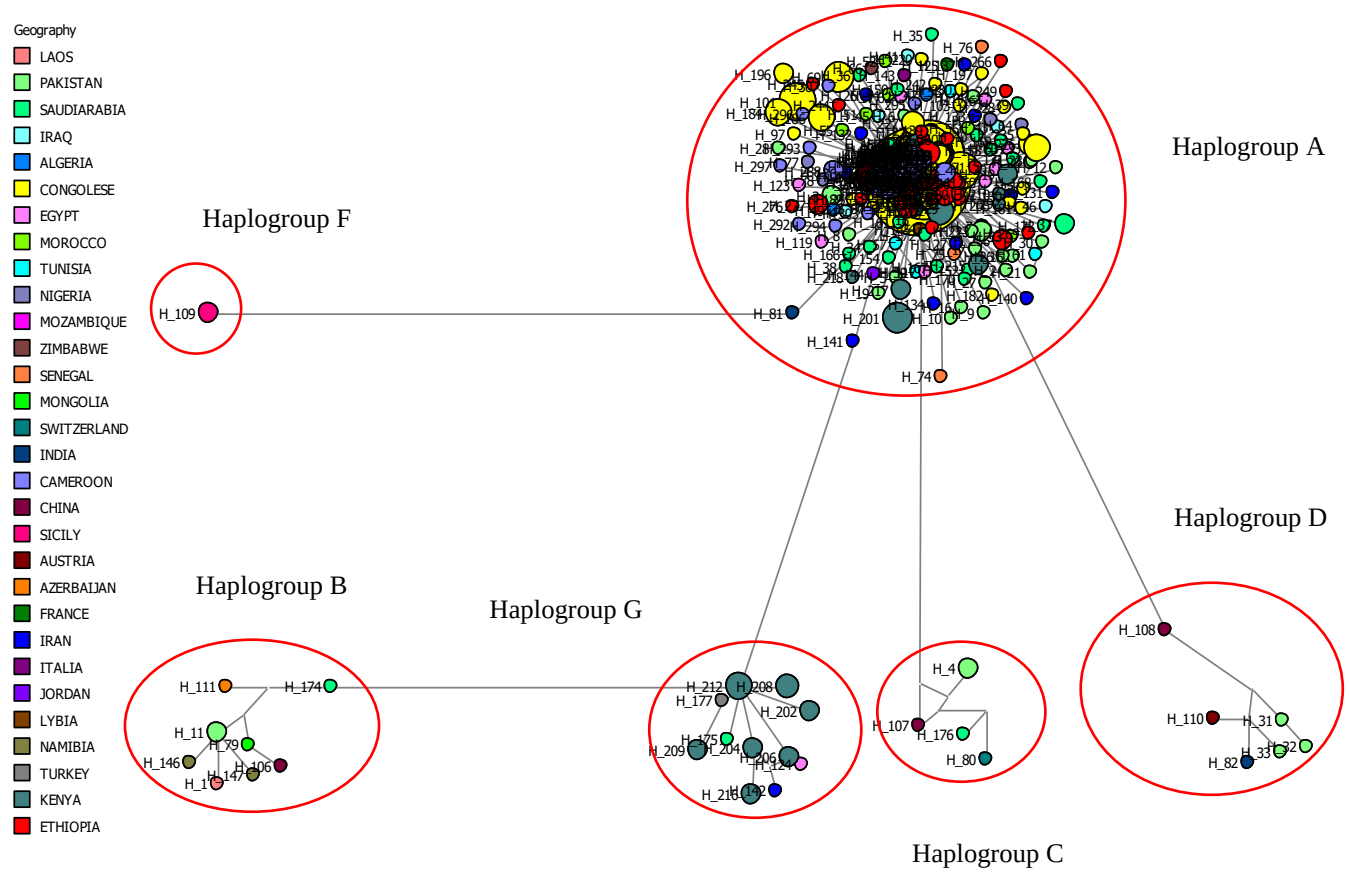


Figure 5. 3: Median-joining network analysis based on HIV region of 339 haplotypes of Congolese indigenous goat populations and 336 haplotypes of reference sequences from 29 different countries (13 African countries, 10 Asian countries, and 6 European countries)

5.4.3. Population genetic structure

The analysis of molecular variance (AMOVA) for the three Congolese indigenous goat populations grouped by AEZs revealed that 5.88% of the total genetic variation was attributed to the genetic differences among populations and the highest proportion (94.12%) to variation within populations (**Table 5.2**). The pair-wise genetic distance (F_{ST}) value revealed low genetic differentiation between the three studied Congolese indigenous goat populations (**Table 5.3**). In the context of the regional and global caprine diversity, low genetic differentiation was observed between Congolese indigenous goats, northern (0.20138) and Cameroonian goats (0.20800)

compared to the F_{ST} value observed between Congolese indigenous goats and Asian goats, European goats and other African goat populations (**Table 5.4**).

Table 5. 2: Analysis of Molecular Variance (AMOVA)

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among populations	2	75.740	0.44749	5.88
Within population	337	2415.254	7.16693	94.12
Total	339	2490.994	7.61442	

Table 5. 3: Population pairwise (FST)

Population	KG	DG	SGC
KG	0.00000		
DG	0.02165	0.00000	
SGC	0.09182	0.04567	0.00000

Table 5. 4: Population pairwise (FST) to estimate the genetic distance between the Congolese and the non- Congolese goat populations

Goat populations	Asia	Europe	North Africa	East Africa	West Africa	Cameroon	Southern Africa	DRC
Asia	0.00000							
Europe	0.23761	0.00000						
North Africa	0.01467	0.32033	0.00000					
East Africa	0.03722	0.33168	0.02473	0.00000				
West Africa	0.00902	0.26289	-0.01361	0.02910	0.00000			
Cameroon	0.16821	0.62990	0.21757	0.18638	0.19294	0.00000		
Southern Africa	0.10085	0.20189	0.15630	0.15075	0.13509	0.30033	0.00000	
DRC	0.23052	0.66525	0.20138	0.23111	0.21406	0.20800	0.35904	0.00000

5.4.4. Population dynamics and history

Mismatch distribution patterns were used to assess the population dynamics of the three Congolese indigenous goat populations grouped into the haplogroup A as revealed by the NJ tree and the MJ network. For each population, the expansion modal of mismatch distribution was unimodal and a valid goodness-of-fit was observed between observed and expected distributions plus their 95% confidence intervals, indicating a demographic expansion signal (Figure 5.4). Either with the demographic or spatial expansion modal, the observed patterns of mismatches significantly deviated from that expected under a null hypothesis except for Kasai goat (**Table 5.5**). However, the variations around the curves were not significant except for dwarf goats if considering the demographic expansion modal (**Table 5.5**). The Fu's *F*s and Tajima's *D* tests were all negative and significant for each population and the global dataset incorporating the three Congolese indigenous goat populations (*P*-value < 0.05). The negative and significant Tajima's D value (-2.189) and Fu's Fs (-20.418) obtained suggests that the three Congolese indigenous goats have expanded demographically in their past. To investigate the demographic history of the three Congolese indigenous goat populations, the Bayesian modelling implemented in DIYABC indicated that the first scenario (**Figure 5.1**) was the best-fitted model in logistic regression (posterior probability > 0.9795) (**Figure 5.5**) indicating that the dispersion of goats towards to DRC following the route from north Africa to central Africa (Cameroon). Based on the estimation of the posterior distribution, the modes values (which are the highest point of the curve) of the divergence time showed that Congolese indigenous goats isolated for the last 354 generations (5% quantile (q050) = 120 generations – 97.5% quantile (q975) = 4570 generations) from northern African goats which is equivalent to 1,062 years assuming that 3 intervals per generation (**Table 5.6**).

Table 5. 5: Population pairwise (FST) to estimate the genetic distance between the Congolese and the non- Congolese goat populations

Population	N	S	Demographic expansion		Spatial expansion		Tajima's D (*p*-value)	Fus' Fs (*p*-value)
			SSD (*p*-value)	Raggedness index (*p*-value)	SSD (*p*-value)	Raggedness index (*p*-value)		
KG	108	187	0.003 (0.510)	0.003 (0.820)	0.007 (0.200)	0.003 (0.850)	-1.980 (0.000)	-24.262 (0.000)
DG	114	495	0.023 (0.000)	0.022 (0.00)	0.249 (0.000)	0.022 (0.080)	-2.705 (0.000)	-12.818 (0.021)
SGC	117	200	0.009 (0.010)	0.008 (0.070)	0.010 (0.000)	0.008 (0.240)	-1.881 (0.014)	-24.173 (0.000)
All	339	568	0.012 (0.173)	0.011 (0.296)	0.014 (0.066)	0.011 (0.390)	-2.189 (0.046*)	-20.418 (0.007*)

* Statistically significant ($p < 0.05$); N= Sample sizes; S= segregating sites; SSD= sum of squared deviations

Table 5. 6: Original parameter estimation and statistics (Mean, median, mode, and quantiles) of the posterior distribution for the scenarios with the highest posterior probabilities.

Parameter	mean	median	mode	q025	q050	q250	q750	q950	q975
N1	8.61e+003	8.94e+003	9.86e+003	5.44e+003	6.16e+003	8.01e+003	9.55e+003	9.91e+003	9.95e+003
N2	7.29e+003	7.54e+003	8.04e+003	3.60e+003	4.36e+003	6.33e+003	8.50e+003	9.42e+003	9.60e+003
N3	5.87e+003	5.99e+003	6.58e+003	2.16e+003	2.67e+003	4.65e+003	7.22e+003	8.59e+003	8.93e+003
N4	4.13e+003	4.07e+003	4.13e+003	8.20e+002	1.14e+003	2.66e+003	5.50e+003	7.41e+003	7.88e+003
N5	1.86e+003	1.48e+003	8.61e+001	7.77e+001	1.38e+002	6.54e+002	2.72e+003	4.87e+003	5.58e+003
t1	1.39e+003	1.05e+003	**3.54e+002**	7.00e+001	1.20e+002	5.00e+002	1.93e+003	3.81e+003	4.57e+003
t2	3.48e+003	3.27e+003	2.62e+003	6.27e+002	8.68e+002	2.05e+003	4.68e+003	6.84e+003	7.54e+003
t3	5.65e+003	5.71e+003	5.50e+003	1.78e+003	2.28e+003	4.17e+003	7.19e+003	8.79e+003	9.16e+003
t4	7.77e+003	8.15e+003	9.77e+003	3.70e+003	4.39e+003	6.72e+003	9.18e+003	9.84e+003	9.91e+003

N1-N2-N3-N4-N5 = North Africa (Morocco, Algeria, Tunisia and Egypt)- East Africa (Ethiopia, Kenya)- West Africa (Senegal, Nigeria) and Cameroon - DRC -Southern Africa (Mozambique, Zimbabwe and Namibia)- goat populations; t1 = time of divergence of Congolese goats from North African goats in generations (3 years per generation in goats).

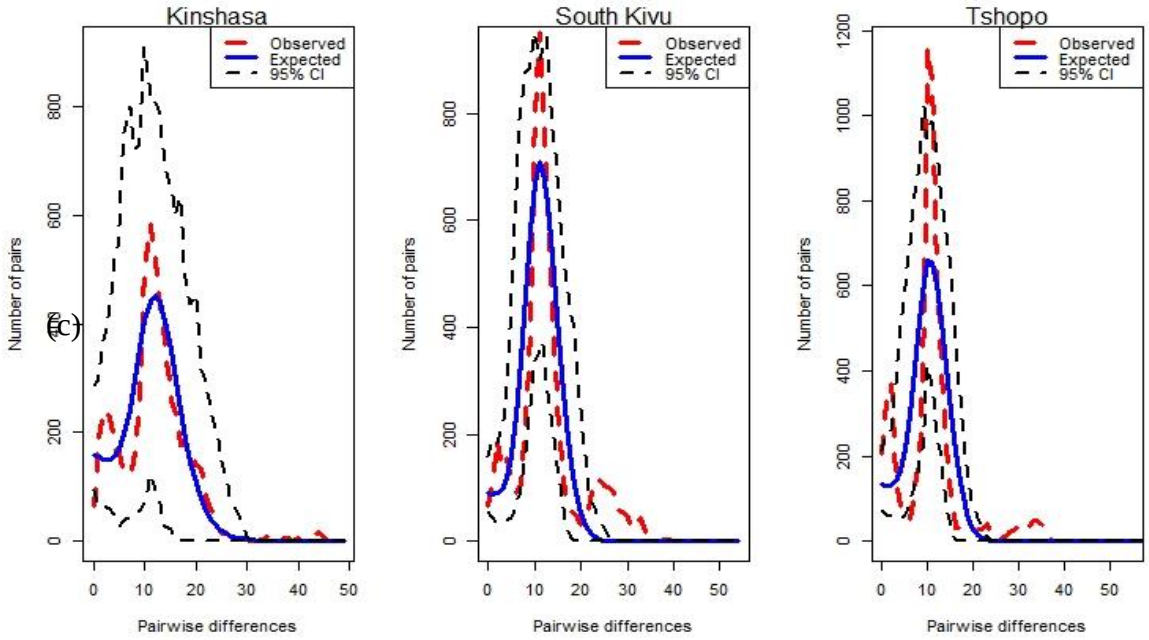


Figure 5. 4: Demographic dynamics among the indigenous goat populations in DRC. The demographic dynamics of each population was inferred from mismatch distribution patterns following 1,000 coalescent simulations. The figure shows a unimodal peak indicating a one origine

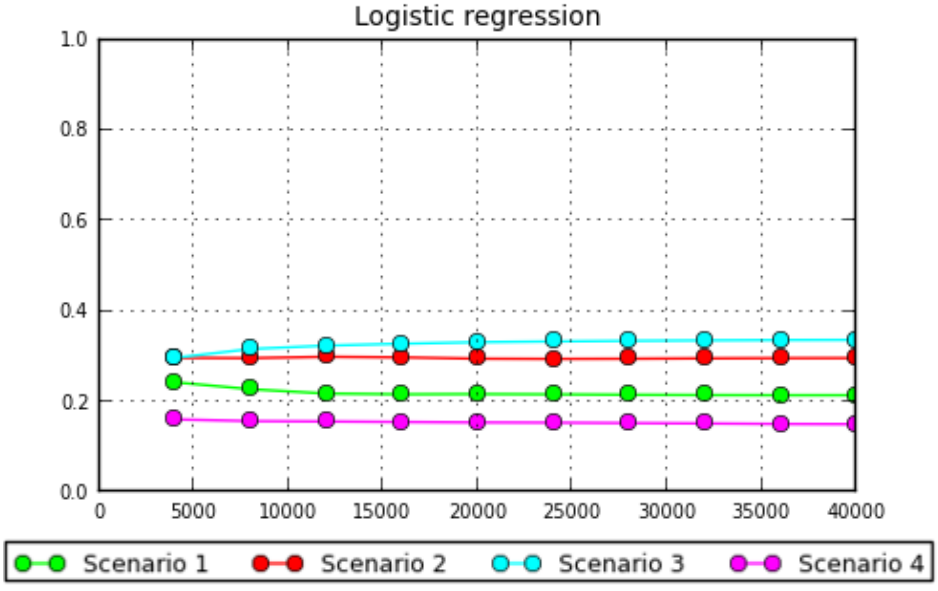


Figure 5. 5: Logistic regression analysis: the scenarios inferred using DIYABC

5.5. Discussion

Sequence variations of the mtDNA *d-loop* region of three Congolese indigenous goat populations from eastern and western DRC were analyzed to assess the genetic diversity, the genetic differentiation, and the demographic historical profiles. The results revealed 192 haplotypes from the analyses of 339 mtDNA *d-loop* sequences.

Average haplotype diversity and nucleotide diversity of 0.987 ± 0.002, and 0.015 ± 0.003 respectively were obtained for the three populations with an average number of nucleotides of 14.740. This average haplotype diversity is similar to that of Kenyan goats (0.981; Kibegwa *et al*., 2016) lower than that of Ethiopian (0.997 ± 0.001; Tarekegn *et al*., 2018), European (0.0994), and Iberian (0.996) but it is higher than that of South and Central American (0.963; Amilis *et al*., 2009), and Sicilian (ranged between 0.86 and 0.969; Sardina *et al.*, 2006) goats. These results assume that the maternal genetic diversity of Congolese indigenous goats is not at risk of loss neither through extinction, nor through genetic erosion (Naderi *et al*., 2007; Tarekegn *et al*., 2018). This assumes that they are potential resources to use in the designing of goat conservation and improvement programs in the Country. The highest level of haplotype diversity and segregating sites in small goat (South Kivu) compared with the two other populations (Kasai goat and dwarf goat) suggests its adaptation ability in wide-range environments of DRC.

Out of 192 haplotypes observed in this study, 7 haplotypes were common to all the three Congolese indigenous goat populations with one haplotype having the highest frequency (42 individuals). This result may be attributed to the evolutionary relationship among the studied goat populations. Relatively divergent haplotypes within breeds and geographical locations suggest that the gene flow has occurred on a regional scale during some time in the recent past and the breeds have not been subdivided by long-term biogeographic barriers (Luikart and Allendorf, 1996). In a study conducted by Vacca *et al.* (2010), it has been reported that goats have shown a

high genetic haplotype diversity, from where more haplotypes are each represented by a single sequence and only a few are shared among animals. Results in this study support that finding and the findings of Naderi *et al*. (2007) showing that it's common to find haplotypes represented by one individual or shared by only a few individuals. Based on the available goat mitochondrial haplogroup classification system (Naderi *et al*., 2007) and by incorporating reference haplotypes, only haplogroup A was found among the three studied Congolese indigenous goat populations. Therefore, Bwihangane *et al*. (2018) have found one individual from the Fizi goat population (0.9%) to be aligned to lineage B in the total individuals considered (110; 100%) in the study. Since Fizi is closed to Tanzania (eastern country) from where the haplogroup B was found in goat populations (data not published), the probability of having the goat that was aligned to the lineage B could be possible. Further investigation is required to confirm the presence of haplogroup B in Congolese indigenous goat populations in that area of South Kivu. The haplogroup A has been shown to have a large geographic distribution (Pereira *et al.,* 2005) and was more reported in large parts of African regions (Luikart *et al.,* 2001). That result could be interpreted as evidence that Congolese indigenous goats come from a unique maternal population with one maternal lineage which could have been introduced from one geographic domestication area (Naderi *et al*., 2008). On the one hand, the fact that 3 and 1 haplotypes were shared between Congolese and Mozambican, and Congolese and Zambian goats respectively, suggest that Congolese goats might have been descended from southern African goat populations. However, scenario 3 (**Figure 5.1**) in this study did not support that assumption (**Figure 5.5**). On the other hand, based on the number of haplotypes shared between Congolese, Cameroonian, Ethiopian, and Kenyan goats, we may deduce that these goats might have the same origin or the Congolese indigenous goats might have been descended either from western or eastern Africa. The posterior probability (< 0.3371) based on the Bayesian modeling implemented in DIYABC showed that the goat population dynamics towards DRC following the route from north Africa region (scenario 3; **Figure 5.1**) in

later times (time of most common ancestor (TMCA) ~1,062 YA; t_1 (**Table 5.6**) considering at least three years generation interval of goat (Al-Atiyat *et al.,* 2013). Based on the result assuming that Cameroonian goat populations descended from northern Africa 1,518 years ago assuming three years generation interval per generation (Tarekegn *et al*., 2019) and the number of haplotypes (3) (in this study) shared with Congolese indigenous goats, we may deduce that the introduction of goats in DRC as in Cameroon (central African countries) followed the same itinerary indicating that the initial gene flow or route of dispersion could be from Egypt following the Nile Delta to Sudan and Ethiopia then to DRC and to Cameroon. This period is closed to the period of the migration of the Bantu population which was believed to happen between 3,000 and 5,000 YP (Vansina 1995; Holden 2002) but later than the arrival time (5000 BC) of goat populations in north Africa from the domestication center (Hassan, 2006) that dispersions southwards are believed to have followed the Nile valley into Sudan and Ethiopia (Clutton-Brock, 2000) then Central Africa. The number of haplotypes (3) shared between Congolese indigenous goats and Cameroonian goats, the closed dates of dispersion of goats toward DRC (~1,062 YA) and toward Cameroon (1,518 YA) (Tarekegn *et al.,* 2019), and the low genetic differentiation (F_{ST}, 0.20800) observed between Congolese and Cameroonian goats (**Table 5.4**), showed that Congolese and Cameroonian (central Africa) goats are genetically closed and may have descended from the same origin in closed periods. The same scenario was mentioned by Flahaux and Schoumaker (2016) showing that the introduction of goat populations in DRC could be linked with the centuries-long movement of the Bantu from west Africa and people known as Nilotes from what is now Sudan into central Africa. Lafleur *et al.* (2018) have mentioned that from Ethiopia, the goats crossed Sudan and some west African countries to reach northeast of Ituri in the north-west of DRC. From that region, the goats spread in the land without tsetse fly, favourable for their development before throughout to take up residence in the whole country. Further investigation is required before firm conclusions are drawn.

The AMOVA showed that 5.88% represented the genetic variation among populations in Congolese indigenous goat populations compared to 94.12% within populations. The higher within-population variation than among populations could be associated with the breeding practices. In addition, it can suggest high levels of female-mediated gene flow (Moritz *et al*., 1987; Tserenbataa *et al*., 2004) and relates to high haplotype diversity implying widespread distribution and diversity to favor for selection within populations (Kibegwa *et al*., 2016). A weak phylogeography with small genetic differentiation (F_{ST}) was confirmed between the three studied Congolese indigenous goat populations (**Table 5.3**). This low genetic differentiation points to a high historical gene flow and intermixing between the three goat populations studied. Consistency is observed between the result in this study and previous reports confirming that weak genetic structure is most observed in small ruminants (sheep and goat) than in large ruminants (cattle) (Luikart *et al*., 2001). Thus, findings in this study suggest that the three Congolese indigenous goat populations share a relatively similar genetic background with the same maternal origin as revealed by the demographic dynamics inferred from the mismatch distribution pattern (**Figure 5.4**). Negative and significant (*P*-value < 0.05) Tajima's D (-2.189) and Fu's D (-20.418) values were obtained for all the three Congolese indigenous goat populations. These results suggest that the studied goat populations were under population expansion because of the predominant departure from neutrality. The significant departure (P-value < 0.05) observed for the Tajima's D and Fu's Fs for all populations explained mainly an excess of new mutations corresponding to the results of evolutionary forces due to either selective sweeps or population growth.

5.6. Conclusion

This study investigated the genetic diversity within and between the three Congolese indigenous goat populations from the east and west of the Democratic Republic of Congo. The analyses of the mtDNA control region (*d-loop*) revealed a high level of genetic diversity in the east and west Congolese indigenous goats with a weak genetic differentiation and a unique maternal origin

belonging to haplogroup A. The three indigenous goat populations share a relatively similar genetic background and have undergone expansion in history. Congolese indigenous goat populations might have separated from Cameroonian goats and may have been descended from North African goats 1,062 YA. These results represent an abundant resource for conservation and selective breeding in the different AEZs of the DRC.

CHAPTER SIX: GENOME-WIDE SCAN FOR SIGNATURES OF SELECTION IN INDIGENOUS GOAT POPULATIONS IN EAST AND WEST OF THE DEMOCRATIC REPUBLIC OF CONGO (DRC)

6.1. Abstract

Both natural and artificial selection are among the main driving forces shaping genetic variation across the genome of livestock species. Selection typically leaves signatures in the genome, which are often characterized by high genetic differentiation across breeds and/or a strong reduction in genetic diversity in regions associated with traits under intense selection pressure. In this study, signatures of selection were evaluated in three indigenous goat populations (Kinshasa, n= 120, South Kivu, n= 120 and Tshopo, n= 80) from east and west of DR Congo. After genotyping and quality control, 45,335 autosomal Single Nucleotide Polymorphism (SNPs) and 298 individuals remained for further analysis. Specific putative signatures of selection were identified across all populations, based on the cross-population expended haplotype homozygosity (XP-EHH) method between Congolese and non Congolese (Cameroon and Keffa in Ethiopia) goat populations. Ten out of the forthy three positive regions were enriched with genes involved in signaling pathways associated directly or indirectly with the body size, growth and muscles development (DEPTOR, MAGEL2), behavior and nervous system (DCDC2, PANK3, ITSN1, COL6A3, ENPP2), reproduction (CMK4), disease control such as the decrease in salmonella proliferation (EIF3J) and hair colour measurement, hair measurement and hair colour (PADI2). The enrichment results for the signatures of selection in this study have provided novel insights into the genetic and physiological architecture of goat's adaptation and reproduction in DRC. However, similar to the relatively poor annotation of the caprine genome, the information provided by GO analysis is limited. Since most of these candidate genes have been previously reported to be under positive selection for several traits in other species; Further research should be conducted on the

significant genomic regions reported in this study to clarify their implication and association with the reported and unreported traits in goat.

Keywords: Adaptation, Candidate genes, Genome wide-scan, Indigenous goats, Reproductive, DRC

6.2. Introduction

Goats (*Capra hircus*) are among the first domesticated species, about 10,500 years ago (Vigne *et al.*, 2011), and disperses to various parts of the world (Hughes *et al.*, 2012). They are the most important livestock species for poverty alleviation and rural development (FAO, 2015). In Africa, goats are an important economic resource. For instance, in the Democratic Republic of Congo (DRC), the goat population is estimated to be 4,111,789 heads contributing up to 72 % to the economy of the rural household that represents 75% of the total population (FAOSTAT, 2018).

Natural selection for the adaptation to a specific environment and resistance to disease had been reported to be more apparent in local breeds and had played important role in their development (Kim *et al*., 2016). Indigenous breeds tend to exhibit tolerance to heat stress, resistance to local diseases, resistance to water scarcity, and the ability to valorize local feeds sources (Onzima *et al*., 2018).

The Democratic Republic of Congo is characterized by three major agro-ecological zones (AEZs) including the high volcanic mountain in the east of the country (South Kivu Province), the stratified savannah zone in the west of the Country (Kinshasa province) and the Equatorial Forest in the central part (Tshopo region) from where indigenous goats breeds are adapted (FAOSTAT, 2018). Adaptation to local conditions is expected to leave distinct signatures in the genome known as "selective sweeps" owing to a rapid increase in the frequency of the desirable alleles or the frequency of neutral markers in linkage disequilibrium (LD) with the favorable alleles (Smith *et al*., 1974).

Both natural or artificial selection are among the main driving forces shaping genetic variation across genomes of livestock species (Onzima *et al*., 2018). In this study, adaptability in the region, disease resistance, and prolificacy were reported by farmers as preferred traits in the selection of goats in the DRC. However, the selection for these traits could have driven genetic variation

across the genome of the goats in the DRC. Accordingly, assessing the signatures of selection related to these traits and others in Congolese indigenous goat populations is important.

The emergence of single nucleotide polymorphism (SNPs) genotyping and whole-genome sequencing facilities coupled with the development of new genomic methodologies have enabled the screening of a large part of the genome to detect the signatures of selection in domestic animals and livestock (Mwacharo *et al*., 2017; Mei *et al*., 2018; Onzima *et al*., 2018; Alshawi *et al*., 2019; Ben-Jemaa *et al*., 2020). Analyses of signatures of selection has the goal of identifying genomic regions or loci showing deviations from neutrality (Onzima *et al*., 2018). Different methods have been established to detect signatures of selection including F-statistic; F_{ST} (Porto-Neto *et al*., 2013), haplotype differentiation statistic- hapFLK (Fariello *et al*., 2013), extended haplotype homozygosity (EHH)-derived statistics (*iHS*, *Rsb* , and *XP-EHH*) based on the decay of haplotype homozygosity as a function of recombination distance, and Bayesian method based on the differentiation of allele frequencies among populations (Weigand and Leese, 2018). Among these statistical methods, the F_{ST} approach is one of the most commonly used to detect signatures of selection for multiple populations. However, it assumes that all populations have a similar effective population size and derive independently from the same ancestral population (Walungembe *et al*., 2019). The hapFLK-statistic measures differences in haplotype frequencies between populations and accounts for the hierarchical structure of the populations (Fariello *et al*., 2013). The cross-population extended haplotype heterozygosity test (XP-EHH) was proposed by Sabeti *et al*. (2007) to identify signatures of selection between populations. It was designed to detect ongoing or nearly fixed signatures of selection by comparing haplotypes from two populations (Sabeti *et al*., 2007). Comparing XP-EHH and F_{ST}-statistics, the XP-EHH statistic based on haplotypes may be less biased than F_{ST} because haplotypes integrate information from patterns of SNPs (Conrad *et al*. 2006; Browning and Weir 2010).

In particular, in the context of this study, the extended haplotype homozygosity method (XP-EHH) was applied through genome-wide scan analysis to assess signatures of selection on candidate genes associated with traits of economic interest in Congolese indigenous goat populations. The extended haplotype homozygosity method (XP-EHH) is based on the decay of haplotype homozygosity as a function of recombination distance to identify genomic regions showing evidence of recent positive selection within and between populations (Zhao *et al*., 2016).

6.3. Materials and methods

6.3.1. Study area

This study was conducted in three AEZs of the DRC, including the alluvial basin or the humid zone (in the northeast and the central part), the savannah or the subhumid zone (in the central, the western, and the south-east parts), and the high-altitude volcanic mountains or the highland zone (in the eastern part) (FAO, 2015). These AEZs were respectively represented by Tshopo, Kinshasa, and South Kivu provinces (presented in **Figure 4.1** and **Table 4.1** in Chapter 4). South Kivu was selected based on the socio-economic importance of goats and its proximity to neighboring countries (Tanzania, Rwanda, and Burundi), which facilitates commercial transactions and animal exchanges. Tshopo was chosen based on its geographic location in the Equatorial Forest, and Kinshasa was chosen because of its proximity to other regions, including Bandundu, Kasai Central, and Congo Central. Due to the environmental variation in these regions, signatures of selection on the adaptability trait could be expected.

6.3.2. Animal resources and sampling

Goat-keeping households were purposively selected based on the following criteria: being a goat keeper for more than 5 years in the local area, own at least 5 mature does which have given birth at least one time, accessibility in the farm, morphological diversity within goats in the farm. To

ensure the representativeness of sampling, 1 to 2 unrelated animals (does) were selected from various farms across the designated AEZs. A total of 320 unrelated does in three local breeds; were selected in 202 farms.

Whole blood (5-10ml) was collected from the jugular vein on adult females into vacutainers tubes containing ethylene diamine tetraacetic acid (EDTA) as an anticoagulant and stored in an icebox at -20°C till DNA extraction. Care was taken to sample unrelated animals, based on information obtained from animals' owners or pedigree records.

6.3.3. DNA extraction and genotyping

The genomic DNA (gDNA) was extracted from the whole blood using a QIAamp® DNA Mini kit (Qiagen) according to the manufacturer's protocol. gDNA was quantified using a Nanodrop spectrophotometer (NanoDrop 2000, ThermoScientific USA) and gel electrophoresis. 30 µl at a concentration of 50ng/µl of the gDNA was genotyped for a total of 320 gDNA samples (120 for Kinshasa goats, 120 for South Kivu goats, and 80 for Tshopo goats) using the Illumina Goat SNP60 Bead Chip (Illumina, Inc. San Diego, CA 92122 USA) containing 53,347 SNPs developed by the International Goat Genome Consortium (IGGC).

6.3.4. SNP filtering and data merging

The SNP panel of the 53, 437 SNPs was converted into genotype calls with GENOME STUDIO software v2011.1. (Park *et al.,* 2015). Only mapped autosomal SNPs was screened and SNPs with minor alleles frequencies (MAFs) less than 5%, more than 5% missing genotypic data (individual genotype call rate lower than 95%), and those falling out of Hardy-Weinberg Equilibrium (HWE *p*-value < 0.001) (Kim *et al*., 2016) were excluded by Plink v1.9 (Chang *et al*., 2015) for analysis. To compare linkage disequilibrium (LD) decay among the three indigenous Congolese goat populations with different sample sizes, the pairwise r^2 values within each population were

calculated with the parameter –ld-window-r^2 set to 0.2 to reduce the size of output from the comparison in PLINK. The combined LD decay of all breeds was calculated using a pruned dataset of 298 goats which excluded SNPs in LD across breeds (PLINK "–indep-pairwise 50 5 0.5"). After the quality check (QC), a total of 259 individuals with 45,335 autosomal SNPs were retrieved for analysis. A relatedness test was performed between individuals within each population using PLINK v1.9 to calculate the extended haplotypes shared between distantly individuals. Genotypic data for Congolese indigenous goat populations were combined with genotypic data for goat populations already published from eastern African (Keffa from Ethiopia, n= 51) and central Africa (Cameroon, n= 102) (Tarekegn *et al*., 2019) for the comparisons. Keffa breed was considered for its reproductive performance for which it is raised by farmers (Farm Africa, 1996). The availability of genotypic data for the Cameroonian Highland goats on the public data management site and the fact that Cameroon is located in the same geographic area (central Africa) as the DRC were the major reasons for considering it in this study pour comparaison.

6.3.5. Genomic diversity and population structure

Genetic diversity indices which included observed (Ho) and expected (H_E) heterozygosity, F statistics (F_{ST}); $F_{ST}= (H_E - H_O)/H_E$ (Nei, 1977), and minor allele frequency (MAF) were calculated within genotypes using Plink v1.9 (Purcell *et al*., 2007). The population structure analysis was done using R software with R packages (*Adegenet* and *pegas*) after converting the pruned raw dataset for reading into R. The Discriminant Analysis of Principal Components (DAPC) multivariate statistical approach (Jombart *et al*., 2010) was used to infer the number of clusters of genetically related individuals. With this approach, variance in the sample is partitioned into between-group and within-group components with the effort of maximizing discrimination between groups. K= 5 was returned for the population clustering. With this approach, data were

first transformed using principal components analysis (PCA) and subsequently, clusters were identified using discriminant analysis (DA). The genetics parameters (H_O, H_E, F_{ST}, MAF, and PHet) were calculated only for the three Congolese indigenous goat populations.

6.3.6. Identification of selection sweeps

To detect the signatures of selection in the three studied Congolese indigenous goat populations which would have resulted from their production or reproductive performances as well as adaptation to the different environment of production or any other important trait, the XP-EHH method was employed for different breed combinations. The combinations were made for the genotypic data between the three Congolese indigenous goats and between the Cameroonian and Ethiopian goat breeds. A total of nine combinations were possible between the population: Kinshasa (Kasai goat) vs South Kivu (small goat), Kinshasa (Kasai goat) vs Tshopo (dwarf goat), South Kivu (small goat) vs Tshopo (dwarf goat), Kinshasa (Kasai goat) vs Keffa, South Kivu (small goat) vs Cameroonian goats, South Kivu (small goat) vs Keffa goats), Tshopo (dwarf goat) vs Keffa goats), Tshopo (dwarf goat) vs Keffa goats). First, the haplotypes extended patterns were phased using Beagle v5.1 for all the considered goat populations (Browning *et al*., 2018). X-EHH scores were then calculated for each comparison using "rehh" R-package (Gautier and Vitalis, 2012). P-values were derived by transforming the XP-EHH scores into -log10[2ϕ-(XPEHH)] in which 2ϕ-(XPEHH) is the cumulative Gaussian distribution function. SNP was considered as significant and as putatively under selection when its -log10 (*p*-value XP-EHH) was superior to the threshold (-log10 (*p*-value XP-EHH) ≥ 3). 0.01 to 0.02% (45 to 90 SNPs) of the total number of SNPs (45,335) of the genomic distribution of standardized XP-EHH scores in pairwise goat populations were considered as SNPs with positive regions identified using a 100 kb scanning window with 50 kb overlap (Kumar *et al*., 2018).

The overlap of the candidate genomic regions detected with https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF_001704415.1 was checked using the *Capra hircus* Genome Data Viewer Assembly ARS1 (GCF_001704415.1). Genes found within the intervals spanning the candidate genome were considered candidate genes.

6.3.7. Annotation of highly significant genomic regions and functional enrichment analysis of candidate genes

Genes were annotated to provide more logical information about their structural features and functional roles (Salzberg, 2019). The annotation was done based on the goat genome information database. For each of the studied goat populations, gene enrichment analyses were performed based on the XP-EHH results with the web-based tool, Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.8 (Huang *et al*., 2009; Jiao *et al*., 2012). DAVID software allowed the investigation of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Kanehisa *et al*., 2012) and of the Gene Ontology (GO) that can be considered for biological function, functional processes, and molecular function (Ashburner *et al*., 2000). GO was considered through the EVIDENCE either Inferred from Biological aspect of Ancestor, from Sequence or Structural Similarity (IBA, ISS) or Direct Assay (IDR) or Electronic Annotation (IEA). Fisher's exact test (*p-value* = 0.05), was applied to identify significantly enriched GO biological function (considered as default for the analysis).

6.4. Results

6.4.1. Genetic diversity indices

In this study, five (H_O, H_E, F_{ST}, MAF, P Het) were calculated to estimate genetic diversity between individuals (**Table 6.1**). H_O varied between 0.27 (Kinshasa) and 0.354 (South Kivu), whereas H_E varied between 0.297 (Kinshasa) and 0.362 (South Kivu). The average F statistics (F_{ST}) measured for each goat population varied between 0.021 (South Kivu) and 0.138 (Tshopo). The minor alleles frequencies varied between 0.211 (Kinshasa) and 0.276 (South Kivu). These results suggest high genetic diversity within the populations. The goat populations from South Kivu were more genetically diverse as compared to goats from Kinshasa and Tshopo. Similar result were obtained with the mtDNA *d-loop* analysis.

Table 6. 1: Genetic diversity indices

Breed	n(before QC)	n(after QC)	H_O	H_E	F_{ST}	MAF	P_{Het}
Kinshasa (Kasai goat)	120	101	0.270	0.297	0.0889	0.211	0.462
Tshopo (Dwarf goat)	80	55	0.307	0.356	0.138	0.269	0.421
South Kivu (Small goat)	120	103	0.354	0.362	0.021	0.276	0.478

6.4.2. Population structure

The first 30 principal components (PCs) of PCA and 4 discriminant functions (DA) were considered for the clustering of the goat populations. Each individual was clustered into a cluster with the proportion of conserved variance = 0.479, and average assignment proportion = 0.996 (high and closed to the assignment values). Five clusters (K = 5) (**Figure 6.1** and **Figure 6.2**) were obtained. Cluster 2 regroups goats from South Kivu, Cluster 3 regroups goats from Kinshasa and Cluster 4 regroups goats from Tshopo. Clusters 1 and 5 regrouped intermixing individuals,

with cluster 5 group intermixing goats from South Kivu and Tshopo, while cluster 1 group intermixes goats from Kinshasa and Tshopo.

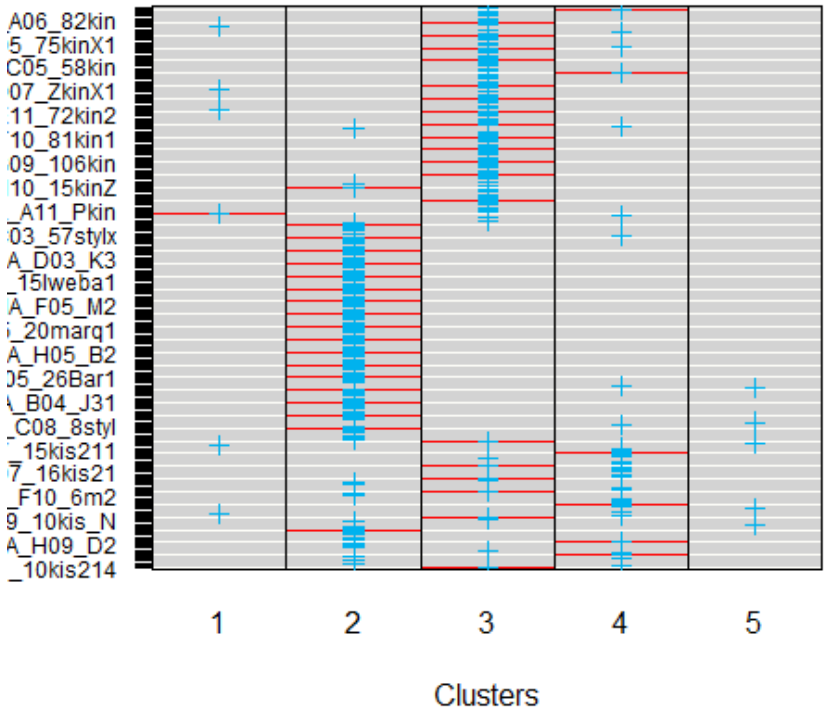


Figure 6. 1: Proportion of assignment of individuals in their clusters visualized using assign plot. Heat colors represent membership probabilities (red=1, white=0); blue crosses represent the prior cluster provided to DAPC. Here DAPC classification is consistent w

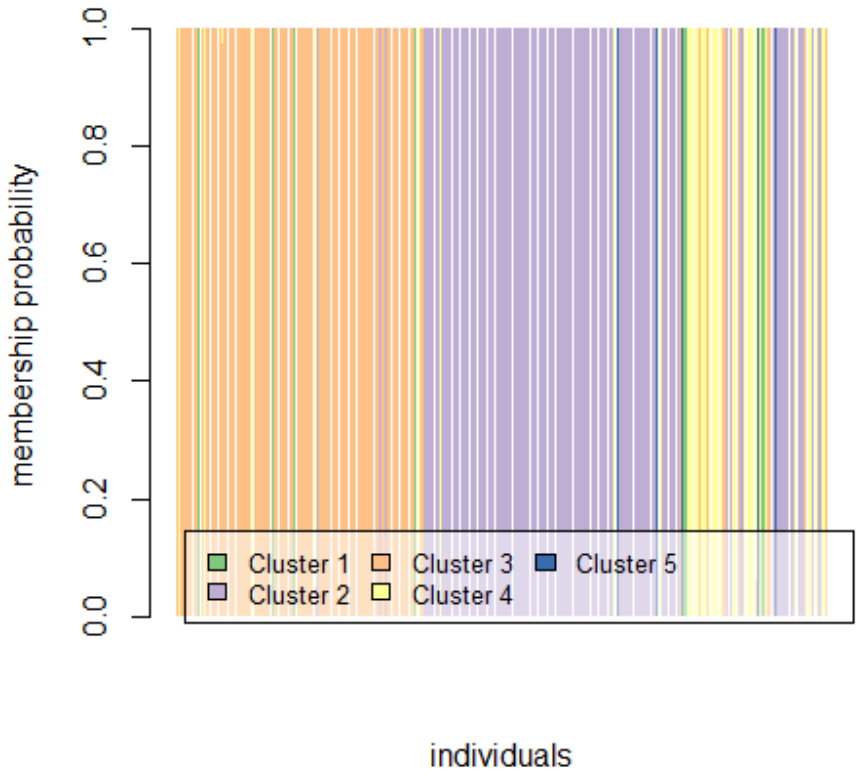


Figure 6. 2: Proportion of assignment of individuals in their clusters plotted in the more common STRUCTURE-like way using compo plot.

6.4.3. Candidate regions under selection based on EHH scores in pairwise goat populations

Candidate genes found in goat populations based on the cross-population haplotype heterozygosity (XP-EHH) method for the 9 combinations are summarized in **Table 6.2** to **Table 6.10.** For all 9 combinations of goat populations, candidate genes were assessed only for significant regions (0.01 to 0.02% of the significant signatures) with a *p*-value of XP-EHH superior to the fixed threshold (*p*XP-EHH> 3) (**Figure 6.3**). Some of these significant signatures were located in the non-coding (introns) region and could not be linked to any gene.

Table 6. 2: XP-EHH between Kinshasa and South Kivu

Chromosome	Location	pXP-EHH	Candidate genes
1	1240437-1273105		PAXBP1
2	928661-952389	4.58	RCC2, PADI2, FBXO42, MRTO4
3	3027235-3088323	3.32	TRAF3IP1
7	3190725-3708301	3.35	FBXL17, FER, CAMK4
8	366581-454508	3.48	ANXA10
10	11985796-13801481	5.21	NRXN3
15	3995909-4038473	3.62	LOC108637647
18	2442206-2464476	3.47	LOC108637948
21	1091977-113163	3.5	SNURF
25	587113-59546	3.33	MSLN

Table 6. 3: XP-EHH between Kinshasa and Tshopo

Chromosome	Location	pXP-EHH	Candidate genes
1	89963-333854	4.31	ITSN1
2	2296748-2347457	4.90	IFFO2, FBXO42
3	3863249-3955497	4.67	COL6A3
8	3848075-5433477	3.45	GALNTL6
10	11985796-13801481	4.93	NRXN3, TERB2, COQ6
14	1150723-1242654	3.61	ENPP2, DEPTOR, MTBP
15	4126164-4144127	3.44	LOC102176906, LOC108637647
18	231700-369311	3.86	DCDC2
19	310988-1145579	4.39	CA10
23	792203-1217301	5,54	GMDS

Table 6. 4: XP-EHH between South Kivu and Tshopo

Chromosome	Location	pXP-EHH	Candidate genes
1	1240437-1273105	3.15	PAXBP1
2	2296748-2347457	3.86	IFFO2
3	3027235-3088323	4.12	TRAF3IP1, SNED, ANKMY1
7	3190725-3708301	3.43	FBXL17,FER, LOC106502376
8	366581-454508	3.53	ANXA10,
9	374763-513326	4.78	CD109
21	1091977-1113163	3.84	SNURF
25	168260-170769	3.65	LOC102186172

Table 6. 5: XP-EHH between South Kivu and Keffa

Chromosome	Location	pXP-EHH	Candidate genes
1	624015 - 653064	4.21	IFNAR1,
4	621406 - 1167657	3.75	PTPRN2
7	624015- 653064	4.10	WDR36
10	11985796- 13801481	3.54	NRXN3
11	10559382- 10593045	3.75	DGUOK, TET3
15	8858- 69851	4.9	NCAPD3
19	310988- 1145579	3.52	CA10
20	506254- 1225801	4.8	SLIT3
28	464,508- 5328400	3.81	GRID1

Table 6. 6: XP-EHH between Kinshasa and Keffa

Chromosome	Location	pXP-EHH	Candidate genes
2	4123297- 4477588	4.33	EIF4G3
6	345389- 363921	4.52	LOC106503885
8	3848075- 5433477	3.84	GALNTL6
10	11985796- 13801481	6.48	NRXN3
21	418902- 423047	3.71	MAGEL2

Table 6. 7: XP-EHH between Tshopo and Keffa

Chromosome	Location	pXP-EHH	Candidate genes
2	287847- 413831	3.24	FBXO42
3	1136915- 1190488	3.18	ANKMY1
6	345389- 363921	6.98	LOC106503885
7	80527- 352060	3.65	CAMK4
8	3848075- 5433477	3.72	GALNTL6
10	11985796-13801481	5.09	NRXN3
14	489696- 717115	4.46	COL14A1, IMPA1, SLC10A5
15	1289317- 1332677	3.07	LOC102176061
18	231700- 369311	3.33	DCDC2
21	1091977- 1113163	4.35	SNURF,

Table 6. 8: XP-EHH between South Kivu and Cameroon

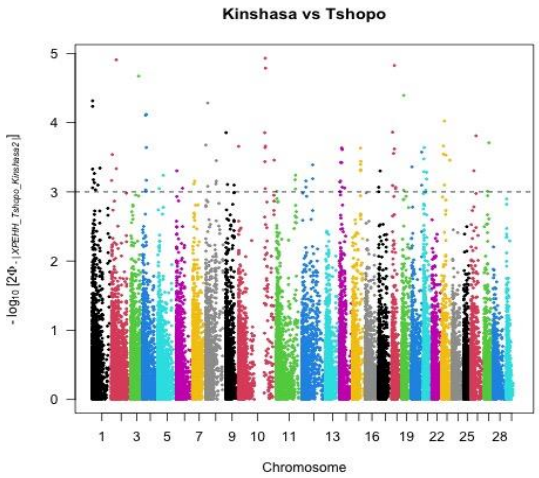
Chromosome	Location	pXP-EHH	Candidate genes
1	624015- 653064	3.54	IFNAR1
2	869828- 902772	3.49	PADI4, FBXO42, MRTO4
3	383387- 399631	3.15	ATG4B
4	621406- 1167657	3.95	PTPRN2
7	913717- 942491	3.41	SLC25A46
8	366581- 454508	3.27	ANXA10
9	374763 -513326	3.10	CD109
11	10053,35- 10081659	3.59	LOC102174666, MAL, DCTN1, CCDC142
17	1320882- 1350824	3.20	RNF185
18	40950- 84986	3.54	KIAA0319
20	506254- 1225801	3.35	SLIT3
24	1349721- 1371239	3.10	LOC108633761

Table 6. 9: XP-EHH between Kinshasa and Cameroon

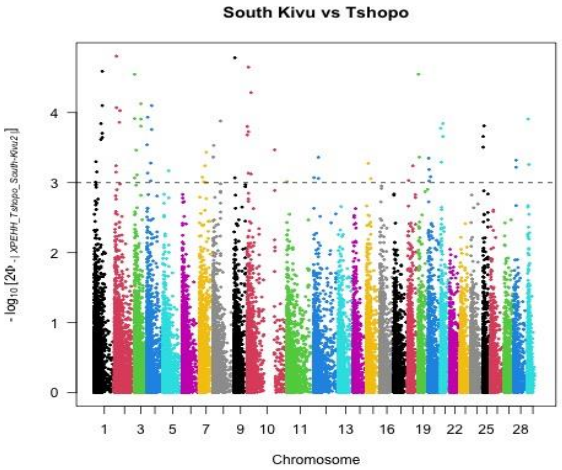
Chromosome	Location	pXP-EHH	Candidate genes
1	473733- 514362	3.96	TMEM50B, PAXBP1
2	629870- 684785	3.24	PADI2
3	352015- 366978	3.89	ING5, FARP2
4	621406- 1167657	3.24	PTPRN2
7	1688786-1883410	3.61	MAN2A1, CAMK4, SLC25A46
9	246951 - 333044	3.27	SLC17A5
10	11985796 - 13801481	8.67	NRXN3, EIF3J
11	191293 - 241641	3.81	TMEM87B, M1AP, LOXL3
12	1985596 - 2514686	4.13	MYO16
13	1494614- 1546842	3.14	LOC102188417
19	310988- 1145579	3.66	CA10
20	356690 - 381538	3.35	PANK3
21	418902- 423047	7.00	MAGEL2

Table 6. 10: XP-EHH signals in the genetic region between Tshopo and Cameroon

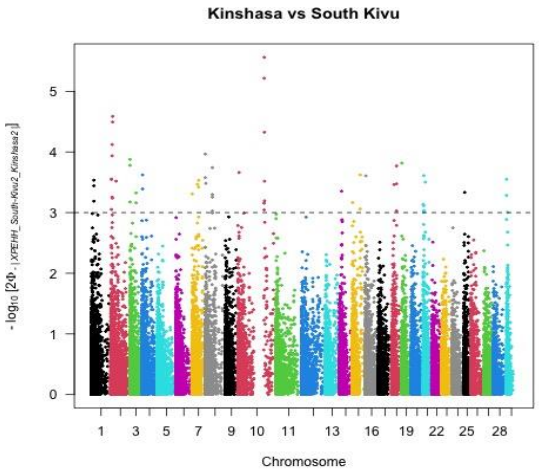
Chromosome	Location	pXP-EHH	Candidate genes
1	473733- 514362	4.47	TMEM50B
2	563281- 570546	4.25	LOC102183471, PAX7, IFFO2, PADI2
3	488714- 588400	3.05	FARP2
5	1717142 - 2170631	3.65	TRHDE
7	80527- 352060	4.60	CAMK4, MAN2A1
8	571937- 953942	3.63	PALLD, SH3RF1
9	374763- 513326	3.14	CD109
10	11985796- 13801481	7.01	NRXN3, EIF3J
11	718502- 891116	3.19	LOC102171344
13	2248987-2364119	3.68	PAK5
19	1717142 - 217063	3.56	CA10
20	506254 - 1225801	5.37	SLIT3, PANK3
21	418902 - 423047	7.40	MAGEL2
25	344179- 402418	3.59	RAB11FIP3
26	639260- 733296	3.16	CFAP46


Kinshasa vs Tshopo
Chromosome

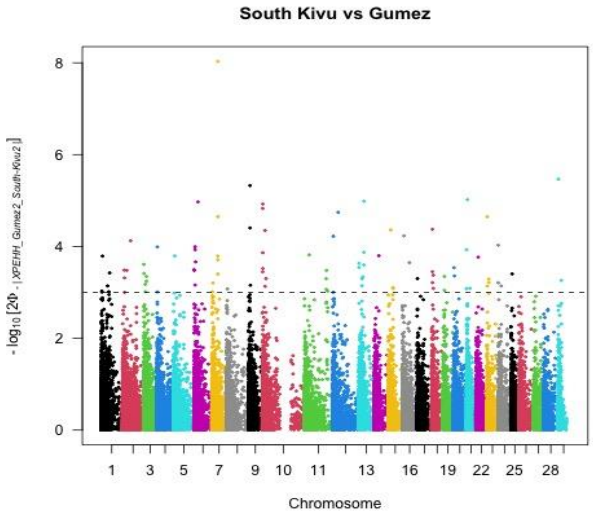
A


South Kivu vs Tshopo
Chromosome

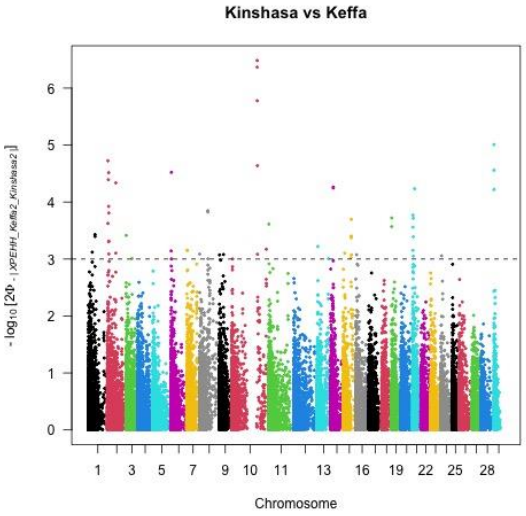
B


Kinshasa vs South Kivu
Chromosome

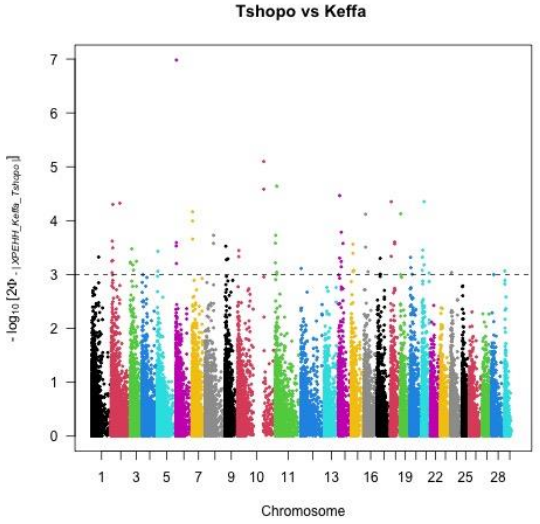
C


South Kivu vs Gumez
Chromosome

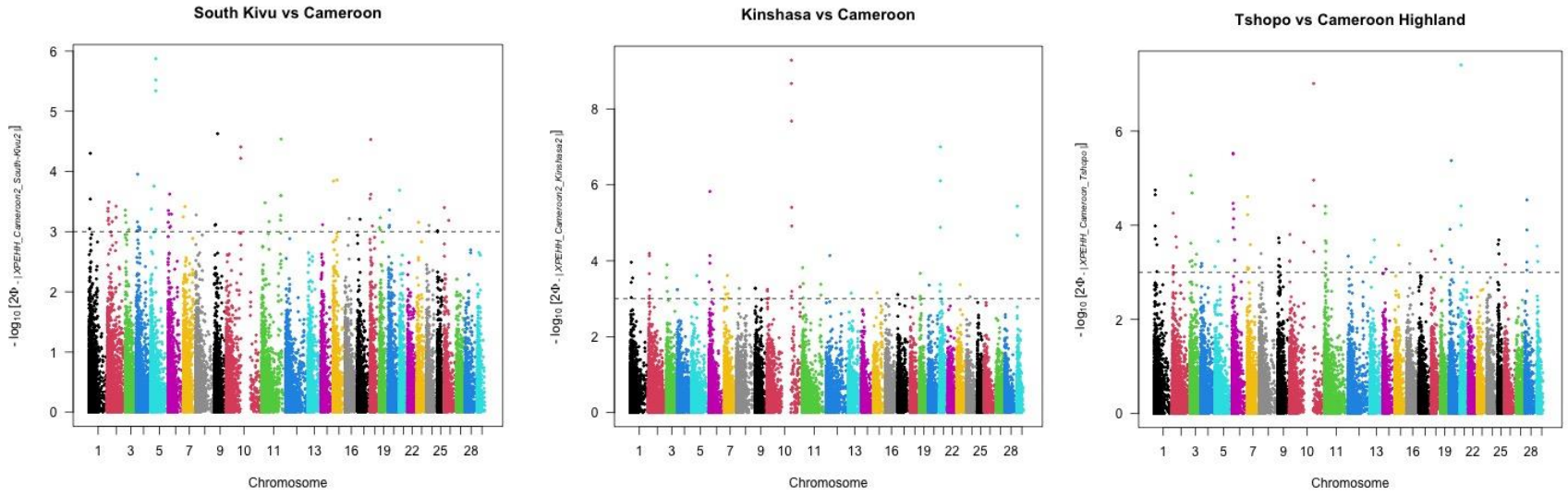
D


Kinshasa vs Keffa
Chromosome

E


Tshopo vs Keffa
Chromosome

F



G H I

Figure 6. 3: Genomic distribution of standardized cross-population extended haplotype homozygosity (XP-EHH) scores in pairwise goat populations: (A): Kinshasa vs Tshopo, (B): South Kivu vs Tshopo, (C): Kinshasa vs South Kivu, (D): South Kivu vs Keffa, (E): Kinshasa

6.4.4. Candidate genes found in the three Congolese indigenous goat populations

Results in **Table 6.11** present the candidate genes found in the three Congolese indigenous goat populations. The candidate genes presented in **Table 6.11** summarise all the identified candidate genes in the three Congolese indigenous goat populations based on the XP-EHH test applied for the comparison between them and between Cameroonian and Ethiopian goats. For each of the three Congolese indigenous goat populations, the highest number of candidate genes found was 6. These were located on chromosome 6 and chromosome 11 (in goats from South Kivu), on chromosome 2 (in goats from Kinshasa), and on chromosome 14 (in goats from Tshopo). 43 following candidate genes were significant and were common in at least two of the three studied goat populations: ITSN1, PAXBP1, TMEM50B (chromosome 1), RCC2, PADI2, FBXO42, MRTO4, IFFO2 (chromosome 2), FARP2, TRAF3IP1, SNED, ANKMY1, COL6A3 (chromosome 3), PTPRN2, (chromosome 4), LOC106503885 (chromosome 6), FBXL17, FER, CAMK4, MAN2A1, SLC25A46, LOC106502376 (chromosome 7), ANXA10, GALNTL6 (chromosome 8), CD109 (chromosome 9), NRXN3, TERB2, COQ6, EIF3J (chromosome 10), ENPP2, DEPTOR, MTBP (chromosome 14), LOC102176906, LOC108637647 (chromosome 15), LOC108637948, DCDC2 (chromosome 18), CA10 (chromosome 19), SLIT3, PANK3 (chromosome 20), MAGEL2, SNURF (chromosome 21), GMDS (chromosome 23), LOC102186172 and MSLN (chromosome 25).

Table 6. 11: Candidate genes found in goat populations from South Kivu, Kinshasa, and Tshopo (DRC)

Region	Chromosome	Candidate genes
South Kivu	1	PAXBP1, IFNAR1,
	2	RCC2, PADI2, FBXO42, MRTO4,
	3	TRAF3IP1, SNED, ANKMY1, ATG4B
	4	PTPRN2
	7	FBXL17, FER, CAMKA, SLC25A46, WDR36, LOC106502376
	8	ANXA10
	9	CD109
	10	NRXN3
	11	DGUOK, TET3, LOC102174666, MAL, DCTN1, CCDC142
	15	LOC108637647, NCAPD3
	17	RNF185
	18	LOC108637948, KIAA0319
	19	CA10
	20	SLIT3
	21	SNURF
	24	LOC108633761
	25	LOC102186172, MSLN
	28	GRID1
Kinshasa	1	PAXBP1, ITSN1, TMEM50B
	2	RCC2, PADI2, FBXO42, MRTO4, IFFO2, EIF4G3
	3	TRAF3IP1, COL6A3, ING5, FARP2
	4	PTPRN2
	6	LOC106503885
	7	FBXL17, FER, CAMK4, MAN2A1, SLC25A46
	8	ANXA10, GALNTL6
	9	SLC17A5
	10	NRXN3, TERB2, COQ6, EIF3J
	11	TMEM87B, M1AP, LOXL3
	12	MYO16
	13	LOC102188417
	14	ENPP2, DEPTOR, MTBP
	15	LOC102176906, LOC108637647
	18	LOC108637948, DCDC2
	19	CA10
	20	PANK3
	21	SNURF, MAGEL2
	23	GMDS
	25	MSLN
Tshopo	1	ITSN1, PAXBP1, TMEM50B
	2	LOC102183471, PAX7, IFFO2, PADI2, FBXO42
	3	FARP2, TRAF3IP1, SNED, ANKMY1, COL6A3
	5	TRHDE
	6	LOC106503885
	7	FBXL17, FER, LOC106502376, CAMK4, MAN2A1
	8	PALLD, SH3RF1, GALNTL6, ANXA10
	9	CD109
	10	NRXN3, TERB2, COQ6, EIF3J
	11	LOC102171344
	13	PAK5
	14	ENPP2, DEPTOR, MTBP, COL14A1, IMPA1, SLC10A5
	15	LOC102176906, LOC108637647, LOC102176061
	18	DCDC2
	19	CA10
	20	SLIT3, PANK3
	21	MAGEL2, SNURF
	23	GMDS
	25	RAB11FIP3, LOC102186172
	26	CFAP46

6.4.5. Functional annotation for candidate genes

Gene enrichment analyses revealed several quantified GO terms for the considered candidate genes. Several positive regions under selection that spanned 43 genes shared between the three studied indigenous goat populations (**Table 6.11**) were identified. Most of these genes were involved in multiple signaling and signal transduction pathways in a wide variety of biological function, cellular and biochemical processes. The most significant GO terms were found in protein signaling, nucleotide biding, signaling receptor binding, calcium ion binding, serine-type endopeptidase inhibitor activity, translation initiation factor activity, guanyl-nucleotide exchange factor activity, and protein-arginine deiminase activity. 13 out of the 43 candidate genes were enriched to be in association with phenotypes of economic interest, including growth, reproductive system, endocrine/exocrine gland, resistance to *Salmonella* contamination, hair color measurement, hair shape measurement, and color (**Table 6.12**).

A total of 53 KEGG pathways were recorded for the 13 candidate genes. 8 of them were found to be significantly enriched at the threshold ($p < 0.05$). The most enriched pathways included "signaling pathway (RAP1)", "metabolic pathways (PANK3)", "calcium signaling pathway (CAMK4)", "RNA transport (EIF3J)", "oxytocin signaling pathway (CAMK4)", "Neurotrophin signaling pathway (CAMK4)", "Esther lipid metabolism (ENPP2)", "protein digestion and absorption (COL6A3)".

Table 6. 12: Phenotypic traits, GO Ids, Quantified GO team for important candidate genes

Gene	Name of gene	Phenotypic Traits	GO IDs	Qualified GO term
FER	Tyrosine-protein kinase Fer	growth /size/body region	GO:0005102	signaling receptor binding
DEPTOR	DEP domain-containing mTOR-interacting protein	growth/size/body region, reproductive system	GO:0005515	protein binding
MAGEL2	Melanoma antigen	growth/size/body region, endocrine/exocrine gland, reproductive system	GO:0005515	Protein binding
ANKMY1	Ankyrin repeat and MYND domain-containing protein1	Decreased epidermal growth factor (EGF), endocytosis	GO:0005515	Protein binding
ANXA10	Annexin A10	homeostasis/metabolism, productive system	GO:0005509	calcium ion binding
CAMK4	Calcium/calmodulin-dependent protein kinase IV	nervous system, immune system, behaviour/neurological, homeostasis/metabolism, growth/size/body region, endocrine/exocrine gland, reproductive system	GO:0000166	Nucleotide-binding
COL6A3	Collagen type VI alpha 3	behaviour/neurological, muscle, growth/size/body region , vision/eye	GO:0004867	Serine-type endopeptidase inhibitor activity
DCDC2	Doublecortin domain containing 2	the nervous system, behavior/neurological	GO:0005515	protein binding
ENPP2	Ectonucleotide pyrophosphatase/ phosphodiesterase 2	nervous system , immune system ,growth/size/body region, pulse pressure measurement, body height	GO:0003676	Nucleotide acid binding
EIF3J	Eukaryotic Translation Initiation Factor 3 Subunit J	Decreased Salmonella proliferation	GO:0003743	contributes to translation initiation factor activity
ITSN1	Intersectin 1	nervous system, behaviour/neurological , growth/size/body region, endocrine/exocrine gland	GO:0005085	guanyl-nucleotide exchange factor activity
PANK3	Pantothenate kinase 3	behavior/neurological, homeostasis/metabolism	GO:0000166	Nucleotide binding
PADI2	Peptidyl Arginine Deiminase 2	Hair color measurement, hair shape measurement, and air color	GO:0004668	Protein-arginine deiminase activity

6.5. Discussion

In this study, the population expended haplotype homozygosity, XP-EHH was calculated to detect genome-wide signatures of selection within and between three goat populations including Kasai goat, small goat, and dwarf goat from East and West of the Democratic Republic of Congo. XP-EHH with REHH statistic approaches are both extensions of Sabeti *et al*.'s statistical approaches (Sabeti *et al*., 2007). REHH has been successfully applied in many species including bovine (Fan *et al*., 2014), chicken (Zhang *et al*., 2012), human (Sabeti *et al.,* 2002). By comparing haplotypes from two populations , it has been revealed that XP-EHH is more powerful for detecting signatures of selection at or near fixation (Sabeti et al., 2007). This study is the first in the DRC to apply XP-EHH statistic approach for detecting signatures of selection in indigenous goat populations. Since their introduction in the DRC, goats have adapted to the country's three AEZs, which include the savannah plateau, the Equatorial Forest, and the high altitude volcanic mountains.Since these livestock populations migrated across the country and adapted to the different environments, they might have been exposed to artificial selection.

A group of genes related to growth, body size, productive system, behavior, nervous system, hair colour, hair shape measurement, decrease in salmonella proliferation, muscle development traits were detected. Four candidate genes including FER and DEPTOR on chromosome 4, MAGEL2 on chromosome 21, and CAMK4 on chromosome 7 linked to the functionality of growth, body size, and reproductive system. Five candidate genes COL6A3 (chromosome 3), DCDC2 (chromosome 18), ENPP2 (chromosome 14), ITSN1 (chromosome 1), and PANK3 (chromosome 20) likely seemed to affect the nervous system and the behavior. One candidate gene; PADI2 (chromosome 2) was associated with colour measurement, hair shape measurement, and hair colour. Some of these genes have been reported in different species including goat breeds from different environments to be linked with the same and different phenotype functionality as observed in this study. Jin *et al*., (2020) reported the association of PADI2 gene with hair follicle

development in cashmere goats in China producing the highest cashmere yield and best fiber quality in the world.

Biological functions of DEPTOR, MAGEL2, COL6A3 were reported in body size and growth in mice, and humans by regulating adipogenesis which is associated with obesity in humans (Bischof *et al*., 2007; McCulloch *et al*., 2015; Poliak and Rajan, 2018). Mercer and Wevrick (2009) have emphasized that gene-targeted mutation of MAGEL2 in mice disrupts circadian rhythm and metabolism causing reduced total activity, reduced weight gain before weaning, and increased adiposity after weaning. Additionally, it has been mentioned that the loss of MAGEL2 in mice reduces fertility in both males and females through extended breeding intervals and early reproductive decline and termination (Mercer and Wevrick, 2009). Female MAGEL2-null mice displayed extended and irregular estrous cycles, while males showed decreased testosterone levels and reduced olfactory preference for female odors. On the other hand, MAGEL2 was reported to influence the testicular size, fertility, and growth in Nellore cattle (Utsunomiya *et al*., 2014), and was involved in circadian rhythms and adaptation to new environments in mouse knockout studies (Fountain *et al*., 2017). Collagen type VI alpha 3 chain gene (COL6A3), which is one of the three genes encoding components of collagen VI, was revealed to play a major role in the maintenance of the strength of muscle and connective tissue (Pan *et al*.., 2013), in meat marbling (Leal-Gutiérrez *et al*., 2019) and was expressed in developing cartilage in the horse (Henson *et al*., 1996). DEPTOR, MAGEL2, and COL6A3 were revealed in Kasai and dwarf goat breeds from Kinshasa and Tshopo respectively. The two regions are among the regions in DRC having a high temperature (28 to 32 ° C) and are geographically located in the stratified savannah plateau and the Equatorial Forest, respectively. However, the possibility of the economic transaction between the two regions and their environmental characteristics could explain the presence of these candidate genes leading to the development of muscles and weight gain that could be important for meat production and adaptation in the environment. Found on chromosome 18 in Kasai and dwarf goats, the Doublecortin domain containing 2 (DCDC2) was annotated to be associated with

behavior and the nervous system. Belonging to the superfamily of doublecortin domain-containing proteins that bind microtubules (Paracchini *et al.*, 2006); DCDC2 was shown to involve in neuronal migration with a potential role in the structure and function of primary cilia in rats and humans (Massinen *et al*., 2011). Sharma *et al*. (2008) reported the association between many human genetic diseases and malfunctions of the primary cilium. However, basing on annotations of the *Sus scrofa* 10.2 genome assembly, DCDC2 was reported with many others candidate genes to have biochemical and physiological roles that were relevant to feeding behavior and feed efficiency traits (Massinen *et al*., 2011). Little information is provided on the probable association of the DCDC2 gene with the feeding behavior and feed efficiency in goats This requires more investigations. ENPP2 gene known as a bifunctional enzyme-linked in tumor and normal cell motility modulation which is expressed in cartilaginous condensations (Williams *et al*., 2005) was reported in both dwarf and Kasai goats.

Zhao *et al*. (2021) have reported that the CAMK4 gene was among genes that deserved intention and further study since it has been reported to be closely related to reproductive traits. Besides the reproductive system, CAMK4 was annotated with the function related to the nervous system, immune system, behavior/neurological, homeostasis/metabolism, growth/size/body region, endocrine/exocrine gland. Akimoto *et al*., (2004) and Zahor (2013) demonstrated that CAMK4 could have an important effect in the adaptation of mammalian skeletal muscles in response to alteration in functional demands by stimulating peroxisome proliferator-activated receptor y-coactivator 1alpha (PGC-1alpha) gene expression, promoting fast-to-slow fiber type switching and augmenting mitochondrial biogenesis in skeletal muscle. PADI2 was discovered to have many potential biological functions; angiogenesis-regulating (Khajavi *et al*., 2017), fatty acid composition regulating in muscle, cell regulation (Horibata *et al*., 2017), potential biomarkers, and therapeutic targets in many diseases, including cancer to humans (Cantariño *et al*., 2016). Additionally, from a study conducted on Epithelial Ovarian Cancer's patients, the expression of PADI2 was found to be in correlation with other co-expressed genes including Bone

Morphogenetic Protein Receptor, type 1B (BMPR1B), which is a member of the Bone Morphogenetic Protein (BMP) involved in endochondral bone formation and embryogenesis and produced in ovaries (Inman *et al*., 2002; Albalbeisi, 2019). In this study, PADI2 was enriched and annotated with biological functions related to hair color measurement, hair shape measurement, and hair color traits. Similar results were obtained from genome-wide association studies identifying candidate genes for coat color and Mohair trait in the Iranian Markhoz goat (Nazari-Ghadikolaei *et al*., 2018).

Despite the significance (*p*-values > to the threshold value = 3) of some SNPs in some genomic regions found in this study, the evidence for their selection was poorly annotated. This observation was consistent with genome-wide analyses of selection signatures in humans (Voight *et al.*, 2006), thoroughbred horses (Gu *et al*., 2009), cattle (Quabari *et al*., 2011), and sheep (Zhao *et al.*, 2016), where some candidate genes were not annotated. Accordingly, noncoding DNA may be suggested to have an important role in adaptative evolution. However, the results of this study may also be justified due to the relatively poor annotation of the goat genome (Onzima *et al*., 2018). These results can also be justified by the fact that no local African goat breeds were considered in the development of the chip for goat genotyping.

Because of the potential importance of the positive signatures of selection of the genomic regions reported in this study, prior should be given to improving their annotation. Genome-wide association studies (GWAS) is an approach to detect genomic variation underlying phenotypic variation (Zhao *et al*., 2016) and was performed using the Linkage disequilibrium-based association mapping from phenotype to genome. Whereas, selection signatures detection involves a population genomic approach to identify likely targets of past selection from genome to phenotype.

In the current study, some well-known, previously reported candidate genes were not identified. This may be due to differences in the statistical methodologies, goat breeds different from these used in the development of the chip for goat genotyping, and effective population size limitations

used. Small effective population size can especially negatively impact statistical power. To fully apply our findings, future studies will require the determination of the functions for the candidate genes that have not yet been documented.

The enrichment results from the signatures of selection analysis in this study provide novel insights into the genetic and physiological architecture of goat's adaptation and reproduction in DRC. However, similar to the relatively poor annotation of the caprine genome, the information provided by GO analysis is limited.

6.6. Conclusion

The present study highlighted, for the first-time positive signatures of selection associated with some genes of economic interest including adaptability and reproduction in three Congolese indigenous goat populations. Information about the location of these regions can now be used as a starting point identifying causal genetic variants that control some growth, reproduction, environmental adaptation, morphology, and disease resistance traits in local goat breeds which can be utilized in the genetic improvement of Congolese indigenous goat populations.

CHAPTER SEVEN : GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1. General discussion

Since the domestication of goat 10,000 years ago, many factors classified into adaptation to different breeding systems and/or purposes and adaptation to different environments, have contributed to the differentiation of goat breeds (Bertolini *et al*., 2018). These breeds differ considerably from one another in term of phenotypic characteristics and are adapted to a wide range of climate conditions (Bertolini *et al*., 2018). Both natural or directional selection events leave footprints across the genome which are known as signatures of selection (Bertolini *et al*., 2018; Onzima *et al*., 2018). Signatures of selection are genomic regions harboring DNA sequences functionally involved in the genetic variation of traits subject to natural or artificial selection (Qambari *et al*., 2012; López *et. al*., 2015). Because of their relevance to evolutionary biology related to the genetic diversity and their association with genes that control phenotypes of interest resulting from directional selection (by farmers), signatures of selection have been intensively studied in recent years (López *et al*., 2015). In this study, the phenotypic and genetic diversity, the typology and the management of goats (farmer-preferred traits for the selection of goats) were characterized, and the signatures of selection accros the genome of three Congolese indigenous goat populations was assessed. High phenotypic and genetic diversity were revealed in the Congolese goat populations. Environmental variation was the major source of that diversity, leading to the adaptation of goats in the three agro-ecological zones in DRC. In connection with that result, Banks *et al.* (2013) have demonstrated how ecological variation influences genetic diversity, while Fariello *et al*. (2014) mention the opportunities offered by genetic diversity in animal domestic populations to study genome response to selection. Selection has certainly been mentioned as an important mechanism of allele-frequency change and as the only mechanism that generates the adaptation and the survival of organisms despite a changing environment (Andrews, 2010).

Selection increases beneficial alleles and removes deleterious one (Buffalo and Coop, 2020).

In this study, it has been demonstrated that farmers participate in the increasing of the adaptation of goats by selecting goats to raise based on their ability to adapt to the region. In addition to the adaptability of goats, farmers focus on the reproduction and disease resistance performances in the selection of goats in the three agro-ecological zones in the DRC. Knowing that selection affects the genome at a specific region, leaving signatures around the selected genes (Smith and Haigh, 1974), the signatures of selection on the candidate genes in association with farmer-preferred traits were assessed. The assessment of the signatures of selection through the genome-wide scan revealed candidate genes for which enrichment and annotation led to phenotypic traits related to adaptation (DEPTOR, MAGEL2, DCDC2, PANK3, ITSN1, COL6A3, ENPP2, PADI2), prolificacy (CMK4) and disease resistance (EIF3J). The frequency of these candidate genes in the three Congolese indigenous goat populations varied according to the agro-ecological zone. Most of the genes related to the adapation and disease resistance were found in Kasai and dwarf goat populations respectively from Kinshasa and Tshopo, while the candidate gene related to the prolificacy was found in small goat populations from South Kivu (**Table 6.11**). These results are in agreement with the results obtained on the reproduction performances variation (**Table 4.3**) and on farmers' selection preferences in the three agro-ecological zones (**Table 4.9**), and justify the presence of farmers' selection pressure for these traits. The selection that occurred for the selection of the three Congolese indigenous goat populations is simulated to a directional selection since fermers tend to fix the expression of particular traits over others. Directional selection occurs when individuals homozygous for one allele have a fitness greater than that of individuals with other genotypes (Holsinger, 2001). It leads to increase over time in the frequency of favored allele (Andrews, 2010; Neiman and Fields, 2016; Buffalo and Coop, 2020). Similar results were obtained in this study showing the presence of candidate genes associated with the motly selected phenotypic traits according to each of the three agro-ecological zones.

In this study, a strong relationship was observed between the variation on phenotypic traits and candidate genes in the three populations of goats in the three agro-ecological zones. The favourable phenotype (high reproductive performances) observed in small goat breed from South Kivu was linked to the genotype of these goat populations by the presence of the candidate gene (CMK4) enriched and annotated for the prolicicacy trait. Same scenario was observed in Kasai goat from Kinshasa from where the higly expressed phenotypic trait (growth) was linked with the genotype by the presence of candidate genes (DEPTOR, MAGEL2, COL6A3, PADI2) enriched and annotated for body size and growth. Dwarf goat from Tshopo was more selected for the adaptability in the region and the disease resistance (farmer preferences phenotypes). The selection pressure for these traits led to the signals of candidate genes enriched and annotated for traits corresponding to the adaptation (DEPTOR, MAGEL2, DCDC2, PANK3, ITSN1, COL6A3, ENPP2), and disease resistance (EIF3J).

7.2. General conclusion

The genetic characterization of animals, a good understanding of their typology and production management, and the identification of candidate genes associated with economic traits offer an opportunity for decision-making in the development of improvement programs for animal genetic resources. The objectives of this study were to characterize the phenotypic and genetic diversity, the typology and production system, and to determine signatures of selection on candidate genes associated with traits of economic importance in three Congolese indigenous goat populations in the east and west of the DRC. The results from this study showed that the goats in DRC are raised in an extensive farming system with no inputs in their management (feeding, reproduction, diseases control), while they are raised for economic purposes. Animal adaptation in the environment of production, prolificacy, and disease resistance are the farmer-preferred traits in the selection of goats to keep. Candidate genes associated with these preferred farmers' traits were identified in the positive genome for signatures of selection assessed through the genome-wide scan analysis. The Congolese goat populations are clustered into three clusters well distinguished

by the reproductive performances mainly the double (twins) and triple kidding with more prolific goats clustered into clusters two and three with more goats from the South Kivu region. The analysis of the mitochondrial DNA *d-loop* region and of the SNPs reaveled high genetic diversity in the three Congolese indigenous goat populations with a weak genetic differentiation. These goats are clustered into the haplogroup A with a single maternal origin and underwent expansion in the history. Considering the number of haplotypes shared between the Congolese goat populations and Cameroonian goats and based on the Approximative Bayesian Computaion Analysis (ABC), the Congolese goat populations might have separated from Cameroonian goats and may have descended from North African goats.

The Genome-wide scan of the three Congolese indigenous goat populations using the Goat 60K SNP chip panel revealed positive regions in their genome whose gene enrichment led to candidate genes associated with traits of economic importance such as growth, body size, and muscle development (DEPTOR, MAGEL2), nervous system and behavior (DCDC2, PANK3, ITSN1, COL6A3, ENPP2), hair color measurement, hair color (PADI2), reproduction (CMK4), and disease control such as the decrease in salmonella proliferation (EIF3J).

Most of these genes have been reported in several other species including humans, rats, pigs, and cattle in which they were associated with the same or other biological functions as reported in this study. The statistical method used for analysis, the low annotation of the goat genome, and the absence of local African goat breeds among the goat breeds used in the development of the Goat 60KSNP chip for goat genotyping could drive and explain low enrichment of several common genes already identified in goats from different environment like in Europe, Asia, and America. The results of this study provide the first information on the genetic potential of goats in the Democratic Republics of Congo and can be used as a basis and reference in the development of local goat improvement programs in DRC.

7.3. Recommendations

1. The use and the comparison of several other methods of SNPs analysis are necessary for a good enrichment and understanding of the positive genomic regions of the studied goat populations.

2. Genome-wide association studies are required for phenotype and genotype association.

3. Particular attention should be paid to the production system of goats in the studied regions to maintain their diversity and to improve their productivity in the adapted local environments.

4. Care should be taken on feeding systems, reproduction methods, health care methods, and the management of goats according to the variation of seasons.

5. The findings of this study have the potential to be applied in research centers and by policymakers involved in the field of animal production as reference for building goat development programs in the DRC.

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APPENDIX

Appendix I

Interview guide for goat farmers

DATE……

Project: Assessment of genetic diversity and signatures of selection in indigenous goat populations in DR Congo.

Current study: Phenotypic characterisation of indigenous goat breeds in DRC to support genetic diversity studies

Introduction

I am a PhD student at University of Nairobi. I would like to obtain information from indigenous goat farmers in to characterize and map the different types of goats that survive in DRC's agro-ecological zones.
The information from this study will be used to help in the description of the unique attributes of different goat types, the environment they survive in and their management practices.
This study will consist of questions about your experience with indigenous goats, and a description of the various goat types you have highlighting their uniqueness, the environment they survive in plus physical measurements of selected goat types. 5ml of blood samples will be taken from each selected goat for genetic studies
You were chosen to participate because you are a farmer of indigenous goats and we request your support in this study
We seek your consent (YES/NO) before we can proceed to study your animals.

A: Location and Description of the farm

Household Number: ……

Village Name……

GPS waypoints:

East: ……..

1. How many goats do you have?

Class	Males	Females	Kids	Weaners	Total

2. What various breeds or types of indigenous goats do you rear? (Select (√), others specify)

Chèvre moyenne du Congo…… b) Chèvre naine du Bandundu…. ……

c) Chèvre du Kasai…… d) La petite chèvre de l'Afrique de l'Ouest…….....

f) Others (Specify)……

3. What is the origin/source of the different types of goats that you rear? (Give origin of each type)

Goat type	Source

Forage species

What are the major feed sources for your goats?

Forage type	% used; for multiple sources	Name	Palatability	Drought effects	Water logging	Other attributes (Specify)
Natural pastures						
Sown pastures						
Crop residues						
Forage trees						
Supplements						

B: Description of the management aspects and benefits

How do you manage the goats in terms of;

Feeding…….

Health care…….

Breeding…….

Are there any unique management aspects of these goats given the environment and the different weather patterns here? (please describe)

What socio-economic benefits are ascribed to goats in this society/ community? Please rank in order of importance (1=most important)

Socio-economic benefits	Rank

What are the marketing characteristics for the goats?

Goat breed	Market orientation	Market targeted	Product marketed (state)
Breed 1			
Breed 2			
Breed 3			

Market orientation: 1: Fully marketed, 2: Subsistence, 3: Mixed; **Market target**: 1: International, 2: Regional, 3: National,

4: Local

C: Description of reproduction and milk production traits

1. Fecundity

a) At what average age are the goats ready for mating? (In months)

Goat type/breed	Females	Males

b) For each type of goats you have, what is the average on the following

Goat type	age at first kidding	Number of kidding before culling	Frequency of kidding per year	Any multiple births (Yes/No)

If yes in above table, complete for each females and birth type, their survival rates

Goat breed	Twins	Survival rate	Triplets	Survival rate	Quadruplets	Survival rate

Survival rate (i) 100% (all survive) (ii) 75% (iii) 50% (iv) 25% (v) 0%

f) Please give a reason(s) for survival rate in each case: ……

2. Growth rate (Can try to estimate)

Growth attribute	Breed 1: ……	Breed 2: …….	Breed 3: ……...	Breed 4: …….
Av. birth weight				
Av. Weaning weight				
Av. Mature weight				
Av. carcass weight				

Note: Relate carcass weight to mature weight

3.Milk production traits

Do you experiment to milk your goats (Yes/No)?

If yes, what is the level of milk production per goat and per day (in litter)

Type of goat	High quantity	Medium quantity	Low quantity
1.			
2.			
3.			

What coping mechanisms do you use to improve the quantity of milk production on your goats?

……

Are all kids born satisfied from the mother 'milk produced? (Yes/No)?

If No, what are you doing for the unsatisfied kid? ……

E: Physical characterisation of the goats (only females)

Request farmer to select three female goats per type that have kidded at least once to represent the best 3, average 3 and poorest 3 for each type. For each give the information below (Use a separate sheet for each type/breed of goats at the farm

GOAT TYPE/BREED:									
Flock size: Bucks: _____ Does: _____ Male kids: _____ Female									
Traits	**Best Does**			**Average Does**			**Poor Does**		
	1:	**2:**	**3:**	**1:**	**2:**	**3:**	**1:**	**2:**	**3:**
Body weight (kg)									
Body length (cm)									
Chest girth (cm)									
Height at withers (cm)									
Height at rump (cm)									
Body condition score (1-5)									
Age (years)/Dentition									
Coat color type									
Color pattern									
Date of last kidding									
Parity number (number of kidding)									
Twinning (in life time)									
Number of kids born (in life time)									
Number of kids weaned (in life time)									
Milk yield per day (if milked)									
Its sire's origin (on farm or external)									
Its dam's origin (on farm or external)									
Disease incidences? (yes/no)									
If yes, what disease(s) and when?									
Did it need treatment to recover? What?									
If you were buying this doe for breeding purposes, how much would you be willing to pay for (knowing its history)?									
What are the reasons for choosing this doe as such?									

Rank the top three reasons.			

Colour type: (1) Black, (2) White (3) Brown (4) Grey. (5) Mixed

Goat Reproduction Traits Appraisal

(Phenotype of Reproduction Potential)

Please collect information about the best 3 does, the average 3 does and the poor 3 does as ranked by the farmer. Use separate sheet for each type/breed

Goat type or Breed……...

Female Goat Descriptors

Rank	Animal ID	Age/Dentition	Parity	Size (?)	Photos N	Mother's ID	Sire ID
Best							
Average							
Poorest							

History of the Female goat

Rank	Animal ID	Born in house hold (Y/N)	Village/Location of Birth	From market (if origin known)
Best				
Average				
Poorest				

Reproductive performances of female goat family (Y/N/NA)

Rank	Animal ID	Did mother have Twins/Triplets	Did mother of Sire have triplets	Has sister have twins or more		
				Sister 1	Sister 2	Sister 3
Best						

Average						
Poorest						

Reproductive performances of the female goat (select one among each rank)

Rank/ ID	Parameters	Current Parity	Previous Parity	Second Parity	First Parity
Best/…….	Number of kids				
	Number of male				
	Number of female				
	Number weaned				
	Buck ID (in known)				
	Origin of the buck				
Average/……	Number of kids				
	Number of male				
	Number of female				
	Number weaned				
	Buck ID (in known)				
	Origin of the buck				
Poorest/……..	Number of kids				
	Number of male				
	Number of female				
	Number weaned				
	Buck ID (in known)				
	Origin of the buck				

Bio Samples

Sample type: Blood sample

Collect samples from all the best 3, average 3 and poorest 3 does as earlier rated by the farmer for each type of goats

Rank	Sample 1 ID No.	Sample 2 ID No.	Sample 3 ID No.

Best			
Average			
Poor			

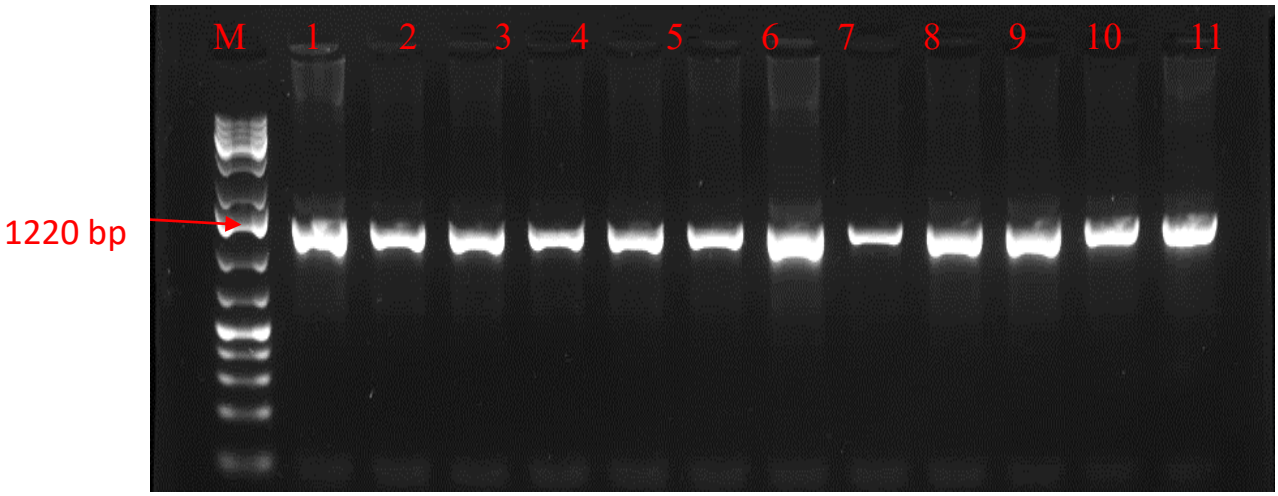
N.B: ***Collect samples of goats from different/unrelated sires and if possible unrelated does for diversity***

<u>ONLY REPRODUCTIVE FEMALES!!!</u>

Appendix II.

II.1. mtDNA quality control check

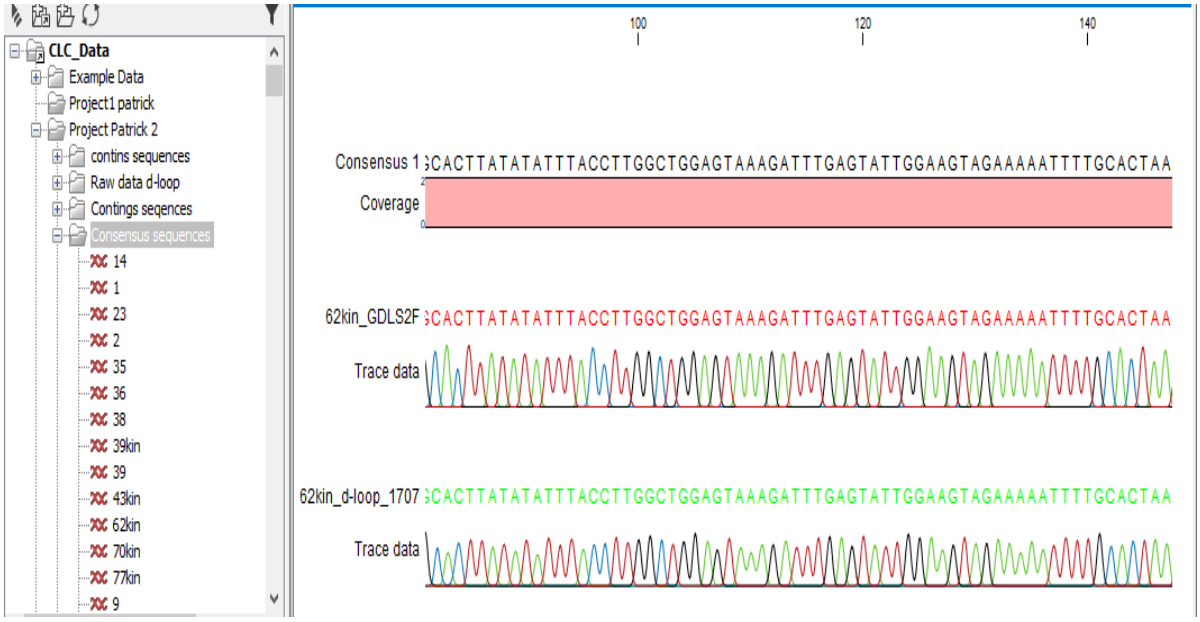
Agarose gel electrophoresis 1.5%, 70V/ 20 cm in 60min



Primers: D-loop 341 F 5'- ACCAGAAAAGGAGAATAGCC- 3' (Tm=56.4)

D-loop 1707 R 5'-ACCAGAAAAGGAGAATAGCC- 3' (Tm=56.4)

Sequences trimming and assembling: CLC Genomics workbench 8.0.3



II.2. Multiple sequence alignments with the reference sequence (RN: GU223571.1): CLC Genomics workbench 8.0.3

Appendix III; Table. Reference sequences employed for haplogroup analysis

Country of origin	Sample size	Haplogroup	Accessioj number	Reference
Laos	1	B	AB044303.1	Mannen et al 2001
Pakistan	36	A, B, C, D	AB110552, AB110553, AB110555, AB110557, AB110558, AB110559, AB110560, AB110561, AB110562, AB110563, AB110564, AB110565, AB110566, AB110567, AB110568, AB110569, AB110570, AB110571, AB110572, AB110573, AB110574, AB110575, AB110575, AB110577, AB110578, AB110579, AB110580, AB110581, AB110582, AB110583, AB110584, AB110585, AB110586, AB110587, AB110588, AB110589	Sultana et al., 2003
Saudi Arabia	39		AJ317752, AJ317753, AJ317754, AJ317755, AJ317757, AJ317758, AJ317759, EF618309, EF618310, EF618311, EF618312, EF618313, EF618314, EF618315, EF618312, EF618313, EF618314, EF618315, EF618316, EF618314, EF618318, EF618319, EF618320, EF618321, EF618323, EF618324, EF618325, EF618327, EF618328, EF618330, EF618331, EF618332, EF618333, EF618334, EF618337, EF618338, EF618339, EF618341, EF618345,	Luikart et al., 2001 ; Naderi et al., 2007
Iraq	6	A	AJ317763, AJ317764, AJ317765, AJ317766, AJ317767, AJ317768	Luikart et al., 2001
Algeria	3	A	AJ317777, AJ317778, AJ317779	Luikart et al., 2001
Egypt	18	A, G	AJ317780, AJ317781, AJ317783, AJ317795, AJ317796, EF617711, EF617712, EF617713, EF617714, EF617715, EF617716, EF617717, EF617719, EF617720, EF617721, EF617723, EF617724, EF617727	Luikart et al., 2001 ; Naderi et al., 2007
Morocco	5	A	AJ317784, AJ317785, AJ317786, AJ317787, AJ317788	Luikart et al., 2001 ; Naderi et al., 2007
Tunisia	5	A	AJ317789, AJ317790, AJ317791, AJ317792, AJ317793	Luikart et al., 2001
Nigeria	4	A	AJ317801, AJ317810, AJ317823, AJ317825	Luikart et al., 2001 ; Naderi et al., 2007
Zimbabwe	2	A	AJ317802, AJ317803	Luikart et al., 2001 ; Naderi et al., 2007

Mozambique	6	A	AJ317804, AJ317805, AJ317806, AJ317807, AJ317808, AJ317809	Naderi et al., 2007
Senegal	3	A	AJ317816, AJ317817, AJ317818,	Luikart et al., 2001
Mongolia	1	B	AJ317833	Luikart et al 2001
Switzeland	1	C	AJ317838.1	Luikart et al 2001
India	2	A, D	AY155721.1, AY155952.1	Joshi et al., 2004
China	3	B, C, D	DQ121578, DQ188892, DQ188893	Liu et al., 2005 and 2006
Sicily	2	F	DQ241349.1, DQ241351.1	Sardina et al 2006
Austria	1	D	EF617701	Naderi et al 2007
Azerbaijan	1	B	EF617706.1	Naderi et al 2007
France	1	A	EF617779.1	Naderi et al 2007
Iran	17	A, G	EF617863, EF617864, EF617865, EF617868, EF617869, EF617870, EF617871, EF617872, EF617873, EF617875, EF617876, EF617878, EF617879, EF617880, EF617945, EF618083, EF618084,	Naderi et al 2007
Italy	1	A	EF618134	Naderi et al 2007
Jordan	1	A	EF618200	Naderi et al 2007
Lybia			EF618220	Naderi et al 2007
Namibia			EF618244, EF618245	Naderi et al 2007
Spain	1	C	EF618413	Naderi et al 2007
Turkey	1	G	EF618535	Naderi et al 2007
Kenya	55	A, G	KP120622, KP120623, KP120624, KP120625, KP120626, KP120627, KP120628, KP120629, KP120629, KP120630, KP120632, KP120634, KP120635, KP120637, KP120638, KP120639, KP120640, KP120641, KP120642, KP120643, KP120646, KP120648, KP120649, KP120650, KP120652, KP120653, KP120654, KP120655, KP120646, KP120648, KP120649, KP120650, KP120652, KP120653, KP120654, KP120655, KP120656, KP120657, KP120658, KP120660, KP120661, KP120666, KP120667, KP120668, KP120669, KP120670, KP120672, KP120673, KP120674, KP120675, KP120677, KP120678, KP120679, KP120680, KP120681	Kibegwa et al., 2015
Ethiopia	75	A, G	KY747687, KY747688, KY747689, KY747690, KY747691, KY747692, KY747693, KY747694, KY747695, KY747696, KY747697, KY747698, KY747699, KY747700, KY747701, KY747702, KY747703, KY747704, KY747705, KY747706, KY747707, KY747708, KY747709, KY747710,	Tarekegn et al., 2018

			KY747711, KY747712, KY747713, KY747714, KY747715, KY747716, KY747708, KY747709, KY747710, KY747711, KY747712, KY747713, KY747714, KY747715, KY747716, KY747717, KY747718, KY747719, KY747720, KY747721, KY747722, KY747723, KY747724, KY747725, KY747727, KY747730, KY747734, KY747738, KY747739, KY747741, KY747743, KY747744, KY747752, KY747756, KY747730, KY747734, KY747738, KY747739, KY747741, KY747743, KY747744, KY747752, KY747756, KY747764, KY747772, KY747773, KY747774, KY747775, KY747778, KY747787, KY747794	
Cameroon	53	A	MH621412, MH621415, MH621418, MH621419, MH621420, MH621421, MH621422, MH621424, MH621425, MH621426, MH621427, MH621428, MH621430, MH621432, MH621433, MH621434, MH621437, MH621438, MH621439, MH621440, MH621441, MH621443, MH621445, MH621446, MH621447, MH621449, MH621451, MH621452, MH621455, MH621456, MH621459, MH621460, MH621463, MH621466, MH621467, MH621468, MH621469, MH621470, MH621471, MH621473, MH621474, MH621477, MH621478, MH621480, MH621483, MH621485, MH621488, MH621490, MH621494, MH621496, MH621497, MH621498, MH621501,	Tarekegn et al., 2019

Appendix IV; Table. Logistic regression analysis of posterior probabilities (confidence intervals) for the scenarios modelled in DIYABC based on mtDNA simulated for 1,000,000 data set

N	Scenario 1	Scenario 2	Scenario 3	
1000	0.9932[0.9687,1.0000]	0.0000[0.0000,1.0000]	0.0068[0.0000,1.0000]	0.0000 [0.0000, 0.0247]
2000	0.9948[0.9822,1.0000]	0.0000[0.0000,1.0000]	0.0052[0.0000,1.0000]	0.0000 [0.0000, 0.0127]
3000	0.9937[0.9816,1.0000]	0.0000[0.0000,1.0000]	0.0063[0.0000,1.0000]	0.0000 [0.0000, 0.0122]
4000	0.9918[0.9783,1.0000]	0.0000[0.0000,1.0000]	0.0082[0.0000,1.0000]	0.0000 [0.0000, 0.0136]
5000	0.9873[0.9674,1.0000]	0.0000[0.0000,1.0000]	0.0104[0.0000,1.0000]	0.0000 [0.0000, 0.0153]
6000	0.9873[0.9706,1.0000]	0.0000[0.0000,1.0000]	0.0127[0.0000,1.0000]	0.0000 [0.0000, 0.0169]
7000	0.9853[0.9674,1.0000]	0.0000[0.0000,1.0000]	0.0147[0.0000,1.0000]	0.0000 [0.0000, 0.0181]
8000	0.9833[0.9646,1.0000]	0.0000[0.0000,1.0000]	0.0167[0.0000,1.0000]	0.0000 [0.0000, 0.0191]
9000	0.9814[0.9618,0.9712]	0.0000[0.0000,1.0000]	0.0186[0.0000,0.9743]	0.0000 [0.0000, 0.0200]
10000	0.9795[0.9591,0.9196]	0.0000[0.0000,0.9881]	0.0205[0.0000,0.9985]	0.0000 [0.0000, 0.0209]