Welwitschia International Journal of Agricultural Sciences (WIJAS)
unam FANR PUBLICATION
W I J A S
http://www.unam.na E.mail: editor4wijas@unam.na (+264) (0) 61 206 3111

Polymorphism of the KAP1.3 and KRT33A genes in the Swakara sheep of Namibia

Nyoni N.F., Itenge T.O.* and Shipandeni M.N.T.

Department of Animal Science, Faculty of Agriculture and Natural Resource, University of Namibia, Windhoek, Namibia

Abstract

The objective of the study was to determine polymorphism in the KAP1.3 and KRT33A genes of Swakara sheep. Blood samples were collected from 40 Swakara sheep randomly selected from four farms; Neudamm, Gellap-Ost, Kalahari and Tsumis. Genomic Deoxyribonucleic acid was isolated using the Inqaba biotech-kit protocol. PCR-Single strand conformational polymorphism gel electrophoresis method was used for genotyping the two genes. The PCR products were sequenced to construct phylogenetic trees for evolutionary analyses. Chi-square test at 5% level of significance was used to analyze the data. Three genotypes were identified at KAP1.3 gene, with genotype frequencies of 0.5 (AA), 0.35 (AB) and 0.15 (CC). The frequency distribution of genotypes across all four farms differed significantly (P=0.05). Two genotypes (AA and BB) of the KRT33A gene were identified. The KRT33A locus was not statistically significant (P=0.118), and the allele frequency was 0.25 (A) and 0.75 (B). The KAP1.3 and KRT33A genes showed no significant deviation from the Hardy Weinberg Equilibrium. The phylogenetic tree showed relatedness of the Swakara sheep found in Namibia. The revealed polymorphism in the KAP1.3 and KRT33A may prompt further studies on KAP genes in Swakara sheep, which may help with the identification of genetic markers linked to superior pelts and strategic selection of breeding stock.

Keywords: Keratin, Pelt, Polymorphism, Sheep.

1. Introduction

Swakara is a fat-tailed breed of sheep that is profoundly bred for the production of high-quality pelts in Namibia (Campbell, 2007). It originated from Uzbekistan in Central Asia and was imported into Namibia

Received 05 March 2020. Received in revised form 28 July 2020. Accepted 29 July 2020. Available online 07 December 2020. *Corresponding author: T. O. Amushendje (Tel: +264 2063180, E-mail: tamushendje@unam.na).

(as Karakul then), in 1907 (Lundie, 2011; Kevorkian et al., 2011; Bravenboer, 2007). It may also be kept for meat and wool (Malesa, 2015; Nsoso and Madimabe, 2003). Soon after its introduction in Namibia, the Karakul sheep were subjected to intensive research and strategic breeding programmes, which has resulted in the production of unique quality pelt characterized by short hair, exceptional patterns and better hair texture.

The Swakara sheep are farmed mainly in Namibia, South Africa and Botswana (Malesa, 2015). Swakara breed plays a vital role in pelt production despite the availability of other pelt breeds globally (Rothauge, 2009). Because of the significant differences between the pelts of the Namibian lambs, when compared to those in the country of origin, characterized by lustrous features of different colours and unmatched quality across the globe, the Namibian authorities granted permission for the change of breed name from Karakul to Swakara in 2012 (Itenge & Shipandeni, 2015). Swakara is the trade name for all pelts produced in three Southern African countries; Namibia, South Africa and Botswana which are auctioned in Copenhagen, Denmark bi-annually in April and September. These sought-after pelts contribute significantly to the Namibian economy (Itenge & Shipandeni, 2015).

Pelts are harvested from lambs by well-trained personnel, adhering to the Swakara industry's Code of Practice (Kruger et al., 2013). Harvesting is done within 48 hours of birth because the curl structure of the pelt deteriorates with increase in age (Martins & Peters, 1992). Farmers are required to practice rigorous husbandry practices. The code of practice focuses on the welfare of Swakara sheep; it set out strict ethical parameters regarding the humane treatment of the Swakara. Induced abortion is illegal in Namibia, and Swakara farms in Namibia are subject to an animal health inspection at least once a year.

Primarily, four natural colour types are found in the Namibian Swakara sheep, namely black, which was the original colour, white, grey and brown (Bravenboer, 2007). The white pelt is the most preferred pelt colour because it can be dyed into several colour tones to satisfy the fashion trends. Currently, visual appraisal technique is being used in the Swakara industry to select animals with superior pelt traits. However, the efficiency of breeding programmes of pelt production may be accelerated through the development of gene-markers linked to superior pelts. Genetic markers can either be genes or non-functional DNA segments such as microsatellites (Abdul-Muneer, 2014) or minisatellites. According to Itenge (2012), a genetic marker for a particular trait can be defined as a piece of DNA that directly affects a phenotype and shows polymorphism. It can also be a piece of DNA that is closely linked to another piece of DNA that affects a phenotype.

The keratins, which are genes of interest in this study, are structural intermediate filamentous proteins that constitute about 90% of the total wool fibre in sheep. The protein gene family is divided into two groups; Keratin Intermediate Filament proteins (KRTs) and Keratin Associated Proteins (KAPs). The KRTs form the skeletal structure of the wool fibre (micro-fibrils) and are embedded in a matrix of KAPs (Powell & Rogers, 1986). Numerous studies showed that there had been an increase in the number of KAP genes defined in humans and sheep species as well as progressive accounts of variation in these genes (Gong et al., 2012). Rogers et al. (2005) illustrated that keratin-associated proteins are encoded by a large number of highly polymorphic genes. Between two to nine alleles have been identified in KAP genes among *Ovies aries* species (Zhou et al., 2012; Gong et al., 2010). Gene expression can be affected by the structure and function of the encoded proteins (Elmaci, 2013), and variation in KAP genes has significantly influenced wool traits (Yardibi et al., 2015) in most ovine species. Keratin associated proteins play a critical role in determining the physicomechanical properties of hair and wool fibres as they form a semi-rigid matrix in which the Keratin intermediate filaments are rooted (Li et al., 2018; Rogers et al., 2007; Powell et al., 1995).

To our knowledge, no genetic markers have yet been associated with pelt quality traits in Swakara sheep. Therefore, the objectives of the study were to determine polymorphism in the KAP1.3 and KRT33A genes, as well as to compare the level of polymorphism of the keratin genes from different study sites situated in three regions of Namibia (Khomas, Hardap, Karas). The identification of polymorphism in the KAPs and KRT genes could facilitate the possible development of genetic markers associated with pelt quality traits.

2. Materials and Methods

2.1 Sheep and blood collection

In this study, we used 40 Swakara sheep sourced from four farms; Neudamm farm in the Khomas region (22.30ºS, 17.22ºE), Gellap-Ost research station in the Karas region (26.27ºS, 18.05ºE), Tsumis research station in Rehoboth, Hardap region (23.73ºS, 17.19ºE) and Kalahari research station in Mariental are located in the Hardap region (25.55ºS, 16.39ºE). Ten Swakara sheep were randomly selected from each of the four farms. The flock comprised of 24 ewes and 16 rams Swakara sheep with age ranging from six months to seven years old. An ethical clearance (FANR/003/2019) for the study was obtained from the University of Namibia through the Centre for Research and Publications. All animals were handled humanely.

Blood samples from each Swakara sheep were collected in four mL K2E-EDTA anti-coagulant vacutainer tubes via jugular vein puncture. Blood samples were immediately placed in a cooler box with ice packs and transported to the laboratory. One hundred µL blood samples were aliquotted into Eppendorf tubes to minimize contamination before refrigeration at 4ºC until DNA extraction was performed.

2.2 DNA Extraction

Genomic Deoxyribonucleic acid (gDNA) was extracted using the Inqaba biotech-kit protocol (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa). Purity and concentration of gDNA were determined by using a Spectrophotometer (Nano-drop 2000). Genomic DNA quality was assessed by using 1% horizontal Agarose gel-electrophoresis stained with 0.1 mg/L ethidium bromide and electrophoresed at 90 volts for 45 minutes. Gels were visualized by trans-illumination on a UV trans-illuminator (Syngene bioimaging, Cambridge, United Kingdom). The gDNA samples with good quality, as shown by intact bands, were used for further analysis.

2.3 PCR amplification of the KAP 1.3 and KRT33A genes

The primer sequences information used to amplify the KAP 1.3 locus were obtained from Rogers et al. (1994) and were as follows: KAP1.3 upstream 5'-GGG TGG AAC AAG CAG ACC AAA CTC-3' and KAP1.3 downstream 5'- TAG TTT GTT GGG ACT GTA CAC TGG C-3', defining a 598 bp amplimer. The primer sequence information used to amplify the KRT33A locus were sourced from Rogers et al. (1993a), and were as follows: upstream 5'-CAC AAC TCT GGC TTG GTG AAC TTG-3' and downstream 5'-CTT AGC CAT ATC TCG GAT TCC CTC-3' and defined a 480 bp region of the KRT33A locus. All primers were synthesised by Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa.

The protocols for amplification of the loci were sourced from Itenge-Mweza et al. (2007). For both KAP 1.3 and KRT33A genes, PCR amplifications were performed in 25 µL reactions containing 200 ng gDNA from whole blood, 1X One Taq Master Mix (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South

Africa), 200 mM of forwarding primer, 200 mM of reverse primer, and nuclease-free water (New England BioLabs@ Inc.).

Amplification consisted of an automated preheating Thermocycler (Thermo Scientific, Arktik thermal cycler) lid at 110 °C for two minutes, an initial denaturation of one minute at 95 ºC, followed by 30 cycles of denaturation at 95 ºC for one minute, annealing at 65 ºC for 1 min and extension at 72 ºC for 1 min, with a final extension of 72 ºC for 7 min. Amplimers were stored at 4 ºC until they were subjected to agarose gel electrophoresis.

2.4 Agarose gel electrophoresis

To confirm that PCR amplification has worked, amplimers were run in 1.0% w/v Agarose gels (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa) prepared with 0.5X Tris-Acetate EDTA (TAE) buffer (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa), containing 0.1 mg/L of ethidium bromide. Seven µL of PCR product was mixed with 1.8X loading dye (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa). A 100 bp molecular weight marker (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa) was included on the agarose gels in order to confirm the size of the expected amplimer (598 bp for KAP1.3 and 480 bp for KRT33A). DNA bands were visualized under a UV trans-illuminator (Syngene bioimaging, Cambridge, United Kingdom).

2.5 Detection of sequence variation using Polymerase chain reaction-Single Strand Conformational Polymorphism (PCR-SSCP) analysis

To detect genetic variation in the KAP1.3 and KRT33A loci, amplified PCR products were subjected to PCR-SSCP analysis according to the method of Itenge (2007), with minor modifications. Polyacrylamide gels (38:2 acrylamide/ bis-acrylamide, (Bio-Rad Laboratories, Ltd, Johannesburg, South Africa) vertical gels (Protean II 16 x 16 cm, 1.0 mm thick spacers, 24 well combs, Bio-Rad Laboratories, Ltd, Johannesburg, South Africa) were prepared to contain 1X TBE, 7 M of Urea, 30% ammonium persulphate (APS) and 30 µL TEMED (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa). The polyacrylamide gels were pre-run for an hour in order to remove any polar impurity in the vertical gel electrophoresis tank. The PCR products were mixed with 50 µL loading dye (95% formamide, ten mM NA_2EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol) (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa), denatured by firstly preheating the Thermo-cycler lid (Thermo Scientific, Arktik thermal cycler) for two minutes followed by denaturing at 95ºC for five minutes and immediately placed on wet ice before loading 10 µL aliquots. The gels were electrophoresed in 1X TBE running buffer (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa) at 200 V for 16 hours 30 minutes, followed by silver staining according to the method of (Sanguinetti et al., 1994).

2.6 DNA Sequencing

DNA sequencing of 20 samples was carried out at the University of Porto in Portugal following the ABI Prism BigDyeTM Terminator v3.1 Sequencing Kit protocol on an ABI3130xl DNA Analyzer (Applied Biosystems, Foster City, California, USA). The sequencing was, however, only performed for one independent PCR.

2.7 Statistical analysis

Chi-square test of association at a 5% level of significance was used to test for allele frequency distribution across all four farms. The Spearman's Rank correlation formulae was used to calculate genotype frequencies of the two loci under study; KRT33A and KAP1.3. Frequency distribution of the different alleles in Swakara sheep was tested for Hardy Weinberg Equilibrium.

The Chi-square test of association equation used:

$$\chi_c^2 = \frac{\sum (O_i - E_j)^2}{E_j}$$

Where;

χ_c^2 is the Chi-square statistic value with c degrees of freedom

O_i is the observed allele frequencies for the ith allele

E_j is the expected allele frequencies for the ith allele

Spearman's Rank correlation defined by (1)–(3): Allele frequency distribution:

$$A = \frac{[2(AA) + (AB) + (AC)]}{2sample\ \text{size}} \quad (1)$$

$$B = \frac{[2(BB) + (AB) + (BC)]}{2sample\ \text{size}} \quad (2)$$

$$C = \frac{[2(CC) + (AC) + (BC)]}{2sample\ \text{size}} \quad (3)$$

The Spearman's Rank correlation a non-parametric test relates the relationship between two genotypes under study; KRT33A and KAP1.3.

Hardy Weinberg Equilibrium equations

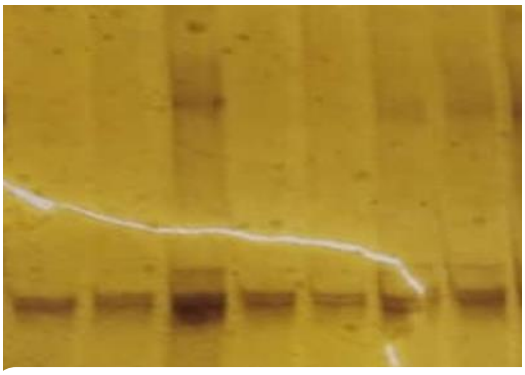
$$p^2 + 2pq + q^2 = 1 \quad (4)$$

$$p^2 + q^2 + r^2 + 2pq + 2qr + 2pr = 1 \quad (5)$$

(https://www.icalcu.com/stat/chisqtest.html)

3. Results

Three different banding patterns (AA, AB and CC) of the KAP1.3 gene were revealed in the PCR-SSCP analysis (Figure 1). Two patterns appeared to be from homozygous sheep, while the third pattern appeared to be from heterozygous sheep. It is important to note that although lanes 1 and 2 seem to be the same as lanes 4 and 5, they were different when observed while in the developer solution at scoring. The A allele (lanes 1 and 2) had no space between the bands while the C allele (lanes 4 and 5) had space between the bands. The alleles were not sequenced.



1	2	3	4	5	6	7
AA	AA	AB	CC	CC	AB	AB

Figure 1: PCR-SSCP analysis of the 598 bp amplimer of the KAP1.3 gene in Swakara sheep. Gels were electrophoresed on a 10% acrylamide/bis-acrylamide, containing 7 M urea, run for 16 hours 30 minutes, 200V at room temperature. Lane one to lane seven denotes the different genotypes at the KAP1.3 gene.

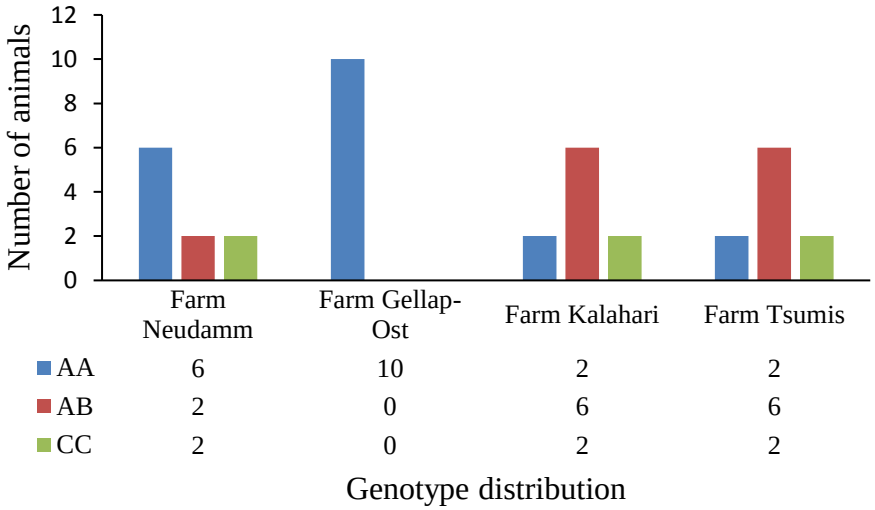


Figure 2: KAP1.3 genotype frequency distribution of Swakara sheep at the four farms

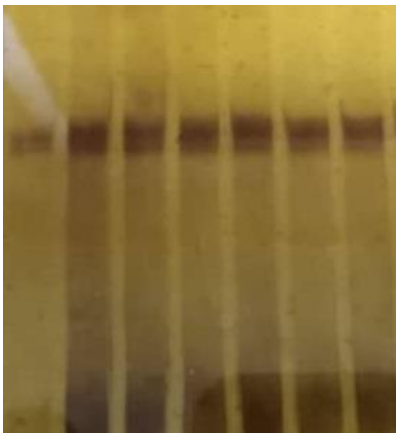


Figure 3: PCR-SSCP analysis of the 480 bp amplimer of the KRT33A gene, in Swakara sheep electrophoresed at 10% acrylamide/bis-acrylamide gel that contained 7 M urea run for 16 hours 30 minutes at 200V at room temperature. Lane one to lane seven denotes the different genotypes at the KRT33A gene.

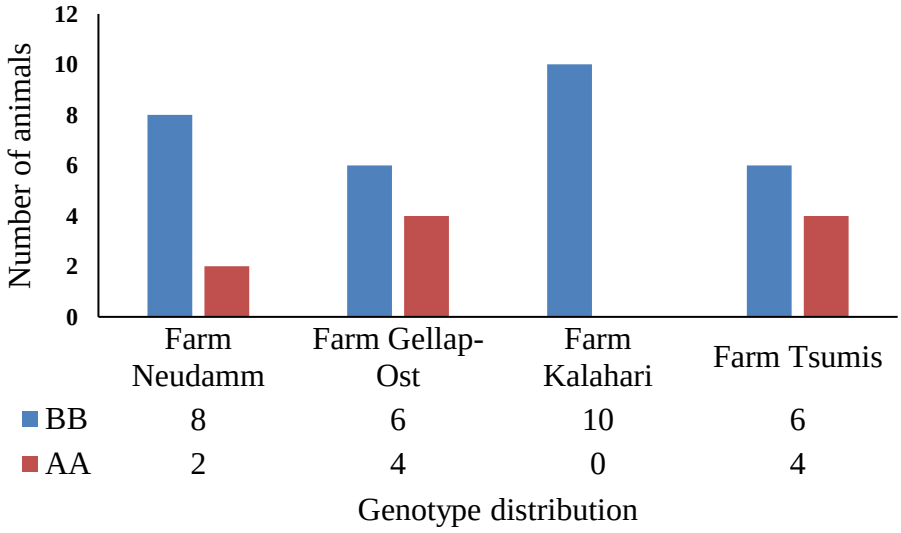


Figure 4: KRT33A genotype frequency distribution of Swakara sheep at the four farms

The frequency of the genotypes AA, AB and CC in the total 40 sheep studied was 0.5, 0.35 and 0.15, respectively (Figure 2). The frequency distribution of genotypes across all four farms differed significantly (P=0.05) and was in Hardy Weinberg Equilibrium. Overall, the KAP1.3 AA genotype was highly distributed in comparison to the AB and CC across the four farms under study. At Gellap-Ost, only the AA genotype was present, whilst Neudamm, Kalahari and Tsumis showed all the three genotypes.

Two different banding patterns (AA and BB) of the KRT33A gene were revealed in the PCR-SSCP analysis (Figure 3). Both banding patterns were from homozygous sheep. The frequency of the genotypes AA and BB in the 40 sheep studied was 0.25 and 0.75, respectively. The frequency distribution of genotypes across all four farms did not differ significantly (P=0.05), but it was in Hardy Weinberg Equilibrium. The genotype frequency distribution of the KRT33A gene is shown in Figure 4.

Phylogenetic trees were constructed to trace evolutionary relationships among the study animals located on the four farms. A pattern of the relative closeness of Swakara sheep located on the four farms in Namibia and other relative sheep breeds around the world was performed. Sample denotes the current study animals whilst AY, KY, KF and number are the similar sheep breeds. The KAP1.3 and KRT33A results are shown in Figure 5.

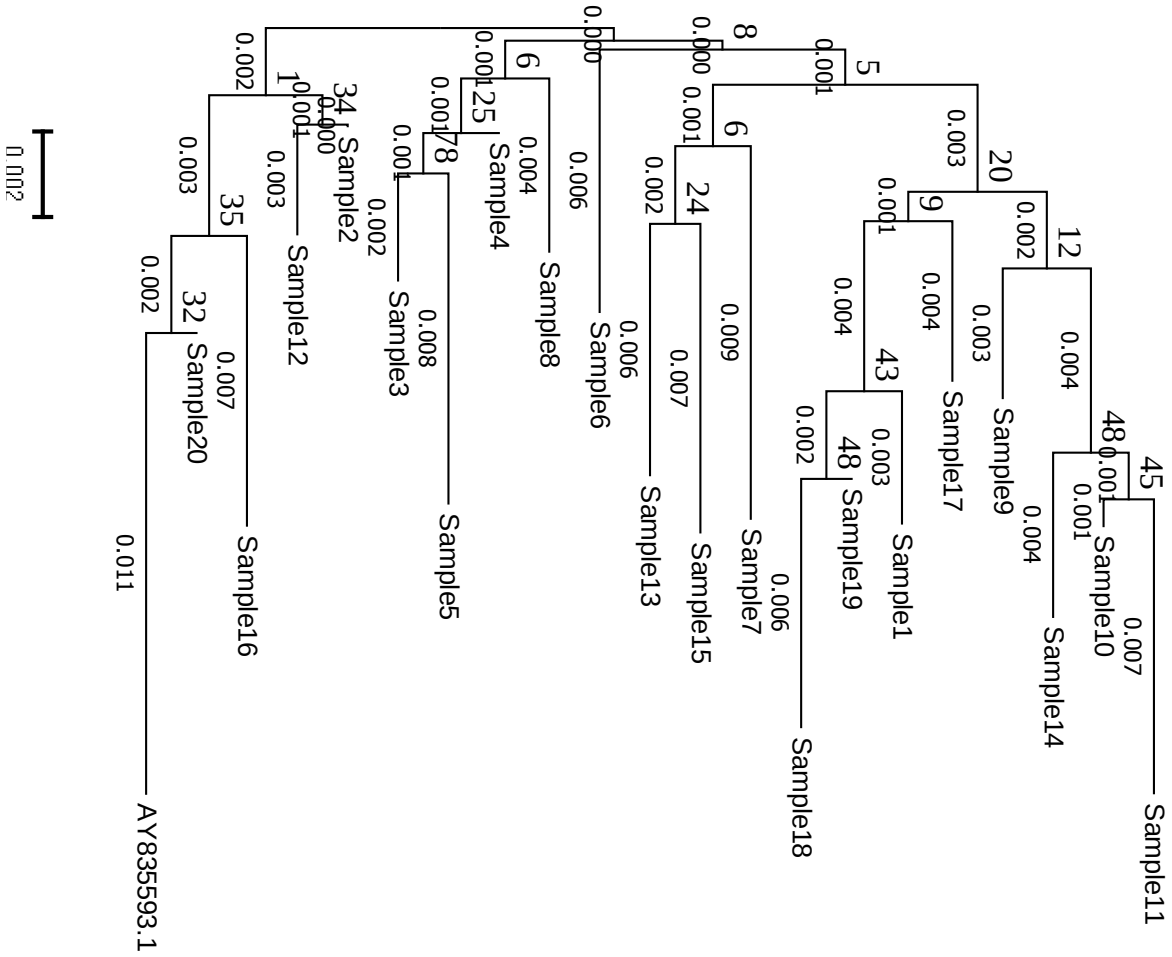


Figure 5: The evolutionary history was inferred using the Neighbor-Joining method, based on a 598 bp sequence and NCBI sequences were used for comparison in the KAP1.3 locus, in Swakara sheep. The optimal tree with the sum of branch length = 0.11929587 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches. The analysis involved 21 nucleotide sequences. KAP1.3 (AY835593.1) Sheep was used as an out-group/root of the tree. Evolutionary analyses were conducted in MEGA version 6.

4. **Discussion**

This study reports three genotypes at the KAP1.3 locus defining a 598 bp amplimer, using PCR-SSCP typing method, denoted as AA, AB and CC. While similar genotype numbers of KAP1.3 have been reported, the typing method used in these studies was not PCR-SSCP, but PCR-RFLP. In a study by Mahajan et al. (2017), three different RFLP patterns were obtained at two restriction sites corresponding to AA, AB and BB genotypes in Rambouillet sheep. The sizes of the RFLP patterns were as follows: AA pattern (350, 225 and 23 bp); AB pattern (350, 307, 225, 43 and 23 bp); BB type (307, 225, 43 and 23 bp). The 43 bp and 23 bp fragments were however not visible on the visualized gels. Different RFLP patterns using *Bsr* I restriction enzyme for the KAP1.3 locus defining a 598 bp amplimer were reported by Rogers et al. (1993b) in six sheep breeds, Xu et al. (2008) in 5 breeds of sheep of Xinjiang and Chen et al. (2011) in Chinese Merino sheep. Kumar et al. (2016) identified three genotypes denoted as XX, XY and YY at the KAP 1.3 gene in 11 Indian sheep breeds using *Bsr* I restriction enzyme. The sizes of the RFLP patterns were as follows: YY genotype (309, 225, 41 and 23 bp); XY genotype (350, 309 and 225 bp). The XX genotype was similar to the AA pattern in Mahajan et al. (2017). Kumar et al. (2016) also reported that smaller fragments of 41 and 23 bp were not visible on the 2 % agarose gel electrophoresis. Besides, Meena et al. (2018) identified three genotypes denoted as XX, XY and YY in the KAP 1.3 gene of Magra sheep, using *Bsr* I restriction enzyme.

Although RFLPs were the first genetic markers developed, they are losing popularity as a screening method to identify genetic markers because they have the disadvantages of not identifying all of the polymorphism within a length of DNA, are time-consuming, and restriction enzymes and consumables tend to be expensive (Itenge 2012). Since the restriction enzymes only target restriction sites, genetic variations that are not within the restriction sites, are therefore not recognizable by restriction enzymes when using this method. Itenge (2012), stated that the PCR-SSCP technique offers a rapid, sensitive, relatively inexpensive and simple way to screen for sequence variation, and has become one of the preferred methods for screening samples to detect polymorphism in genes. Numerous studies have reported at least six alleles of the KAP1.3 gene, using PCR-SSCP typing method. Rogers et al. (1994) reported six alleles in a 598 bp KAP1.3 amplimer from Romney sheep of New Zealand using PCR-SSCP typing method. Nine unique KAP1.3 SSCP patterns (designated A-I) were identified in Merino sheep of New Zealand (Itenge-Mweza et al., 2007) and in Chios, Kivircik and Awassi sheep (Yardibi et al., 2015).Furthermore, Gong et al. (2015); Mahajan et al. (2017) and Sulayman et al. (2017) reported six KAP1.3 alleles. In two different studies by Farag et al. (2018a) and (2018b), seven KAP1.3 alleles (A, B, C, D, F, G, T) in Egyptian sheep, and ten patterns in 112 tested sheep were identified, respectively using PCR-SSCP analysis of amplified 598 bp KAP1.3 gene. Since only three genotypes of the KAP1.3 gene were detected in this study, it is possible that the PCR-SSCP protocol may not have been optimized well enough to detect all the alleles present. While the PCR-SSCP protocol in this study was performed at room temperature, the vertical gel electrophoresis tanks used by Itenge (2007) were connected to a circulating water chiller to maintain a constant gel temperature. This cooling system was, however, not accessible in the current study. The detection of only three genotypes and not more could also be attributed to the sample size of animals used in the study (n=40). It is also possible that only three alleles are present at the KAP1.3 locus. It may also be possible that selection (which is most likely to occur in these flocks) may have reduced the frequency of some of the alleles. With selection for pelt quality taking place every generation, it would be expected that marker alleles associated with ‘inferior’ pelt quality would likely be reduced or even be eliminated from the

population over time. Therefore, we recommend that the sample size is increased to more than 100 animals in future studies, which include both commercial and communal Swakara sheep flocks.

The frequency of the genotypes KAP1.3 AA, AB and CC, was 0.5, 0.35 and 0.15, respectively. Overall, the KAP1.3 AA genotype was highly distributed in comparison to the AB and CC across the four farms under study. It was observed that the AA genotype was inherent mostly among ewes in comparison to rams. Gellap-Ost farm only had the AA genotype, whilst Neudamm, Kalahari and Tsumis showed all the three genotypes. Mahajan et al. (2017) observed the genotype frequencies of 0.41 (AA), 0.47 (AB) and 0.12 (BB), while Meena et al. (2018) observed the genotype frequencies in three genotypes; XX, XY and YY of 0.12, 0.17 and 0.71, respectively. In contrast, Kumar et al. (2016) had a higher frequency of XY genotype in comparison to the YY genotype in Indian sheep breeds. The A and B alleles in the study by Mahajan et al. (2017) were the same as the X and Y alleles in described by Kumar et al. (2016), though in different sheep breeds located in two different countries.

It should be noted that the AA genotype of Mahajan et al. (2017) is not similar to the AA genotype in this study, since the typing methods used were different. Variation in genotype frequencies could be as a result of selective breeding schemes, breed differences and mere evolutionary pressure (Mahajan et al., 2017).

Two different banding patterns (denoted as AA and BB) were detected at the KRT33A locus defining a 480 bp amplimer, using PCR-SSCP analysis, with genotype frequency of 0.25 and 0.75, respectively. A di-allelic polymorphism was reported at the KRT1.2 locus defining a 480 bp amplimer, using PCR-RFLP in Romney sheep by Rogers et al. (1993a). The two alleles were identified as a result of the presence/absence of an *Msp* I recognition site at nucleotide 259 bp (allele M/N respectively). In a study on 15 Indian native sheep breeds, Arora et al. (2008) found three genotypes (MM, MN, NN) in the 480 bp amplimer of the KRT1.2 locus across the breeds investigated. The sizes of the RFLP patterns were as follows: MM genotype (159, 126, 100 and 95 bp); MN genotype (259, 159, 126, 100 and 95 bp); NN genotype (259, 126 and 95 bp). Ahlawat et al., (2014) reported similar KRT1.2 genotype findings in Patanwadi, Marwari and Dumba sheep breeds of India, with overall genotypic frequencies at KRT 1.2 locus for MM, MN and NN being 0.64, 0.34 and 0.02, respectively. Furthermore, Kumar et al. (2016) reported three genotypes (MM, MN, NN) in Patanwadi and Nali sheep breed with an allele frequency distribution of 0.778 (M) and 0.222 (N), with genotype frequencies of 0.648 (MM), 0.260 (MN) and 0.092 (NN). The same three genotypes (MM, MN, NN) were observed by Meena et al. (2018) in the Magra sheep.

The number of alleles observed in the present study, and observed only in the homozygous form is not comparable to the number of alleles reported at the KRT1.2, using PCR-SSCP method. Itenge-Mweza et al. (2007) identified five alleles (A, B, C, D and E) in the KRT33A gene, (previously referred to as KRT1.2 gene) of the Merino sheep in New Zealand using PCR-SSCP protocol. The banding patterns in the stained gels were also not comparable. It is possible that there were more than two alleles within the KRT33A gene of the Swakara sheep, but the PCR-SSCP genotyping method used may not have detected all the variations. We recommend further studies which will optimize the PCR-SSCP protocol in order to pick up more alleles which may be available. It is also recommended that the sample size is increased to more than 100 animals in future studies, which include both commercial and communal Swakara sheep flocks. We recommend that polymorphism reported in the KAP1.3 and KRT1.2 in the current study through PCR-SSCP typing method is confirmed in other independent studies. Besides, we recommend that the PCR-RFLP typing method is carried out by digesting KAP1.3 and KRT33A loci with *Bsr* I and *Msp* I restriction enzymes, respectively in the Swakara sheep.

The evolutionary history was inferred using the Neighbor-Joining method, based on a 598 bp sequence and NCBI sequences were used for comparison in the KAP1.3 locus, in Swakara sheep (Figure 5), as well as on a 480 bp sequence and NCBI sequences for comparison in the KRT1.2 locus, in Swakara sheep. Inferences made using phylogenetic tree analysis entail the relatedness of the Swakara sheep found in Namibia. Figure 5 shows gaps between clads in the tree, which entails uniqueness between individuals sharing a common ancestor.

5. Conclusion

Swakara sheep are primarily kept for pelt production in Namibia, which is sought after in the fashion industry. The present study reveals the presence of genetic variation at the KAP1.3 and K33A genes in Swakara sheep. Results obtained may prompt further studies on KAP genes in Swakara sheep, which may help with the identification of genetic markers linked to superior pelts and strategic selection of breeding stock.

The use of genetic markers in the selection of breeding stock would complement the current visual appraisal technique used during pelt sorting and grading of Swakara pelts. It may also potentially improve the accuracy of selection for exceptional lustrous pelt pattern. This work has the potential to provide crucial baseline information that other researchers can use in better utilization and improvement of the Swakara sheep

Acknowledgements

The authors are grateful for the financial support from National Commission on Research Science and Technology (NCRST) in Namibia. We would also like to acknowledge the Department of Animal Science at the University of Namibia for technical support and the Department of Biotechnology at the Ministry of Agriculture, Water and Forestry (MAWF), particularly Mr Filemon Nghitila Shindume and Dr Gerhard Haitembu for their guidance with laboratory work.

Disclosure of conflict of interest

The authors declare no conflict of interest

References

Abdul-Muneer, P. M. (2014). Application of Microsatellite Markers in Conservation Genetics and Fisheries Management: Recent Advances in Population Structure Analysis and Conservation Strategies. *Genetics Research International*, 2014:1-11. http://dx.doi.org/10.1155/2014/691759

Ahlawat, A. R., Gajbhiye, P. U., Prince, L. L. L., Singh, A. and & Gajjar, S. G. (2014). Polymorphism of keratin intermediate filament (kif) type I gene association of wool quality traits in Patanwadi, Marwari and Dumba breeds of sheep. *Indian Journal of Animal Sciences*, 84 (3): 316–319.

Arora, R., Bhatia, S. Sehrawat, A., Pandey, A. K., Sharma, R., Mishra, B. P., Jain, A. and Prakash, B. (2008). Genetic Polymorphism of Type 1 Intermediate Filament Wool Keratin Gene in Native Indian Sheep Breeds. *Biochem Gene*, 46:549–556. DOI 10.1007/s10528-008-9169-3

Bravenboer, B. (2007). *Karakul: Gift from the Arid Land: Namibia 1907-2007*. Karakul Board of Namibia, Windhoek, Namibia.

Campbell, L.J. (2007). Evaluation of two indigenous South African sheep breeds as pelt producers. *MSc Thesis*. University of Pretoria, South Africa.

Chen, H. Y., Zeng, X. C., Hui, W. Q., Zhao, Z. S., & Jia, B. (2011). Developmental expression patterns and association analysis of sheep KAP8. 1 and KAP1. 3 genes in Chinese Merino sheep. *Indian Journal of Animal Sciences*, *81*(4), 391-396.

Elmaci, C., Sahin, S., & Oner, Y. (2013). Distribution of different alleles of aromatase cytochrome P450 (CYP19) and melatonin receptor 1A (MTRN1A) genes among native Turkish sheep breeds. Kafkas Univ Vet Fak Derg. 19, 929-933. DOI: 10. 9775/kvfd.2013.8900

Farag, I. M., Darwish, H. R., Darwish, A. M., Eshak, M. G., & Ahmed, R. W. (2018a). Genetic Polymorphism of KRT1. 2 Gene and its Association with Improving of Some Wool Characteristics in Egyptian Sheep. *Asian Journal of Scientific Research*, *11*, 295-300. **DOI:** 10.3923/ajsr.2018.295.300

Farag, I. M., Darwish, H. R., Darwish, A. M., El-Shorbagy, H. M. and Ahmed, R. W. (2018b). Effect of Genetic Polymorphisms of the KAP1.1 and KAP1.3 Genes on Wool Characteristics in Egyptian Sheep. *J. Biol. Sci.*, 18 (4): 158-164, 2018

Gong, H., Zhou, H., Hodge, S., Dyer, J. M., & Hickford, J. G. (2015). Association of wool traits with variation in the ovine KAP1-2 gene in Merino cross lambs. *Small Ruminant Research*, *124*, 24-29.

Gong, H., Zhou, H., & Hickford, J.G.H. (2010). Polymorphism of the ovine keratin-associated protein 1-4 gene (KRTAP1-4). *International Journal Molecular Biology Report*, (2010) 37: 3377. Doi.org/10.1007/s11033-009-9925-4. https://www.icalcu.com/stat/chisqtest.html

Gong, H., Zhou, H., McKenzie, G. W., Yu, Z., Clerens, S., Dyer, J. M., Plowman, J.E., Wright, M.W., Arora, R., Bawden, C.S. & Chen, Y. (2012). An updated nomenclature for keratin-associated proteins (KAPs). *International journal of biological sciences*, 8(2), 258.

Itenge, T. O. (2007). Identification of Genetic Markers Associated with Wool Quality Traits in Merino Sheep. (PhD thesis). Lincoln University, Christchurch, New Zealand.

Itenge, T. O. (2012). Identification of polymorphism in the keratin genes (KAP3.2, KAP6.1, KAP7, KAP8) and microsatellite BfMS in Merino sheep using polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis. Chapter publications in: *Electrophoresis*. Edited by Kiumars Ghowsi, pp. 193-220. InTech- ISBN 980-953-307-117-1.

Itenge-Mweza, T. O., Forrest, R. H. J., McKenzie, G. W., Hogan, A., Abbott, J., Amoafo, O., & Hickford, J. G. H. (2007). Polymorphism of the KAP1. 1, KAP1. 3 and K33 genes in Merino sheep. *Molecular and cellular probes*, *21*(5-6), 338-342.

Itenge, T.O., & Shipandeni, M.T.N. (2015). Sale trends of Swakara pelt offered at the Copenhagen fur auction from 1994-2013. *Applied Animal Husbandry & Rural Development*, 8.

Kevorkian, S. E. M., Zaulet, M., Manea, M. A., Georgescu, S. E., & Costache, M. (2011). Analysis of the ORF region of the prion protein gene in the Botosani Karakul sheep breed from Romania. *Turkish Journal of Veterinary and Animal Sciences*, *35*(2), 105-109.

Kumar, R., Meena, A. S., Kumari, R., Jyotsana, B., Prince, L. L. L., & Kumar, S. (2016). Polymorphism of KRT 1.2 and KAP 1.3 Genes in Indian Sheep Breeds. *Indian Journal of Small Ruminants (The)*, *22*(1), 28-31.

Li, W., Gong, H., Zhou, H., Wang, J., Liu, X., Li, S., ... & Hickford, J. G. H. (2018). Variation in the ovine keratin-associated protein 15-1 gene affects wool yield. *The Journal of Agricultural Science*, 156(7), 922-928.

Lundie, R. S. (2011). The genetics of colour in fat-tailed sheep: a review. *Tropical animal health and production*, 43(7), 1245-1265.

Martins, C, and K. J. Peters. (1992). Alternative use of Karakul sheep for pelt and lamb production in Botswana. I. Reproduction and growth performance. *Small Ruminant Research*, 9: 1-10.

McLaren, R. J., Rogers, G. R., Davies, K. P., Maddox, J. F. and Montgomery, G. W. (1997) Linkage mapping of wool keratin and keratin-associated protein genes in sheep. *Mammalian Genome*, 8:938-940.

Mahajan, V., Das, A. K., Taggar, R. K., Kumar, D. and Sharma, R. (2017) Association of polymorphic variants of KAP 1.3 gene with wool traits in Rambouillet sheep. *Indian Journal of Animal Sciences*, 87 (10): 1237–1242.

Malesa, M. T. (2015). Population genetics of Swakara sheep inferred using genome-wide SNP genotyping. *MSc Thesis*. University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Meena, A. S., Kumar, R., Jyotsana, B., Narula, H. K., & Kumar, S. (2018). Genetic polymorphism of KRT 1.2, KAP 1.3 and THH gene in Magra sheep. *Indian Journal of Small Ruminants* (The), 24(1), 27-30.

Ministry of Agriculture Water and Forestry (MAWF): Namibia livestock catalogue. (2013). John Meinert Print, Windhoek. ISBN: 978-99945-0-067-3.

Nsoso, S. J., & Madimabe, M. J. (2003). A survey of Karakul sheep farmers in Southern Kalahari, Botswana: management practices and constraints to improving production. *South African Journal of Animal Science*, 4, 23-27.

Powell, B. C., Arthur, J., & Nesci, A. (1995). Characterization of a gene encoding a cysteine-rich keratin associated protein synthesized late in rabbit hair follicle differentiation. *Differentiation*, 58(3), 227-232.

Powell, B. C, and G. E. Rogers. (1986). Hair keratin: Composition, structure and biogenesis. In Biology of the integument. *Springer*, pp. 695-721.

Rogers, M. A, L. Edler, H. Winter, L. Langbein, I. Beckmann, and J. Schweizer. (2005). Characterization of new members of the human type II keratin gene family and a general evaluation of the keratin gene domain on chromosome 12q13. 13. *Journal of investigative dermatology*, 124(3): 536-544.

Rogers, M. A., Winter, H., Langbein, L., Wollschläger, A., Praetzel-Wunder, S., Jave-Suarez, L. F., & Schweizer, J. (2007). Characterization of human KAP24. 1, a cuticular hair keratin-associated protein with unusual amino-acid composition and repeat structure. *Journal of investigative dermatology*, 127(5), 1197-1204.

Rogers, G. R., Hickford, J. G. H., & Bickerstaffe, R. (1994). Polymorphism in two genes for B2 high sulfur proteins of wool. *Animal genetics*, 25(6), 407-415.

Rogers, G. R., Hickford, J. G. H., & Bickerstaffe, R. (1993a). MspI RFLP in the gene for a Type I intermediate filament wool keratin. *Animal genetics*, 24(3), 218-218.

Rogers, G. R, Hickford, J. G, Bickerstaffe R and Woods, J. L. (1993b). *Bsr* I RFLP in the gene for the ovine B2C high-sulphur wool protein. *Animal Genetics*, 24: 69.

Rothauge A. (2009). *Increasing the efficiency of Karakul pelt production by accelerated lambing*. In: AGRA Co-operative LTD Member's Newsletter. RING, Namibia.

Sanguinetti, C. J., Dias, E. N., & Simpson, A. J. (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques*, 17(5), 914-921.

Sulayman, A., Mamat, A., Taursun, M., Huang, X. X., Tian, K., Tian, Y., & Fu, X. (2017). Identification of polymorphisms and association of five KAP genes with sheep wool traits. *Asian-Australasian journal of animal sciences*.

Xu, H. F., Zhao, Z. S., Xue, A. Y., Amina, L. G. L., Ban, Q., & Zhang, J. (2008). Studies on the relation between KAP1.3 gene and 5 breeds of sheep, and with some wool traits. *Journal of Shihezi University (Natural Science*), 26(1): 60-63.

Yardibi, H., Gursel, F.E., Ates, A., Akis, I., Hacihasanoglu, N., & Oztabak, K.O. (2015). Polymorphism of the Kap1.1, Kap1.3 and K33 Genes in Chios, Kivircik and Awassi. 21(4): 535-538. DOI: 10.9775/kvfd.2014.12885.

Zhou, H, H. Gong, W. Yan, Y. Luo, and J. G. Hickford. (2012). Identification and sequence analysis of the keratin associated protein 24-1 (KAP24-1) gene homologue in sheep. *Gene*, 511: 62-5.