

Phenotypic and genetic diversity of domestic yak (*Bos grunniens*) in high-altitude rangelands of Gilgit-Baltistan, Pakistan

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Abstract

Due to climate change, irrigated agriculture may become restricted in the mountain valleys in northern Pakistan in the future. Hence, the importance of yak (*Bos grunniens*) keeping in the mountain ranges as risk-mitigating strategy for mountain dwellers will potentially increase. However, little is known about the current status of the domestic yak in this region. We therefore used phenotypic characteristics and 13 microsatellite loci to determine the phenotypic differences and the level of genetic differentiation between populations of six valleys. Larger body measures and partially different physical appearance were observed in Shimshal and Khaplu yaks, especially when compared with yaks in the Chapurson valley. Overall, the mean observed heterozygosity was similar to the mean expected heterozygosity. Average genetic diversity was highest in the Hopar population and lowest in the Haramosh population. A low F_{IS} value indicated that individuals were less related than expected under a model of random mating. Three distinct genetic clusters were found for the six yak populations under study. Genetic distances were largest between Shimshal and Khaplu populations, and lowest between populations of Phandar and Hopar. It is concluded that yaks of Shimshal, Khaplu and Haramosh valleys were genetically distinct from yak populations in Chapurson, Hopar and Phandar valleys, indicating that the free-range conditions and pastoral yak rearing system in the region have preserved the underlying genetic diversity of the yak populations.

KEYWORDS

genetic diversity, microsatellites, phenotypic diversity, yaks

This paper is dedicated to the memory of Asif Hameed who died in an accident.

†Died December 15, 2020.

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1 | INTRODUCTION

In 2003, the global yak (*Bos grunniens*) population was estimated at around 14.2 million, of which most were concentrated in China and Mongolia. A small remainder is found in the Himalayan region, and a few central Asian countries (Wiener et al., 2003). In China, at least 12 yak breeds have been recognized based on the differences in their outer appearance and performances (Wiener, 2013), and molecular studies proved that nine of these breeds are genetically distinct (Zhang et al., 2008). Various yak types have been described in India that differ in their body size, hair type and coat colour (Pal & Madan, 1996). Similarly, two types of Bhutanese yaks have been differentiated based on their physical characteristics (Dorji & Tshering, 2006) that have been confirmed by microsatellite data (Dorji et al., 2000). In Pakistan, the Hindu Kush-Karakoram-Himalayan and Pamir mountains are key yak habitats. Yaks are usually found at 2000–5000 m above sea level, in areas bordering China, India and Afghanistan (Khan et al., 2016). The region is a major water tower for India and Pakistan, providing water for human consumption, irrigation of agricultural land, generation of energy and for further ecosystem services (Scott et al., 2019). With decreasing glacial melt contributions to river flows (Mukhopadhyay & Khan, 2015), irrigation of agriculture in the mountain valleys may become restricted in the future. Hence, yak keeping at higher altitudes will likely continue to serve as an important risk-mitigating strategy for mountain dwellers, especially because in the Asian context altitudes above 4000 m can only be used by yak due to its specific oxygen metabolism (Ding et al., 2014). The Gilgit-Baltistan (GB) region of Pakistan has an estimated population of 25,000 pure yaks and more than 100,000 yak hybrids (Cai & Wiener, 1995). Especially, the Tibetan/Balti-speaking Muslim population in the Baltistan region is well known for its hybridization practices between yak and cattle (Khan et al., 2016; Rasool et al., 2000). The F1 hybrid offspring is considered a well-adapted animal for lower elevations, suited for ploughing crop fields and threshing purposes. Furthermore, female hybrids are valued for their milk yield that is considerably higher than in pure yaks (Rasool et al., 2000; Wiener et al., 2003). Domestic yaks are important and sometimes the only means of livelihood of transhumant agropastoralists in these areas, providing milk, meat, wool, hairs and hides (Khan et al., 2016; Rasool et al., 2000). Moreover, yaks are of high agro-ecological and socio-cultural heritage value in this system. Nevertheless, their population numbers are declining (Ali, 2015). Lack of a well-structured breeding programme and organization (Wiener et al., 2003), as well as lack of accurate information on the genetic and phenotypic diversity and the status of the existing yak genetic

resources (ILRI, 2014) may lead to an irreversible loss of the genetic diversity and unique external characteristics of yaks, thereby threatening the agro-ecological stability and socio-cultural heritage of the whole system. Hence, there is an urgent need to document the genetic and phenotypic diversity of yak populations and to design strategies for their sustainable conservation in GB. Proving the existence of different yak populations in the area could form the basis for developing a conservation policy and a genetic improvement programme for commercial yak husbandry. This study therefore aimed to assess the morphological and genetic variability of yaks in GB by considering morphological traits and information from microsatellite markers, respectively. Thereby, specific objectives were to (a) compare the body measurements and other phenotypic features of yak populations in GB, (b) assess genetic diversity patterns of yaks among different valleys and (c) evaluate the genetic differentiation of yak populations in the study area.

2 | MATERIALS AND METHODS

2.1 | Ethics statement and consent to participate

This research received ethics approval from the International Centre for Development and Decent Work at the University of Kassel, and written consent from livestock authorities in GB, Pakistan. Before taking body measurements of yaks and sampling tail hair, each herd owner or herder, respectively, was informed about the purpose of the research and how the results would be used and gave oral consent to participate.

2.2 | Study area

Field surveys were conducted from May to November 2018 in the high mountains of the Hindu Kush and Karakoram, in the federally administered GB region of Pakistan, formerly known as “Northern Areas.” The region borders Afghanistan in the Northwest, Tajikistan in the North, China in the Northeast, India in the East and the Chitral district of Khyber-Pakhtunkhwa (KPK) province in Pakistan in the West (Figure 1). It covers a total land area of 72,496 km² and hosts a very diverse and dispersed human population of over 1.8 million. The land-use pattern is dominated by rangelands and seasonal pastures that account for nearly 52% of the total land area, followed by glaciers and mountains (34%), whereas natural forests (<4%) and irrigated agricultural land (<1%) are less common (Beg, 2010). The climatic conditions in GB

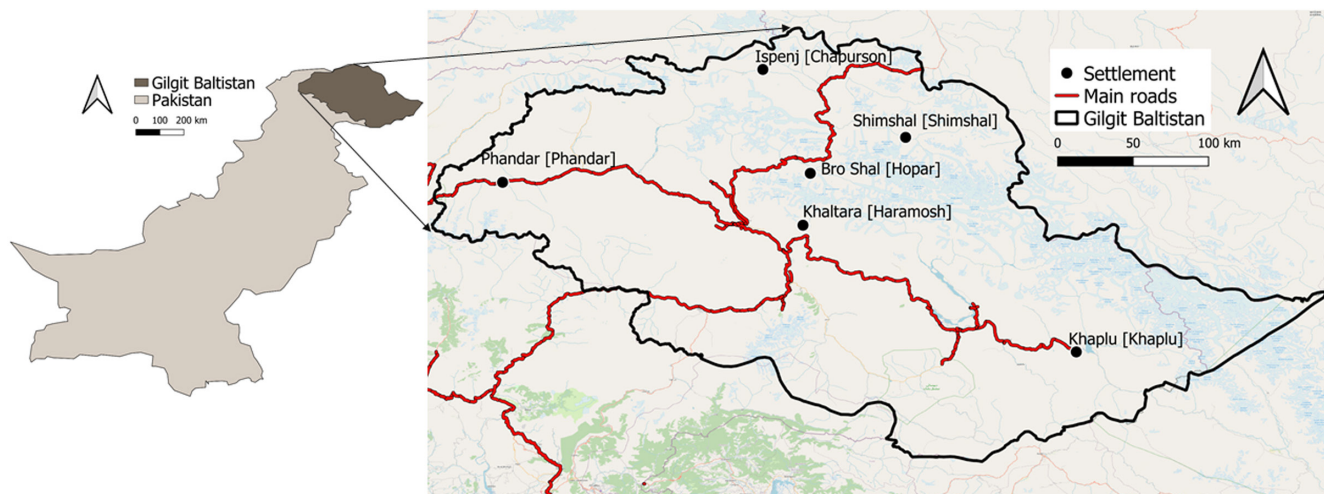


FIGURE 1 Outline of Pakistan and Gilgit-Baltistan (brown) region (left) and regional map (right) depicting the central villages of each of the six sample valleys, with valley names given in parentheses. OpenStreetMap (OSM) data. <https://www.openstreetmap.org/wileyonlinelibrary.com>; <https://planet.openstreetmap.org/wileyonlinelibrary.com> [Colour figure can be viewed at wileyonlinelibrary.com]

are quite harsh, with very dry, extremely cold winters in the higher settlements and hot summers in the western part of GB. Given that less than 2% of the land area is favourable for crop cultivation, there are also resource constraints to agricultural exploitation of the land (Beg, 2010; Khan et al., 2016).

The surveys were conducted in six valleys in GB (Figure 1), which were identified based on information of regional key persons, namely Gilgit-Baltistan Agriculture Department practitioners in all valleys and team members of the Agha Khan Rural Support Programme. The Chapurson valley (comprising the settlements Ispenj, Reshit, Sher-e-Sabz, Shitmirg and Zoodkhun) is located in the North, bordering Afghanistan. The valley is characterized by yak movements through trade with the Wakhan corridor. The Shimshal valley (settlements Aminabad, Shimshal, Farmanabad and Khizrabad) is also located in the North, bordering China. The Haramosh valley (settlement Khaltara) and the Hopar valley (settlement Hakalshall) are located in the centre of GB. The Phandar valley (settlements Barsat, Handarab, Hilti, Phander and Teru) is located in the western part of the region and borders the KPK province. Finally, the Khaplu valley (settlements Baraq Boustash, Jharbango, Ahlli, Kaldag and Kholi) is located in the eastern part of the region bordering the Indian part of Kashmir.

2.3 | Animals

A total of 120 animals (20 yaks/valley; 15 female animals, 5 male animals) were randomly selected for phenotypic description and collection of hair samples for genetic analysis. The sampling only included pure adult yaks

(5.7 ± 0.20 years old) from 20 individual family herds per valley, and their owners or yak herders confirmed that the sampled animals were no yak hybrids. Just one animal per herd was selected to ensure that sampled animals were not closely related to each other. As animals gain considerable weight during the summer season (Xue et al., 2005), it is important to mention the exact months of the sampling for the different valleys, namely May–June for Chapurson, June–July for Hopar, June and September for Haramosh, June and September–October for Phandar, August for Khaplu, and October–November for Shimshal. During summer (May–August), animals were mostly sampled during grazing on the mountain pastures, with the exception of animals in the Hopar valley that were all sampled in the settlement. During autumn (September–November), also the animals from the other valleys were sampled in the settlements.

2.4 | Phenotypic description

Phenotypic description of yaks was based on the FAO guidelines for animal phenotypic characterization studies (FAO, 2012). At the end of the assessment, a digital picture of each animal was taken.

2.4.1 | Linear body measurements

Linear body measurements (in centimetres) included heart girth (HG), height at withers (HW), height at rump (HR), body length (BL), horn length (HoL), interval between ends of the horn (IBH), ear length (EL), hair length (HL) and muzzle circumference (MC).

Measurements were taken with a measurement tape; vertical measurements were supported with a sliding ruler. Measurements were realized early in the morning to avoid the effect of feeding and watering on body measures.

2.4.2 | Qualitative traits

Qualitative traits included coat colour pattern (uniform/plain, spotted and patchy), dominant skin colour (black, blue, brown, grey, yellow and white), ear shape (drooping, erected and horizontal), the presence of horns (yes and no), horn shape (straight and stumpy), horn colour (six colours as stated above), horn direction (backward, forward, inwards, lateral and upwards), backline profile (straight, dipped, slopes up towards rump and slopes down from withers), tail length (short, medium and long), hair type (coarse, normal and glossy) and head profile (straight, concave and convex).

2.5 | Genetic description

At the end of the phenotypic description, hair samples from the yaks' tail were collected for DNA extraction and microsatellite analysis. Each animal was given a unique ID for proper identification of data, samples and photographs.

2.5.1 | DNA extraction from hair samples

Genomic DNA was extracted from hair roots using the NucleoSpin Tissue Kit (Macherey-Nagel) following the manufacturer's instructions. DNA with a ratio of absorbance at 260 and 280 nm of ~1.8, standing for pure DNA was standardized to 50 ng/ μ l using ND-1000 NanoDrop spectrophotometer (NanoDrop Technologies, Inc.).

2.5.2 | Polymerase chain reaction and fragment analysis

Polymerase chain reactions (PCRs) were performed using 12.5 μ l Qiagen[®] PCR Master Mix (Qiagen, N.V.), 10 pM of each primer and 50 ng bovine genomic DNA and filled up to a final volume of 25 μ l with H₂O. The reverse primer was labelled with a fluorescent dye at the 5' end. Microsatellite genotype analysis was performed on an Applied Biosystems 3130 Genetic Analyser (Applied Biosystems, Thermo Fisher Scientific, Inc.) using the Gene Mapper™ software version 4.0 (Applied Biosystems).

2.5.3 | Selection of microsatellite markers

Autosomal microsatellites isolated from most livestock species are abundant and FAO/ISAG lists of recommended markers for genetic characterization studies are publicly available (FAO, 1998). Unlike other livestock species, there is no recommended list of microsatellite markers for yaks, for which cattle microsatellite markers are commonly used for genetic studies (Dorji et al., 2000; Liao et al., 2008; Minqiang et al., 2003; Nguyen et al., 2005; Qi et al., 2010; Reissmann et al., 2017; Sharma et al., 2018; Sujun et al., 2004; Xuebin et al., 2005; Zhang et al., 2008). Based on these studies, 13 highly polymorphic microsatellite loci (BM1824, BM2113, CSSM066, ETH152, ETH185, ETH225, HEL1, HEL13, INRA005, SPS115, TGLA52, TGLA122 and TGLA227) were selected for this study and tested with the total of the sample ($n = 120$ yaks).

2.6 | Data analysis

R version 4.1.3 was used for statistical analyses of phenotypic data (R Core Team, 2022). Linear body measurements were tested for normality by Shapiro–Wilk test and by visual observation of the shape of the frequency histograms, and for variance homogeneity by Levene's test. The effect of the sex, the valley and their interaction on the linear body measures was evaluated by analysis of variance with interaction, followed by Tukey posthoc test for pairwise comparison of yaks in different valleys (linear body measures with equal variances, namely HG, BL and HoL), or by Welch's t test (effect of sex) and Kruskal–Wallis test (effect of valley, valley by sex interaction effect), followed by pairwise Wilcoxon test to compare yaks in different valleys (linear body measures with unequal variances, namely HW, HR, IBH, EL, HL and MC), respectively. Qualitative traits were analysed by Chi-squared-test (effect of sex and of valley on ear shape), or by Fisher's exact test in the case that one or more expected values were <5 (effect of sex, valley and sex by valley interaction on coat colour pattern, dominant coat colour, hair type, head profile, backline profile, tail length; interaction effect of sex and valley on ear shape). Pairwise Fisher's exact test was used to detect differences in the distribution of qualitative traits between yaks (total, male yaks and female yaks) in different valleys. Significance was declared at $p < 0.05$.

Genetic variation parameters such as the number of alleles per locus (N_a), observed (H_o) and expected (H_e) heterozygosity, and the index of fixation of an individual relative to its population (F_{IS} , hereafter referred to as inbreeding coefficient) and their 95% confidence

intervals (Weir & Cockerham, 1984) were determined for each locus and population, using the *adegenet* (Jombart, 2008; Jombart & Ahmed, 2011) and *hierfstat* (Goudet & Jombart, 2015) package within the R environment. Besides, deviations from the Hardy–Weinberg equilibrium (HWE) were checked by Chi-squared-test for each locus in each population with the *hw.test* function from the *pegas* package (Paradis, 2010). GenAlEx software (6.5 version) was employed to calculate the number of effective alleles (N_e) per locus. To determine differentiation between populations, pairwise F_{ST} values (Weir & Cockerham, 1984) and their 95% confidence intervals were estimated with the *hierfstat* package in R using the default parameters. Furthermore, the F'_{ST} value over all loci was calculated using the *basic.stats* function of the *hierfstat* package (Goudet & Jombart, 2015). Additionally, Nei's genetic distances among different populations were estimated using the *dist.genpop* function from the *adegenet* package, and a neighbour-joining tree was computed to visualize the results. All results are depicted as arithmetic mean \pm standard error of the mean unless otherwise stated.

Genetic structure analysis was performed using the STRUCTURE 2.3.4 software (Pritchard et al., 2000) with the parameter values set as follows: length of burnin period, 200,000; number of MCMC (Markov Chain Monte Carlo) repetitions after burnin, 500,000; use admixture model; 20 replicates per K and $K = 1$ –10. Barplots visualizing the average individual cluster membership estimates were created using the R package POPHELPER (Francis, 2017) after aligning the cluster results from separate runs (function *alignQ*) and merging replicate runs for each K (function *mergeQ*). The most likely number of K was determined according to the ΔK method implemented in the R package POPHELPER (Francis, 2017) by identifying K with the highest value of ΔK and the lowest value of the mean posterior probability of the data ($\text{Ln}p[D]$) (Evanno et al., 2005) (see also Figure 3b). In addition, genetic clusters in the data set of six populations were also inferred using the k-means clustering algorithm based on a principal component analysis (PCA, *find.clusters* function in *adegenet*). The optimal value of K , that is the actual number of genetic clusters in the full dataset, was inferred based on comparisons of the Bayesian information criterion (BIC) by identifying the K value corresponding to the lowest BIC (see also Figure 3c) and compared with the most likely number of K based on the Evanno method. As the inferred number of K diverged between the two methods, barplots for K values of $K = 2$ to $K = 4$ are presented for discussion. Microsoft Excel was used for calculation and graphical representation of the results obtained by STRUCTURE 2.3.4.

3 | RESULTS

3.1 | Linear body measurements

According to the Shapiro–Wilk test and visual observation of frequency histograms, the following linear body measures were normally distributed: HG (total sample $p = 0.81$, males $p = 0.48$, females $p = 0.16$, Chapurson $p = 0.31$, Haramosh $p = 0.62$, Hopar $p = 0.59$, Khaplu $p = 0.32$, Phandar $p = 0.55$, Shimshal $p = 0.83$); HW (males $p = 0.11$, females $p = 0.81$, Chapurson $p = 0.81$, Hopar $p = 0.09$, Phandar $p = 0.54$, Shimshal $p = 0.50$); BL (total sample $p < 0.11$, females $p < 0.62$, Chapurson $p = 0.20$, Haramosh $p = 0.24$, Hopar $p = 0.58$, Khaplu $p = 0.36$, Phandar $p = 0.40$, Shimshal $p = 0.25$); HR (males $p = 0.06$, females $p = 0.56$, Chapurson $p = 0.44$, Hopar $p = 0.39$, Khaplu $p = 0.11$, Phandar $p = 0.57$, Shimshal $p = 0.29$); HoL (males $p = 0.07$, Haramosh $p = 0.67$, Khaplu $p = 0.32$, Phandar $p = 0.18$, Shimshal $p = 0.84$); IBH (males $p = 0.15$, females $p = 0.36$, Chapurson $p = 0.78$, Hopar $p = 0.68$, Khaplu $p = 0.52$, Phandar $p = 0.48$, Shimshal $p = 0.39$); EL (Chapurson $p = 0.06$, Haramosh $p = 0.09$, Phandar $p = 0.22$); MC (Chapurson $p = 0.08$, Haramosh $p = 0.55$, Hopar $p = 0.33$, Khaplu $p = 0.28$, Phandar $p = 0.81$, Shimshal $p = 0.58$); and HL (Chapurson $p = 0.79$, Haramosh $p = 0.52$, Khaplu $p = 0.47$, Shimshal $p = 0.92$). There was homogeneity of variances for HG, BL, and HoL for both, male and female yaks ($p = 0.44$, $p = 0.16$, $p = 0.47$, respectively) and yaks in the six valleys ($p = 0.27$, $p = 0.56$, $p = 0.55$, respectively); for HW, HR, BL, and IBH for yaks in the six valleys ($p = 0.28$, $p = 0.17$, $p = 0.57$, and $p = 0.12$, respectively); and for EL, MC, and HL for male and female yaks ($p = 0.77$, $p = 0.15$, and $p = 0.50$, respectively), as assessed by Levene's test for equality of variances. The assumption of normality was violated for HW (total sample, yaks in Haramosh and in Khaplu); BL (males); HR (total sample, Haramosh); HoL (total sample, females, Chapurson, Hopar); IBH (total sample, Haramosh); EL (total sample, males, females, Hopar, Khaplu, Shimshal); MC (total sample, males, females); and HL (total sample, males, females, Hopar, Phandar) (all $p < 0.05$), and variances were unequal for HW, HR, and IBH for male and female yaks, and for EL, MC, and HL for yaks for the six valleys (all $p < 0.05$).

Mean HG, HW, BL, HR, HoL, IBH, EL, MC, and HL across yak populations are presented in Table 1. Linear body measures were influenced by the sex of the animal (HG, BL, HoL $p < 0.001$, analysis of variance; HR, IBH, MC $p < 0.001$, HW and HL $p < 0.05$, Welch's t test), by the valley (HG, BL, HoL $p < 0.001$, analysis of variance; HR, HL $p < 0.001$ and HW, MC $p < 0.01$, Kruskal–Wallis test), and by the interaction between valley and

TABLE 1 Linear body measurements (all in cm) of yaks ($n = 120$) in Gilgit-Baltistan, Pakistan

Linear body measurement	Abbreviation	Mean \pm SEM	Sex (S)	Valley (V)	V \times S
			<i>p</i> -value		
Heart girth ^a	HG	159.5 \pm 1.06	<0.001	<0.001	<0.001
Height at withers ^b	HW	113.0 \pm 0.84	<0.05	<0.01	<0.01
Body length ^a	BL	117.7 \pm 1.01	<0.001	<0.001	<0.001
Height at rump ^b	HR	110.9 \pm 0.83	<0.001	<0.001	<0.001
Horn length ^a	HoL	33.6 \pm 0.91	<0.001	<0.001	<0.01
Interval between horn ends ^b	IBH	47.5 \pm 1.06	<0.001	0.14	<0.05
Ear length ^b	EL	11.0 \pm 0.09	0.08	0.64	0.18
Hair length ^b	HL	24.7 \pm 0.90	<0.05	<0.001	<0.001
Muzzle circumference ^b	MC	39.9 \pm 0.37	<0.001	<0.01	<0.001

Note: V \times S, valley by sex interaction.

Abbreviation: SEM, standard error of the mean.

^aAnalysis of variance with interaction.

^bWelch's *t* test (sex) and Kruskal–Wallis test (valley, sex \times valley interaction) followed by pairwise Wilcoxon test to compare yaks (total, male and female) in different valleys.

sex (HG, BL $p < 0.001$ and HoL $p < 0.01$, analysis of variance; HR, HL, MC $p < 0.001$, HW $p < 0.01$ and IBH $p < 0.05$, Kruskal–Wallis test). Only for EL, no effect of the sex, the valley and V \times S was observed ($p > 0.05$, Welch's *t* test for sex, Kruskal–Wallis test for valley and valley by sex interaction). Similarly, the valley had no effect on IBH ($p > 0.05$, Kruskal–Wallis test for valley) (Table 1).

In general, the measured body parameters were larger in male yaks than in female yaks. The average body measures and *p*-values are given in Table S1. Furthermore, the largest HG was found in yaks from Shimshal and the smallest HG in yaks from Chapurson and Hepar valley (both $p < 0.001$, Tukey HSD test), while the highest values for HW, HR and BL were observed in yaks from Khaplu valley, with significant differences to yaks in Chapurson valley (HW and HR $p < 0.01$), and in Chapurson ($p < 0.001$) and Haramosh ($p < 0.01$, pairwise Wilcoxon rank sum test) valley (Table S2). Yaks in Khaplu valley also had the highest MC, showing significant differences to yaks in Phandar valley ($p < 0.01$, pairwise Wilcoxon rank sum test). The HL was significantly smaller for yaks in Phandar than in Shimshal, Chapurson, Haramosh ($p < 0.001$), and Khaplu ($p < 0.01$; pairwise Wilcoxon rank sum test) (Table S2). A pairwise comparison of linear body measures of male and female yaks across the six valleys showed that in particular female yaks in Chapurson valley tended to be smaller than female yaks in other valleys, while male yaks in Shimshal valley tended to be larger than male yaks in the other valleys. The mean values for linear body measures and the statistical test results of pairwise comparisons are presented in Table S1.

3.2 | Qualitative traits

Plain was the dominant coat pattern observed in most yaks, while patchy and spotted coat colour patterns were less common. No differences in the coat colour pattern were observed between valleys and sexes (both $p > 0.05$, Fisher's exact test), but the valley by sex interaction was significant ($p < 0.01$, Fisher's exact test) (Table 2). Overall, six coat colours were observed in the six studied yak populations, with black being the dominant one. Brown, white, blue, yellow and grey were also observed, but to a much lesser extent. Some differences in the distribution of the dominant coat colour were observed between valleys ($p < 0.001$, Fisher's exact test), and also a significant valley by sex interaction effect was found ($p < 0.01$, Fisher's exact test), while no differences in the dominant coat colours were observed between male and female yaks ($p > 0.05$, Fisher's exact test). Mostly, the body hair was found glossy and normal. The profile of the head was either concave or straight, while convex head profile was less frequently observed. Again, a significant valley effect ($p < 0.05$ and $p < 0.01$, respectively, Fisher's exact test), as well as valley by sex interaction effect (both $p < 0.05$, Fisher's exact test) on the hair type and head profile of yaks was found. Similar to the body hair, male and female yaks had similar hair types and head profiles (both $p > 0.05$, Fisher's exact test). Overall, the most frequent backline profile was dipped/curved (52%), followed by 35% of yaks with a straight back. The backline profile differed according to sex ($p < 0.05$), valley ($p < 0.05$), and valley by sex interaction ($p < 0.01$; all Fisher's exact test). Erected ears were dominant, with significant differences between valleys ($p < 0.05$, Chi-squared-test), whereas no sex and valley by

TABLE 2 Qualitative trait description for yaks ($n = 120$) in Gilgit-Baltistan, Pakistan

Qualitative trait and trait expression	Frequency (%)	Sex (S) <i>p</i> -value	Valley (V)	V × S
Coat colour pattern		0.05	0.35	<0.01
Plain	91 (75.8)			
Patchy	28 (23.3)			
Spotted	1 (0.9)			
Dominant coat colour		0.68	<0.001	<0.01
Black	83 (69.2)			
Brown	12 (10.0)			
White	10 (8.3)			
Blue	7 (5.8)			
Yellow	5 (4.2)			
Grey	3 (2.5)			
Hair type		0.09	<0.05	<0.05
Glossy	53 (44.2)			
Normal	49 (40.8)			
Coarse	18 (15.0)			
Head profile		0.31	<0.01	<0.05
Concave	59 (49.2)			
Straight	48 (40.0)			
Convex	13 (10.8)			
Backline profile		<0.05	<0.05	<0.01
Dipped	62 (51.7)			
Straight	42 (35.0)			
Slopes down from withers	15 (12.5)			
Slopes up towards rump	1 (0.8)			
Ear shape		0.65	<0.05	0.09
Erected	82 (68.3)			
Horizontal	38 (31.7)			
Tail length		0.11	<0.05	<0.05
Long	56 (46.7)			
Medium	49 (40.8)			
Short	15 (12.5)			

Note: Chi-squared-test (effect of sex and of valley on ear shape) or Fisher's exact test (effect of sex, valley and sex by valley interaction on coat colour pattern, dominant coat colour, hair type, head profile, backline profile, tail length; effect of sex by valley interaction on ear shape). V × S: valley by sex interaction.

sex differences were observed in the distribution of ear shapes ($p > 0.05$, Chi-squared-test and Fisher's exact test, respectively). In general, tail length was long or medium, with no differences between sexes ($p > 0.05$, Fisher's exact test). By contrast, the valley and valley by sex interaction significantly influenced the tail length (both $p < 0.05$, Fisher's exact test) (Table 2).

Male yaks in Khaplu and Shimshal valley and female yaks in Chapurson valley tended to have a higher proportion of patchy coat colour pattern; yet all pairwise comparisons were non-significant ($p > 0.05$, Fisher's exact

test) (Table S3). Dominant coat colours were more equally distributed in yaks from Chapurson valley than in yaks from the other valleys (all pairwise comparisons $p < 0.05$, Fisher's exact test). In contrast, yaks in Phandar valley were the least diverse in coat colour, with significant differences to yaks in Chapurson and Haramosh valleys (both $p < 0.05$, pairwise Fisher's exact test). The distribution of dominant coat colours of yaks in the latter valley also significantly differed from that of yaks in Khaplu ($p < 0.05$, pairwise Fisher's exact test) (Table S3). The distribution of hair type was relatively similar between yaks in different valleys,

with the exception of yaks in Haramosh and Phandar ($p < 0.05$, pairwise Fisher's exact test). Concave head profile was predominantly seen in Chapurson and Haramosh valley, with significant differences between the head profile of yaks in Chapurson and Hopar, Khaplu, Phandar and Shimshal, respectively (all comparisons $p < 0.05$, pairwise Fisher's exact test) (Table S3). A higher proportion of dipped backline profiles was observed in female yaks (59% vs. 30%), whereas straight backs (47% vs. 31%) and backs that sloped down from withers (23% vs. 9%) were more common in male yaks ($p < 0.05$, all pairwise Fisher's exact test). Furthermore, the backline profile of yaks in Hopar valley was predominantly straight, whereas in Shimshal valley, a higher proportion of yaks with a sloping down backline were identified ($p < 0.05$, pairwise Fisher's exact test) (Table S3).

The distribution of coat colour pattern was different for male yaks in Phandar valley. Furthermore, a comparatively higher proportion of female yaks in Shimshal had coarse hair, while the predominant hair type of male yaks in Hopar and Phandar valleys was normal. Especially, female yaks in Chapurson tended to have shorter tails than male yaks and female yaks in the other valleys. Yet, pairwise comparisons of valley by sex interactions were not significant (Table S3). Yet, all pairwise comparisons between male and female yaks in different valleys were non-significant ($p > 0.05$, pairwise Fisher's exact test). In particular, a higher proportion of male yaks in Chapurson had a straight backline, while none of them had a dipped backline. Similarly, a dipped backline was less frequently observed in female yaks in Hopar. However, pairwise comparisons were again non-significant ($p > 0.05$, pairwise Fisher's exact test; Table S3).

Horns were present in 84% of the studied yaks, with no sex and valley by sex differences, but the presence of horns depended on the valley ($p < 0.01$, Fisher's exact test). In Haramosh, 35% of the studied yaks had no horns, while in Khaplu, Chapurson and Shimshal valley, polled yaks were not (0%) or barely (5%, 10%) observed. However, pairwise comparisons were not significant ($p > 0.05$, pairwise Fisher's exact test). The horns were mostly lyre-shaped (99%), only one female yak in Chapurson had stumped horns. The majority of horns were directed upwards (71%), while horns that were directed backwards (19%), forwards (5%), laterally (4%) or inwards and backwards (1%) were less frequent. No sex, valley or valley by sex effect on the direction of horns was observed (all $p > 0.05$, Fisher's exact test). Overall, the predominant horn colour was grey (47%), followed by brown (31%) and black (22%), while only one yak had white horns. The horn colour was influenced by the valley and valley by sex interaction ($p < 0.05$ each, Fisher's exact test), while the sex had no effect ($p > 0.05$, Fisher's exact test). Yaks in Shimshal valley

were more likely to have black horns (40%) than yaks in Phandar (5%) and Hopar (0%) (both $p < 0.05$, pairwise Fisher's exact test), whereby in Hopar, a higher proportion of yaks had grey horns (55% vs. 30% in Shimshal; $p < 0.05$, pairwise Fisher's exact test).

3.3 | Genetic diversity of microsatellite markers

A total of 91 alleles were observed for the 13 studied microsatellite loci in the yaks from six valleys in GB. Overall, missing values averaged 0.25%, with the highest value in locus TGLA122 (total 2.5%; Haramosh, Hopar and Phandar 5.0% each) and ETH185 (total 0.83%; Shimshal 5.0%). The mean number of alleles per locus averaged 7 ± 0.49 and ranged from 4 (HEL1) to 11 (TGLA122), while that of effective alleles averaged 2.9 ± 0.23 and ranged from 2 to 4 (Table 3). H_e and H_o varied between 0.520 (SPS115) and 0.791 (TGLA122) and between 0.508 (ETH185) and 0.992 (SPS115), respectively. Hence, the inbreeding coefficient F_{IS} was lowest for SPS115 (-0.904) and highest for ETH152 (0.282). The overall mean for F_{IS} was -0.008 ± 0.077 (Table 3). Overall, more than half of the loci deviated from HWE. Only BM1824, CSSM066, HEL1, HEL13 and TGLA227 were in HWE. Four of these loci (BM1824, CSSM066, HEL1 and TGLA227) were in HWE in all yak populations. There is one locus (SPS115) which was not in HWE in all the studied yak populations (Table S3). The F'_{ST} over all loci was 0.041.

3.4 | Genetic diversity and differentiation of yak populations

The total number of alleles in individual yak populations ranged from 58 (Haramosh) to 69 (Khaplu; Table 4); overall, the total number averaged 62. A total of 19 private alleles were detected, six, four, three, two, and one in the yak populations of Khaplu, Chapurson, Hopar and Shimshal, and Haramosh and Phandar, respectively (Table 4). The highest N_a was observed in yaks in Khaplu (5.3 ± 0.51), and the lowest N_a in yaks in Haramosh (4.5 ± 0.24) and in Phandar (4.5 ± 0.51). The lowest mean H_o (0.618 ± 0.049) and H_e (0.617 ± 0.029) were estimated for yaks in Haramosh, while the yaks in Khaplu and Hopar had the highest mean H_o (0.681 ± 0.047) and H_e (0.669 ± 0.029), respectively. All observed population-specific F_{IS} values significantly differed from zero ($p < 0.05$). It was largest in Hopar (0.039 ± 0.109 , 95% confidence interval -0.1443 to 0.2182) and smallest in Shimshal population (-0.049 ± 0.083 , 95% confidence interval -0.1696 to 0.1114 ; Table 4).

TABLE 3 Characteristics of 13 microsatellite markers used for yak populations in Gilgit-Baltistan, Pakistan

Locus	Size (bp)	Na	Ne	He	Ho	F_{IS}
BM1824	179–195	6	3	0.726	0.717	−0.015
BM2113	125–150	9	3	0.698	0.717	−0.057
CSSM066	171–191	6	4	0.757	0.725	0.004
ETH152	199–211	7	4	0.727	0.521	0.282
ETH185	214–224	7	2	0.690	0.508	0.238
ETH225	140–162	6	3	0.708	0.617	0.078
HEL1	105–111	4	3	0.703	0.592	0.141
HEL13	180–200	8	2	0.613	0.558	−0.012
INRA005	125–151	8	2	0.598	0.583	−0.036
SPS115	232–260	6	2	0.520	0.992	−0.904
TGLA227	69–81	5	2	0.588	0.583	−0.017
TGLA53	148–170	8	4	0.744	0.658	0.097
TGLA122	144–166	11	4	0.791	0.701	0.097
Mean	—	7	2.9	0.682	0.652	−0.008
SEM	—	0.49	0.23	0.021	0.034	0.077

Note: Na: number of alleles, Ne: number of effective alleles, He: expected heterozygosity, Ho: observed heterozygosity, F_{IS} : inter-population fixation index.

Abbreviation: SEM, standard error of the mean.

TABLE 4 Genetic diversity and population structure of six yak populations studied in Gilgit-Baltistan, Pakistan

Population	Nt	Np	Na	Ho	He	F_{IS} (95% confidence interval)
Chapurson	67	4	5.2 ± 0.50	0.646 ± 0.041	0.654 ± 0.027	0.023 ± 0.075 (−0.0819 to 0.1353)
Haramosh	58	2	4.5 ± 0.24	0.618 ± 0.049	0.617 ± 0.029	0.005 ± 0.089 (−0.1230 to 0.1698)
Hopar	61	3	4.6 ± 0.43	0.648 ± 0.064	0.669 ± 0.029	0.039 ± 0.109 (−0.1443 to 0.2182)
Khaplu	69	6	5.3 ± 0.51	0.681 ± 0.047	0.663 ± 0.038	−0.030 ± 0.089 (−0.1449 to 0.1081)
Phandar	59	1	4.5 ± 0.51	0.657 ± 0.044	0.636 ± 0.026	−0.039 ± 0.095 (−0.1674 to 0.1631)
Shimshal	60	3	4.6 ± 0.35	0.660 ± 0.035	0.627 ± 0.017	−0.049 ± 0.083 (−0.1696 to 0.1114)

Note: Nt: total number of alleles, Np: number of private alleles, Na: mean number of alleles per locus, He: expected heterozygosity, Ho: observed heterozygosity, F_{IS} : fixation index. 95% confidence interval based on bootstrapping.

Genetic distances were largest between the Shimshal and Khaplu yak populations (0.244), and the Shimshal and Haramosh populations (0.224; Table 5; Figure 2). The smallest genetic distances were observed between the Phandar and Hopar populations (0.072), and between the Phandar and Chapurson populations (0.079). Also, the genetic distances between the Khaplu and Chapurson (0.122), and the Khaplu and Phandar populations (0.114) were comparatively small (Table 5; Figure 2). Pairwise F_{ST} values generally confirmed the patterns, with the highest value between the Shimshal and Haramosh (0.086, 95% confidence interval 0.045–0.136) and between the Shimshal and Khaplu populations (0.084, 95% confidence interval 0.031–0.146), and the smallest between the Hopar and Phandar populations (0.011, 95% confidence interval −0.002 to 0.024; Table 5).

The STRUCTURE analysis revealed the subdivision of the six yak populations in most likely two to four

genetic clusters (Figure 3a), depending on the method used for inference. Using the Evanno method based directly on the STRUCTURE results, Figure 3b shows the peak for ΔK at $K = 2$ but also high values for $K = 3$ and $K = 4$. Using the k -means clustering based on PCA, the smallest BIC value corresponding to the most likely value of K was estimated for $K = 4$, although $K = 3$ showed an almost identical value. Directly comparing the results from $K = 2$ to $K = 4$ shows that the while two genetic clusters are basically sufficient to describe the overall genetic structure in the sample dataset, $K = 3$ and $K = 4$ display further substructure that is also reflected in the estimated genetic distances. In this regard, especially the estimated genetic distance between the Shimshal and Haramosh valleys is not reflected in the results for $K = 2$.

In more detail, for $K = 3$, the results for the average individual estimated cluster membership proportions

TABLE 5 Pairwise Nei's genetic distance (below the diagonal) and F_{ST} values with 95% confidence interval (above the diagonal) of six yak populations of Gilgit-Baltistan, Pakistan

Population	Chapurson	Haramosh	Hopar	Khaplu	Phandar	Shimshal
Chapurson		0.030 (0.010–0.053)	0.017 (0.006–0.028)	0.032 (0.006–0.055)	0.015 (0.005–0.024)	0.040 (0.016–0.065)
Haramosh	0.106		0.058 (0.027–0.102)	0.057 (0.030–0.093)	0.575 (0.040–0.083)	0.086 (0.045–0.136)
Hopar	0.091	0.174		0.029 (0.003–0.054)	0.011 (–0.002–0.024)	0.031 (0.006–0.045)
Khaplu	0.122	0.160	0.122		0.031 (0.017–0.045)	0.084 (0.031–0.146)
Phandar	0.079	0.161	0.072	0.114		0.042 (0.009–0.071)
Shimshal	0.130	0.224	0.097	0.244	0.128	

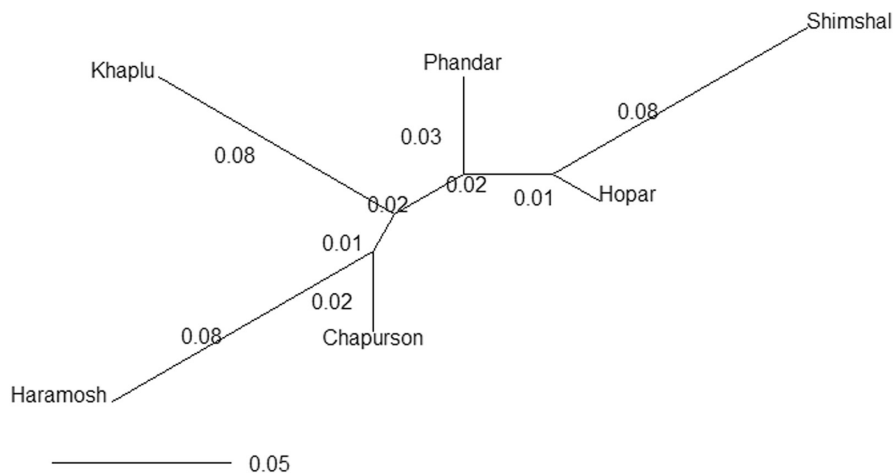


FIGURE 2 Nei's genetic distances between six yak populations of Gilgit-Baltistan, Pakistan: Neighbour-joining relationship tree of sampled yak populations

generally confirmed the results from the neighbour-joining tree based on Nei's genetic distances. The sample populations in the Shimshal, Khaplu and Haramosh valleys build largely homogenous genetic groups with the majority of animals assigned to either Cluster1 (Shimshal), Cluster2 (Khaplu), or Cluster3 (Haramosh). A minority of samples shows mixed ancestry mainly from Cluster1 and Cluster3 with varying contributions. Only the sample populations from the Khaplu valley showed a substantial number of animals clearly assigned to Cluster2. In contrast, in the Phandar, Shimshal and Hopar valleys, only single individuals showed noticeable ancestry contributions from Cluster3.

In contrast to the more homogenous populations in the Shimshal, Khaplu and Haramosh valleys, the sample populations from all other valleys (Chapurson, Hopar and Phandar) show varying degrees of admixture between two genetic clusters. The individuals from the Phandar and Hopar valleys show admixture between Cluster1 and Cluster3 including individuals with equal ancestry contributions from both clusters. Notwithstanding, in the Chapurson and Khaplu valleys, the sample populations show admixture between Cluster3 and Cluster1 or Cluster2, respectively.

4 | DISCUSSION

4.1 | Phenotypic variation

The body measurements indicated that yaks from the Shimshal, Khaplu and Chapurson valleys of the GB region were phenotypically different to the other yak populations. In cattle, another bovid species, hip height, body length and heart girth are moderately to highly heritable (Kamprasert et al., 2019). The same may apply to yaks, and hence, the highly significant differences in body measurements between yaks in the studied valleys may be due to genetic differences. This is also in line with the clustering results which indicate that yaks from Shimshal and Chapurson valleys most likely belong to different genetic groups. Besides the genetic contribution, environmental factors may have contributed to phenotypic differences between the studied yak populations. For instance, the availability of adequate grazing resources has been reported as an important cause for differences in the body size among yaks in different regions (Gyamtscho, 2002). Similarly, the large body size of yaks from Qinghai province in China was related to the abundance of green pastures in these areas (Nivsarkar et al., 1997). The yaks in

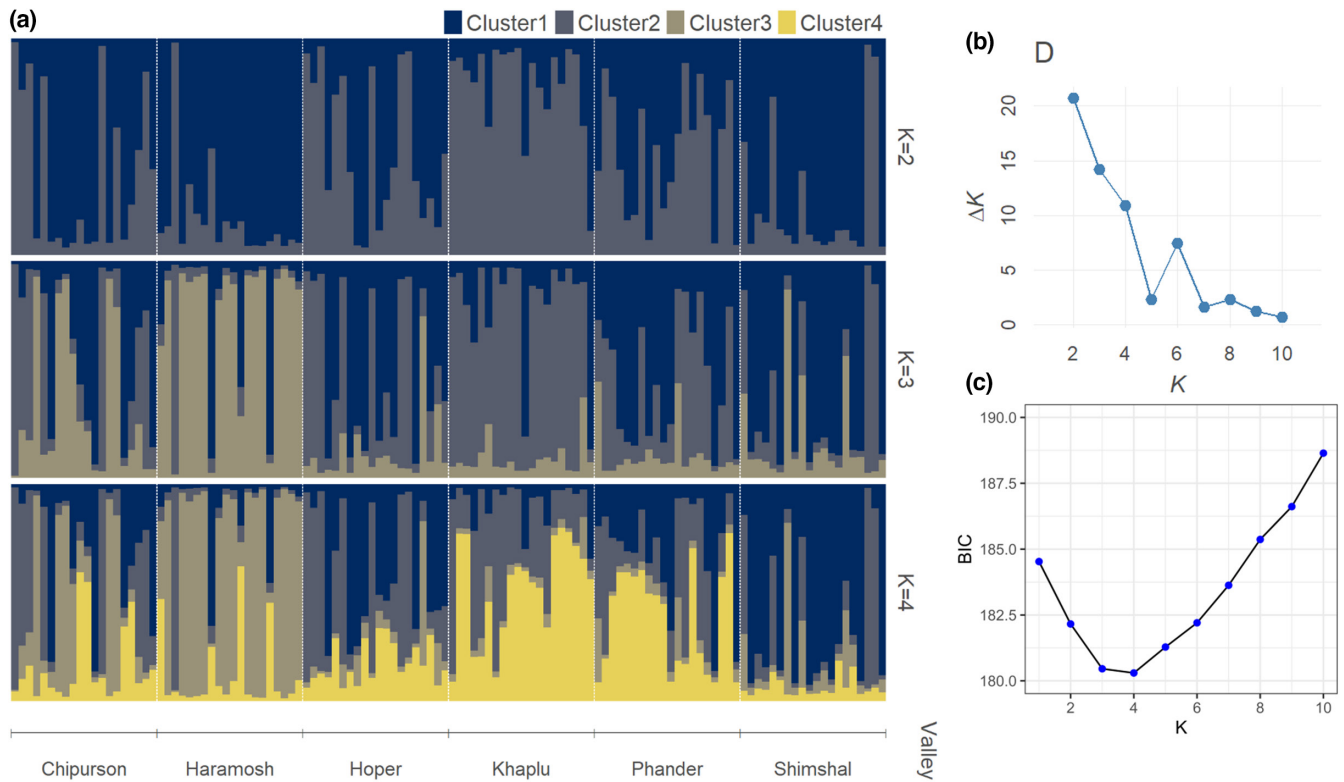


FIGURE 3 Bayesian inference of most likely clusters ($K = 2\text{--}4$) of six yak populations sampled in Gilgit-Baltistan, Pakistan. (a) Each animal is represented by a single vertical bar. Colours in individual vertical bars reflect the likelihood of individual yaks to belong to one of the $K = 2$ to $K = 4$ clusters. Populations are separated by black lines. (b) ΔK and (c) Bayesian information criterion (BIC) showing the most likely number of clusters [Colour figure can be viewed at wileyonlinelibrary.com]

the Shimshal valley had access to good winter pastures, whereas the other populations were brought back to areas with limited grazing resources in winter, there being fed with hay and sometimes concentrate feed. It is unfortunate that the nutritious value of rangeland vegetation in different yak herding zones of northern Pakistan has been barely described (Joshi et al., 2013). In addition, sampling in the Shimshal valley was realized in late autumn, whereas body measurements in the Chapurson valley were taken in spring. Hence, the yaks in Shimshal may have been able to develop a thick layer of subcutaneous fat due to abundant grazing in summer and early autumn (Wiener et al., 2003), which potentially contributed to larger body measurements in Shimshal yaks besides genetic differences.

Variation in qualitative phenotypic traits of yaks in GB region was low. The results from our study are generally consistent with those from studies in other countries. Plain black and brown were the two predominant coat colours observed in our study. Similarly, 68% of Mongolian (Bat-Erdene, 1996) and about 65% of Nepalese yaks (Namikawa et al., 1992) have black coat colour. Black-brown was also reported to predominate in yak breeds of China (Wiener et al., 2003). According to (Cai & Wiener, 1995), dark coat colours of yak are a natural adaptation to protect the

animals against the effects of solar radiation at high altitudes. In Baltistan, black coat colour is a common selection criterion for yak bulls due to a higher tolerance of the cold (Ali, 2015). Hence, black and brown fur coloration are the two main variants in yaks (Zhang et al., 2014). The same authors (Zhang et al., 2014) described white spotting with three different patterns, namely white body stripe, white face with variable patches on the torso and completely white, as another category of fur coloration in yaks. Accordingly, about one-quarter of yaks had white patches in our study, which was similar to 40% of Indian yaks with black and white patched colour (Pal & Madan, 1996).

Lyre-shaped and upwards directing horns were the most common feature found in yaks in our study, and have been also documented in Huanhu yaks of Qinghai (China); similarly, wild yaks possess big black upward- and backward-curving horns (Rhode et al., 2007; Wiener et al., 2003). The overall proportion of polled yaks observed in our study region was 16%, which suggests that there is no directed selection for polled yaks in GB region. Again, this result agreed well with findings of other yak studies. For instance, from Tibet 17% polled yaks were reported, and in India about 10%–12% of yaks are hornless (Pal & Madan, 1996) without directed selection for polled animals (Liang et al., 2016; Liu et al., 2014; Nivsarkar

et al., 1997). In contrast, polled yaks are preferred for easy handling in Mongolia, where 84% of the yaks were without horns (Bat-Erdene, 1996). Likewise, polled bulls were preferably selected for breeding in the Gansu province of China, where the proportion of such animals was as high as 80% (Cai & Wiener, 1995). Besides long hair, large horns have been described as a key external characteristic of yaks in traditional husbandry systems (Liu et al., 2014), whereas the polled phenotype is an economically important trait in more modern commercial yak husbandry systems (Liang et al., 2016).

4.2 | Genetic diversity and differentiation

Genetic diversity and variability provide the basis for any breeding and conservation programme, and microsatellite analysis is a well-established tool for evaluating the genetic relationships between and within-population (Hansen et al., 2002; Liu et al., 2014; Sharma et al., 2018). In the past, different sets of cattle-specific microsatellite markers were used to study population and evolutionary genetics of yaks (Liu et al., 2014; Nguyen et al., 2005). Only recently, a first scientific assessment of molecular phylogeny and genetic diversity of Pakistani yaks was conducted using microsatellite markers (Hussain et al., 2021), which underlines the persistent importance of microsatellite markers for genetic diversity studies, especially for countries in the Global South that often lack the financial and technical capacities for genetic diversity studies based on single-nucleotide polymorphism markers. In our study, we found a total of 93 alleles across 13 loci. Similarly, Xuebin et al. (2005) reported 115 alleles for 15 microsatellite loci in Russian and Mongolian yak populations, while 159 alleles of 16 microsatellites were found in five native Chinese yak breeds (Liao et al., 2008). Considering the number of samples ($n = 120$) and microsatellites used ($n = 13$), GB yak populations showed a similar range of allele abundance as reported in other studies. The highest number of alleles ($N_a = 11$) was found for TGLA122. This is similar to the number of alleles reported for western Mongolian and southeastern Russian yak populations ($N_a = 10$) (Xuebin et al., 2005), but higher in comparison with the number reported for Swiss and Kirghiz yak populations ($N_a = 5$) (Nguyen et al., 2005; Reissmann et al., 2017), as well as for yaks in the Sayan-Altai region in Russia and Mongolia ($N_a = 7$) (Oyun et al., 2018). Another study with Pakistani yak populations in GB and Skardu regions that used 27 microsatellite markers also reported the highest number of alleles for TGLA122 ($N_a = 12$) (Hussain et al., 2021). Eleven loci showed at

least one private allele per population, which is in line with genetic diversity studies of Mongolian and Russian yak populations (Xuebin et al., 2005). However, the number of private alleles per population in our study were generally lower than that reported for Mongolian and Russian yak populations (Oyun et al., 2018). Furthermore, high heterozygosity indicated high genetic variability of yak populations in GB. The mean H_o value (0.652) in our study was similar to that reported for two Chinese yak populations using 12 bovine microsatellite markers (0.64 and 0.66) (Minqiang et al., 2003), as well as for three Bhutanese yak populations using eight microsatellite markers (0.598–0.621) (Dorji et al., 2000). By contrast, lower mean H_o values were found in Pakistani yak populations in GB and Skardu regions (0.483) (Hussain et al., 2021), in Arunachali yak population in India (0.553) (Sharma et al., 2018) and in Kirghiz yaks (0.554) (Reissmann et al., 2017), respectively. The highest H_o value (0.681) in our study was found in the Khaplu population. The mean H_e (0.682) in the studied yak populations was close to the mean H_o , which also confirms that there is no heterozygote deficiency and inbreeding. This is further supported by a negative F_{IS} value (-0.008 ± 0.077). Our results contradict results of a previous study in Pakistani yaks in GB and Skardu regions that showed an average heterozygosity deficit of 0.205 (Hussain et al., 2021). The low inbreeding is probably the result of seasonal transhumance and constant mixing of animals (i.e. random mating) from other populations. Before the demarcation and closure of international borders in the course of the 20th century, traditional transhumance to China, Afghanistan and India had been practiced by the yak herders in GB region for decades (Jasra et al., 2016). More recently, farmers from the northern GB region were involved in yak trading with their Kirghiz neighbours in Afghanistan, and similarly, yaks have been imported from the Chinese Pamirs (Wiener et al., 2003). Farmers in GB specifically obtained good breeding animals from these imports (Wiener et al., 2003), which may have limited the rate of inbreeding in the GB yak populations and contributed to the overall high diversity in the sample population. The low overall F'_{ST} indicates a low genetic differentiation of yak populations across different valleys due to genetic structure. Pairwise F_{ST} values and their 95% confidence interval confirm a poor population structuring.

Genetic distances were largest between the yaks from Haramosh and the other five valleys. The relatively large genetic distances between the yak populations in the Haramosh valley and the yak populations in the other five valleys were not unexpected due to the geographical isolation of this valley which limits the movement of yaks and imposes reproductive isolation (Dorji et al., 2000; Dorji &

Tshering, 2006). The Haramosh valley, albeit centred and geographically close to the yak populations in Hopar and Shimshal (Figure 1), is separated from the other valleys by high mountain ranges and few passages, and hence difficult to access.

The individual estimated ancestry coefficients from the STRUCTURE analysis allowed a more detailed view in this regard. Especially, the Haramosh but also the Shimshal and to a lesser degree the Khaplu populations form mainly homogenous, distinct genetic groups with different ancestry (Figure 3). However, a limited number of individuals with a diverging ancestry compared with the rest of the population in the two valleys are most likely the result of animal trade rather than deliberate crossbreeding. All other valleys show varying contributions of the three genetic groups and higher numbers of admixed animals. Yet, also patterns of local isolation are visible in these valleys (Figure 3). Hence, purebred individuals from Cluster2 are almost absent in the other valleys except Khaplu and Chapurson, where purebred individuals from Cluster1 are substantially less frequent. In this regard, traditional grazing routes are different among the valleys and may serve as an explanation for the exchange of animals and between genetic groups by trade or mating. For example, the grazing routes for the Khaplu and Shimshal valleys extend towards the Leh region of Indian administered Kashmir, and towards China, respectively (Khan et al., 2016). By contrast, yak herders in the Chapurson and Phandar valleys have their traditional trading routes towards Afghanistan (Khan et al., 2016). A significant gene flow from Indian, Chinese or Afghani yak populations into yak populations in Khaplu, Chapurson and Phandar populations may explain the mixed and inhomogeneous ancestry in these three yak populations. Especially, the almost exclusive presence of (purebred) individuals from Cluster3 in the Chapurson and Khaplu valleys should be further investigated using reference samples from regions in the neighbouring countries to answer the question of the original ancestry groups in the yak populations in GB, which is a shortcoming of this study.

5 | CONCLUSIONS

The free-range conditions and pastoral yak rearing system in GB have preserved the underlying genetic diversity of the yak populations. Yaks of the Shimshal, Khaplu and Haramosh valleys in the GB region were genetically distinct from yak populations in Chapurson, Hopar and Phandar valleys. These genetic differences could explain the larger body measures and partially different physical appearance of Shimshal and Khaplu yaks, especially

when compared with yaks in the Chapurson valley. These results provide valuable information for regional and national livestock institutions with respect to planning and implementing of conservation strategies for yaks in this region.

AUTHOR CONTRIBUTIONS

Andreas Buerkert and Eva Schlecht secured funding, and Andreas Buerkert conceptualized the study. Eva Schlecht contextualized the sampling design and supervised its implementation in the field by Asif Hameed; the actual field data collection of Asif Hameed was academically supported by Muhammad Tariq; Asif Hameed analysed the data with the help of Regina Roessler and Carsten Scheper, and compiled a first complete draft of the manuscript; Regina Roessler performed additional statistical analyses, corrected the draft manuscript and compiled the final manuscript for submission. Sven König and Carsten Scheper supported the additional statistical analysis and compilation of the final manuscript. All authors read and approved the submission of the final manuscript. The senior author (Regina Roessler) approved the final manuscript for the first author (Asif Hameed) as the latter died suddenly before the final manuscript was completed. She obtained the written consent of Asif Hameed's widow for publication of the data and findings of the present study.

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CONFLICTS OF INTEREST


The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data can be obtained from the corresponding author upon request.

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