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REGULAR ARTICLES

Genetic characterization of domestic pigs in the core zone of swine production of Argentina

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Abstract

Argentina is a small player in the global pork market, contributing only 0.7% of the total production. With increasing global demand for meat, there is an opportunity for countries with an agricultural profile to grow their pork production. However, there is a need to understand the current state of the pork production sector in all aspects to inform decision-making. The aim of this study was to genetically characterize pig herds from different production strata in the primary region for pork production in the country. For this purpose, phylogenetic and genetic variability analyses were performed using the mitochondrial control region marker (n=95 pig samples). Moreover, genotyping of ryr1 and PRKAG3 genes (n=108 pig samples) were performed to evaluate the frequency of deleterious alleles for meat quality traits in the region. The results showed high levels of genetic variability in the pig herds (Hd= 0.840 ± 0.031 and π= 0.010 ± 0.001), with a creole sow and Iberian lineage standing out in the phylogeny. The genotyping of the ryr1 marker revealed the presence of the deleterious t allele in all analyzed strata. However, the RN-allele of the PRKAG3 gene was detected only in the two lower strata. This study represents the first analysis of the phylogenetic relationships among domestic pigs from Argentina and provides an initial assessment of genetic variability in the region. Additionally, the results present, for the first time, the frequency of deleterious alleles for pig production in the productive core area, demonstrating their prevalence.

Keywords Pigs · Genetic variation · Animal production · Meat quality · ryr1 · PRKAG3

Introduction

The pig is a ubiquitous domesticated animal with global importance, and its meat is the most widely consumed protein source worldwide. In Argentina, pig production reaches approximately 695 thousand tons per year, being utilized for both domestic consumption (99% of national production) and exportation purposes (Ministerio de Agricultura, Ganadería y Pesca 2021). Regarding foreign trade, Argentina contributes 0.7% to the world's production, exporting approximately 8000 tons of meat in 2022. China, Russia, South Africa, and Paraguay are the main destinations for Argentine pork (Secretaría de Agricultura, Ganadería y Pesca 2022). The highest production is located in the central region of the country, specifically in the provinces of Buenos Aires (23.74% of the animal stock), Córdoba (23.53%), and Santa Fe (14.10%). Within this area lies the nucleus of the country's agricultural production, characterized by robust infrastructure development encompassing roads, enterprises, and slaughterhouses facilities (Ministerio de Agricultura, Ganadería y Pesca 2021).

In the core region, registered production units with less than 10 breeding sows in Buenos Aires, Córdoba, and Santa Fe represent 79.96%, 78.50%, and 69.54% of the units in these provinces, respectively (Ministerio de Agricultura, Ganadería y Pesca 2021). According to the last National Agricultural Census conducted in 2018, near 62% of the agricultural operations in this region engage in pig farming for self-consumption, while the remainder is commercially oriented, primarily for meat production, and to

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a lesser extent, for genetic purposes (1%) (INDEC 2020). These values reflect the high percentage of producers in self-consumption scales who, due to multiple factors, have not yet been able to grow in the activity.

Since the beginning of pig production in Argentina, the genetic material introduced has been diverse. The first pigs introduced were Iberian breeds (Spanish and Portuguese) brought by colonizers throughout the sixteenth century (Freitas and Rosado 2014). At the start of the twentieth century, modern European and Asian breeds replaced their predecessors due to superior performance (Benítez Ortiz and Sánchez 2001; Revidatti 2009). The patterns of genetic variability produced from their introduction, crossbreeding, and adaptation to different environments have been poorly explored. In this scenario, it is interesting to emphasize the role of small producers as possible reservoirs of such variability, as they have less access to the acquisition of improved hybrids (Lemus 2008; Salcedo and Guzmán 2014).

Over the decades, molecular markers have been used to address these issues. Numerous studies have reported data on genetic variability and phylogeny in pig populations worldwide (Larson et al. 2005; Scandura et al. 2011; McCann et al. 2014). Specifically, the use of different fragments of the mitochondrial DNA control region (CR) allowed the identification of three mitochondrial clades: two European (E1 and E2) and one Asian (A). The E1 clade is comprised of the majority of European wild boar sequences and domestic pig breeds from the same continent (Landrace, Large White, Berkshire, Pietrain, Hampshire, and Iberian breeds), whereas the E2 clade is composed of sequences from wild boars in Italy, Sardinia, and Croatia (Scandura et al. 2008). In turn, it has been proposed that E1 can be divided into two groups or sub-clades: E1A and E1C. E1A, most commonly founded in Italy, France, Germany, and Austria, and E1C distributed throughout the European continent, but more frequent in Spain, Portugal, Poland, and Hungary. Finally, the A clade includes domestic pig breeds from China and Japan and wild pigs from Asia (Giuffra et al. 2000; Kijas and Andersson 2001; Watanobe et al. 2001; Scandura et al. 2011; Kusza et al. 2014). In Argentina, CR has been used to evaluate patterns of genetic variability and phylogenetic relationships in wild pig populations (Sagua et al. 2018; Acosta et al. 2019; Figueroa et al. 2022).

In the last few decades, consumer demands have driven pig breeders to focus on obtaining a product with high values of organoleptic quality. Improvement efforts aimed at lean meat have shifted towards the pursuit of meat with better texture, color, and marbling (intramuscular fat content of quality) for the production of cured meats (Gjerlaug-Enger et al. 2010). This has sparked a wave of genetic research aimed at identifying the variables that influence these traits (Otsu et al. 1992; Estrade et al. 1993; Choi et al. 2012). The most studied genes influencing meat quality are the ryanodine receptor gene (ryr1), or halothane gene (hal), and the PRKAG3 or Napole yield gene (rn).

Specifically, the mutation in ryr1 is produced by a nucleotide substitution at position 1843 (pArg615Cys) in the ryanodine receptor gene located on chromosome 6. The receptor is responsible for regulating the flow of Ca2+ ions across the sarcoplasmic reticulum membrane of skeletal muscle. It is responsible for the syndrome of malignant hyperthermia, as well as for reducing the organoleptic evaluations of meat. Pigs carrying the mutant allele (t) show shorter carcasses, lower final *pH* in the muscles, and low water-holding capacity, a phenotype known as PSE (pale, soft, exudative meat) (Britt 1991; Pommier et al. 1998; Hamilton et al. 2000).

The adenosine monophosphate-activated protein kinase, gamma 3 subunit gene (PRKGA3) encodes an isoform of the regulatory subunit of the adenosine monophosphate-activated protein kinase (AMPK). Two non-synonymous mutations associated with meat quality traits have been detected in this gene: the R200Q substitution (found only in populations with Hampshire ancestry) and the I199V mutation (found in the Landrace, Berkshire, Hampshire, Large White, Duroc breeds, and wild pigs) (Miller et al. 2000; Ciobanu et al. 2001; Josell et al. 2003; Martínez-Quintana et al. 2006). The negative effects are caused by the RN- allele (199V/200Q), which leads to an increase in glycogen in muscle cells resulting in meat with reduced water-holding capacity, low muscle protein content, and decreased pH at 24 h *post-mortem* (Miller et al. 2000; Ciobanu et al. 2001; Lindahl et al. 2004).

The frequency of these genes in Argentina has been little explored. Marini et al. (2012) reported a high allele frequency of the mutant allele t (0.196) of ryr1 in a population of commercial hybrids from the provinces of Córdoba, Santa Fe, Chaco, and Tucumán. The same trend is observed in pigs from the northeast (Chaco and Entre Ríos) with allele frequencies of 0.149 and 0.173, respectively (Lagadari et al. 2019; Rodriguez et al. 2022). In turn, a high number of animals carrying the mutant allele (RN-) of the PRKGA3 gene were detected in these provinces. In Buenos Aires, the only record belongs to wild populations, where Acosta et al. (2021) identified wild animals carrying the mutant allele t as a result of crossbreeding with domestic pigs.

In this context, characterizing current pig productions constitutes the basis for decision-making in the sector, aimed at efficient and inclusive production (Revidatti et al. 2005; McManus et al. 2010). To attain this objective, it is imperative to comprehend the genetic variability within the domestic pig populations in the country, for which pertinent data has not been reported until now. Additionally, there exists a dearth of knowledge concerning the prevalence of genes deleterious to meat quality in the central region of the country. Then, the aim of this work is to carry out a genetic characterization of the pig herds in northern Buenos Aires, a core

zone of swine production, using nuclear molecular markers (ryr1 and PRKAG3) and mitochondrial marker (CR). This information will enable make decisions in the management of pig herds, protection of genetic variability, and strategies for the elimination of deleterious genes by decision-makers, as a basis to improve the national production.

Materials and methods

The study area extends from 33°42′43″ to 34°47′75″ south latitude and from 61°52′30″ to 60°20′38″ west longitudes, located in the northern region of the Buenos Aires province (Fig. 1). Specifically, it encompasses the districts of Colón, Rojas, Junín, Salto, Gral. Arenales, Gral. Pinto, and Gral. Viamonte. This region is known geographically as "Humid Pampa" and has been heavily modified by human activity due to agriculture and livestock production, which record the highest levels of cereal production in the country, enabling related pig farming activities (Table 1) (Calzada and Corina 2019).

The breeding farms were stratified following the classification used by Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) from Argentina, based on the number of mother sows. This is one of the most used indicators for stratifying pig production, which allows for a comparison of self-consumption productions with those more developed or already integrated into the national pig production chain (Benés and Cendon 2013). In this regard, farms were grouped into three categories: small farms (S), less than 10 sows; medium farms (M), between 11 and 100 sows; and large farms (L), more than 100 sows (Benés and Cendon 2013). A total of 43 establishments were sampled, of which 19 belonged to the S category, 14 to the M category,

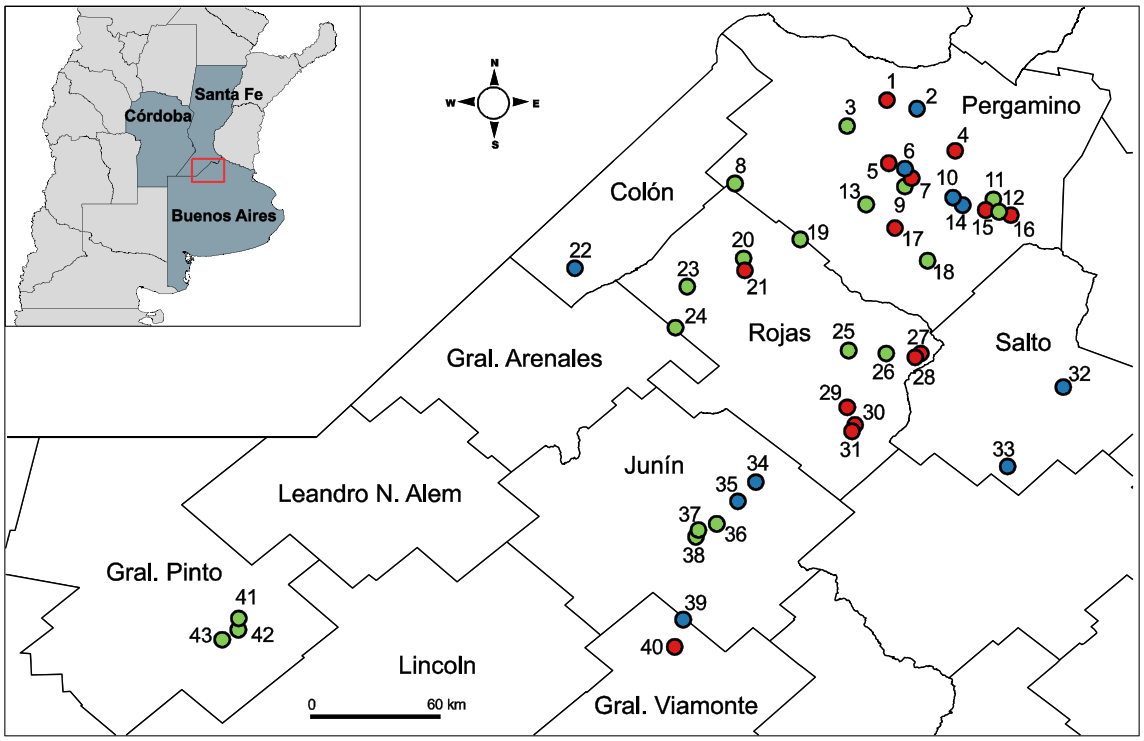

Fig. 1 Geographical distribution of sampled productions. The green, blue, and red circles indicate the productions of the small (S), medium (M), and large (L) strata, respectively. In the reference map, the three provinces that make up the productive nucleus of the country are shown in dark gray. BA, Buenos Aires; C, Córdoba; SF, Santa Fe

Table 1 General indicators of pork production in the province of Buenos Aires and Argentina during the year 2022. Modified from Ministerio de Agricultura, Ganadería y Pesca (2022) based on data from SENASA

	Productive units	Sow stock	Total pigs	Animals sent to slaughter	Slaughterhouses
Argentina	97,680	925,196	5,477,107	7,709,536	186
Buenos Aires province	16,759	225,898	1,338,815	2,098,155	47

and 10 to the L category (Fig. 1, Online Resource 1). It was decided to include all breeding farms with more than 100 sows within the same category to ensure a minimum of 10 farms in all strata (Online Resource 1).

A total of 109 muscle samples of non-related animals that were previously slaughtered were collected (Fig. 1, Online Resource 1). The samples were transported in 15-mL Falcon tubes with 96% alcohol and stored at −20°C in the tissue bank of the Centro de Bioinvestigaciones (UNNOBA, Pergamino, Buenos Aires) for further processing. It should be noted that these animals were already destined for slaughter and were not sacrificed specifically for the purposes of this study. The collection was carried out with prior authorization and supervision of a veterinarian and/or the responsible person of each establishment.

From each sample, between 50 and 100 ng/µL of genomic DNA was extracted using the phenol-chloroform-isoamyl alcohol extraction method (Sambrook and Russell 2006). The DNA was diluted in a final volume of 100 µL of Tris-EDTA buffer solution and stored at −20°C under sterile conditions.

For the genetic variability analyses using CR, 95 sequences of 734 bp of the marker (Online Resource 2) were amplified by PCR using the primers implemented by Kusza et al. (2014). The thermocycling conditions were set at 94°C for 2 min, followed by 30 cycles of 45 s at 94°C, 61°C for 45 s, and 74°C for 1 min with a final extension of 74°C for 10 min. PCR products were sequenced by Macrogen Co. Ltd. (South Korea).

The sequences obtained were visualized and corrected manually using the BioEdit v.7.0.5 program (Hall 1999). In this editing process, the initial fragments of 734 bp were reduced to 521 bp. New sequences were deposited in the GenBank nucleic acid sequence repository under accession numbers OQ802787-OQ802809 (Online Resource 2). The number of polymorphic sites, haplotype diversity, and nucleotide diversity were calculated using DNAsp v5 software (Librado and Rozas 2009).

For phylogenetic analyses, the sequences generated in this study (n=95) were combined with 139 sequences from the GenBank nucleotide database corresponding to domestic pig breeds from the USA, Europe, and Asia and wild pig populations from Argentina, Europe, and Asia (Online Resource 2). A javan warty pig (*Sus verrucosus*) sequence was added as outgroup.

Multiple-sequence alignments were performed using the ClustalW algorithm in the MEGA v.7 software (Kumar et al. 2016). The mutation model that best fit the data was calculated based on corrected Bayesian information criteria using JModelTest v2.1.4 software (Darriba et al. 2012). For the tree reconstruction, a strict clock as molecular clock rate variation model and a 100,000,000 generations Monte Carlo Markov Chain length, sampling every 1000 were set in BEAUti 2.6.3 (Bouckaert et al. 2019). Calculations were performed in BEAST2 (Bouckaert et al. 2019). To generate the consensus tree, first 20% of the sampling trees and estimated parameters were discarded as burn-in with TreeAnnotator v2.6.3 (Bouckaert et al. 2019).

Genotyping of nuclear markers for meat quality were obtained for 108 pig samples (Online Resource 2). For allele identification, the PCR-RFLP technique was performed (Otsu et al. 1992; Meadus et al. 2002). Specifically, the C1843T mutation (M9145.1:g.1843>T) was identified for ryr1, while two mutant alleles were identified for PRKAG3: one at codon 199 and another at codon 200 (NM_214,077.1:c596G>T: I>V and NM_214,077.1:c.599G>C: R>Q). Fragments of 659 bp and 249 bp were amplified for ryr1 and PRKAG3, respectively through the primers: Forward: 5′-TCCAGTTTGCCACAG GTCCTACCA-3′ and Reverse: 5′-ATTCACCGGAGTGGA GTCTCTGAG-3′ and Forward: RN + 1639F 5′-AAATGT GCAGACAAGGATCTCG-3′ and Reverse: RN + 1888R 5′-ACGAAGCTCTGCTTCTTGC-3′ for ryr1 and PRKAG3, respectively (Otsu et al. 1992; Meadus et al. 2002).

PCR was carried out in a final volume of 20 µL containing 25–100 ng template DNA, 1.0 mM $MgCl_2$, 0.2 µM each primer, 0.2 mM each dNTP, 1x reaction buffer, 0.5 U of Taq T-Plus DNA polymerase (Inbio Highway), and ultrapure sterilized water to complete the final volume. Thermocycling was performed as follows: a denaturation step at 94°C for 2 min, followed by 35 cycles of 45 s at 94°C, annealing step at 61°C for 40 s, and 62°C for 30 s for ryr1 and PRKAG3, respectively; and extension at 74°C for 1 min. A final extension step at 74°C for 10 min was added.

For ryr1, the C1843 mutation generates a recognition site for the HgiA1 endonuclease, so after digestion with this enzyme, the wild-type genotype (CC) is manifested with two bands, one at 524 bp and another at 135 bp (the cleavage occurs at a basal restriction site of the enzyme that is used as an internal control for digestion); the mutant genotype (tt) is manifested with three bands of 358, 166, and 135 bp due to the combination of the basal restriction site and the mutated site, while the heterozygote (Ct) is manifested with four bands of 524, 358, 166, and 135 bp. For digestion, 1 µg of PCR product was incubated with 0.5 µL of HgiA1 endonuclease (New England BioLabs) and 1X NEBuffer in a final volume of 40 µL at a temperature of 65°C for 15 min.

In the case of PRKAG3, the R200Q mutation changes the recognition site of the BsrBI enzyme in the wild-type allele, preventing cleavage. Therefore, after incubation, the homozygous wild-type genotype will show two fragments, one at 215 bp and another at 34 bp, while the homozygous mutant allele genotype (RN-/RN-) remains undigested. Heterozygous individuals manifest three bands of 249, 215, and 34 bp. It is important to note that the sample was considered heterozygous only if the signal of the undigested band was

greater than the digested band. As this fragment does not have an internal control, a positive control was used to evaluate enzyme efficiency.

The I199V mutation is identified by a cleavage at position 119 of the sequence. Homozygous wild-type individuals show a banding pattern with one fragment of 164 bp and one of 85 bp; homozygous mutant individuals exhibit three bands of 119, 85, and 45 bp; and heterozygous individuals show a combination of both patterns. The 85 bp fragment is generated due to a basal recognition site of the BsaHI enzyme, which serves as an internal control for digestion. For digestion, 1 µg of PCR product was incubated with 0.5 µL of BsrBI and BsaHI endonucleases (New England BioLabs) for R200Q and I199V mutations, respectively, and 1X NEBuffer in a final volume of 30 µL. In both cases, digestion was performed at 37°C for 60 min and 80°C for 20 min to inactivate the enzyme. It is worth noting that due to the linkage between V199I and R200Q, the 200Q variant is always found together with 199V, so the gene has three possible haplotypes: the wild-type allele rn+ (199V/200R), the rn* (199I/200R), and RN- (199V/200Q) (Milan et al. 2000).

The reaction mixture was mixed with Seeding Buffer in a 1:1 ratio and loaded onto a 2% (w/v) agarose gel. The gel was then stained with ethidium bromide (10 mg/ml).

The genotypic and allelic frequencies of both loci were calculated for the entire dataset and for each stratum separately. Given the observed negative additive effect between the t and RN- alleles, the combined genotype frequencies were also evaluated by stratum.

To assess the statistical significance of differences in frequencies between strata, a chi-square test was performed. Similarly, the Fisher test was used to evaluate the statistical significance between pairs of strata.

Results

A multiple alignment of the 95 CR sequences from this study revealed 22 polymorphic sites. Furthermore, a variable site represented by a gap was discovered at position 112 of the sequence. The sequences were grouped into 23 haplotypes (Online Resource 2). The total haplotypic diversity (Hd) was calculated to be 0.840 ± 0.031, and the nucleotide diversity (π) was estimated to be 0.010 ± 0.001. Variability data for each stratum can be found in Table 2.

The phylogenetic tree presents the E1A, E1C, A and NE clades previously described by Giuffra et al. (2000) and Scandura et al. (2011) based on the geographic location of the most frequent haplotypes within the European and Asian continents (Fig. 2).

Within the E1A clade, sequences from international modern breeds, such as Duroc, Landrace, Pietrain, Berkshire, Hampshire, and Large White, were grouped together with 51.72%, 55.55%, and 63.33% of sequences from the small, medium, and large strata, respectively. The sequence of a hybrid pig between wild boar and domestic pig, maintained in a small-scale breeding facility, is noteworthy within this clade (Online Resource 2). In the E1C clade, sequences from Iberian pigs, as well as the Duroc and Landrace breeds, were collapsed together with 24.14%, 27.78%, and 13.33% of sequences from the S, M, and L strata, respectively. Notably, the clade included a sequence from a wattle pig that was sampled from a small-scale backyard production (Online Resource 1 and 2).

Table 2 Genetic diversity indices calculated for CR in the three study strata (n=95). *Hd*, haplotypic diversity, π, nucleotide diversity, *SD*, standard deviation. *S* small stratum, *M* medium stratum, *L* large stratum

Strata	*n*	Polymorphic sites	*N*° haplotypes	Hd ± SD	π ± SD
S	29	18	11	0.828 ± 0.051	0.011 ± 0.002
M	36	16	13	0.892 ± 0.030	0.009 ± 0.002
L	30	20	14	0.754 ± 0.085	0.011 ± 0.002

In the Asian clade (A), sequences from domestic pigs from China and Japan, as well as modern breeds such as Duroc, Large White, Pietrain, and Berkshire, were grouped together with 24.14%, 16.67%, and 23.34% of sequences from the S, M, and L strata, respectively. A total of 7 exclusive haplotypes were identified among the populations studied in this work, with one belonging to the S stratum (Hap 18), four to the M stratum (Hap 2, Hap 9, Hap 15, Hap 17), and six in the L stratum (Hap 14, Hap 16, Hap 20–23).

Gene variants of ryr1 and PRKAG3 segregated in the three strata (Table 2).

The genotype frequencies calculated for ryr1 in the total sample set were 0.787, 0.1852, and 0.028 for the CC, Ct, and tt genotypes, respectively, with an allele frequency of $t = 0.120$. When comparing between strata, the genotypic frequency of tt and the allele frequency of t were higher in the S stratum, whereas no homozygotes for the mutation were detected in the L stratum (Table 2).

A total of 35.71% of farms (n=42) presented at least one copy of the ryr1 mutant allele. Specifically, the S (n=18), M (n=14), and L (n=10) strata showed 38.89%, 42.86%, and 20% of establishments with at least one copy of the mutant allele, respectively. The differences between strata were not significant according to the chi-square test (chi-square=1.466, p-value=0.481).

Regarding the PRKAG3 gene, it was observed that 50% of farms included in the study have at least one copy of the RN- allele on their farms. Specifically, the S (n=18) and M (n=14) strata presented 38.89% and 50% of establishments with at least one copy of the allele, respectively. The allele

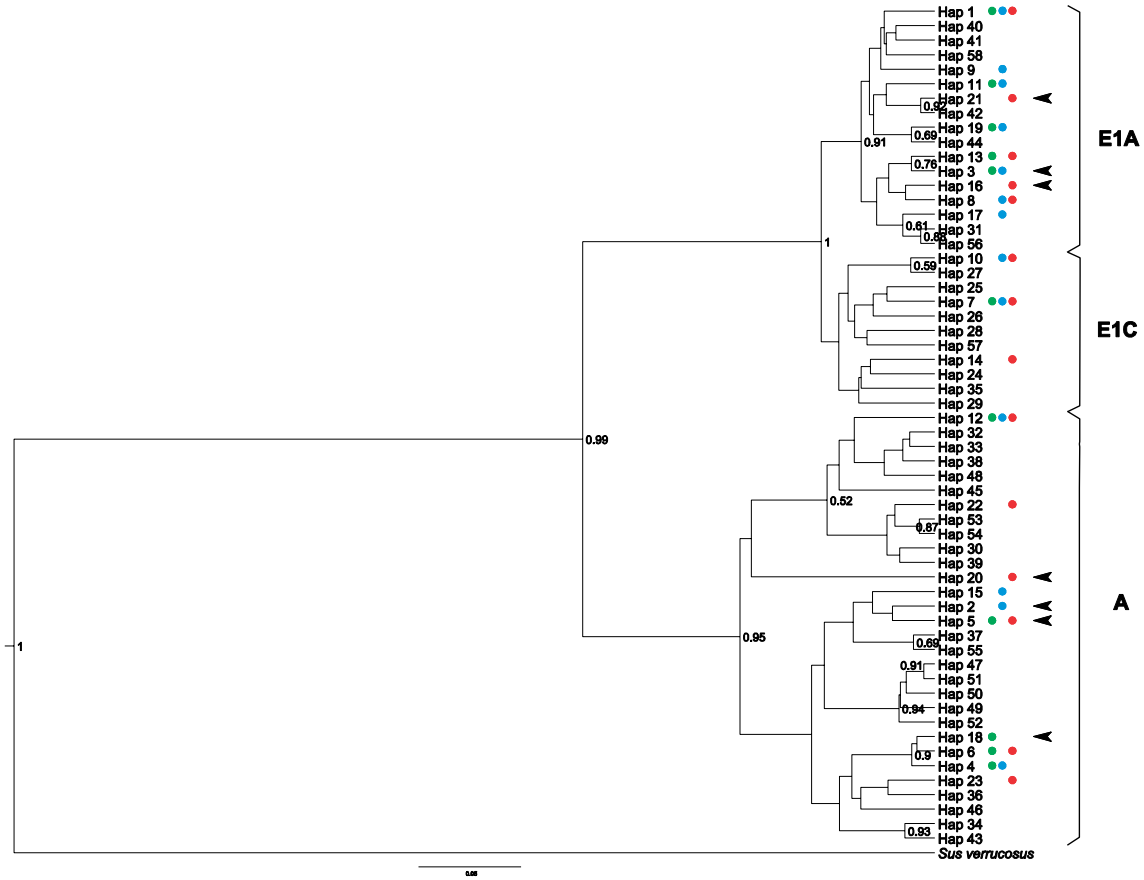

Fig. 2 Bayesian phylogenetic tree built from 59 control region haplotypes based on 521 pb sequences from this work (n=95) and GenBank database (n=139). The nodes show posterior probability values greater than 0.5. The green, blue, and red circles show haplotypes made up of sequences from the S, M, and L strata, respectively. Arrows point to haplotypes unique to this study. E1A, European clade, side A; E1C, European clade, side C; A, Asian clade. *Sus verrucosus* was used as outgroup

was not detected in the L stratum. Statistically, the difference in the number of farms with at least one copy of the RN- allele between the large stratum and the two remaining strata was significant (Fisher's test: p-value=0.019 and p-value=0.030 compared with the medium stratum and small, respectively).

The genotypic and allele frequencies for both PRKAG3 mutations, considering the total sample set, are presented in Table 3. The genotypes composed of at least one copy of the deleterious alleles t and RN- were detected at low frequencies (less than 10%) in both the small and large strata (Table 4).

Discussion

The results obtained in this study represent the first analysis of the phylogenetic relationships among pigs from the Argentine herds and the initial assessment of the genetic variability in the region. Additionally, for the first time, the identification and frequency of deleterious alleles for pig production in the productive core area are presented, demonstrating their prevalence in small, medium, and large herds.

In the context of global pig production, market dynamics require countries to make constant advances in technology and genetics. To achieve this goal, such characterization is necessary, which allows for informed management decisions. In this sense, this study aims to contribute to the knowledge of the current situation in Argentina.

According to the available literature, the estimated genetic variability indices for CR in this work could be considered high (Alves et al. 2010; Huo et al. 2014; Zhang et al. 2016). Alves et al. (2010) reported Hd values of 0.87 for local breeds (Alentejano and Bísaro) from the Iberian Peninsula. In that study, the authors analyzed populations with an apparent lower selection pressure compared to modern breeds; hence, comparable values could be deemed indicative of high genetic variability. Huo and colleagues (2014) presented lower Hd values from domestic pig breeds in China, ranging from 0.34 to 0.82. The high levels of

Table 3 Allele and genotypic frequencies of the ryr1 and PRKAG3 genes by stratum and in the total population, considering the three strata (n=108 pig samples). The frequencies of the records available in Argentina are included. *S* small stratum, *M* medium stratum, *L* large stratum

		This work				Lagadari et al. (2019)	Rodriguez et al. (2022)		Marini et al. (2012)
		Argentina				Argentina	Argentina		Argentina
		S	M	L	Total population		Hybrids	Creole	
RYR1 frequencies	CC	0.730	0.763	0.879	**0.787**	0.651	0.701	0.552	0.650
	Ct	0.243	0.184	0.121	**0.185**	0.349	0.299	0.448	0.308
	tt	0.027	0.053	-	**0.028**	-	-	-	0.042
	C	0.852	0.855	0.940	**0.080**	0.825	0.851	0.776	0.804
	t	0.148	0.145	0.060	**0.120**	0.175	0.149	0.224	0.196
PRKAG3 frequencies	rn+/rn+	0.406	0.342	0.394	**0.380**	0.060	0.162	0.092	
	rn+/rn*	0.027	0.079	0.091	**0.065**	0.163	0.234	0.149	
	rn*/rn*	0.378	0.158	0.515	**0.343**	0.072	0.123	0.046	
	rn+/RN-	0.108	0.210	-	**0.111**	0.169	0.110	0.184	
	rn*/RN-	0.081	0.158	-	**0.083**	0.530	0.364	0.506	
	RN-/RN-	-	0.053	-	**0.018**	0.006	0.007	0.023	
	rn+	0.473	0.487	0.439	**0.468**	0.166	0.334	0.259	
	rn*	0.432	0.276	0.561	**0.417**	0.419	0.422	0.373	
	RN-	0.095	0.237	-	**0.115**	0.415	0.244	0.368	

Table 4 Multilocus genotypic frequencies of the ryr1 and PRKAG3 genes by stratum. *S* small stratum, *M* medium stratum, *L* large stratum

Genotype	Frequencies			
	S	M	L	Total
CC/rn+rn+	0.298	0.263	0.485	0.343
CC/rn*rn*	0.270	0.079	0.364	0.231
Ct/rn*rn*	0.081	0.079	0.030	0.065
Ct/rn+rn+	0.108	0.053	0.030	0.065
CC/rn*RN-	0.054	0.158	-	0.074
CC/rn+RN-	0.081	0.131	-	0.074
Ct/rn+RN-	0.027	0.053	-	0.028
CC/RN-RN-	-	0.053	-	0.018
Ct/rn*RN-	0.027	-	-	0.009
tt/rn+RN-	-	0.026	-	0.009
tt/rn+rn+	0.027	0.026	-	0.019
Ct/rn*rn+	-	-	0.061	0.019
CC/rn+rn*	0.027	0.079	0.030	0.046

diversity obtained in our study may be due to the heterogeneity of breeds present in the region, including both international commercial breeds such as Landrace and local breeds such as Che Tapuy, even a hybrid between wild boar and domestic pig (Online Resource 2). Evidence supporting this hypothesis comes from the work of Fang and Andersson (2006), who studied 19 local breeds and different lines of international commercial breeds in populations from Europe and China. Their results show a greater number of variable sites and haplotypes than estimated in this study. Genetic variability levels such as those obtained in the study area are striking because commercial breeds are expected to have a wide representativeness in pig production in the region, replacing any local type. These exotic breeds in general present outstanding performance (especially under confinement) and optimal values in meat quality characters, but their massive use results in the erosion of genetic resources, and the loss of genetic variability (Rege and Gibson 2002).

The diversity indices by stratum showed slightly higher values in the small and medium (Table 2). This can be explained by the variety of sources that the lower strata maintain compared with productions of the higher stratum, which maintain a homogeneous gene pool. This supports the hypothesis of lower access to the purchase of commercial hybrids by small producers. Although in these strata, producers can acquire genetic material from the discarding of animals from larger strata, or mainly through exchange between members of the same stratum (Online Resource 2). Additionally, numerous producers have reported utilizing pigs from local herds, particularly the Che Tapuy breed, due to their commendable performance under the prevailing climatic conditions. These results support the idea that small productions can function as potential reservoirs of genetic variability and reinforce the need to replicate these studies in other regions to unravel this variability (McManus et al. 2010).

The phylogenetic tree shows that the sequences from this study, from all strata, are grouped into the three clades (Figure 2). The largest proportion of sequences from the three strata, along with the modern domestic breeds Large White, Duroc, Landrace, Pietrain, Berkshire, and Hampshire, are all currently present in Argentine territory and are grouped in clade E1A. These results agree with the origin of these breeds, which is Germany and England.

The sequences of local breeds from the Iberian Peninsula such as Negro Canario, Negro Lampiño, or Iberian Retinto are grouped in the E1C clade. A sequence from the control region of a wattle pig located in a family production stands out in this clade. This sequence corresponds to haplotype 7 belonging to the sub-clade E1C (Fig. 2). The phylogenetic lineage supports the hypothesis of a possible criollo origin for these pigs, with ancestry in Iberian breeds. Wattles are hanging appendages located at the base of the neck in some species such as pigs or goats. The origin of these appendages is not entirely clear, but it is believed to have originated from the Iberian pig lineages (Spain and Portugal) and animals belonging to the Mediterranean trunk (Castro et al. 2003). For this reason, the presence of wattle pigs in the American continent has traditionally been related to criollo strains, descendants of the pigs introduced by the colonizers in the fifteenth century.

In South America, there are known creole breeds described with this particular morphology in Uruguay (Uruguayan Mamellado pig), Brazil (some specimens of the Nilo breed), and Cuba (Cuban Creole pig) (Santana 2001; Mariante et al. 2003). Due to the replacement of these varieties with modern breeds in commercial farms, the presence of animals descended from creole strains and the presence of wild boar hybrids in the productive core region of the country reflect a reduced utilization of pedigree material by small producers. This could play a significant role in the conservation of animal genetic resources and serve as a basis for the generation of value-added strategies as a commercial grow option.

Just as with the Large White, Duroc, and Landrace modern breeds, the presence of Asian haplotypes in this study can be explained by the introgression that occurred during the eighteenth century when Asian pigs were introduced to Europe with the aim of increasing litter size in Large White or phenotypic changes such as black color in Large Black breed (Kijas et al. 1998; Chen et al. 2020). Genetic studies carried out since the early 2000s demonstrate how this introgression manifests in European pig breeds with the appearance of the Asian lineage in mitochondrial DNA haplotypes (Giuffra et al. 2000; Kijas and Andersson 2001; Scandura et al. 2008; Kusza et al. 2014).

Contextualizing the results obtained for the nuclear marker ryr1 with studies from Argentina, Marini et al. (2012), in a study carried out with commercial hybrids (Landrace × Yorkshire females and Duroc × Pietrain × Hampshire × Yorkshire males) from the provinces of Córdoba, Santa Fe, Chaco, and Tucumán, obtained higher genotypic frequencies of heterozygotes (Ct) and mutant homozygotes (tt) than those obtained in this study (Table 3). A similar scenario is presented in populations from the provinces of Entre Ríos and Chaco, where Ct genotypic frequencies ranging from 0.299 to 0.448 are reported without identifying tt homozygotes (Lagadari et al. 2019; Rodriguez et al. 2022). Although this study found the presence of mutant homozygotes, the t allele frequency was lower, partly due to the high frequency of heterozygotes found in those provinces where the mutant allele remains masked. The authors suggest that the high incidence of the mutant allele may be due to the lack of knowledge of producers, insufficient breeding planning, and the use of pure carrier lines for hybrid generation (Marini et al. 2012).

Comparing our results for ryr1 with those obtained for wild populations from the Buenos Aires coast, a higher frequency of heterozygotes is observed in the population analyzed in this study. Acosta et al. (2021) indicate that 6.6% of wild pigs are carriers of the deleterious allele. Historically, the increase in frequency of this allele has been associated with selection for lean meat and greater muscular development, specifically in breeds such as Pietrain, Poland China, or Landrace (Marini et al. 2012). This fact could explain the higher frequency in populations of hybrids developed from these breeds than in wild pigs (Acosta et al. 2021).

In a regional context, there is limited data on ryr1 genotype frequencies in neighboring countries, except for Brazil, where much of the work has been done only with commercial hybrids. Generally, the published results in that country show higher frequencies of heterozygotes than in this study, which is not expected for a global pork producer like Brazil, with 4.9 million tons produced per year and being the fourth largest exporter in the world. Brazil provides approximately 646 thousand tons to a wide range of countries in Europe, North and South America, Africa, and Asia (Gorga 2018). This positioning allows for updated technological infrastructure and excellent genetic material. It is estimated that 70% of Brazilian sows are artificially inseminated, which is practical for the selection of breeders free from harmful alleles (Fávero and Pereira de Figueiredo 2009).

This study focused on small to medium-scale production for self-consumption or small-scale commercialization, which provides a novel and necessary perspective for regional planning. Along these lines, it is interesting to highlight the results of Montenegro et al. (2010) for pig populations in Uruguay that include the local Pampa Rocha breed in the analysis. These authors estimated allele frequencies similar to those reported in Brazil for modern breeds and commercial hybrids, without finding the mutation in the local Creole breed.

Regarding the PRKAG3 gene, all three genotypes carrying the deleterious RN- allele were found (RN-/RN-, RN-/rn+, RN-/rn*), although they were found at low frequencies compared to other studies in Argentina (Lagadari et al. 2019; Rodriguez et al. 2022). Lagadari et al. (2019) reported similar frequencies for rn* and RN- in Entre Ríos, with low frequency of the wild type allele rn+ (Table 3). In our study, the results are opposite, with frequencies of rn* and rn+ being similar and exceeding 40% in both cases. The fact that the rn* allele has a frequency similar to the wild type is a positive indicator of quality, as a previous study showed a glycogen-reducing effect of rn* in non-RN- carriers, the latter being dominant (Lindahl et al. 2004).

Rodriguez et al. (2022) reported similar findings to those of Entre Ríos in the Chaco ecoregion. They determined RN- frequencies of 0.244 and 0.368 for hybrids and creole pigs, respectively, which were both higher than the frequencies observed in this study. The higher frequency in the creole population is unexpected, as it has no genetic linkage to the Hampshire breed. In contrast, the higher frequency in hybrids from various regions of the country suggests a low selection pressure for eliminating the mutation in genetic improvement programs. Our study revealed a significant difference in these frequencies between the large stratum and the other two, possibly due to successful genetic improvement efforts in the area, which have eliminated the RN- allele from commercial productions. Commercial productions have been reported to have a low frequency of the RN- allele, according to Cherel et al. (2010).

The scarcity of regional data emphasizes the significance of this study. Compared to other populations globally, the frequencies of the three genotypes RN-/RN-, RN-/rn+, and RN-/rn* were lower. The frequencies reported for Hampshire x Finnish Landrace crosses in Sweden were higher (RN-RN-=0.23, RN-rn+=0.24, RN-rn*=0.33, rn+rn+=0.08, rn+rn*=0.09, and rnrn=0.02) (Lindahl et al. 2004), as well as for American Hampshire populations (RN-RN-=0.397, RN-rn+=0.466, and rn+rn+=0.137) (Miller et al. 2000). This further underscores the importance of conducting similar studies in various populations within and among countries to have a more comprehensive understanding of the distribution and frequency of the RN- allele.

Our findings suggest that the deleterious RN- allele has low prevalence in the study area, although half of the productions still carry at least one copy of the variant. Notably, the major stratum showed no presence of this allele. This suggests a scenario in which higher strata with greater technology and access to RN- free material are achieving the elimination of RN- from their establishments, while the lower strata are eliminating it at a slower pace.

The difference in frequencies between the two genes (ryr1 and PRKAG3) studied may be attributed to their dominance relationships with respect to the traits being evaluated. RN- is dominant over rn+ and rn*, whereas the t allele in ryr1 has a recessive effect, making its elimination and detection within an improvement plan challenging (Martínez-Quintana et al. 2006; Cherel et al. 2010)

Previous studies have reported the additive effect of the ryr1 and PRKAG3 genes on the meat's ultimate pH, color, and water holding capacity, leading to lower organoleptic value in pigs carrying both harmful copies of the genes (Hamilton et al. 2000; Salas and Mingala 2016). Hamilton et al. (2000) reported that the Hampshire breed's CC/rn+rn+ pigs had the best intramuscular fat score. Based on this background, we calculated the ryr1/PRKAG3 multilocus frequency in our study, which showed no animals with deleterious copies of both genes in their genotype (tt/RN-RN-). However, it's worth noting that heterozygotes with both harmful alleles were present in both the small and medium strata (Table 4).

In developing countries like Argentina, it is crucial to develop tools that aid producers in technological advancement and participate in the national production chain. Genotyping is one such tool that can help identify animals carrying deleterious alleles and eliminate them from the productive chain to avoid segregation within the herd. This task can be carried out in state laboratories or state-dependent agencies like SENASA and incorporated into their routine analyses to help improve the efficiency and quality of the national production chain. Furthermore, the dissemination of artificial insemination practices and the provision of training by the government could potentially enable small-scale producers to access free materials. Data from the last National Agricultural Census (2018) reveals that only 10.8% of Argentina's pig productions employ artificial insemination, a significantly low figure in comparison to the world's leading pig producers (Knox 2015; INDEC 2020).

It is well known that both deleterious variants (RN- and t) are associated with optimal values of daily gain in pigs or better carcass characteristics, including quality traits like tenderness (Lundström et al. 1996). This could explain why these variants have rapidly segregated in the pig industry (Windig et al. 2004). However, the effects of malignant hyperthermia can result in significant economic losses, particularly for small producers. Additionally, studies indicate that the removal of RN- does not greatly impact productivity (Closter et al. 2011). These findings highlight the importance of elimination plans for these variants and the need to replicate these studies in other parts of the country to obtain a comprehensive national overview of Argentine swine production concerning these economically impactful aspects.

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Author contribution All authors contributed to the study conception and design. Material preparation and data collection were performed by all authors. Analyses were performed by Carlos Ezequiel Figueroa. The first draft of the manuscript was written by Carlos Ezequiel Figueroa, and all authors contributed, checked, and approved the final version of the manuscript.

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Data availability Control region sequences used for variability calculations and phylogeny construction are available in the GenBank database, under accession number OQ802787 to OQ802809.

Declarations

Ethics approval Animals from which tissue samples were obtained were not sacrificed specifically for the purposes of this study. No ethical approval is required.

Competing interests The authors declare no competing interests.

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