

# PRELIMINARY ANALYSIS OF MITOCHONDRIAL AND AUTOSOMAL SNP GENOTYPING DATA SUGGESTS AN ADMIXED GENETIC ORIGIN OF BAZNA PIGS FROM ROMANIA

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## Abstract

*Bazna breed originates in a Transylvanian local pig populations created by crossing of Mangalitza sows with a Berkshire boar in 1872. Subsequently, these populations were improved in different episodes with British (ex. Hampshire) or German (ex. Angeln Saddleback) white belted breeds and cosmopolitan (ex. Large White, Landrace). It was officially recognised as breed in 1958. In the last years the number of Bazna pigs increased from several dozen animals to several hundred, due to an increased market demand for quality pork traditional products. To preliminary verify its presumed admixed origin, the mitochondrial CytB gene was sequenced in Bazna, Mangalitza and Vietnamese pigs (carriers of Asian haplotypes) and Romanian wild boar. Additionally, we analysed with Porcine SNP60 BeadChip few Bazna pigs and some presumed contributing breeds i.e. Mangalitza, Hampshire or cosmopolitan breeds. The NJ tree generated using mitochondrial DNA data evidenced a European (Mangalitza and wild boar) and an Asian cluster (Vietnamese). Some of the Bazna pigs clustered within the Mangalitza group, suggesting its maternal contribution. Other formed distinct sub-clusters suggesting the existence of additional European maternal lineages. Several Bazna pigs clustered in the Asian group, an observation consistent with the presence of Asian haplotypes introgressed via contributing British breeds. The principal component and admixture analysis using SNP data confirmed the admixed origin of Bazna breed.*

**Key words:** *Bazna pigs, mitochondrial DNA, SNP, diversity, admixed origin*

## INTRODUCTION

In the last 50 years the majority of European local pig breeds have experienced a sustained demographic decline due to: an unequal competition with cosmopolitan breeds (ex. Large White, Duroc, Pietrain), indiscriminate cross-breeding, progressive abandonment of rural activities or to some virus outbreaks.

The origin of the European pig breeds is complex and several events shaped their current genetic background. Archaeological evidence suggests that pigs were first domesticated 10,500-10,000 years ago in South-Eastern Anatolia. Analysis of mitochondrial DNA in modern pigs and wild boars revealed that current European pig

breeds do not carry Near Eastern mitochondrial DNA haplotypes (Larson et al. 2005). In contrast, DNA analysis of ancient pig remains from Romania, Germany and France revealed the presence of Near Eastern pigs mitochondrial haplotypes (Larson et al. 2007). The current view is that Near Eastern pigs entered in Europe 7,000-8,000 years BC *via* human migration routes, but afterwards they were rapidly replaced (in a few hundred years) by pigs locally domesticated in Europe (Larson et al. 2005; Frantz et al. 2019). Therefore this first gene flow had a minor influence on the genetic background of the European local pigs.

A second important gene flow from Chinese pigs to British breeds occurred in 18<sup>th</sup>-19<sup>th</sup> Centuries *via* importation, aiming to increase fatness, reproductive maturity and prolificacy. This Far Eastern Asian gene flow had a major influence on the genetic

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background of British breeds. It was spread afterwards in the majority of European pigs breeds (Larson et al., 2005; Fang et al., 2006; Ramirez et al. 2009; Yang et al. 2017). However, it was shown that Mangalitza and Iberian pigs do not carry Near or Far Eastern genetic signature, which supports the theory that Mangalitza is a locally developed European pig breed (Manunza et al. 2013).

A third event that contributed to the current genetic background of some European pig breeds was the gene flow from wild boars. In European local pigs such admixture events, which probably occurred on natural pastures, were documented in Iberian pigs by mtDNA (Van Asch et al. 2012) and autosomal DNA (Manunza et al. 2013) analyses. Such events are also documented in Asia (Ji et al. 2011) and USA (Smyser et al. 2020), where these hybrids represent a real threat for the ecosystems.

In Romania, two local fatty pig breeds *i.e.* Red Mangalitza and Bazna are critically endangered (Figure 1).



Fig. 1 Pictures of Bazna and Red Mangalitza pigs breeds from Romania

Bazna breed (a black coated pig breed with a white belt), originates from a local pig population from Transylvania created by a first crossing of Mangalizia sows with a Berkshire boar in 1872. Subsequently, these populations were improved in different episodes with British (ex. Hampshire, Wessex) or German (ex. Angeln Saddleback) white belted breeds and other cosmopolitan breeds (ex. Large White, Landrace). It was officially recognised as breed in 1958.

In the last few years the number of Bazna-like pigs increased from several dozen animals to several hundred due to an

increased market demand for high quality pork traditional products. However, from conservation point of view this raises a fundamental question: Which are the real Bazna pigs?

Although historical data suggest an admixed origin of Bazna pigs, their genetic background was not properly investigated so far. Therefore, the objective of this work was to obtain preliminary information regarding the genetic history of this breed, using genomic analysis tools.

## MATERIAL AND METHODS

To verify this hypothesis we sequenced the mitochondrial cytochrome B (*CytB*) gene in Bazna pigs (BR; N=14) *versus* Red Romanian Red Mangalitza (RedR; N=12), Vietnamese (ViR; N=9) (carriers of Asian mitochondrial haplotypes) pigs and Romanian wild boar (wR; N=36), collected from different regions.

PCR amplification was performed in 25  $\mu$ l reactions using a 2X GoTaq G2 master mix (Promega, USA), 10 pmol of each specific primer (Alves et al. 2003) and 50 ng of genomic DNA. The amplification protocol was: 94 °C for 3 min followed by 35 cycles of 94°C for 1 min, 58 °C for 1 min, 72 °C for 1 min. Purification was performed with ExoSAP-IT and the sequencing reactions with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific, USA). The sequencing products were analysed after purification on a SeqStudio apparatus (ThermoFisher Scientific, USA).

The analysis of the sequencing data was performed using the BioEdit software. A fragment of 800 bp from *CytB* gene was used to generate the Neighbour Joining (NJ) Tree with MEGA7 software. DnaSP software was used to assess haplotypes diversity.

Additionally, we used a Porcine SNP60 BeadChip data set to preliminary compare the genetic background of few Bazna pigs (BR; N=4) *versus* some presumed contributing pig breeds *i.e.* Red Mangalitza from Romania (RedR; N=25), Mangalitza with Hungarian origin *i.e.* Red (RedH; N=18) and Blond (BlondH; N=24) and British breeds *i.e.* Hampshire (Ha; N=11), Landrace

(La; N=25), Large White (LW; N=23), and Pietrain (Pi; N=16). The bioinformatics analysis of the autosomal SNP data was performed using several tools: PLINK v. 1.07, Admixture v. 1.23, FastStructure, Structure and Structure Harvester.

## RESULTS AND DISCUSSIONS

Mitochondrial DNA variation (CytB) was absent in Romanian Red Mangalitza (RedR) pigs, only one haplotype being identified. This was not unexpected since the analysed pigs were sampled in a small and inbred population (less than 100 pigs), which was reproductively managed relatively closed.

Surprisingly, a very low diversity was noticed as well in Romania wild boar (wR). This information is in contrast with the current estimated number of wild boars (over 50,000). In Romania wild boars is the most successful game specie, flourishing in extremely diverse wild or agricultural ecosystems from Carpathian basin. However, in the last two centuries this specie suffered an important demographic decline due to overhunting, diseases outbreaks or habitat destruction, which explains the reduced diversity and inbreeding.

In contrast, in Bazna pigs a high diversity of CytB gene was noticed, with 6 different haplotypes out of 14 being identified. This suggests the existence of several maternal lineages. This is surprising information, since this ancient population is composed today of less than 100 animals and has been closed reproductively for several decades. This important mitochondrial haplotype diversity could suggest an admixed origin of this breed, which is plausible.

The NJ tree generated using mitochondrial CytB data evidenced two clusters *i.e.* European (Mangalitza and wild boar) and Asian (Vietnamese) (Figure 2). The majority of Romanian wild boar (wR) clustered tightly in two close sub-clusters, observation which is in agreement with the reduced haplotypes diversity (Figure 2).

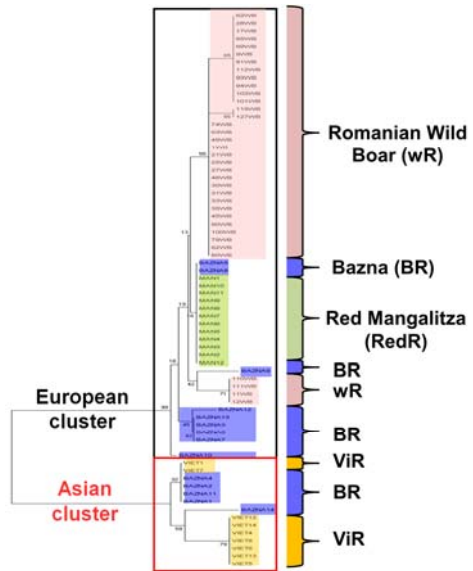


Fig. 2 The NJ tree generated using mitochondrial DNA (CytB) data depicting the genetic relationships amongst Bazna pigs (BR) versus Red Romanian Mangalitza (RedR) Vietnamese (ViR) pigs and Romanian wild boar (wR)

Four Romanian wild boars (wR) clustered in a Bazna (BR) pigs sub-cluster, suggesting a possible gene flow between pigs and wild boars. This was not unexpected since these samples were collected from Dobrogea region near Black Sea, where hybridization often occurs.

Mangalitza pigs (RedR) formed one cluster, an observation in agreement with the absence of diversity at CytB level (Figure 2).

Bazna (BR) pigs were distributed in both European and Asian cluster. In the European cluster some of the Bazna pigs clustered within the Mangalitza group. This observation suggests a maternal contribution of Mangalitza to Bazna breed genetic makeup. Other Bazna pigs formed distinct sub-clusters suggesting the existence of additional European maternal lineages. Several Bazna pigs clustered in the Asian group as the Vietnamese pigs (Figure 2). This observation is consistent with the presence of Asian haplotypes in Bazna breed, which were introgressed in the past in Bazna breed *via* contributing British breeds.

The  $F_{ST}$  value (coefficient of genetic differentiation) indicated strong genetic differentiation between Bazna (BR) and Romanian wild boar (wR). Similar values were observed between Mangalitza (RedR) and wild boar (Table 1).

At the same time Bazna pigs (BR) were more differentiated from Vietnamese pigs (ViR) and less differentiated from Mangalitza (RedR) (Table 1).

Table 1  $F_{ST}$  values (CytB data) depicting the genetic differentiation between Bazna versus other European pigs (Mangalitza) or Romanian wild boar and Asian pigs (Vietnamese).

BR	ViR	RedR	wR	
0				BR
0.410	0			ViR
0.272	0.873	0		RedR
0.933	0.970	0.981	0	wR

However these  $F_{ST}$  indices values are high enough to sustain an important level of genetic differentiation between these breeds.

The autosomal SNP genotyping data were used to perform the population genetics analyses. A MDS plot was built based on genome-wide identity-by-state pairwise distances calculated with PLINK, in order to evaluate the genetic relationships between investigated pig breeds and Romanian wild boar (Figure 3).

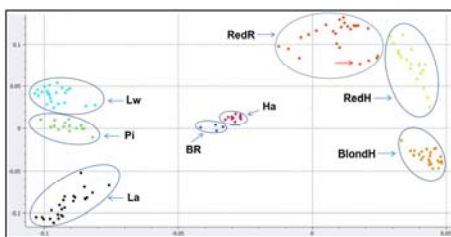


Fig. 3 Multidimensional scaling plot (MDS) generated using autosomal SNP data depicting the genetic relationships amongst Bazna pigs versus Red and Blond Mangalitza, Hampshire and cosmopolitan breeds (Landrace, Large White and Pietrain)

The principal component analysis (PCA) separated the analysed breeds in three main groups. The cosmopolitan breeds (Landrace, Large White and Pietrain) formed a distinct cluster, which is clearly differentiated from

Mangalitza pigs (RedR, RedH and BlondH) cluster (Figure 3). Bazna and Hampshire pigs clustered very close to each other (but in two different groups) and occupied an intermediate position between the other two main clusters (Figure 3). This observation confirms that these breeds share a common genetic background, which is expected since belted British pig breeds had a significant contribution to Bazna breed formation.

In the Mangalitza group, BlondH pigs formed a distinct sub-cluster compared with RedR and RedH, which clustered closer, but independently, in two separate groups (Figure 3). In RedR group four individuals (indicated with a red arrow in Figure 3) show a very close affinity to RedH group, suggesting a common genetic background.

The findings are supported by the calculated values of the coefficient of genetic differentiation ( $F_{ST}$ ) (Table 2). In the cosmopolitan breeds (Landrace, Large White and Pietrain) this coefficient ranged from 0.108-0.125, evidencing a close relatedness between these breeds. The same observation was noticed in the case of the two Red Mangalitza populations (RedR and RedH), although a  $F_{ST}=0.115$  is high enough to sustain a certain level of genetic differentiation (Table 2). From conservation point of view, this is a significant find considering the high inbreeding coefficients found in both RedR and RedR populations (data not shown). This information could be further exploited in breeding schemes to widen the diversity of both populations. In BlondH the  $F_{ST}$  value was higher (0.170) compared with RedR, but lower compared with RedH (0.132) (Table 2). These findings are in agreement with the clustering observed in MDS plot (Figure 2).

Bazna pigs show a significant level of genetic differentiation in relation with cosmopolitan breeds (Landrace, Large White and Pietrain), ranging from 0.202-0.207 (Table 2). This coefficient in cosmopolitan breeds was higher in relation with Mangalitza (0.234-0.259). An intriguing high  $F_{ST}$  value (0.329) was obtained in Bazna pigs versus Hampshire, although they clustered closely in the MDS plot (Figure 2). This might signify that even they share a common gene pool, the contribution of other breeds might have been more significant.

Table 2 Populations  $F_{ST}$  values (SNP data) depicting the genetic differentiation between Bazna pigs versus Red and Blond Mangalitza, Hampshire and cosmopolitan breeds (Landrace, Large White and Pietrain)

RedH	RedR	BlondH	BR	Ha	La	LW	Pi	
0								<b>RedH</b>
0.115	0							<b>RedR</b>
0.132	0.170	0						<b>BlondH</b>
0.259	0.234	0.259	0					<b>BR</b>
0.279	0.259	0.274	0.329	0				<b>Ha</b>
0.201	0.180	0.212	0.207	0.241	0			<b>La</b>
0.204	0.175	0.223	0.202	0.238	0.125	0		<b>LW</b>
0.206	0.181	0.218	0.207	0.243	0.108	0.115	0	<b>Pi</b>

Population structure analyses evidenced the admixed genetic origin of Bazna pigs. At  $K=2$  several common genetic backgrounds were observed: 1) Landrace, Large White and Pietrain; 2) Bazna and Hampshire; 3) Red Mangalitza from Romania and Hungary and Blond Mangalitza (Figure 4).

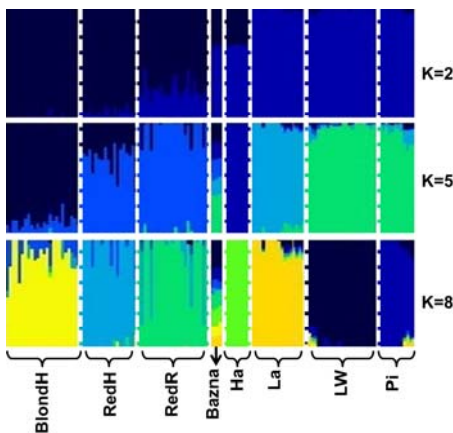


Fig. 4 Admixture analysis generated using autosomal SNP of Bazna pigs versus Red and Blond Mangalitza, Hampshire and cosmopolitan breeds (Landrace, Large White and Pietrain) at different K values

The first signs of admixture in Bazna pigs were clearly noticed at  $K=5$ , visible as a multicolour admixture diagram (Figure 4). This signifies a common genetic background shared between Bazna breed and the presumed contributing breeds analysed.

A significant find is the contribution of Mangalitza pigs to the formation of Bazna breed, observation in agreement the results obtained with mitochondrial CytB data (Figure 2). At the same time, the contribution of British pigs gene pool (ex. Hampshire) is

evident, in agreement with historical data. This observation was persistent until  $K=8$  (Figure 4), which according to the calculations is the most probable number of clusters. This is in agreement with the number of analysed populations / breeds *i.e.* eight. Indeed, Red Mangalitza from Romania (RedR) and Hungary (RedH) might be judged phenotypically as belonging to the same variety / breed, but as was shown earlier they display a surprisingly high level of genetic differentiation ( $F_{ST}=0.115$ ; Table 2). This could be probably due to their past isolation or to a distinct origin. Indeed, RedR Mangalitza pigs analysed in this study were sampled in an old population, which was reproductively managed relatively closed until recently. Surprisingly, in four RedR individuals we found signs of RedH introgression (Figure 4, blue lines). This might be explained by a recent use of RedH boars to reduce inbreeding of the RedR population. This observation explains the clustering of the four RedR pigs closer to the RedH group (Figure 3). These preliminary results are the basis of a more comprehensive on-going study, aiming to clarify the complex origin of the Bazna pigs.

## CONCLUSIONS

Both types of genetic markers (mitochondrial and autosomal) employed in this study confirmed the admixed origin of Bazna pigs. The NJ tree generated using mitochondrial DNA data evidenced a European (Mangalitza and wild boar) and an Asian cluster (Vietnamese). Bazna pigs were distributed in both. In the European cluster, a significant find was the clustering of some Bazna pigs in Mangalitza sub-cluster, which

is consistent with the contribution of Mangalitza maternal lineages to its genetic makeup. Several Bazna pigs formed distinct sub-clusters, suggesting the contribution of additional European maternal lineages. On the other hand, several Bazna pigs clustered in the Asian group together with Vietnamese pigs. This observation is consistent with the presence of Asian haplotypes in Bazna breed introgressed *via* contributing British breeds.

The autosomal SNP data analysis confirmed the admixed origin of Bazna breed. A significant find was the contribution of Mangalitza pigs to the formation of Bazna breed, in agreement with mitochondrial results. At the same time, the contribution of the British gene pool (ex. Hampshire) to Bazna pigs genetic background was evident.

Due to its superior meat quality Bazna can well be the next fatty pig success story, similar to Iberian and Mangalitza, if reliable conservation plans are implemented.

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