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GENETIC STRUCTURE OF THREE ALBANIAN LOCAL GOAT BREEDS BASED ON MICOSATELLITE MARKERS

SUMMARY

Goats are an important livestock species in the hilly and mountainous area of Albania. The aim of the study was to estimate the genetic diversity of three local goat breeds in Albania by the use of 10 microsatellite markers. A total of 90 animals were selected as representative of three local goat breeds Velipoja, Dragobia and Smokthina. The molecular data were analyzed by different bioinformatics tools to estimate the genetic structure and genetic distances between the selected breeds. A total of 78 alleles were identified for 10 microsatellite loci. The number of alleles ranged from 4 (MAF209) to 11 (MAF70 and SRCRP5). The mean observed and expected heterozygosity values across all polymorphic loci in all populations were 0.573 and 0.67, respectively. The overall value for the polymorphic information content of all microsatellite loci was 0.637, indicating their suitability for genetic diversity analysis. The populations displayed heterozygosity deficit indicating the presence of inbreeding (F_{IS}=0.143). Global breed differentiation (F_{ST}) was 0.018, indicating a very poor genetic differentiation. The Factorial Component Analysis (FCA) and Bayesian based clustering structure analysis indicated the admixture between breeds. AMOVA indicated that only 1.89% of the variation can be explained by the differences between breeds. All breeds display a high genetic diversity, but the differentiation between them is very poor. The high level of inbreeding and admixture between breeds can be explained with the breeding practices, with the small population size and Whalund effect.

Keywords: goats, genetic diversity, microsatellites, genetic differentiation, structure analysis, heterozygosity

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INTRODUCTION

The population of goat in Albania is estimated at 774 thousand (Instat, 2020). Meat production (live weight) on 2020 was 18 thousand tones and milk production 80 thousand liters. The most frequent system of livestock management in Albania is the extensive system, which is characterized by the small-scale family farms with a small number of animals of local breeds. The main purpose of productive activities in these farms is the production of agricultural and livestock products to meet the family's food needs (Jani & Kume, 2018) . In Albania are raised mainly local goat breed. Goats are very important in the socio-economic life of community that raises them. Goats are spread out especially in hilly and mountainous area of Albania. They are resistant to diseases and very well adapted to the climate and local conditions of the region where are raised. Goat biodiversity of different populations is described using qualitative and quantitative traits (Kume *et al.*, 2012), or based on the visible profile (Bozgo *et al*., 2012). Genetic diversity of six local goat breeds is estimated previously by the use of different markers such as microsatellites (Hoda, Hyka, *et al*., 2011), AFLP (Hoda *et al*., 2012), mtDNA (Hoda *et al.*, 2014) in the frame of Econogene project. The present study is focused on three local goat breeds: Dragobia, Velipoja and Smokthina. The population size of these breed is decreasing (Leka, 2019). These goat breeds are raised by the local farmers for meat and milk production. The aim of this study is to estimate genetic diversity of these local goat breeds that are not previously characterized, using microsatellite markers. Microsatellite markers due to their availability, low cost, and sufficient information content remain one of the most common markers for studies of genetic resources of farm animals (Selionova *et al.*, 2021). Microsatellite markers are widely used for genetic characterization of different species such as cattle (Misrianti *et al*., 2022), water buffalo (Ünal *et al*., 2021), goat (Cañón *et al.*, 2006; Lenstra *et al*., 2017), sheep (Cinkulov *et al.*, 2008; Hoda *et al.*, 2009; Peter *et al*., 2007), carp (Biba *et al*., 2015), horse (Machmoum *et al*., 2020) *etc*.

There are many studies focused on the estimation of genetic diversity of goat breeds by the use of microsatellite markers. (Saitbekova *et al*., 1999) have estimated the genetic diversity of eight swiss goat breeds by the use of 20 bovine microsatellites. In order to analyse the genetic relationship among twelve Chinese indigenous goat breeds, twenty six microsatellite markers are used (Li *et al*., 2002). Genetic diversity and relationship between twenty Indian goat breeds were investigated based on 25 microsatellite markers (Dixit *et al*., 2012). Microsatellites are used for the genetic diversity analysis of Gohiliwari (Kumar *et al*., 2009), Ardi (Aljumaah *et al.*, 2012), Mehsana (Aggarwal *et al*., 2007), three Egyptian (Egyptian Baladi, Barki and Zaraibi) and two Italian (Maltese and Montefalcone) goat breeds (Agha *et al*., 2008), Yannan indigenous goat breed (E *et al*., 2019).

MATERIAL AND METHODS

Microsatellite genotyping

Blood samples were collected from 30 unrelated animals per each goat breed: Velipoja, Dragobia and Smokthina, according to the information provided by the farmer. Blood samples were collected with Vacutainer® system, in tubes with EDTA as anticoagulant. DNA was isolated by DNA salt out procedure (Gaaib *et al*., 2011).

A total of 10 microsatellites were used for this study and genotyped as previously described for 6 other Albanian goat breeds (Hoda, Hyka, *et al*., 2011). This panel of microsatellite loci was part of the panel used for the characterization of goat breeds from Europe and Middle East (Cañón *et al*., 2006).

Statistical analysis

The total number of alleles per locus, allelic frequencies, the private allele list, observed (HO) and expected (HE) heterozygosity for each microsatellite loci were computed using the GenAlEx 6.5 (Peakall & Smouse, 2012). Exact tests for Hardy–Weinberg equilibrium (HWE) were applied using the Markov Chain Monte Carlo method (20 batches, 5000 iterations per batch and a dememorisation number of 10,000) as implemented in GENEPOP 4.0 software (Raymond & Rousset, 1995). Polymorphic information content (PIC) was estimated for all markers in all breeds using the Cervus software (Marshall *et al*., 1998).

The Nei's gene diversity (HT), the diversity between populations (DST), the coefficient of gene differentiation (GST) values and allelic richness (AR) per each locus and population were calculated with FSTAT 2.9.4 (Goudet, 1995). The same software was used to compute F-statistics with 1000 permutations. Reynold's genetic distance, gene flow, pairwise FST values were calculated by GENETIX® software (Belkhir *et al*., 2004). STRUCTURE version 2.2 (Pritchard *et al*., 2000) was used for Bayesian clustering assignment, with 5 independent runs for each K between 2-4, applying 300000 MCMC repetitions. The most likely number of K was estimated by comparing the log-likelihood of each K-value and the estimated delta K (DK) were plotted using Evanno method (Evanno *et al*., 2005) as implemented in the Structure Harvester software (Earl & vonHoldt, 2012),. Graphic presentation of these statistics was obtained with the web-based Structure Harvester v 0.6.8 (Earl & vonHoldt, 2012). The factorial correspondence analysis (FCA) for the evaluation of the number of genetic groups was performed by GENETIX® software (Belkhir *et al*., 2004). The population assignment analysis was performed using the procedure proposed by Baudouin *et al*. (2004) as implemented in the GeneClass2 program (Piry *et al*., 2004).

A hierarchical analysis of genetic diversity using the analysis of molecular variance (AMOVA) is carried out by ARLEQUIN v 3.01.(Excoffier *et al*., 2007) in order to determine the partitioning of genetic variation between and within groups and populations. The significance levels were obtained by 1000 permutations.

RESULTS AND DISCUSSION

Genetic variation within and among breeds

The number of alleles (NA) per each locus in three goat populations are shown in Table 1. All markers were polymorphic in each population. The lowest value of expected heterozigosity were displayed by InraBern185 *at* Velipoja (0.341) and Smokthina breeds (0.388). MAF209 displayed the lowest expected heterosigosity *at* Dragobia breed (0.438), P19 is the locus that has the highest expected heterosigosity in all populations (Table 1). The FIS parameter displayed negative and positive values in different loci in each population.

Table 1. Number of alleles (NA), expected heterozygosity (H_E), allelic richness (AR) and inbreeding coefficient (F_{IS}) in each locus and population

	Velipoja				Dragobia				Smokthina			
Loci	NA	H_E	AR	F_{IS}	NA	H_E	AR	F_{IS}	NA	H_E	AR	F_{IS}
INRA023	7	0.801	6.903	0.195	9	0.792	8.863	0.032	9	0.7	8.86	-0.19
INRA063	6	0.63	5.806	-0.076	5	0.625	4.933	-0.173	5	0.645	4.867	0.225
MAF70	9	0.827	8.798	0.181	8	0.736	7.733	0.23	8	0.82	7.93	0.065
SRCRSP5	8	0.775	7.887	-0.083	10	0.833	10	0.143	10	0.688	10	0.066
ILSTS005	5	0.583	4.933	0.199	5	0.648	5	-0.102	6	0.699	5.999	0.211
P19	7	0.857	7	0.586	9	0.848	8.927	0.214	9	0.846	8.93	0.488
MAF209	3	0.444	3	0.127	4	0.438	3.933	0.01	3	0.504	3	0.074
SRCRSP7	6	0.616	5.887	0.005	6	0.72	5.93	0.259	6	0.67	5.933	0.253
ILSTS029	5	0.694	4.997	0.424*	5	0.673	4.999	0.231	5	0.657	5	0.391*
INRABER185	5	0.341	4.806	-0.039	8	0.559	7.797	-0.134	5	0.388	4.966	-0.067
*p<0.05												

Table 2. Total Number of identified alleles per locus (TNA), observed (H_O) heterozygosity, H_T (total expected heterozygosity), D_{ST} (gene diversity between populations), and G_{ST} (genetic diversity among populations), polymorphism information content (PIC), Allelic richness (AR), number of effective alleles (Ne), Shannon's information index (I).

Locus	TNA	Ho	Hs	H_T	D_{ST}	G_{ST}	PIC	AR	Ne	I
INRA023	10	0.748	0.764	0.831	0.067	0.1	0.809	9.072	5.614	1.916
INRA063	6	0.637	0.633	0.631	-0.002	-0.004	0.558	4.942	2.620	1.136
MAF70	11	0.67	0.794	0.797	0.003	0.005	0.763	8.488	4.649	1.681
SRCRSP5	11	0.732	0.765	0.771	0.006	0.009	0.739	9.292	4.241	1.708
ILSTS005	6	0.578	0.643	0.644	0.001	0.002	0.604	5.448	2.699	1.232
P19	10	0.485	0.85	0.863	0.013	0.019	0.840	9.072	6.082	1.947
MAF209	4	0.429	0.462	0.459	-0.003	-0.004	0.414	3.308	1.879	0.769
SRCRSP7	6	0.549	0.669	0.671	0.002	0.003	0.621	5.627	2.891	1.270
ILSTS029	5	0.439	0.675	0.67	-0.005	-0.007	0.614	4.924	2.817	1.219
INRABER185	9	0.467	0.429	0.431	0.002	0.002	0.409	5.932	1.796	0.898
Overall	78	0.573	0.669	0.677	0.008	0.012	0.637	6.611	3.529	1.378

A total number of 78 alleles were found for 10 loci in whole goat population (Table 2). Number of alleles ranged from 4 (MAF209) to 11 (MAF70 and SRCRSP5). In table 2 is shown genetic diversity according to Nei for each marker at the level of whole population. Observed heterozygosity (HO) ranged from 0.429 (MAF209) to 0.748 (INRA02) with a population mean value of 0.573. The values of Nei's genetic diversity range from 0.431 (INRABERN185) to 0.863

(P19) with a mean value 0.677. Diversity within populations (H_S) is 0.669 and between populations (D_{ST}) is 0.008. Meanwhile the G_{ST} value, which indicates the differentiation within breeds compared to the whole population is 0.012. This value of index indicates a very poor genetic differentiation of Albanian goat breeds, where 98.2% of genetic variation is due to the differences between individuals.

The polymorphism information content (PIC) per locus ranged from 0.409 (INRABERN) to 0.840 (P19). That means that all markers are informative except of INRA023 and INRABERN185 that have a PIC value lower than 0.5. Allelic richness varied from 3.308 (MAF209) to 9.292 (SRCRSP).

The Shannon's information index (I) ranged from 0.898 (InraBern185) to 1.947 (P19) with a mean value of 1.378. The number of effective alleles varied from 1.796 (Inrabern185) to 6.082 (P19).

In table 3 is summarized the list of private alleles by goat breeds with their respective frequency. A total of 13 private alleles were found. Only one private allele (MAF70-142) has a frequency of 5%, all other alleles have a very low frequency. In Smokthina breed only two private alleles are found.

Table 3. Summary of private alleles by goat breed

Pop	Locus	Allele	Freq
Velipoja	INRA023	201	0.017
Velipoja	MAF70	162	0.033
Velipoja	P19	172	0.017
Velipoja	INRABERN185	274	0.033
Velipoja	INRABERN185	286	0.033
Dragobia	INRA063	182	0.016
Dragobia	MAF70	164	0.016
Dragobia	MAF209	100	0.016
Dragobia	INRABERN185	266	0.017
Dragobia	INRABERN185	268	0.017
Dragobia	INRABERN185	284	0.017
Smokthina	MAF70	142	0.050
Smokthina	ILSTS005	188	0.033

The mean number of alleles (MNA) ranged from 6.1 (Velipoja) to 6.9 (Smokthina) (Table 4). Mean allelic richness (AR) ranged from 6.158 to 6.874. Each population revealed observed (H_O) and expected (H_E) heterozygosity values higher than 0.5. The values of H_E were greater than H_O in each breed, indicating the presence of inbreeding. All populations displayed heterozygosity deficit (F_{IS}), which confirms the presence of inbreeding. The highest value of F_{IS} was found in Velipoja (17.5%) and the lowest in Dragobia (8.6%). All populations have similar values of allelic richness. The mean effective number of alleles is 3.387 and Shannon index 1.374.

Table 4. Mean Number of Alleles (MNA), Number of Effective Alleles (Ne), Information Index (I), Observed (H_O), Expected (H_E) and Unbiased Expected (uHE) Heterozygosity, Fixation Index allelic (F_{IS}), Allelic richness (AR), and number of private alleles per population

Breed	MNA	Ne	I	Ho	He	uHe	F_{IS}	AR	NPA
Velipoja	6.100	3.413	1.334	0.542	0.644	0.655	0.175	6.158	5
Dragobia	6.900	3.513	1.428	0.628	0.675	0.686	0.086	6.874	6
Smokthina	6.600	3.235	1.362	0.551	0.649	0.660	0.168	6.332	2

Genetic differentiation

Heterozygosity deficit within breed (F_{IS}) ranged from -0.088 (INRABERN185) to 0.432 (P19) with a total of 0.143 for all loci (Table 5).

Table 5. Wright's F-statistics (F_{IT}, F_{IS}, F_{ST}) for each locus and all loci in three Albanian goat breeds

LocName	F_{IS}	F_{IT}	F_{ST}
ILSTS005	0.106	0.108*	0.003
ILSTS029	-0.016*	-0.011*	0.351*
INRA023	0.023	0.137	0.116
INRA063	-0.006	-0.012	-0.006
INRABER185	-0.088	-0.082	-0.006
MAF209	0.072	0.063	-0.009
MAF70	0.157*	0.161*	0.006
P19	0.432	0.444*	0.022
SRCRSP5	0.039	0.050	0.011
SRCRSP7	0.177	0.181*	0.005
Overall	0.143*	0.158*	0.018*

Table 6. Reynold's D_R genetic distance matrix (below diagonal), pairwise F_{ST} distance between breeds (above diagonal) and gene flow (Nm) (in bracket)

	Velipoja	Dragobia	Smokthina
Velipoja		0.016	0.029
Dragobia	0.017 (14.94)		0.009
Smokthina	0.03 (8.09)	0.009 (28.80)	

The highest statistically significant ($p<0.005$) contribute was provided by MAF70 (0.157). F_{IT} values ranged from -0.082 (INRABERN185) to 0.444 (P19), with a value of global heterozygosity deficit of 0.158. Genetic differentiation between breed is measured by F_{ST}. Global breed differentiation (F_{ST}) was 0.018, that is almost equal with G_{ST} value. The only marker that statistically contributed to breed differentiation was ILSTS005 (0.351) ($p<0.005$). All estimates of F statistics were significantly different from zero ($p<0.005$). This is line with the

similar frequency distribution of common allele in each microsatellite locus between populations.

Reynold's genetic distances (D_R) are shown in table 6 (below the diagonal). The smallest distance is between Velipoja and Dragobia. Pairwise F_{ST} values are very low, indicating a poor genetic differentiation between breeds. The smallest differentiation is between Dragobia and Smokthina, which also have the highest gene flow (28.80.).

In the FCA, the first Principal Component accounted for 55.8% of the variance and the second component contributed 44.2% of the variance (Figure 1). The figure 1 shows some level of admixture between breeds. This can be explained with the gene flow between breeds.

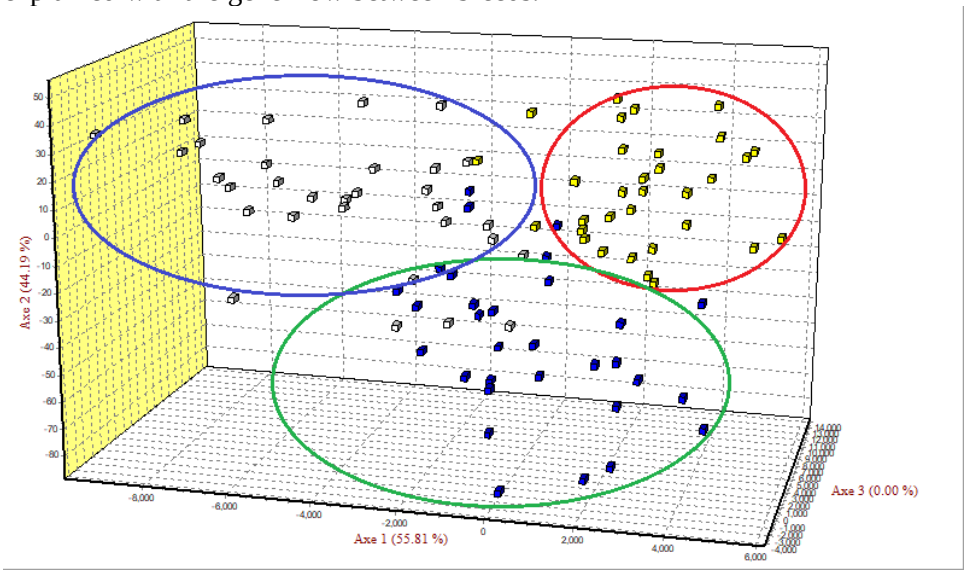

Figure 1. Factorial Correspondence Analyses (FCA) between individuals of three goat populations

In order to investigate breed differentiation, Bayesian clustering analysis was carried out as implemented by the software STRUCTURE. According to Evanno method (Evanno *et al.*, 2005), implemented in the Structure Harvester software (Earl & vonHoldt, 2012), it was assumed that k = 3 is the most likely number of ancestral populations that contribute to the genetic diversity of Albanian goat breeds (Figure 2). Results show admixture between populations, which are in line with the results of FCA.

Results of AMOVA analysis are shown in table 7 about 85.11% of variation is within individuals and variation among goat populations is 1.78%.

Population assignment analysis is performed by the use of GeneClass2 programme (Piry *et al.*, 2004), according to the procedure of (Baudouin *et al.*, 2004). The analysis indicated that 56% of individuals were correctly assigned to the population of origin, with a quality index of 42.60%.

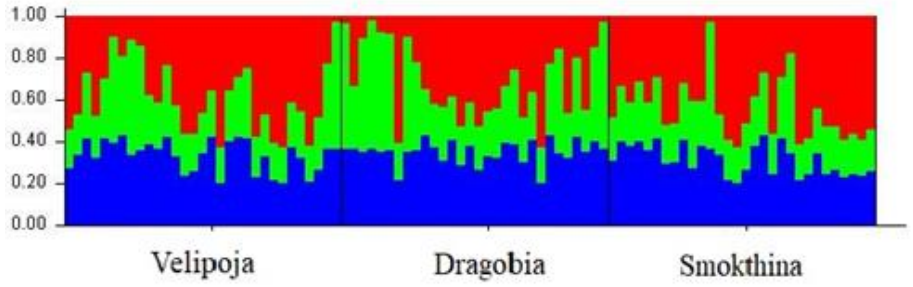

Figure 2. Genetic structure of goat breeds based on microsatellite markers under an assumption of K = 3 using the STRUCTURE software.

Table 7. AMOVA analysis results for all goat populations

Source of variation	Sum of squares	Variance components	Percentage of variation
Among populations	14.996	0.063 Va	1.89
Among individuals within populations	325.109	0.432 Vb	13.00
Within individuals	257.500	2.829 Vc	85.11
Total	597.604	3.324	

Genetic characterization of goat breeds is essential for conservation strategy as well as for genetic improvement (El-Sayed *et al*., 2017). The microsatellite markers used in this study displayed a good level of polymorphism and high values of heterozygosity. The number of alleles (Na) in a population are very important measure of genetic variability. The mean number of alleles was higher than 6 in each breed. All populations displayed similar number of alleles and number of effective alleles. MAF209 have the lowest number of alleles in each population. The average PIC value was 0.637 and only two markers have PIC values less than 0.5. Expected heterozygosity in each population is higher than 0.6 and the values of observed heterozygosity were higher than 0.5. These finding of number of alleles and the heterozygosity demonstrate high genetic diversity of the studied populations. Heterozygosity is essential for populations ant the heterozygous population is usually provides a more genotypes that are able to adapt to the harsh environment. The results indicate that the breeds had high genetic diversity. Some of the reasons for the high genetic diversity observed in a breed might be the overlapping generations, mixing of populations from different geographical locations (Serrano *et al*., 2009). The number of males used for mating is very low. The herd book are lacking and the breeding programes do not exist (Hoda *et al*., 2012). Therefore mixing of population realized by the farmers in each breed may have played role in the poor

differentiation of the goat breeds. The observed heterozygosity was less than expected heterozygosity for ach population. The deviation from Hardy Weinberg equilibrium can be explained by Wahlund effect, inbreeding, selection (Hoda, Hykaj, *et al*., 2011). Another reason of deviation from HWE might be the presence of null alleles. This is supported with the positive F_{IS} values at different marker (P19, ILSTSO) in each population. The level of inbreeding was high (14.3%). Only two loci contribute significantly to this value. Therefore in the high value of inbreeding might contribute the effect of null alleles, the Wahlund effect. Positive values of F_{IS} indicate the presence of inbreeding in each population. Dragobia displayed the lowest inbreeding level. Inbreeding is reported previously (Hoda, Hyka, *et al.,* 2011; Hykaj *et al*., 2013) in other albanian goats populations. The F_{IT} indicate 15.8% general deficit of heterozygous individuals. The positive F_{IS} values are in line with the decreasing of the population size reported by (Leka, 2019).

Genetic differences between populations based on F_{ST} values are low. Pairwise F_{ST} values in all cases are lower than 0.05. Genetic differentiation might be affected by migration and random genetic drift. F_{ST} index indicated a poor differentiation between all breeds (F_{ST} = 1.8%), which is in line with the small genetic distance according to Reynold's and with the high values of gene flow. These breeds are geographically distant and the high gene flow can be explained with the breeding practices. The males are selected by the farmers without having any information about the genealogy, due to lack of herd book. The factorial correspondence analysis and structure analysis indicated admixture between breeds. The most appropriate number of clusters according to structure analysis was 3. AMOVA is applied to estimate the differentiation and genetic similarity between populations. Estimation of genetic diversity within and between populations is fundamental for designing appropriate breeding and conservation programs. AMOVA revealed that most of variation was found within individuals than among populations (1.89%). The gene flow between populations was high which might be the principal source of genetic similarity between populations (Hoda, Hykaj, *et al.,* 2011). The percentage of individuals correctly assigned to the population of origin was not very high (56%), which indicate the existence of genetic similarities between populations The microsatellite markers used in this study were sufficiently informative to estimate the genetic structure and similarity between these breeds. It is very important to maintain genetic diversity therefore breeding strategies that aim to increase effective population size and minimize genetic drift effect must be designed (Serrano *et al.,* 2009). Actually no selection schemes are applied for genetic improvement of the goat breeds. Menezes *et al.,* (2020) suggested efficient herd management for the conservation of genetic resources by avoiding breeding with related individuals, exchanging individuals among herds, and increasing the effective number, which are very appropriate also for our local goat breeds. The data provided in this study must be used for the development of breeding strategy.

CONCLUSIONS

Three local goat breeds are characterized for the first time by the use of 10 microsatellite markers. The set of markers used in this study are satisfactory informative. All goat breeds show high genetic diversity. Genetic differentiation between breeds is very poor. High level of admixture was found between breeds. The level of inbreeding is high due to small population size and Wahlund effect. The results obtained in this study must be used for the development of breeding strategy and for designing the policy how to conserve these valuable genetic resources.

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