

GENETIC CHARACTERISTICS OF THE LAST AUTOCHTHONOUS HERD OF MYRHOROD PIG BREED BASED ON MITOCHONDRIAL DNA ANALYSIS

K.F. Pochernyaev¹, S.M. Korinnyi¹, A.A. Getya², V.G. Tsybenko¹, P.A. Vashchenko³

¹ The Institute of Pig Breeding and Agroindustrial production
of the National Academy of Agrarian Science of Ukraine,
1, Shvedska Mohyla Str., Poltava, 36013, Ukraine

² The National University of Life and Environmental Sciences of Ukraine
of the Ministry of Education and Science of Ukraine,
15, Heroiv Oborony Str., Kyiv, 03041, Ukraine

³ The Poltava State Agrarian University of the Ministry of Education and Science of Ukraine,
1/3, Hryhoriia Skovorody Str., Poltava, 36003, Ukraine

E-mail: * k.f.pochernyaev@gmail.com, korinny_sergey@ukr.net, getya@ukr.net,
dpdgim.dekabristiv@gmail.com, p.a.vashchenko@gmail.com

ORCID: <https://orcid.org/0000-0001-9973-6429>,
<https://orcid.org/0000-0002-1649-3079>,
<https://orcid.org/0000-0002-4747-9261>,
<https://orcid.org/0000-0003-3932-8508>,
<https://orcid.org/0000-0002-9287-819X>

Received May, 22, 2026; revised August, 11, 2026; accepted August, 24, 2026

Aim. To identify mitochondrial haplotypes in the last autochthonous herd of Myrhorod pigs, to assess haplotype diversity, evolutionary divergence, and the evolutionary history of the breed in order to adjust the strategies for its restoration. **Methods.** The biological material was collected in 2011 and 2017 ($n=25$) and ($n=84$), respectively. Mitochondrial haplotypes of pigs were determined using a multisite PCR-RFLP assay. The population and statistical analyses were performed using GENALEX 6 software. The evolutionary analysis was conducted in MEGA12. **Results.** Over two generations in the last autochthonous herd of the Myrhorod breed, which was raised under intensive conditions, the total number of mitochondrial haplotypes decreased from 4 to 3. The disappearance of the genealogical family with haplotype *I* does not eliminate the m.15714C allelic variant from the population, as it persists in sows with haplotypes *A* and *C*. The loss of the maternal line with haplotype *I* did not reduce the level of haplotype diversity in the Myrhorod breed population; on the contrary, it increased from ($h=0.077$) in 2011 to ($h=0.153$) in 2017. An analysis of the number of base substitutions per site between sequences revealed evolutionary divergence among the sequences defining the haplotypes. The lowest evolutionary divergence (number of substitutions per site, $d=0.058$) was observed between the sequences defining haplotypes *A* and *C*. The greatest divergence ($d=0.298$) was observed between haplotypes *O*, *C*, and haplotype *JI*, which was used as the outgroup. An assessment of the divergence among the haplotypes of Myrhorod pigs and their evolutionary history demonstrated their affiliation with European domestication events. The haplotype of the Asian wild boar (AF136564) and the Meishan breed (AF136567), used as an outgroup in the phylogenetic analysis, formed a separate branch on the optimal tree with a total branch length ($d=0.359$) clearly demonstrating the differentiation between European and Asian domestication events. Three of the six SNPs studied — m.15616T>C, m.15714T>C, and m.15758T>C — influenced the level of haplotype diversity in the Myrhorod breed population. Among the polymorphic allelic variants of mitochondrial DNA, SNP m.15714C ($q=0.960$) was the most frequent in 2011, and m.15758C ($q=0.917$) — in 2017. A significant deviation was observed between the expected variance and the observed variance ($V_o^{2011}=0.157$, $V_e^{2017}=0.487$) and ($V_o^{2017}=0.161$, $V_e^{2017}=0.509$). **Conclusions.** 1. An as-

assessment of the evolutionary divergence among the haplotypes of Myrhorod pigs and their evolutionary history demonstrates their connection to European domestication events. The presence of four mitochondrial haplotypes in this local breed suggests the influence of introgression from domesticated pigs or breeds that evolved in different geographic regions of Europe. **2.** While developing the Myrhorod pig breed, boars of the Berkshire, Middle White, Large White, Large Black, and Tamworth breeds were used, none of which could pass on Asian mitochondrial haplotypes. **3.** Over two generations in the last autochthonous herd of the Myrhorod breed, which was kept in conditions of competition with modern transboundary breeds, the total number of mitochondrial haplotypes decreased from 4 to 3. Paradoxically, the level of haplotype diversity in the Myrhorod breed population increased after the loss of the maternal line with haplotype *I*. In light of this, preserving maternal lines with different haplotypes is of greater practical importance for the genetic characterization and management of the local breed than haplotype diversity. **4.** To preserve and restore the Myrhorod pig breed, given the current situation, the most effective approach may be one based not on searching for the original local genetic material, but rather on identifying and utilizing the marker haplotypes we have identified that have been introgressed into the Large White, Poltava Meat, and Red White-Belted breeds.

Keywords: pigs, mitochondrial haplotypes, haplotype diversity, Myrhorod breed, local breed, breed restoration, genetic characterization.

DOI: <https://doi.org/10.15407/agrisp13.02.003>

INTRODUCTION

The diversity of animal genetic resources for food production and agriculture is a global public good that must be properly managed to ensure that livestock farming makes a sustainable contribution to global food security and nutrition (Ajmone-Marsan P et al. 2023). Local breeds are generally less productive than highly developed international cross-border breeds, but they are adapted to the local climate and often have excellent consumer qualities in terms of meat and fat products. One of Ukraine's local breeds is the Myrhorod pig breed. Unfortunately, in August 2018, the only breeding herd of the Myrhorod breed was culled due to an outbreak of African swine fever. A small number of Myrhorod pigs remained uninfected and were preserved. Specifically, 9 animals were kept at the research station of the Institute of Pig Breeding and AIP of the NAAS, and 14 animals were kept on private farms.

The overall goal of the restoration is to clarify the relationships among a group of local breeds and their relationships with other breeds in the same geographic region, especially if these breeds are the ancestors of one or more local breeds (Ajmone-Marsan et al. 2023, Gao et al. 2023, Nguyen et al. 2026).

For example, research by (Simčič et al., 2015) suggested that a conservation and restoration approach that does not rely exclusively on identifying the original, ancestral genetic basis, but rather on identifying and transferring common introgressed haplotypes, would be more effective. The successful implementation of such an approach must be based on a com-

bination of phenotypic data, pedigree data, and genome-wide haplotypes of the breed of interest and a range of reference breeds that have potentially made a direct or indirect historical contribution to the genetic composition of the breed being restored. Today, the origins of domestic pigs can be traced back to specific European and Asian domestication events (Zhang et al. 2022, Jain et al. 2024, Han et al. 2025).

For example, the studies of phylogenetic relationships between Asian and European pig breeds using mitochondrial DNA D-loop sequences, conducted by (Kim et al., 2002) demonstrated that indigenous Asian pig breeds are clearly different from European-type pigs. European pig breeds consist of animals with both Asian- and non-Asian-type mitochondria. For example, Asian pigs contributed to the development of the Berkshire and Large White breeds. The presence of Asian pig mtDNA sequences in some major European breeds (Large White and Landrace) most likely reflects the documented introgression of Asian germplasm into European stocks during the 18th and 19th centuries (Kijas et al., 2001). Fossil evidence shows that even in the Neolithic, European pigs had a small proportion of genes from Southwest Asian origins (Erwen et al. 2025).

The history, unique features, and genetic characteristics of the Myrhorod breed were described based on nuclear gene analysis (Vashchenko et al. 2019). Historical records of the Myrhorod breed development indicate crossbreeding of local short-eared Black-and-White pigs from the Poltava region with Berkshire, Middle White, and Large White breeds,

and to a lesser extent with Large Black and Tamworth breeds; therefore, it is possible to assume the presence of Asian-type mitochondria as well. Since mitochondrial DNA is inherited exclusively through the maternal line and does not undergo recombination, this allows us to trace phylogenetic relationships and maternal lines and to understand how the breed has evolved over the centuries.

The aim of the work. To identify mitochondrial haplotypes in the last autochthonous herd of Myrhorod pigs, to assess haplotype diversity and evolutionary divergence among haplotypes, and the evolutionary history of the breed in order to obtain data that will help adjust the approaches to its restoration.

MATERIALS AND METHODS

The biological material was collected in 2011 and 2017 ($n=25$) and ($n=84$), respectively, at the breeding farm of the State Enterprise «Experimental Farm named after the Decabrists» of the Institute of Swine Breeding and Agro-Industrial Production of the National Academy of Agrarian Sciences of Ukraine, Poltava Oblast, Myrhorod District, village of Velykyi Bairak. DNA was extracted from blood using Chelex-100

ion-exchange resin according to (Walsh et al. 1991). The blood samples remaining after DNA extraction were applied to paper and dried for long-term storage. The detailed information about dry blood samples can be found at <https://docs.google.com/spreadsheets/d/118TkR8v9wMS2r4il-Lnk4bmFVcvRtC4KA4Ra-LNUZKOQ/edit?pli=1&gid=0#gid=0>

The mitochondrial haplotypes of pigs were determined using a multisite PCR-RFLP assay, as described by (Pochernyaev 2016). The multisite PCR-RFLP allows for the differentiation of domestic pig breeds and subspecies of the wild boar into 15 mitochondrial haplotypes based on six SNPs (m.15556T>C, m.15580C>T, m.15616T>C, m.15714T>C, m.15758T>C, m.15917A>T). If, upon depositing the sequence in GenBank, the reference point is specified as the start of the D-loop control region, the SNPs are localized as follows (m.121T>C, m.145C>T, m.180T>C, m.279T>C, m.323T>C, m.478A>T) (**Table 1**).

The population and statistical analyses were performed using GENALEX 6 software (Peakall et al. 2012). The evolutionary analysis was performed in MEGA12 (Kumar et al. 2024).

Table 1. SNPs of the mitochondrial genome that allow differentiation of domestic pig breeds and wild pig subspecies into 15 mitochondrial haplotypes

Haplotype	Variable positions of the mitochondrial genome are indicated by the reference sequence AJ002189*						GenBank access codes and domestic pig breed and wild pig subspecies determined by 6 SNPs
	15556	15580	15616	15714	15758	15917	
	Variable positions of the mitochondrial genome are indicated by the reference sequence EF545567**						
	121	145	180	279	323	478	
<i>A</i>	T	C	C	C	C	A	Hampshire (AF136558), Duroc (FJ236997), Mangalitsa (AF136557, JN601068), Turopolje (JN601072)
<i>B</i>	C	T	C	C	C	A	Korean native breed Jeju (AY879793)
<i>C</i>	T	C	T	C	C	A	European wild boar (AF304203), Hampshire (AF136559), Landrace (AF486866), Pietrain (KC469587), Iberian (FJ236991), Mangalitsa Blonde (JN601067)
<i>E</i>	T	C	C	T	C	A	European wild boar (FJ236998)
<i>G</i>	T	C	T	T	C	A	Italian wild boar (AF304201), European wild boar (AF136563), Duroc (FJ236996), Large White (KC250275)
<i>H</i>	G	C	C	C	T	A	Wild boar Ryukyu (AB911704)
<i>I</i>	T	C	C	C	T	A	Landrace (AF136561)

Haplotype	Variable positions of the mitochondrial genome are indicated by the reference sequence AJ002189*						GenBank access codes and domestic pig breed and wild pig subspecies determined by 6 SNPs
	15556	15580	15616	15714	15758	15917	
	Variable positions of the mitochondrial genome are indicated by the reference sequence EF545567**						
	121	145	180	279	323	478	
<i>J1</i>	T	T	C	C	T	A	Asian wild boar (AF136564), Meishan (AF136567), Yorkshire (JN601075), Landrace (AF304202)
<i>J2</i>	T	T	C	C	T	T	Large White (AY574048)
<i>K</i>	T	T	C	T	C	A	Wild boar Russia (KX982635)
<i>L</i>	T	C	T	C	T	A	Large White (AF136562)
<i>M</i>	T	T	T	C	T	A	Duroc (AF136568)
<i>N</i>	T	T	C	T	T	A	Wild boar China (KP681245), Meishan (JN601071), Berkshire (AY574045), Large White (AF486874), Yorkshire (KF752550, JN601074, JN601074)
<i>O</i>	T	C	T	T	T	A	European wild boar (AJ002189), Landrace (AF136560)
<i>P</i>	T	T	T	T	T	A	Wild boar Ryukyu (AB015090)

Notes: * The reference point (Position 1) when depositing the sequence in GenBank is indicated as the beginning of the tRNA-Phe gene [16].

** The reference point (Position 1) when depositing the sequence in GenBank is indicated as the beginning of the D-loop control region [17].

RESULTS

The polysite method for haplotyping the porcine mitochondrial genome involves the determination, using PCR-RFLP, of six single-nucleotide substitutions in the region between nucleotide positions 15531 and 15958 of the mitochondrial genome using a single *TasI* endonuclease restriction reaction (**Table 1**). The nucleotide substitutions m.15560T>C, m.15580C>T, m.15616T>C, m.15714T>C, and m.15758T>C are specified relative to the reference sequence AJ002189.1. Since the analysis of GenBank NCBI data revealed that the SNP m.15917indelA was present in only one animal of the Large White breed (AY574048), we did not take it into account in our studies.

The analysis of mitochondrial DNA from Myrhorod pigs performed using this method identified the presence of three haplotypes: **A**, **C**, and **O** (**Fig. 1**). Compared to the 2011 study of mitochondrial genome SNPs in the Myrhorod breed, haplotype **L** is found in Large White (AF136562), Duroc (MF183225,

OM363288), and Iberian native pig (LN885364) populations. As of 2011, the frequency of mitochondrial haplotype **L** was 8% (**Fig. 1**).

In our study, the haplotype was determined based on a combination of five mitochondrial genome SNPs (**Table 2**).

These haplotypes are characteristic of the following domestic breeds and subspecies of the wild boar. Haplotype **A** corresponds to sequences Hampshire (AF136558), Duroc (FJ236997), Mangalitsa (AF136557, JN601068), Turopolje (JN601072), deposited in GenBank. Haplotype **C** — to European wild boar (AF304203), Hampshire (AF136559), Landrace (AF486866), Pietrain (KC469587), Iberian (FJ236991), Mangalitsa Blonde (JN601067), haplotype **O** — to European wild boar (AJ002189), Landrace (AF136560) and haplotype **L** — to Large White (AF136562), Duroc (MF183225, OM363288), Iberian native pig (LN885364).

To address the issue of differentiating mitochondrial haplotypes in the Myrhorod pig breed into Euro-

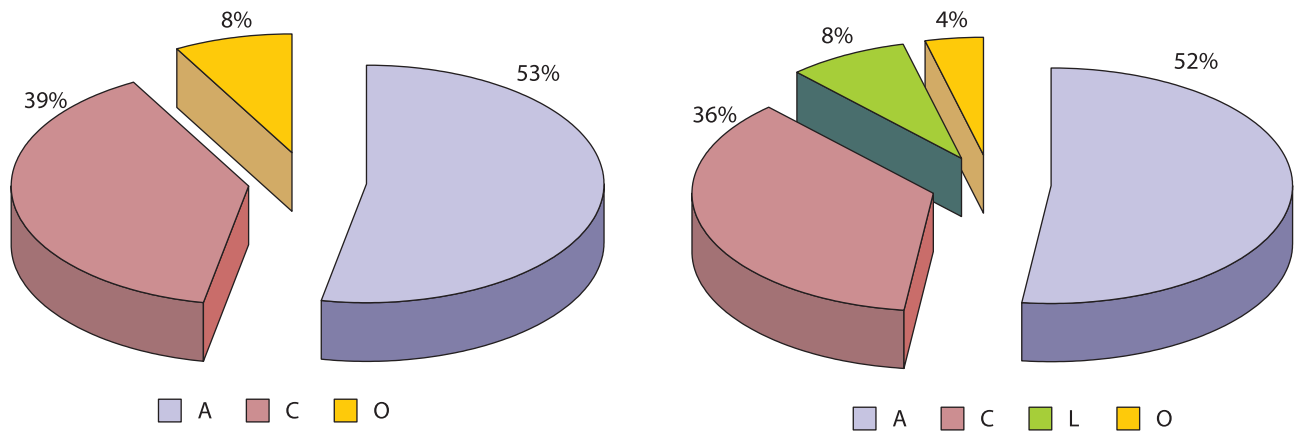


Fig. 1. The concentration of mitochondrial haplotypes of the Myrhorod pig breed as of 2011 and 2017 was determined using the polysite PCR-RFLP haplotyping method

Table 2. Combination of five SNPs of the mitochondrial genome (allelic variants) that determines a specific haplotype

SNP	m.15560T>C	m.15580C>T	m.15616T>C	m.15714T>C	m.15758T>C
<i>A</i>	t	c	c	c	c
<i>C</i>	t	c	t	c	c
<i>I</i>	t	C	T	C	T
<i>O</i>	t	c	t	t	t
<i>JI</i>	t	T	C	C	T

pean and Asian ones, the evolutionary divergence between the combinations of nucleotide sequences defining the four mitochondrial haplotypes in pigs was assessed (**Table 3**).

The number of base substitutions per site between sequences was shown. The analyses were performed using the maximum composite likelihood model. The analytical procedure covered 5 combinations of nucleotide sequences. The pairwise deletion option

was applied to all ambiguous positions for each pair of sequences, resulting in a final dataset containing 5 positions.

To differentiate the mitochondrial haplotypes of the Myrhorod breed as of 2011, an evolutionary history was constructed using the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean). The optimal tree with the sum of branch length was demonstrated ($d=0.359$). The tree is drawn to scale,

Table 3. Estimates of evolutionary divergence between sequences of the five SNPs of the mitochondrial genome

Haplotypes	<i>A</i>	<i>C</i>	<i>L</i>	<i>O</i>	<i>JI*</i>
<i>A</i>	0.000**				
<i>C</i>	0.058	0.000			
<i>L</i>	0.143	0.058	0.000		
<i>O</i>	0.298	0.143	0.058	0.000	
<i>JI*</i>	0.143	0.298	0.143	0.298	0.000

Notes: * Haplotype JI Asian wild boar (AF136564) and Meishan breed (AF136567) were used as outgroup.

** Number of nucleotide substitutions per site between sequences is shown.

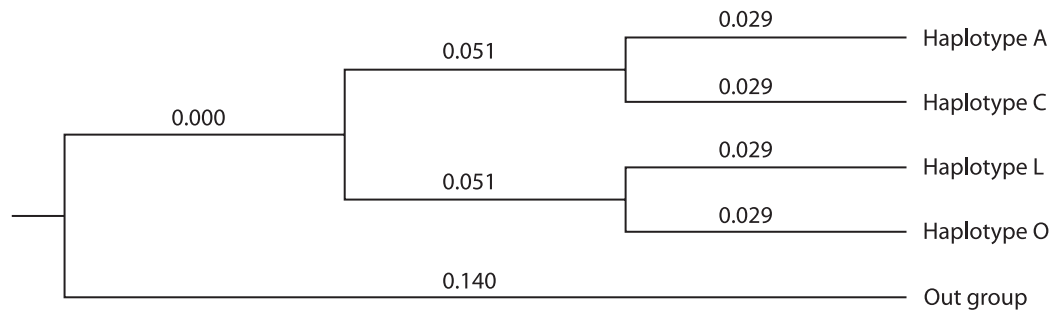


Fig. 2. Evolutionary history was derived using the UPGMA method for four mitochondrial haplotypes of the Myrhorod pig breed and one haplotype of the Asian wild pig, and the Meishan pig breed used as an outgroup

with branch lengths (next to the branches) in the same units of measurement as the evolutionary distances used to construct the phylogenetic tree. The haplotype of the Asian wild boar and the Meishan breed were used as the outgroup (**Fig. 2**).

The assessment of the evolutionary divergence between the combinations of nucleotide sequences defining the four mitochondrial haplotypes among the Myrhorod pig breed, as well as their evolutionary history, demonstrates their affiliation with European domestication events. The combination of nucleotide sequences from the Asian wild boar (AF136564) and the Meishan breed (AF136567), which was used as an outgroup, demonstrates an affiliation with the Asian domestication event.

The analysis of evolutionary divergence among the sequences of five mitochondrial genome SNPs allowed us to differentiate the mitochondrial haplotypes of the Myrhorod pig breed into two European clusters.

Haplotype diversity, which is a measure of genetic variation within a population, is calculated based on

the frequencies of allelic variants of the mitochondrial genome. The frequencies of allelic variants in a sample of Myrhorod breed pigs as of 2011 and 2017 are presented in **Table 4**.

The haplotype diversity reflects the probability that two randomly selected haploid alleles are different. The genetic diversity of the Myrhorod breed as of 2011 and 2017 was determined based on the frequencies of haploid alleles (**Table 5**). A test for haploid disequilibrium ($Vr = Vo/Ve$) was not performed because haploid alleles at two or more different loci of the mitochondrial genome are always inherited together.

DISCUSSION

Genetic characterization of local (autochthonous) pig breeds using the mitochondrial genome which is inherited maternally and does not undergo recombination is important for several reasons. Photographs of animals of the Myrhorod pig breed, characteristic of their exterior, are presented in **Fig. 3–6**.

Table 4. Frequencies of allelic variants of the mitochondrial genome and sample size of the Myrhorod pig breed as of 2011 and 2017

Year	Allel	SNPs of the mitochondrial genome (haploid alleles)					
		m.15560 T>C	m.15580 C>T	m.15616 T>C	m.15714 T>C	m.15758 T>C	m.15917 A>T
		Frequencies of allelic variants of mitochondrial DNA					
2011 (<i>n</i> =25)	1	1.000	0.000	0.480	0.040	0.120	0.000
	2	0.000	1.000	0.520	0.960	0.880	0.000
2017 (<i>n</i> =84)	1	1.000	0.000	0.464	0.083	0.083	0.000
	2	0.000	1.000	0.536	0.917	0.917	0.000

Table 5. Haplotype diversity, observed and expected variance of the sample of Myrhorod pig breed as of 2011 ($n=25$) and 2017 ($n=84$)

SNP/year	h		V_o		V_e	
	2011	2017	2011	2017	2011	2017
m.15560T>C	0.000	0.000	0.157	0.161	0.487	0.509
m.15580C>T	0.000	0.000				
m.15616T>C	0.499	0.497				
m.15714T>C	0.077	+0.153				
m.15758T>C	0.211	-0.153				

Notes: h — haplotype diversity; V_o — observed variance (N — average population diversity); V_e — expected variance.

First, it allows us to trace phylogenetic relationships between maternal lines, helping us understand how the Myrhorod breed evolved over the centuries from subspecies of wild pigs to domesticated pigs and, subsequently, to a local breed. The analysis of mitochondrial DNA from Myrhorod pigs as of 2011 identified the presence of four haplotypes: **A**, **C**, **L**, and **O** (**Fig. 1**). These haplotypes corresponded to specific subspecies of wild pigs and breeds of domestic pigs, as determined by comparison with GenBank sequences.

Unlike the interpretation of nuclear gene variants, the use of mtDNA phylogeny in combination with minor allele frequencies is crucial for the correct interpretation of mtDNA variants. Although many haplotypes block also exist in nuclear DNA, the number of mtDNA haplogroups is relatively limited due to the small size of the mitochondrial genome (McCormick et al. 2020). The analysis using the maximum composite likelihood model made it possible to determine the number of base substitutions per site between the sequences that define haplotypes. The analytical procedure covered 5 combinations of nucleotide sequences. The lowest evolutionary divergence, in terms of the number of substitutions per site (0.058), was observed between the sequences defining haplotypes **A** and **C**. The greatest divergence (0.298) was observed between haplotypes **O**, **C**, and haplotype **JI**, which was used as an outgroup.

An assessment of the evolutionary divergence among the haplotypes of Myrhorod pigs and their evolutionary history demonstrates their affiliation with European domestication events. The haplotype of the Asian wild boar (AF136564) and the Meishan breed (AF136567), used as an outgroup in the phylogenetic analysis, formed a separate branch on the

optimal tree with a total branch length ($d=0.359$) clearly demonstrating the differentiation between European and Asian domestication events. The presence of four haplotypes in the local breed suggests the influence of introgression from domesticated pigs or breeds that evolved in different geographic regions of Europe. Research indicates multiple centers of domestication in Eurasia, with modern European domestic pigs primarily descended from European wild boars rather than Middle Eastern ones (Larson et al. 2014).

The identification of at least six centers of wild pig domestication has been made in India (Desai et al. 2025).

Genetic studies have proven that the spread of domesticated pigs was facilitated by humans. For example, sequence analysis of mitochondrial control regions of the Formosan wild boar (*Sus scrofa taiwanus*) and the local Lanyu pig breed found in Taiwan showed that the Formosan wild boar was the ancestor of the Lanyu pigs domesticated in Taiwan (Li et al. 2017). Ultimately, Lanyu pigs became widespread in Taiwan and the Philippines.

Since historical records of the Myrhorod breed development indicate crossbreeding of local short-eared Black-and-White pigs from the Poltava region with Berkshire, Middle White, and Large White breeds, and, to a lesser extent, with Large Black and Tamworth breeds, it was possible to hypothesize the presence of Asian-type mitochondria as well. However, in our studies, only European haplotypes were identified among the Myrhorod pig breed. Thus, haplotype **A** corresponded to those deposited in GenBank for such local European breeds as Mangalitsa and Turopolje. A study showed that Mangalica pigs descended from primitive breeds that may be direct descendants of

the wild boar (Georgescu et al. 2012). The absence of Asian haplotypes in local European pig breeds was also reported (Alves et al. 2003). Asian mtDNA types were not detected in Iberian pig populations; therefore, the maternal contribution of Asian pigs to the Iberian breed is considered to be unlikely (Alves et al. 2003). Finally, haplotypes specific to the European wild boar, rather than the Asian wild boar, are found among Myrhorod pigs. This indicates that the evolutionary history of Myrhorod pigs can be traced back to European domestication events.

The analysis of archaeological data indicates that domesticated pigs arrived in Europe alongside farmers approximately 8,500 years BCE (Frantz et al. 2020). However, several thousand years after the introduction of Middle Eastern pigs to Europe, their characteristic mtDNA signature disappeared and was replaced by haplotypes associated with European wild boars. Attribute this substitution to a significant influx of genes from local European wild boars, although it is also possible that European wild boars were domesticated independently, without any genetic contribution from the Middle East (Frantz et al. 2020).

Thus, the identified haplotypes serve as genetic markers that help confirm the authenticity of the Myrhorod breed, highlight the uniqueness of the breed gene pool, and enable the management of the pedigree structure based on maternal lines.

Another genetic characteristic of the local Myrhorod pig breed was its haplotype diversity. Three of the six SNPs studied — m.15616T>C, m.15714T>C, and m.15758T>C — influenced the level of haplotype diversity in the Myrhorod breed population. The other three SNPs were monomorphic within the sample of animals studied. SNP m.15714C ($q=0.960$) was the most frequent among the polymorphic allelic variants of the mitochondrial DNA in 2011, and m.15758C ($q=0.917$) — in 2017. There is a high risk of losing this allelic variant in local populations completely with time. For this reason, it is advisable to artificially maintain its frequency in breeding programs by retaining sows that are hosts of these allelic variants.

Currently, the information on mitochondrial DNA allelic variants in pigs is limited. Even for the human mitochondrial genome, where population analysis is conducted on a larger scale compared to animals, such information is considered insufficient. To date, mitochondrial genome variants have not been included in most large human genomic variation databases, such as the Exome Aggregation Consortium — ExAC

and the Genome Aggregation Database — gnomAD (Laricchia et al. 2022). However, the population frequency data are extremely useful for the clinical interpretation of variants of uncertain significance (VUS) (McCormick et al. 2020).

The assessment of haplotype diversity of a herd across several generations has become particularly important. This makes it possible to assess the effectiveness of the chosen breeding strategy for the purpose of breed conservation. Over two generations in the last autochthonous herd of the Myrhorod breed, which was kept in conditions of competition with modern transboundary breeds, the total number of mitochondrial haplotypes decreased from 4 to 3. We investigated the effect of a reduction in the number of mitochondrial haplotypes on haplotype diversity. Haplotype diversity, which is a measure of genetic variation within a population, is calculated based on the frequency of mitochondrial DNA allelic variants. A mitochondrial haplotype is a specific combination of alleles. For example, the m.15714C* allele is found in sows with haplotypes *A* (TCCC*CA), *C* (TCTC*CA), and *I* (TCTC*TA). The disappearance of a genealogical family with haplotype *I* does not eliminate the m.15714C allelic variant from the population. Moreover, sows with haplotypes *A* and *C* were the most abundant in the herd. Thus, the loss of the maternal line with haplotype *I* did not reduce the level of haplotype diversity in the Myrhorod breed population; on the contrary, it increased from ($h=0.077$) in 2011 to ($h=0.153$) in 2017. This may also be related to a very small size of the herd. Under such conditions, even a minimal change in herd size could have had very significant consequences.

The disappearance of the genealogical family with haplotype *L* (TCTCT*A) did not significantly affect the frequency of the m.15758T* allele, since this allele is also present in the unique allele combination of haplotype *O*.

Within the herd, the frequency of the m.15758T allele ($q=0.120$) carried by animals with haplotype *I* in 2011 was nearly offset by animals with haplotype *O* ($q=0.083$) in 2017. Paradoxically, due to this, the level of haplotype diversity in the Myrhorod breed population increased from ($h=0.077$) in 2011 to ($h=0.153$) in 2017. This may also be a consequence of the small population size. Under such conditions, each individual has a very large influence on allele frequency fluctuations. A significant change in haplotype diversity was observed only for SNP m.15714T>C



Fig. 3. A sow with piglets of the last autochthonous herd of the Myrhorod breed on pasture (State Enterprise «Research Farm named after Decembrists» of the Institute of Pig Breeding and Agricultural Production of the NAAS, 2011)

($h^{2011}=0.077$; $h^{2017}=0.153$). As noted above, this is associated with the disappearance of a genealogical lineage carrying the I haplotype (TCTCT*A).

The observed variation in mitochondrial nucleotide sequences in the Myrhorod pig herd reflects the actual genetic diversity. We determined a considerable deviation from the expected variance ($Vo^{2011}=0.157$, $Ve^{2017}=0.487$) and ($Vo^{2017}=0.161$, $Ve^{2017}=0.509$). Clearly, this is a general characteristic of the mitochondrial DNA control region (D-loop).

CONCLUSIONS

1. The assessment of the evolutionary divergence among the haplotypes of Myrhorod pigs and their evolutionary history demonstrates their affiliation with European domestication events. The presence of four mitochondrial haplotypes in this local breed suggests the influence of introgression from domesticated pigs or breeds that evolved in different geographic regions of Europe.

2. In the development of the Myrhorod pig breed, boars of the Berkshire, Middle White, Large White, Large Black, and Tamworth breeds were used, none of which contributed Asian mitochondrial haplotypes.

3. Over two generations in the last autochthonous herd of the Myrhorod breed, which was kept in conditions of competition with modern transboundary breeds, the total number of mitochondrial haplotypes decreased from 4 to 3. The loss of the maternal line with haplotype I did not reduce the level of haplotype diversity in the Myrhorod breed population; on the contrary, it increased from ($h=0.077$) in 2011 to ($h=0.153$) in 2017. In light of this, the preservation of maternal lines with different haplotypes is of greater practical importance for the genetic characterization and management of small populations than haplotype



Fig. 4. Repair pigs of the last autochthonous herd of the Myrhorod breed on pasture (State Enterprise «Research Farm named after the Decembrists» of the Institute of Pig Breeding and Agricultural Production of the NAAS, 2011)

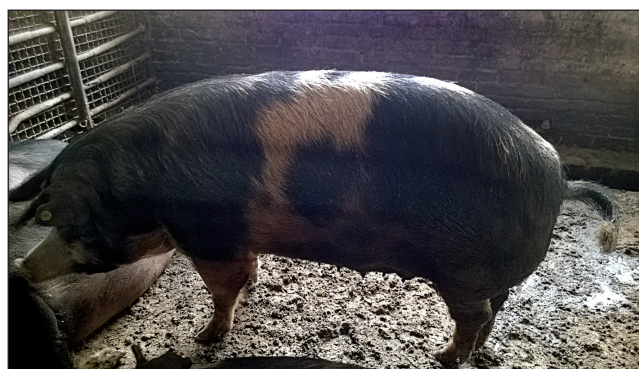


Fig. 5. A sow of the last autochthonous herd of the Myrhorod breed (State Enterprise «Research Farm named after Decembrists» of the Institute of Pig Breeding and Agricultural Production of the NAAS, 2014)



Fig. 6. A sow of the restored Myrhorod breed with piglets (experimental station of the Institute of Pig Breeding and Agricultural Production of the NAAS, 2024)

diversity. To conserve and restore the Myrhorod pig breed, given its current status, measures based on the search for original native genetic material of the Myrhorod breed which has been introgressed into the Large White, Poltava Meat, and Red White-Belted breeds using the marker haplotypes we have identified may prove effective.

The sources of financing. The work was funded by FAO grant GF.REUTD.RA20101OORIE1, «Development of a Comprehensive Program for the Conservation of the Local Myrhorod Pig Breed Based on Molecular-Genetic Analysis.»

Conflict of interests. The authors declare the absence of any conflicts of interests.

REFERENCES

- Ajmone-Marsan P, Colli L, Ginja C, Kantanen J, Lenstra JA, eds (2023) Genomic characterization of animal genetic resources. FAO Animal Production and Health Guidelines 32: Rome, FAO. <https://doi.org/10.4060/cc3079en>
- Alves E, Ovilo C, Rodríguez MC, Silió L (2003) Mitochondrial DNA sequence variation and phylogenetic relationships among Iberian pigs and other domestic and wild pig populations. *Anim Genet* 34(5):319–24. <https://doi.org/10.1046/j.1365-2052.2003.01010.x>
- Desai S, Singh PP, Pandey RK, Mishra RK, Chaubey AK, Kumar A, Tiwary SK, Gupta SK, Achilli A, Rambaldi Migliore N, Chaubey G (2025) Multiple Domestication Centers of the Indian Pig Population. *Genome Biol Evol* 17(3):evaf030. <https://doi.org/10.1093/gbe/evaf030>
- Erven JAM, Mattiangeli V, Dreshaj M, Mullin VE, Rossi C, Daly KG, Jackson I, Parker Pearson M, Bradley DG, Frantz LAF, Madsen O, Raemaekers D, Çakırlar C (2025) Archaeogenomic insights into commensalism and regional variation in pig management in Neolithic northwest Europe. *Proc Natl Acad Sci U S A* 122(12):e2410235122. <https://doi.org/10.1073/pnas.2410235122>
- Frantz LAF, Haile J, Lin AT, et al (2020) Ancient pigs reveal a near-complete genomic turnover following their introduction to Europe. *Proc Natl Acad Sci U S A* 117(25):14610–11. <https://doi.org/10.1073/pnas.2008793117>
- Gao J, Sun L, Pan H, Zhang S, Xu J, He M, Zhang K, Zhou J, Zhang D, Wu C, Dai J (2023) Genomic Dissection through Whole-Genome Resequencing of Five Local Pig Breeds from Shanghai, China. *Animals* (Basel) 13(23):3727. <https://doi.org/10.3390/ani13233727>
- Georgescu SE, Manea MA, Dudu A, Costache M (2012) Phylogenetic relationships of the Mangalitsa Swine breed inferred from mitochondrial DNA variation. *Int J Mol Sci* 13(7):8467–81. <https://doi.org/10.3390/ijms13078467>
- Han Y, Zhao Z, Stanton DWG, Lei XL, Wu Z, Liu Y, Yu C, Chen X, Li W, Wang J, You Y, Li Y, Lei S, Yi H, Fan W, Cai Q, Min R, Hu C, Chen C, Cui Y, Guo J, Zhang H, Song H, Guo X, Ruan Q, Tan Y, Li Z, Zhang X, Shi X, Zhou X, Zhuang Y, Manin A, Frantz LAF, Alves JM, Pan Y, Wu X, Luo SJ, Larson G, Yu H (2025) Ancient Genomics Reveals the Origin, Dispersal, and Human Management of East Asian Domestic Pigs. *Mol Biol Evol* 42(9):msaf214. <https://doi.org/10.1093/molbev/msaf214>
- Jain K, Panigrahi M, Nayak SS, Rajawat D, Sharma A, Sahoo SP, Bhushan B, Dutt T (2024) The evolution of contemporary livestock species: Insights from mitochondrial genome. *Gene* 927:148728. <https://doi.org/10.1016/j.gene.2024.148728>
- Kijas JM, Andersson L. A phylogenetic study of the origin of the domestic pig estimated from the near-complete mtDNA genome (2001). *J Mol Evol* 52(3):302–8. <https://doi.org/10.1007/s0023900101585>
- Kim KI, Lee JH, Li K, Zhang YP, Lee SS, Gongora J, Moran C (2002) Phylogenetic relationships of Asian and European pig breeds determined by mitochondrial DNA D-loop sequence polymorphism. *Anim Genet* 33(1):19–25. <https://doi.org/10.1046/j.1365-2052.2002.00784>
- Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S, Tamura K. (2024) MEGA12: Molecular Evolutionary Genetic Analysis Version 12 for Adaptive and Green Computing. *Mol Biol Evol* 41(12):msae263. <https://doi.org/10.1093/molbev/msae2639>
- Laricchia KM, Lake NJ, Watts NA, et al (2022) Mitochondrial DNA variation across 56,434 individuals in gnomAD. *Genome Res* 32(3):569–82. <https://doi.org/10.1101/gr.276013.121>
- Larson G, Dobney K, Albarella U, Fang M, Matisoo-Smith E, Robins J, Lowden S, Finlayson H, Brand T, Willerslev E, Rowley-Conwy P, Andersson L, Cooper A (2005) Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science* 307(5715):1618–21. <https://doi.org/10.1126/science.1106927>
- Li KY, Li KT, Yang CH, Hwang MH, Chang SW, Lin SM, Wu HJ, Basilio EB, Vega RSA, Laude RP, Ju YT (2017) Insular East Asia pig dispersal and vicariance inferred from Asian wild boar genetic evidence. *J Anim Sci* 95(4):1451–66. <https://doi.org/10.2527/jas.2016.1117>
- McCormick EM, Lott MT, Dulik MC, et al (2020) Specifications of the ACMG/AMP standards and guidelines for mitochondrial DNA variant interpretation. *Hum Mutat* 41(12):2028–57. <https://doi.org/10.1002/humu.24107>
- Nguyen TV, Ba NV, Pham LD, Do DN (2026) Conservation of Indigenous Pig Breeds in Vietnam: Genetic Characterization, Conservation Strategies, and Future Perspectives

tives. *Biology* (Basel) 15(9):730. <https://doi.org/10.3390/biology15090730>

- Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research — an update. *Bioinformatics* 28(19):2537–39. <https://doi.org/10.1093/bioinformatics/bts460>
- Pochernyaev KF (2016) Genetic structure of Ukrainian large white pigs, estimated using mitochondrial DNA-markers. *Agric Sci Pract* 3(1):61–5. <https://doi.org/10.15407/agrisp3.01.061>
- Simčič M, Smetko A, Sölkner J, Seichter D, Gorjanc G, Kompan D, Medugorac I (2015) Recovery of native genetic background in admixed populations using haplotypes, phenotypes, and pedigree information — using Cika cattle as a case breed. *PLoS One* 10(4):e0123253. <https://doi.org/10.1371/journal.pone.0123253>
- Ursing BM, Arnason U (1998) The complete mitochondrial DNA sequence of the pig (*Sus scrofa*). *J Mol Evol* 47(3):302–6. <https://doi.org/10.1007/pl00006388>
- Vashchenko PA, Balatsky VM, Pochernyaev KF, Voloshchuk VM, Tsybenko VH, Saenko AM, Oliynychenko YeK, Buslyk TV, Rudoman HS (2019) Genetic characteristic of the Mirhorod pig breed by analysing single nucleotide polymorphisms. *Agricultural Science and Practice* 6(2); 47–57. <https://doi.org/10.15407/agrisp6.02.047>
- Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10(4):506–13. PMID:1867860.
- Wu CY, Jiang YN, Chu HP, Li SH, Wang Y, Li YH, Chang Y, Ju YT (2007) The type I Lanyu pig has a maternal genetic lineage distinct from Asian and European pigs. *Animal Genetics* 38:499–505. <https://doi.org/10.1111/j.1365-2052.2007.01646.x>
- Zhang M, Yang Q, Ai H, Huang L (2022) Revisiting the Evolutionary History of Pigs via De Novo Mutation Rate Estimation in A Three-generation Pedigree. *Genomics Proteomics Bioinformatics* 20(6):1040–52. <https://doi.org/10.1016/j.gpb.2022.02.001>

UDC 636.4.082: 575.113

ГЕНЕТИЧНА ХАРАКТЕРИСТИКА ОСТАНЬОГО АВТОХТОННОГО СТАДА СВИНЕЙ МИРГОРОДСЬКОЇ ПОРОДИ НА ОСНОВІ АНАЛІЗУ МІТОХОНДРІАЛЬНОЇ ДНК

К.Ф. Почерняєв¹, С.М. Корінний¹, А.А. Гетья²,
В.Г. Цибенко¹, П.А. Ващенко³

¹ Інститут свинарства і агропромислового комплексу
Національної академії аграрних наук України,
вул. Шведська Могила, 1, м. Полтава, 36013, Україна

² Національний університет біоресурсів
і природокористування України
Міністерства освіти і науки України,
вул. Героїв Оборони, 15, м. Київ, 03041, Україна

³ Полтавський державний аграрний університет,
Міністерства освіти і науки України
вул. Григорія Сковороди, 1/3, м. Полтава,
36003, Україна

E-mail: * k.f.pochernyaev@gmail.com,
korinny_sergey@ukr.net, getya@ukr.net,
dpgim.dekabristiv@gmail.com,
p.a.vashchenko@gmail.com

ORCID: <https://orcid.org/0000-0001-9973-6429>,
<https://orcid.org/0000-0002-1649-3079>,
<https://orcid.org/0000-0002-4747-9261>,
<https://orcid.org/0000-0003-3932-8508>,
<https://orcid.org/0000-0002-9287-819X>.

Мета. Ідентифікувати мітохондріальні гаплотипи в останньому автохтонному стаді свиней миргородської породи, оцінити гаплотипове різноманіття, еволюційну дивергенцію та еволюційну історію породи для коригування стратегій її відновлення. **Методи.** Біологічний матеріал було зібрано у 2011 та 2017 роках ($n=25$) та ($n=84$) відповідно. Мітохондріальні гаплотипи свиней визначали за допомогою мультилокусного ПЛР-ПДРФ аналізу (PCR-RFLP). Популяційний та статистичний аналізи проводили за допомогою програмного забезпечення GENALEX 6. Еволюційний аналіз здійснювали в MEGA12. **Результати.** За два покоління в останньому автохтонному стаді миргородської породи, яке вирощувалося в інтенсивних умовах, загальна кількість мітохондріальних гаплотипів зменшилася з 4 до 3. Зникнення генеалогічної родини з гаплотипом *I* не усуває алельний варіант m.15714C з популяції, оскільки він зберігається у свиноматок з гаплотипами *A* і *C*. Втрата материнської лінії з гаплотипом *I* не зменшила рівень гаплотипового різноманіття в популяції миргородської породи; навпаки, він зріс з ($h=0.077$) у 2011 році до ($h=0.153$) у 2017 році. Аналіз кількості замін азотистих основ на сайт між послідовностями виявив еволюційну дивергенцію серед послідовностей, що визначають гаплотипи. Найнижча еволюційна дивергенція (кількість замін на сайт, $d=0.058$) спостерігалася між послідовностями, що визначають гаплотипи *A* і *C*. Найбільша дивергенція ($d=0.298$) спостерігалася між гаплотипами *O*, *C* та гаплотипом *JI*, який використовувався як зовнішня група (аутгрупа). Оцінка дивергенції серед гаплотипів свиней миргородської породи та їхньої еволюційної історії продемонструвала їхню приналежність до європейських подій одомашнення. Гаплотип азійського дикого кабана (AF136564) та породи мейшан (AF136567), що використовувалися як зовнішня група у філогенетичному аналізі, утворили окрему гілку на оптимальному дереві із загальною довжиною гілки

($d=0.359$), чітко демонструючи диференціацію між європейськими та азійськими подіями одомашнення. Три з шести досліджених SNP — m.15616T>C, m.15714T>C та m.15758T>C — вплинули на рівень гаплотипового різноманіття в популяції миргородської породи. Серед поліморфних алельних варіантів мітохондріальної ДНК SNP m.15714C ($q=0.960$) був найчастішим у 2011 році, а m.15758C ($q=0.917$) — у 2017 році. Спостерігалось значне відхилення між очікуваною та спостережуваною дисперсіями ($V_o^{2011}=0.157$, $V_e^{2017}=0.487$) та ($V_o^{2017}=0.161$, $V_e^{2017}=0.509$). **Висновки. 1.** Оцінка еволюційної дивергенції серед гаплотипів свиней миргородської породи та їхньої еволюційної історії демонструє їхній зв'язок з європейськими подіями одомашнення. Наявність чотирьох мітохондріальних гаплотипів у цій локальній породі свідчить про вплив інтрогресії від одомашнених свиней або порід, які еволюціонували в різних географічних регіонах Європи. **2.** Під час виведення миргородської породи свиней використовували кнурів беркширської, середньої білої, великої білої, великої чорної та темвортської порід, жоден з яких не міг передати азійські мітохондріальні гаплотипи. **3.** За два покоління в останньому автохтон-

ному стаді миргородської породи, що утримувалося в умовах конкуренції із сучасними транскордонними породами, загальна кількість мітохондріальних гаплотипів зменшилася з 4 до 3. Парадоксально, але рівень гаплотипового різноманіття в популяції миргородської породи зріс після втрати материнської лінії з гаплотипом *I*. З огляду на це, збереження материнських ліній з різними гаплотипами має більше практичне значення для генетичної характеристики та управління локальною породою, ніж гаплотипове різноманіття. **4.** Для збереження та відновлення миргородської породи свиней у нинішній ситуації найбільш ефективним підходом може бути той, що ґрунтується не на пошуку оригінального локального генетичного матеріалу, а на ідентифікації та використанні виявлених нами маркерних гаплотипів, які інтрогресували до великої білої, полтавської м'ясної та червоної білопоясої порід.

Ключові слова: свині, мітохондріальні гаплотипи, гаплотипове різноманіття, миргородська порода, локальна порода, відновлення породи, генетична характеристика.