



Original investigation

## Genetic diversity and phylogenetic relationships in feral pig populations from Argentina

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### ABSTRACT

In Argentina, domestic pigs (*Sus scrofa* Linnaeus 1758) were introduced during the first Buenos Aires foundation, in the year 1536. Their provenance was mainly from the Iberian Peninsula, the Canary Islands and Cape Verde. In 1541 those pigs were released and, consequently, the first feral populations were originated. Thereafter, the species propagated both naturally and through human action, reaching a distribution that covers most of the Argentinian territory. The objective of this study is to genetically characterize the oldest feral pig populations in Argentina, making use of the mitochondrial control region (CR) and the amelogenin gene (AmelY), in order to determine their phylogenetic origin and corroborate its consistency with the historic information. The obtained results indicate that most of the feral pigs in Corrientes and Buenos Aires populations are positioned in the European subclades, E1-A and E1-C for CR, and HY1 and HY2 for AmelY. Despite this fact, a low frequency of individuals of Asian origin was found in populations from Buenos Aires, whereas none of them disclosed African ancestry. Furthermore, given that a large proportion of feral pigs found in the species' original sites in Argentina have European ancestry, we can partially corroborate the historical records.

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### Introduction

Domestic pigs (*Sus scrofa* Linnaeus 1758) were introduced in America during Christopher Columbus' second trip to the continent in 1493, through the island La Española (currently, Republic of Haiti and Dominican Republic). These first individuals came from La Gomera Island (Canary Islands) and expanded from there towards the new Caribbean colonies and the north of South America (Donkin, 1985; Río Moreno, 1996; Zadić, 2005).

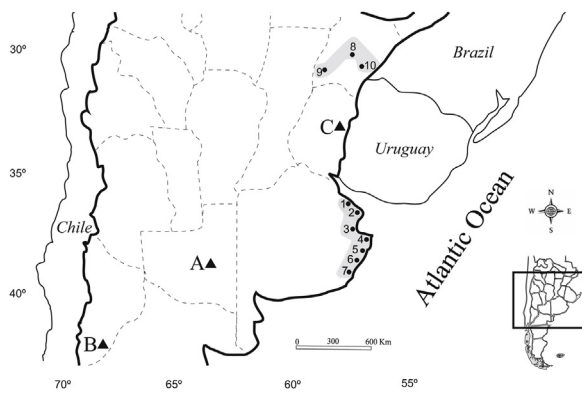
In the case of the South American Atlantic coast, the first pigs arrived in 1532, at San Vicente harbour, in what is currently the State of Sao Paulo (Brazil). They were originally from the Iberian Peninsula, the Canary Islands and Cape Verde, where the Por-

tuguese and Spanish conquerors did their stops, prior to their arrival to South America (Crossby, 2003; Donkin, 1985; Wernicke, 1938).

In 1536, during the first foundation of Buenos Aires by Pedro De Mendoza, the first domestic pigs arrive to the Río de la Plata river (Argentina). In 1541, the settlement was abandoned due to the strong famine produced by lack of food resources, added to the frequent attacks of the indigenous population (Schmidl, 2010). As a result, some of the animals were released, and this first free ranging pigs gave rise to the first feral pig populations, which rapidly occupied the plains and the hills of the current Buenos Aires Province (Cardiel, 1930; Giberti, 1985; Iriart, 1997; Morris, 1956; Sánchez Labrador, 1936; Schmidl, 2010). These first free ranging animals belonged to Hispanic Iberian breeds (negra lampiña, rubia andaluza, gallega, manchado de jabugo and perigordina), Portuguese breeds (alentejana and bísara) and those breeds local to the Canary Islands, Cape Verde and the Portuguese settlements in Rio de Janeiro, all stops in De Mendoza's travel towards the Río de la Plata river (Freitas and Rosado, 2014).

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**Fig. 1.** Sampling sites in populations of feral pigs from Buenos Aires and Corrientes provinces, Argentina. The sampling sites are indicated with numbers; letters indicate the wild boar introduction sites in Argentina. 1. Da. A. Juan Blanco, Magdalena, Buenos Aires; 2. Verónica, Punta Indio, Buenos Aires; 3. Bahía Samborombón, Buenos Aires; 4. Gral. Lavalle, Buenos Aires; 5. Pavón, Gral. Lavalle, Buenos Aires; 6. Reserva Laguna Salada Grande, Gral. Madariaga, Buenos Aires; 7. Reserva Provincial Mar Chiquita, Mar Chiquita, Buenos Aires; 8. Rincón del Socorro, Mercedes, Corrientes; 9. Maruchas, Goya, Corrientes; 10. Loma Alta, La Cruz, Corrientes. A. Gral. Acha, Conchelo, La Pampa; B. Lago Espejo, Los Lagos, Neuquén; C. Parque Nacional El Palmar, Colón, Entre Ríos.

During the next centuries, feral pigs' populations expanded through the Argentinian territory, especially in the Atlantic coast, the Paraná and Uruguay rivers margins, the Negro and Colorado rivers outfalls and the plateaus in the territory of the Río Negro Province (Cardiel, 1930; Hudson, 1956; Maeder, 1981; Musters, 1997; Villarino, 1783).

Otherwise, in the beginning of the XX century, the European wild boar (*Sus scrofa scrofa*) was introduced in La Pampa Province (Argentina) to fulfil a hunting purpose. From this site and other subsequent ones in the Provinces of Neuquén and Entre Ríos (Fig. 1), the wild boar presented a major geographic expansion, both natural and driven by human activity. This situation promoted mating between wild boars and feral pigs, giving origin to a complex interrelation among the species' different morphotypes (Navas, 1987; Novillo and Ojeda, 2008; Sagua et al., 2018).

Currently, feral pig populations inhabit a broad region of the country; the largest and oldest populations being found in the Bahía Samborombón region located in the east of the Buenos Aires Province and Corrientes Province (Fig. 1). These feral populations have gone through more than 400 years of environmental adaptation and constitute a genetic reservoir, with a direct bond to the breeds originally introduced by the Spanish conquerors.

The feral pig population from Bahía Samborombón, of an estimated size of 10,000 individuals, was established in the early onset of the Spanish conquest. Therefore, the individuals of this population are direct descendants of those originally introduced 150 km away from Bahía Samborombón, in the site of the first foundation of Buenos Aires (Merino and Carpinetti, 2003; Pérez Carusi et al., 2009). Feral pigs have been proved to present good maternal ability and resistance to diseases of great sanitary relevance, such as trichinosis, classic swine pest and apthous fever, among others. Consequently, the settlers in the population's surrounding areas, usually use feral pigs as a source of genetic improvement for their domestic pig productions (Carpinetti et al., 2016; Serena et al., 2015).

On the other hand, the feral pig populations from Corrientes were developed from individuals introduced in the XVI century, which rapidly became wild, forming an important food source, both for the natives and the growing colonial population (Maeder, 1981).

Before our research the only records in South America, as to genetic diversity in feral pig populations, are from the studies of

Aravena et al. (2015) and Grossi et al. (2006), for populations from Tierra del Fuego (Chile) and Pantanal (Brazil), respectively.

Nowadays, the advance of molecular techniques coupled with phylogenetic analysis for *Sus scrofa*, using the control region (CR) of mitochondrial DNA and the Y chromosome marker, are key to unravel the history of the introduction of wild populations, variability levels and population structure (Aravena et al., 2015; Gongora et al., 2004; Iacolina et al., 2016; Kim et al., 2002; Larson et al., 2010; Ramírez et al., 2009; Scandura et al., 2008; Veličković et al., 2015). Particularly the use of CR sequences, have allowed the identification of the geographic distribution for the different clades: E1 (widely distributed in the European continent), subdivided in E1-A (Italy, France, Germany, and Austria), and E1-C (Iberian Peninsula and Central Europe), E2 (restricted to Italian Peninsula, Sardinia and Croatia), NE (Near Eastern) and A (Asian) (Alexandri et al., 2012; Fang and Andersson, 2006; Giuffra et al., 2000; Kusza et al., 2014; Ramírez et al., 2009). On the other hand, studies based on the amelogenin gene present in the Y chromosome (AmelY), have allowed the identification of three present haplogroups: HY1, HY2 and HY3. In the HY1, we frequently found domestic pigs and wild boars of European and Asian ascendancy; in HY2, European wild boars and domestic pigs, as well as African wild boars; at last, in HY3, African pig breeds are predominant (Ramírez et al., 2009).

The aim of this work is to obtain the first description of the genetic variation for the oldest populations of feral pigs in Argentina, through the mitochondrial CR and AmelY markers. These results are analysed and discussed, in order to clarify the phylogenetic origin of these populations, and corroborate their consistency with the historical records.

## Material and methods

### Study area and sampling

A total of N = 136 samples of epithelial and muscular tissues were collected from feral pigs from Buenos Aires (N = 102) and Corrientes (N = 34) Provinces, Argentina (Fig. 1; Table 1). Samples were obtained from the different localities through collaborators. They were stored in 96% ethyl alcohol at  $-20^{\circ}\text{C}$  in the sample bank of the Centro de Bioinvestigaciones (Pergamino, Argentina) until processing.

Buenos Aires' populations are located in the Pampa ecoregion (Matteucci, 2012), characterized by a prairie ecosystem and a temperate sub-humid climate, with medium annual temperatures between  $14$  and  $16^{\circ}\text{C}$  and a rainfall of  $700$ – $1200$  mm/year (Iriondo, 1995). The predominant vegetation is grass steppe or pseudo-steppe, with floodable plains comprised by brackish substrate and halophilic vegetation.

Populations from Corrientes are distributed over two different ecoregions. The locality of Loma Alta, is located in the Mesopotamian savannah ecoregion, have a landscape composed of natural humid grassland sites on flat lowlands, locally known as "malezales" (Carnevali, 1994; Di Giacomo and Casenave, 2010). The preponderance of one or a few tall grass species is characteristic to this ecoregion (Etchepare et al., 2013). The climate is humid subtropical without a delimited dry season; medium temperatures are of  $19$ – $20^{\circ}\text{C}$  and rainfalls of  $1200$  mm per year. Otherwise, the locality of Rincón del Socorro and Maruchas, are located in the Iberá marshes ecoregion, which comprises a set of functionally related ecosystems, among which, the marsh habitats are predominant (Neiff, 2004). The landscape is dominated by lagoons, dams of floating vegetation and marshes. The medium temperatures in this humid subtropical climate are between  $16$  and  $28^{\circ}\text{C}$ , with rainfalls of  $1800$  mm per year. Given the different habitats found in this ecoregion, vegetation associations are found, with *Cyperus gigan-*

**Table 1**  
Sampling sites, indicating sample ID, Origin (locality), geographic coordinates, number of samples (N), Status (feral pig or wild boar) and Reference.

ID site	Locality	Province	Geographical Coordinates	N	Status	Reference
1	Da. A. Juan Blanco, Magdalena	Buenos Aires	35°4'54.86"S 57°18'17.366"W	1	Feral pig	This study
2	Verónica, Punta Indio	Buenos Aires	35°21'54.495"S 58°17'14.283"W	1	Feral pig	This study
3	Bahía Samborombón	Buenos Aires	36°17'35"S 57°19'03"W	81	Feral pig	This study
4	Gral. Lavalle	Buenos Aires	36°21'18.439"S 56°23'23.703"W	5	Feral pig	This study
5	Pavón, Gral. Lavalle	Buenos Aires	36°42'50.04"S 56°44'20.04"W	3	Feral pig	This study
6	Reserva Laguna Salada Grande, Gral. Madariaga	Buenos Aires	36°57'34.3"S 56°57'43.6"W	3	Feral pig	This study
7	Reserva Provincial Mar Chiquita, Mar Chiquita	Buenos Aires	37°40'40.001"S 57°30'0"W	8	Feral pig	This study
8	Rincón del Socorro, Mercedes	Corrientes	28°38'46.94"S 57°25'52.59" W	25	Feral pig	This study
9	Maruchas, Goya	Corrientes	29°10'0.12"S 59°4'0.48"W	3	Feral pig	This study
10	Loma Alta, La Cruz	Corrientes	29°3'0"S 57°4'59.88"W	6	Feral pig	This study
A	Gral. Acha, Conhelos	La Pampa	37°22'41.472"S 64°36'15.494W	–	Wild boar	Sagua et al., 2018
B	Lago Espejo, Los Lagos	Neuquén	40°41'25"S 71°41'41"W	–	Wild boar	Sagua et al., 2018
C	Parque Nacional El Palmar, Colón	Entre Ríos	31°40'17.5"S 58°14'2.7" W	–	Wild boar	Sagua et al., 2018
Total				136		

teus communities, dams with water hyacinths and other aquatic plants that form floating islets.

Therefore, the characteristics given by these three ecoregions to the feral pigs are fundamental for the survival and reproduction of the species, leading to an exponential growth of the population.

#### Laboratory analysis and sequencing

Genomic DNA was extracted following the "phenol-chloroform protocol" (Sambrook and Russell, 2006). DNA from each specimen was eluted in 100 µl of Tris-EDTA buffer solution and stored at –20 °C, under sterile conditions to preclude contamination until sample use for polymerase chain reaction (PCR) analysis.

For the complete set of sequences (N = 136), a 734 bp fragment of CR between sites 15,390 and 16,124 was amplified by PCR using Rf 5'-CGCCATCAGCACCCAAAGCT-3' and RCr 5'-ACCATTGACTGAATAGCACCT-3' primers (Alves et al., 2003). A subset of N = 50 samples was used to identify polymorphic sites in the Y chromosome. For this, a 543 bp fragment of the Amely gene was amplified by PCR using the primers described by Ramírez et al., 2009 (Amely-proFW 5'-GCGTTACATGCATATTGCCTTG-3' and Amely-E1Rv 5'-TCAAGGATGCTGGAGCTTTT-3').

PCR reaction was set to a final volume of 20 µL, containing: 25–100 ng of template DNA, 1.5 mM Cl<sub>2</sub>Mg, 0.2 µM of each primer, 0.2 mM of each dNTP, 1X reaction buffer, 0.5U of Taq T-Plus DNA polymerase and ultrapure sterile water to come to final volume. Thermocycling conditions were set at 94 °C for 5 min, followed by 30 cycles of 45 s at 94 °C, 62 °C CR and 55 °C Amely for 45 s, and 45 s at 74 °C, with a final extension at 74 °C for 5 min. All amplifications were performed in conjunction with a negative control (distilled water). DNA fragment amplification was confirmed by electrophoresis on 1% agarose gel, stained with ethidium bromide and visualized under UV light.

Amplification products were purified using 10U of Exonuclease I and 1U of FastAp thermosensible alkaline phosphatase, incubating at 37 °C for 15 min and then at 85 °C for another 15 min to stop the reaction. These purified PCR products were sequenced by Macrogen Co. Ltd. (South Korea).

#### Data analysis

The sequences obtained for both molecular markers were visualized and manually edited using BioEdit v.7.0.5 (Hall, 1999), resulting in fragments of 641 bp and 510 bp, for CR and Amely, respectively.

Haplotype and nucleotide diversities and number of polymorphic sites were calculated making use of DnaSP 5.10.1 (Librado and Rozas, 2009). Haplotype sequences were loaded into the GenBank nucleic acid sequence database (<http://www.ncbi.nlm.nih.gov/genbank>) with accession number MN539114–MN539137 for CR and MN544275–MN544278 for Amely.

To perform the phylogenetic analysis, as well as to determine the haplotype relationships, 148 CR and 32 Amely sequences were taken from the GenBank nucleic acid database, which include European and Argentinian wild boars, European and Asian domestic pigs and feral pigs from Chile (Appendix A). In the case of wild boars in Argentina, we employed only four haplotypes from the introduction centres, located in the provinces of La Pampa, Entre Ríos and Neuquén, analysed in the research of Sagua et al., 2018 (Fig. 1; Table 1). Feral pigs from Chile were employed in order to evaluate their relationship with feral populations in Argentina.

A multiple alignment was performed for the complete set of sequences, using ClustalW algorithm in the MEGA v.6 software (Tamura et al., 2013). This alignment was used to obtain the phylogenetic trees, which were built with Bayesian and Neighbour Joining (NJ) methods of statistical inference. For the NJ phylogeny, the nodes' confidence degree was assigned by bootstrapping with 1000 replicates through the software MEGA v.6 (Felsenstein, 1985). In the Bayesian analysis, the mutational model that best fits the data set was determined through JModelTest software v2.1.4 (Darriba et al., 2012; Hasegawa et al., 1985). The data was subsequently converted into BEAST XML format through BEAUti 1.7.5 (Drummond et al., 2012). For the tree reconstruction for both molecular markers, the following settings were used: strict clock as molecular clock rate variation model and 50,000,000 generations Monte Carlo Markov Chain length, sampling every 1000. All calculations were performed in BEAST (Drummond et al., 2012); the first 25% of the sampling trees and estimated parameters were discarded as burn-in with TreeAnnotator v1.7.5 (Drummond et al., 2012). FigTree v1.4.0 was employed to visualize the phylogenetic tree (Rambaut, 2012).

The relationships between haplotypes were attained making use of the Median-Joining algorithm through the PopART v1.7 software (Bandelt et al., 1999; Leigh and Bryant, 2015). In order to evaluate population expansion through the CR marker, the pig population was divided in the Corrientes and Buenos Aires groups, tested for neutrality with Tajima's D and Fu F<sub>s</sub> tests in Arlequin 3.5 (Excoffier and Lischer, 2010).

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## Results

#### Control region

For the complete set of *Sus scrofa* sequences (N = 284), of the 93 found haplotypes, 24 contain sequences of feral pigs obtained in this study, 21 of them being new haplotypes and the remaining three (H1, H3 and H4), previously reported for Europe and Caucasus (Table 2; Appendix A). For these 24 haplotypes, 36 variable sites were found, of which, 28 are parsimony-informative sites, 5



singletons and 3 sites with gaps or missing data (Table 2). The haplotype diversity was  $Hd = 0.710 \pm 0.033$  and the nucleotide diversity  $Pi = 0.00612 \pm 0.00078$ .

Regarding the phylogenetic analysis, both the Bayesian and the NJ trees, showed the same topology (Fig. 2; Appendix B). From the Bayesian inference, it transpires that HKI+G is the mutational model that best explains our data (Hasegawa et al., 1985). The different haplotypes, obtained from the Buenos Aires' and Corrientes' populations of feral pigs, can be observed in the phylogenetic tree in the subclades previously reported as E1-A, E1-C and the A clade.

In the E1-A subclade, the found haplotypes are H1, H2, H4, H11, H12, H16-H18 and H21-H23. In the E1-C subclade, the haplotypes are H3, H5-H10, H13 and H24. At last, in clade A, H14, H15, H19 and H20 are found.

For the Buenos Aires feral pig populations, 78.21% of the analysed sequences correspond to the subclade E1-A (H1, H2, H4, H11, H12, H16, H21-H23), while 13.59% belong to the E1-C subclade (H3, H7, H13, H24) and the remaining 8.2%, to the A clade (H14, H15, H19, H20) (Fig. 3).

As to the feral pig populations from Corrientes, 79.4% of the analysed sequences are found in the E1-C subclade (H3, H5-H10, H13), while the other 20.6% belongs to the E1-A subclade (H4, H17, H18) (Fig. 3).

The Median-joining haplotype network supports the relationships suggested by the phylogenetic analysis, where the link between feral pig and wild boars' haplotypes from Argentina can be visualized, as well as their relative place and relationships into the subclades E1-A, E1-C and A clade (Fig. 4). Moreover, 13 mutational steps separating clades E1 and A can be visualized.

Tajima's D neutrality test for the Buenos Aires and Corrientes groups was not significant (-0.79 and -0.362 respectively); however, Fu's FS test resulted in values of -0.27 for Buenos Aires and -3.13 for Corrientes, being significant ( $p \leq 0.05$ ) for this last population.

### AmelY

For the analysed set of *Sus scrofa* sequences (N=80), seven haplotypes were found, four of which contain sequences of feral pigs generated in this study, A6 and A7 correspond to new haplotypes, while A1 and A4 being previously reported (Table 2; Appendix A). Two variable sites are detected among the four haplotypes found in the samples from Argentina, with a value of  $Hd = 0.47 \pm 0.0016$  for haplotype diversity and a nucleotide diversity of  $Pi = 0.00092 \pm 0.00008$  (Table 2).

Both the Bayesian and the NJ phylogenetic trees present the same topology (Fig. 5, Appendix C). According to the Bayesian inference, our data fits best to the HKI+I mutational model (Hasegawa et al., 1985).

In the Bayesian phylogenetic tree, the four obtained haplotypes can be visualized as part of the groups previously reported as HY1 and HY2 (Fig. 5, Appendix C). Notably, only sequences from the Buenos Aires feral pig populations are found in the HY1 haplogroup (A4, A6); while the haplogroup HY2 contains sequences from both provinces in the same frequency (A1 and A7) (Fig. 5; Appendix A).

### Discussion

In this study, we present the first results regarding genetic variability and phylogenetic origin in feral pig populations from Argentina, particularly from the largest and oldest Buenos Aires and Corrientes populations.

Historical records indicate that part of the domestic pigs that arrived in South America during the XV century, coming from the Iberian Peninsula, Canary Islands and Cape Verde (Burgos-

Paz et al., 2013; Crossby, 2003; Donkin, 1985; Wernicke, 1938), originated the feral populations that expanded throughout the Argentinian territory later on (Cardiel, 1930; Giberti, 1985; Iriart, 1997; Morris, 1956; Sánchez Labrador, 1936). In the XX century, with the wild boar arrival to Argentina, a more complex interrelations pattern arose, presenting interactions among domestic pigs (including hybrid breeds), feral pigs and wild boars (Navas, 1987; Novillo and Ojeda, 2008; Sagua et al., 2018).

The phylogenetic analysis for the CR marker, partially support the historic records, finding feral pigs from Buenos Aires populations of both, European and Asiatic ascendancy (Mar Chiquita population and few individuals from Bahía Samborombón); this suggests the possibility of different colonization events. In this sense, the greatest haplotype diversity in feral pig populations from Buenos Aires, compared to those from Corrientes, is probably due to the European (E1-A and E1-C) and Asian lineage contribution. Furthermore, most individuals from Buenos Aires populations were grouped in the E1-A subclade, while those from Corrientes were mainly found on the E1-C subclade, supporting the hypothesis of more than one colonization event.

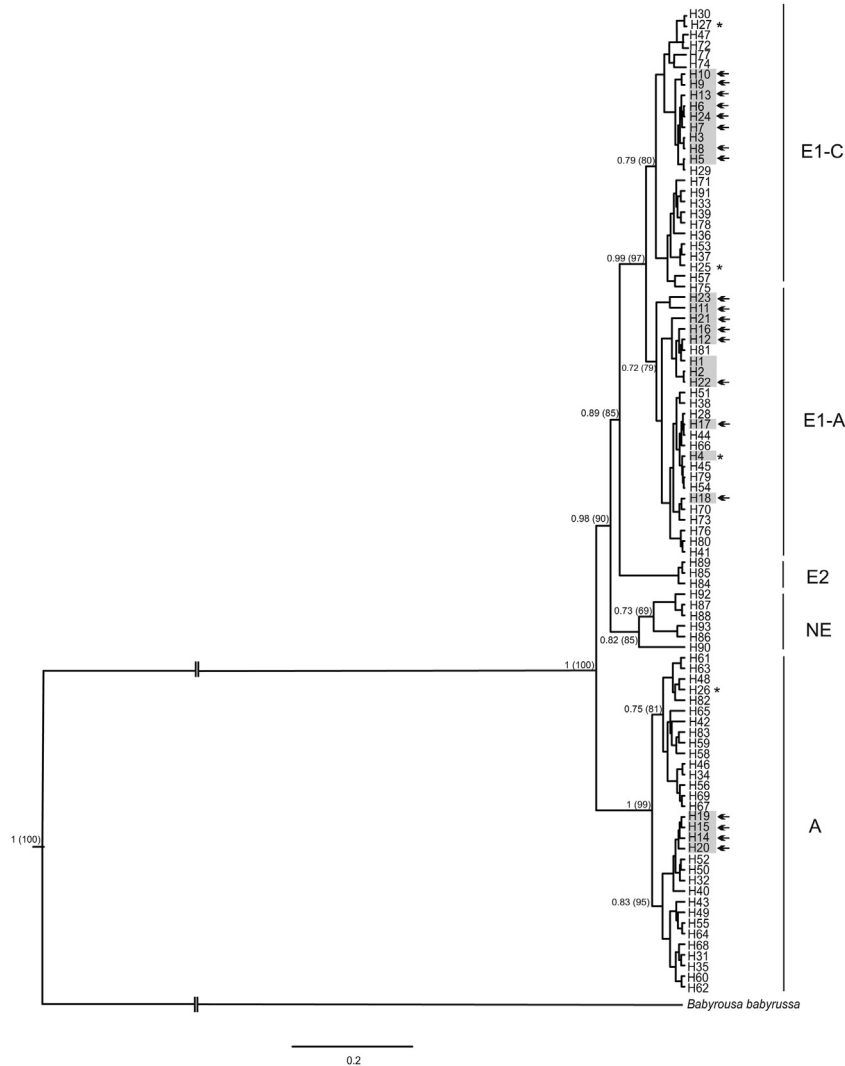
The E1-A subclade is mainly comprised with domestic pigs from the Portugal and Canary Islands, while subclade E1-C holds individuals from the Iberian Peninsula (Alves et al., 2003, 2010; Fang and Andersson, 2006; Kim et al., 2002; Kuzsa et al., 2014; Larson et al., 2005; Watanobe et al., 2003). Based on these records, our genetic results are in concordance with the historical information.

Previous studies on genetic diversity making use of the CR marker in feral pig populations in Chile and Brazil, showed European ascendancy on all individuals studied (Aravena et al., 2015; Grossi et al., 2006). On the other hand, there is evidence that feral pig populations from Tierra del Fuego (Chile) are more recent than the Argentinian populations, being established over the XIX century (Aravena et al., 2015; Gade, 1987). In our study, feral pigs from Tierra del Fuego presented haplotypes (H28 and H29) related to the ones from Corrientes (H17 and H5), and showing haplotype identity with Iberian breeds as well. The genetic closeness between this geographically distant feral populations, may be ought to the scarce or absent intervention of other morphotypes, entailing to a gene pool nearly identical to that of Iberian breeds (Aravena et al., 2015; Gade, 1987).

There are controversies as to the Pantanal feral pig population (Brazil), which is known to be over 200 years old. Sollero et al. (2009) indicates that the first individuals arrived in 1864, after the Paraguay war; on the other hand, Gonela (2003) argues that this feral "porcos monteiros" are descendants of the pigs brought by the conquerors in 1778, during Alburquenque's foundation (currently Corumbá). Given that the "porcos monteiros" sequences are not available in GenBank, we were unable to include them in our study.

Regarding the origin of individuals of feral pigs in the clade A is difficult to explain, considering that the original Iberian pig breeds did not present Asian gene introgressions (Alves et al., 2003) and given that, up to date, the only evidence of a direct introduction of Asian pigs to the American continent is the case of the Cuino pig in Mexico (Burgos-Paz et al., 2013; Lemus and Ly, 2010). However, it is known that there was an indirect Asian contribution to European breeds can be traced to the XVIII-XIX centuries, when genic Asian introduction was utilized for breeding purposes. Therefore, those European domestic pigs that arrived to Argentina might have carried an Asian component in their gene pool (Alves et al., 2003; Giuffra et al., 2000; Kim et al., 2002).

Other possible explanation as to the presence of Asian variants in feral populations, could be the mating of local feral pigs with modern domestic breeds. Currently, the most spread breeds in Argentina are Landrace, Large White, Hampshire and Duroc; through CR and Cytochrome *b* markers, previous studies identified



**Fig. 2.** Bayesian phylogenetic tree obtained for the 641 bp fragment (94 haplotypes) in the CR marker. Haplotypes containing the study's feral pigs ( $N = 24$ ) are presented in colour. Arrows indicate new Argentinian haplotypes. Haplotypes that contain sequences of wild boar from Argentina are indicated as an asterisk. Posterior probability  $>0.50$  is expressed in the nodes. Parenthesis specify bootstrap values  $>50\%$ .

them as related to the Asian clade (Alves et al., 2003; Giuffra et al., 2000; Kim et al., 2002).

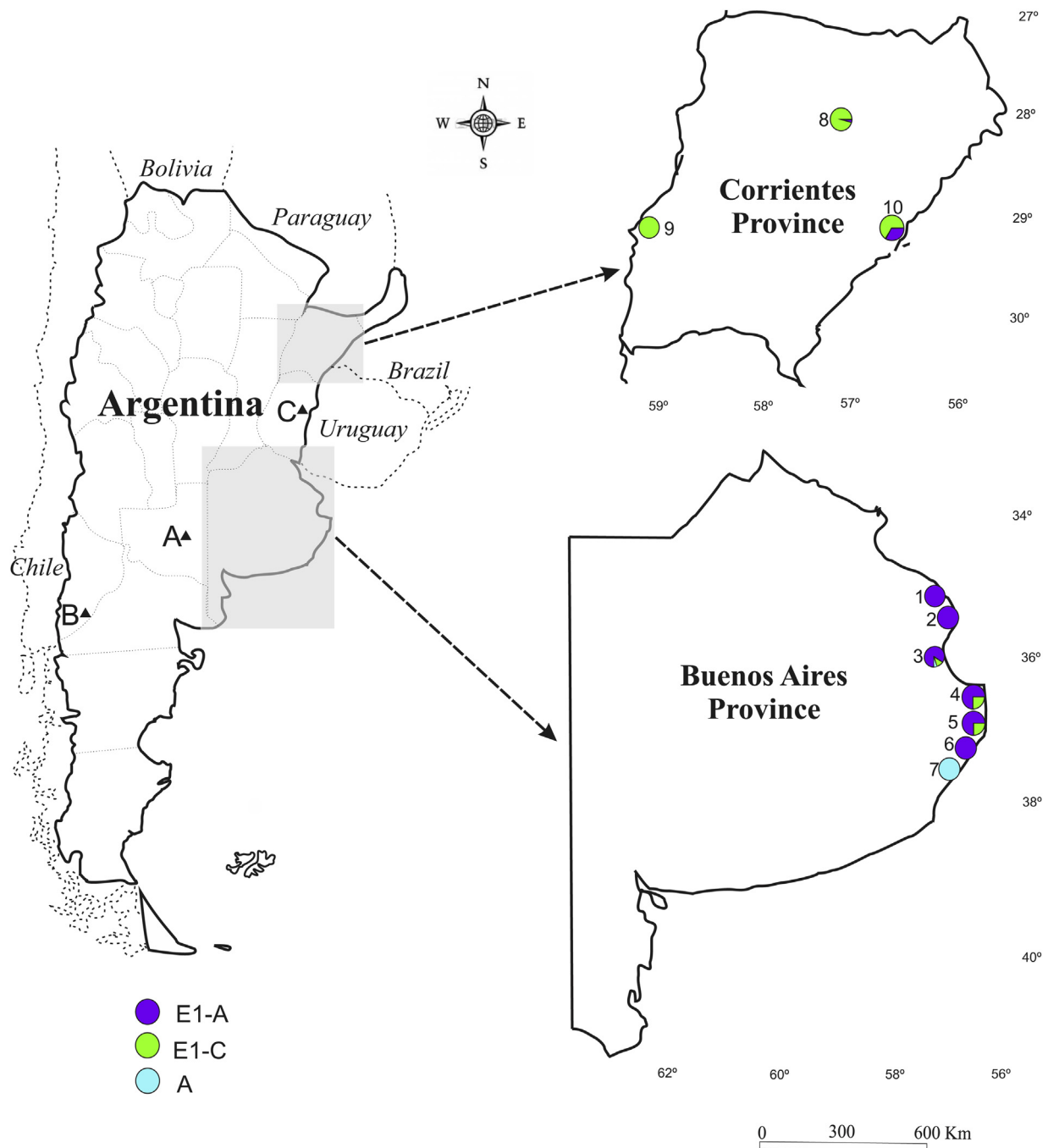
Another contribution to Buenos Aires feral pig population's major genetic diversity, might be introduction of wild boar coming from the province of La Pampa in 1973 to "El Destino" natural reserve (Magdalena county) at the north of Bahía Samborombón (Giménez-Dixon, 1991).

In other respects, historical records report that some of the individuals to enter the country, were of African provenance (Cape Verde islands). Hence, we employed the AmelY marker to explore African lineage presence in feral pig populations from Argentina. Previous studies with mitochondrial (CR) and nuclear markers (UTY, SRY, NRY y MC1R), report that African pigs have a particularly complex ancestry, given that their origin can be traced back to Europe, Asia's Southeast and India (Adeola et al., 2017; Noce et al., 2015; Osei-Amponsah et al., 2017).

Through this analysis we do not detect any African component in the studied populations of feral pigs, because the found haplotypes in Buenos Aires' populations belong to the HY1 and HY2 haplogroups, while the Corrientes populations were grouped in the HY2 haplogroup, indicating strong European and Asian ascendancy. These results differ from those obtained by Ramírez et al. (2009), who reported an individual

with African origins (HY3) in Entre Rios, Argentina. Moreover, this marker allowed the recognition of greater haplotype diversity in Buenos Aires populations, concurrent with the results for the CR marker.

The CR-estimated gene diversity for Argentinian feral populations was lower than that reported for domestic breeds and European wild boars (Alves et al., 2010; Van Asch et al., 2012). Particularly, for Argentina's data set of feral pigs, the haplotype diversity ( $Hd = 0.710 \pm 0.033$ ) is lower than reported for Argentina's wild boars ( $Hd = 0.827 \pm 0.017$ ), European domestic breeds ( $Hd = 0.886 \pm 0.008$ ), European feral pigs ( $Hd = 0.791 \pm 0.089$ ) and European wild boars ( $Hd = 0.912 \pm 0.007$ ), both in the Iberian Peninsula ( $Hd = 0.92 \pm 0.01$ ) and in Central Europe ( $Hd = 0.89 \pm 0.07$ ) (Alves et al., 2010; Sagua et al., 2018; Van Asch et al., 2012). In the same manner, the nucleotide diversity value for the feral pigs from this study ( $Pi = 0.00612 \pm 0.00078$ ) was lower to the ones reported for wild boars from Argentina ( $Pi = 0.007 \pm 0.001$ ), European domestic ( $Pi = 0.016 \pm 0.008$ ) and feral pigs ( $Pi = 0.009 \pm 0.005$ ), as well as European wild boars ( $Pi = 0.012 \pm 0.006$ ) (Alves et al., 2010; Sagua et al., 2018; Van Asch et al., 2012). The reduced genetic diversity observed for Argentinian feral pig populations, in contrast to ancestral European domestic pig populations, is probably a consequence of the founder effect



**Fig. 3.** Distribution of the subclades previously reported as E1-A, E1-C and A in the feral pig populations from Buenos Aires and Corrientes, for the 641 bp CR fragment. Numbers indicate sampling sites and letters show main wild boar introduction sites. Colours illustrate subclades E1-A, E1-C and A.

from the small amount of individuals that arrived to the country in the XV century and gave origin to these populations.

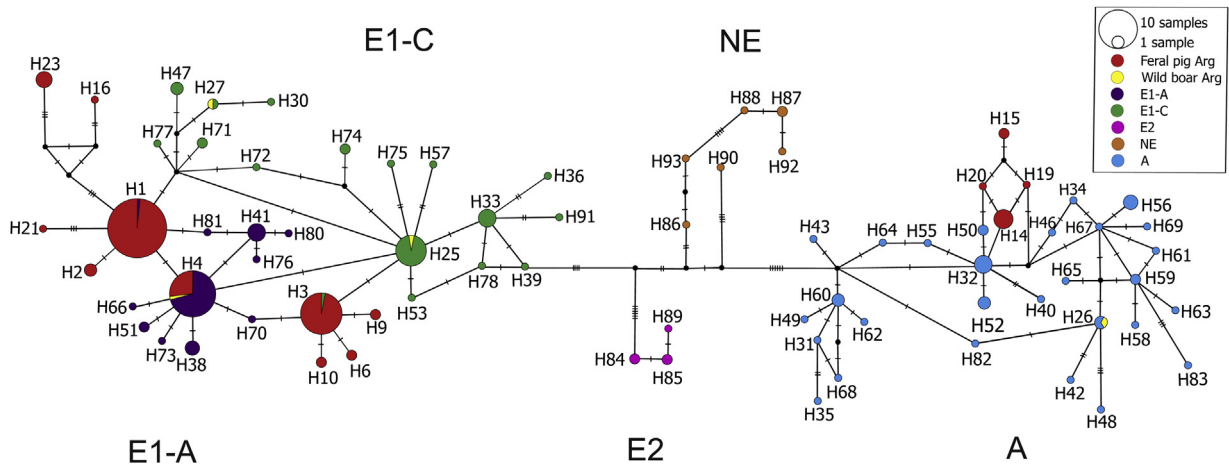
On the other hand, our results are compatible with a scenario of recent expansion in both population groups studied of feral pigs, but most visibly, in populations from Corrientes. The aforementioned expansions might be induced by the humid and highly productive habitats, as well as the lack of predators, other than the human-inflicted hunting pressure (Carpinetti, 2015; Merino and Carpinetti, 2003; Neiff and Poi de Neiff, 2006; Volpedo et al., 2005).

In conclusion, this is the first study to analyse phylogenetic origin in Argentina's oldest feral pig populations. Our results are consistent with the available historical records, building up to a preliminary confirmation of the European ascendance for most

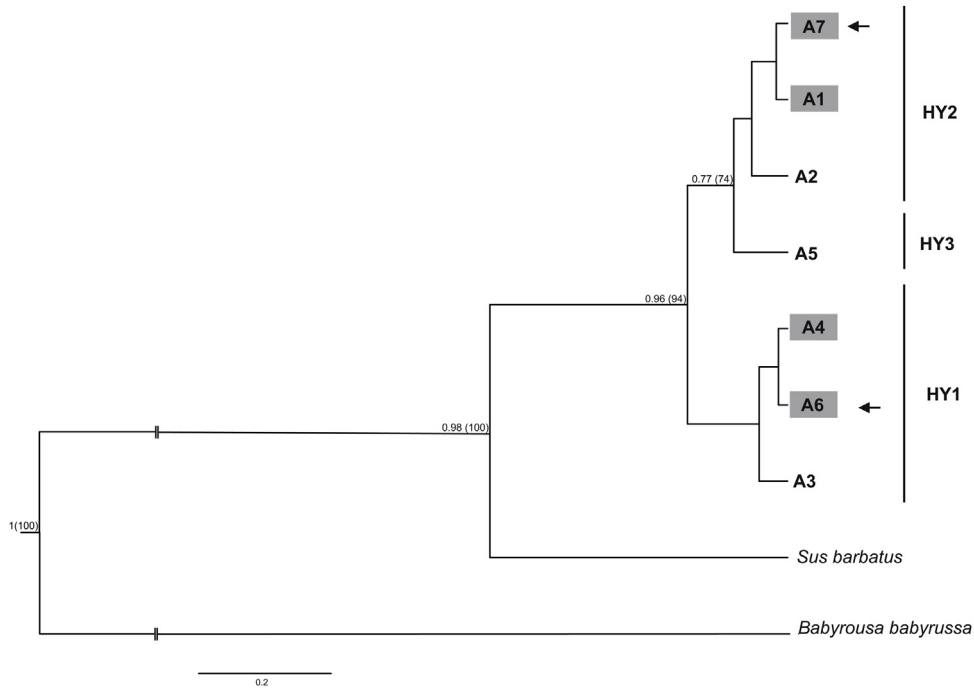
individuals studied, with exception of those cases where Asian origin was identified. Up to this moment, African lineage presence hasn't been detected.

In order to shed more light as to the population dynamics for feral pigs in Argentina, future studies should contemplate additional sampling sites inside the species' distribution, as well as the use of different molecular markers, such as microsatellites. Furthermore, it is important to point out these populations' economic importance to the national livestock activity, given their use as a genetic resource for low budget domestic productions.

On the other hand, in all areas where naturalized populations of *Sus scrofa* are found, they are known to generate a large negative impact on flora and fauna. For the flora, it is known that



**Fig. 4.** Median-joining haplotype network of the 94 haplotypes obtained in this study, for the 641 bp CR fragment. The colours allow the identification of feral and wild boar sequences from Argentina in the context of the total haplogroups previously reported (E1-A, E1-C, E2, NE, A). Circle size is proportional to haplotypes frequencies and transversal lines between haplotypes represent the number of mutations that separate them.



**Fig. 5.** Bayesian phylogenetic tree obtained from the 510 bp fragment corresponding to the AmelY marker, for the 7 haplotypes obtained in this study. Coloured haplotypes contain our sequences of interest. Arrows mark newly reported haplotypes in Argentina. Posterior probability values ( $>0.50$ ) are indicated in the nodes. Parenthesis specify bootstrap values  $>50\%$ .

modifications of species composition, local plant extinction, diversity reduction and soil cover alteration facilitate the colonization of exotic plants. Additionally, fauna is affected by predation, nest destruction, food competition and habitat destruction (Ballari et al., 2016; Carpinetti et al., 2014).

Currently in Argentina, there is a process of species control in certain protected areas, where the most used and effective technique is hunting, and the following are traps (Ballari et al., 2014). As the species is distributed throughout the entire country, this task in such a restricted area is not sufficient for control, since in the other sites they are in full expansion. Therefore, to increase efficiency, it is essential to develop a strategic project that includes the use of various techniques and seeing it through, with participation of regional, provincial and national institutions, to effectively visualize a reduction of the species (Ballari et al., 2014, 2016).

Consequently, knowledge on this species is a fundamental starting point for the design of preservation strategies for natural ecosystems and the genetic resources that these populations represent.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.mambio.2019.09.013>.

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