GENETIC CHARACTERIZATION OF SOUTH AMERICA DOMESTIC GUINEA PIG USING MOLECULAR MARKERS[1]

[CARACTERIZACIÓN GENÉTICA DEL CUY DOMÉSTICO EN AMÉRICA DEL SUR USANDO MARCADORES MOLECULARES]

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SUMMARY

Twenty specific primers were used to define the genetic diversity and structure of the domestic guinea pig (*Cavia porcellus*). The samples were collected from the Andean countries (Colombia, Ecuador, Peru and Bolivia). In addition, samples from Spain were used as an out-group for topological trees. The microsatellite markers were used and showed a high polymorphic content (PIC) 0.750, and heterozygosity values indicated microsatellites are highly informative. The genetic variability in populations of guinea pigs from Andean countries was (He: 0.791; Ho: 0.710), the average number of alleles was high (8.67). A deficit of heterozygotes (F_{IS}: 0.153; $p<0.05$) was detected. Through the analysis of molecular variance (AMOVA) no significant differences were found among the guinea pigs of the Andean countries (F_{ST}: 2.9%); however a genetic differentiation of 16.67% between South American populations and the population from Spain was detected. A poor genetic structure was found among the Andean countries with high genetic variability. The results suggest that it is necessary to take urgent measures to prevent further genetic erosion of native guinea pigs in the Andean countries with plans for recovery and conservation of this important genetic resource in South America.

Keywords: DNA markers, *Cavia porcellus*, South America, genetic diversity.

RESUMEN

Se utilizaron 20 cebadores específicos para definir la diversidad genética y la estructura del cobayo doméstico (*Cavia porcellus*). Las muestras fueron recolectadas de los países andinos (Colombia, Ecuador, Perú y Bolivia). Además, se utilizaron muestras de España como grupo externo de los árboles filogenéticos. Los marcadores microsatélites mostraron un alto contenido de información polimórfica (PIC) 0.750, y los valores de heterocigosidad indicaron que los microsatélites son altamente informativos. La variabilidad genética en las poblaciones de cuyes de los países andinos fue (He: 0.791, Ho: 0.710), el número promedio de alelos fue alto (8.67). Se detectó un déficit de heterozigotos (FIS: 0.153; $p<0.05$). A través del análisis de varianza molecular (AMOVA) no se encontraron diferencias significativas entre los cuyes de los países andinos (FST: 2.9%); Sin embargo, se detectó una diferenciación genética del 16,67% entre las poblaciones sudamericanas y la población española. Se encontró una estructura genética deficiente entre los países andinos con alta variabilidad genética. Los resultados sugieren que es necesario tomar medidas urgentes para prevenir una mayor erosión genética de cuyes nativos en los países andinos con planes para la recuperación y conservación de este importante recurso genético en América del Sur.

Palabras clave: Marcadores de ADN, *Cavia porcellus*, América del Sur, diversidad genética.

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INTRODUCTION

The guinea pig (*Cavia porcellus*) is a native animal to the Andes (Wing, 1986). This animal plays an important role in the economic income of rural families and, at the same time, it is strongly connected to cultural and religious Pre-Inca traditions (Avilés *et al.,* 2014). The guinea pig and South American camelids are a source of food due to their ability to convert poor vegetable resources to good protein (Avilés *et al.,* 2015). Guinea pig meat contains about 70% dry matter, 21.4% crude protein, 3.0% fat, 0.5% carbohydrate and 0.8% minerals, while chicken meat contains 70.2% dry matter, 18.3% crude protein, 9.3% fat, 1.2% carbohydrates and 1% minerals, which reaches commercial maturity at 3.5 months of age with an average between 800 g to 1200 g (Manjeli, 1998; Zumárraga, 2011). Since the sixteenth century, guinea pig has been introduced in Europa as a pet or scientific experimentation (Guerrini, 2003) and is now widespread in Central and South America. It even has been introduced to sub-Sahara Africa (SSA) where it has an extensive distribution and plays an important role with smallholder farmers in better nutrition and poverty reduction (Manjeli *et al.,* 1998; Matthiesen *et al.,* 2011; Maass *et al.,* 2016).

During the 1970s, in the four Andean countries (Colombia, Ecuador, Peru and Bolivia), the three commercial lines of domestic guinea pigs introduced from Peru, have been phenotypically characterized, but never using molecular marker while several studies have been conducted on guinea pig breeding with the aim to increase meat production performance (INIA, 2005). Native guinea pigs in Ecuador but also in the other Andean countires, due their lower meat production are being substituted by commercial animals without any breeding plan.

Nowadays, there exist studies on genetics of *Cavia porcellus* and its close relatives covered the phylogenetic of living lineages and domestication effects in Latin America (Spotorno *et al.,* 2004; 2006; 2007; Brust and Guenther, 2015), molecular assess of systematics, taxonomy and biogeography of the genus Cavia (Dunnum and Salazar-Bravo, 2010); and differentiation of cryptic genetics differences in wild cavies (Trillmich *et al.,* 2004); no complete genetic study has been carried out on the domestic guinea pig to understand the pattern of genetic variation in the Andean countries. Only one study has been performed with small marker panel of microsatellites in Colombia (Burgos-Paz *et al.,* 2011).

Microsatellite markers have been used, among others, for the characterization, genetic diversity and differentiation assessment, the reciprocal influence of the genetic relationships between one or more breeds' populations on each other, paternity testing and kinship studies. Currently, it is also used as a tool for genetic differentiation between domestic species (Martínez *et al*., 2000).

In order to inquire about the genetic diversity and structure of seven domestic guinea pig populations reared in the four Andean countries; this study included one Spanish commercial population from an experimental population as an out-group. The aim of this work was to evaluate the diversity and genetic structure of guinea pig, using microsatellite markers, so as to undertake a program of genetic resources' conservation.

MATERIALS AND METHODS

Sample collection

Hair from the back part of 476 animals from four South American Andean countries were analyzed, 282 samples corresponded to the three different commercial lines: Andean (AND, 94), Inti (INTI, 94) and Peru (LPR, 94) (Chauca, 1997); these samples were obtained from ten Andean provinces from Ecuador: Carchi, Imbabura, Pichincha, Cotopaxi, Tungurahua, Bolivar, Chimborazo, Cañar, Azuay, and Loja. Samples from native Andean guinea pigs from currently guinea pig meat consuming countries were obtained: Ecuador (NTVE, 94), Colombia (COL, 17), Peru (PERU, 41) and Bolivia (BOL, 13) (Figure 1). As an out-group, 29 samples of guinea pig from Spain (SPAIN) were included, because there might be a genetic variation due to the adaptation of the environment of guinea pigs carried 500 years ago. All these native samples were obtained from the BIOCUY consortium, established within the CONBIAND Network (http://www.uco.es/conbiand/Bienvenida.html).
Molecular marker analysis were carried out at the Applied Molecular Genetics laboratory from Animal Breeding Consulting Company S.L. (ABC) of the University of Cordoba, Spain.

Molecular Markers

The marker set was previously studied in Avilés *et al.* (2015). The final panel is listed in Table 1.

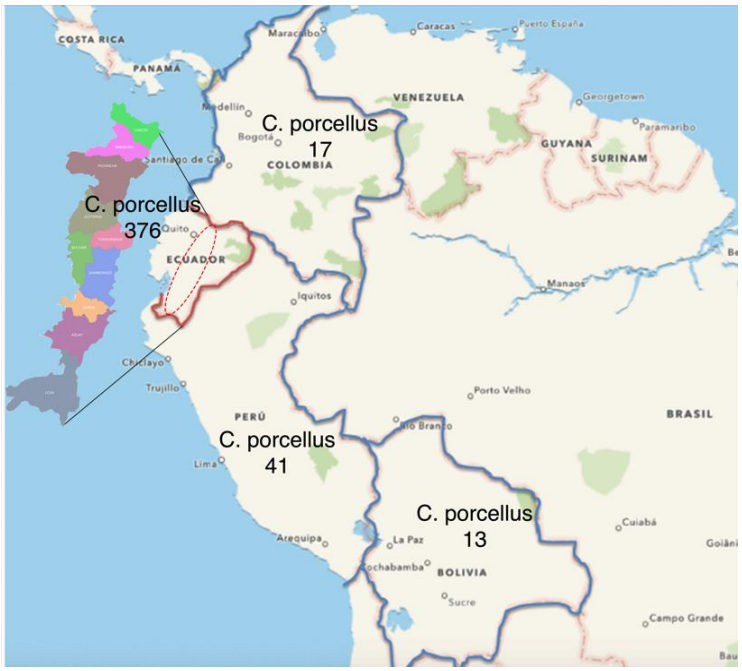

Figure 1. Geographical map of samples from Andean countries that consume guinea pig meat

Table 1. General characteristics of microsatellites

Locus	GB	RP	MX	Tm	SR	Forward	Reverse
CUY01	KP115879	GT	2	55	271-285	CTTTCAGGCAATAGGCATCC	GCAGCTTGGACTACAGAGCA
CUY02	KP115880	CA	2	55	250-262	CAAGATGCCATCAACTTTCGT	TGTTGCTGAGATGCTGCTTT
CUY03	KP115881	GT	1	55	212-252	GCAAGTCAAATTCATCCCTGA	GAGTCCTGCCAAGCAAAATC
CUY04	KP115882	GT	2	55	210-230	TCATCTCGCTTCAGCATTTG	AATGGTCAGGGGCTAGGATT
CUY05	KP115883	CA	2	55	141-163	GGCCAAAGCAGGAATGTCTA	TAGGGCAAGCATTGATGATG
CUY06	KP115884	CA	4	55	158-168	TGGCTTGCTTTCTCTTTGGT	CTGTGCTCAGCATTGCATTT
CUY07	KP115885	CA	2	55	183-197	GATGCAGTGCAGAGGAGTCA	TGTGTGGTTTTGTGTGTGAGG
CUY08	KP115886	TC	1	55	181-217	TGATTGCACCTGAGAAGTGG	CCAAGTGTTCTTGGTGCTTG
CUY09	KP115887	GT	2	55	116-130	GCTGGAATGCAAGACAAGC	TGAGTTTTCAGCTGTGATGAGT
CUY10	KP115888	GT	1	55	106-128	TTCCAAGCATTTCAGAAAACA	TGACTTCCCAACCAAGGAAA
CUY12	KP115889	AG	4	55	232-250	GGAATGGTGGCAAACTCCTA	TCTCCTCCTCCTCCTCCTTC
CUY16	KP115890	AT	3	60	223-247	TTTGAGTCAAGCCGTGAACA	GCCTGTTTTGAAACTGTTTTACTG
CUY17	KP115891	TC	4	55	152-170	TGATGGACAATATACTGGGAACC	TAGCATGCATGAAGCCCTAA
CUY18	KP115892	CA	2	55	176-214	TGTCACTTCTCACTCCACCA	TCCCAAACCTCTTGTTTGCT
CUY20	KP115893	AT	4	55	218-258	TCTTGGAAATGGCCTACATTTT	TGGTCTCTAGGGGTATCCATT
CUY22	KP115894	TC	4	55	206-232	CGAACATGCCAAGCAGATTA	ACACCAGTTCCTTGCCACAT
Cavy02*	AJ496560	AC	2	55	124-154	GGCCATTATGCCCCCCAAC	AGCTGCTCCTTGTGCTGTAG
Cavy03*	AJ496561	CT	1	55	195-225	ACAGCGATCACAATCTGCAC	GCAGTGGTAACCCAGAATGG
Cavy11*	AC192015	CT	1	55	140-180	CCGTGCTTTTCCTGTCTTTG	TGGACCCCAATCTGACATAG
Cavy12*	AC182323	AG	1	55	143-187	AGAATGCCTTTGGGACTGG	AGATCTTGCCTCTGCACTTG

GB: GenBank accession number; RP: microsatellite repeat motive; MX: polymerase chain reaction multiplex reaction where the locus amplified; Tm: annealing temperature of polymerase chain reaction; SR: size range in base pairs. * Selected Loci from Kanitz *et al.* (2009) and Asher *et al.* (2008)

Microsatellite analysis

Genomic DNA was extracted by incubating 3 hair roots in the presence of 100 μL of 5% Chelex® (Biorad, Göttingen, Germany) resin suspension at 95°C for 15 minutes, 60°C during 20 minutes and 99°C for 3 min. Twenty microsatellite loci were amplified in four multiplex PCRs divided into three electrophoresis sets (Avilés *et al.,* 2015). The PCR products were separated through electrophoresis using a 3130Xl Genetic Analyzer® (Life Technology, Madrid, Spain), using a POP7 polymer and the internal size standard GeneScan500-Rox® (Applied Biosystems, Carlsbad, CA, USA). The allelic typification was achieved through Genescan® 3.1.2 and Genotyper® 3 software packages (Applied Biosystems, Carlsbad, CA, USA).

Statistical analyses

Mean number of alleles, observed, and unbiased expected gene diversity estimates and their standard deviations were obtained with the MS® Excel Microsatellite Toolkit software (Park, 2001) (Dublin, Ireland). The distributions of gene variability within and between breeds were studied through the analysis of F-statistics (Weir and Cockerham, 1984) as implemented in Genetix® 4.05 (Belkhir *et al.,* 2003) (Montpellier, France). The within-breed inbreeding coefficient (F_{IS}) in each population was calculated with a 95% confidence interval. Deviations from Hardy–Weinberg equilibrium (HWE) were assessed by means of using Genepop® 3.4 software (Raymond and Rousset, 1995). To determine the structure and genetics differentiation among populations (South American and European), an analysis of molecular variance was performed (AMOVA), calculations were assessed with Arlequin® 3.01 (Excoffier and Lischer, 2010) (Lausanne, Switzerland). Genetic distances were calculated (Reynolds *et al.,* 1983) using Populations® 1.2.28 software (Langella, 1999) (Boston, MA, USA).

A distance tree (NeighborNet) was developed from the obtained matrix D_A of Nei et al. (1983) with Splits Tree4® software (Huson & Bryant, 2006) (Tübingen, Germany) to represent the relationships between breeds graphically, as well as to depict the evidence of admixture. The version 2.3.4 of Structure® software (Pritchard et al., 2000) (Stanford, CA, USA) was used to identify the genetic structure, which identifies clusters of related individuals from multilocus genotypes and assigns individuals to identified clusters using a Bayesian algorithm based on the Markov chain Monte Carlo method. The analysis involves an admixture model with correlated allele frequencies. Eight independent runs were conducted with 50,000 interactions during the burn-in phase and 1,000,000 interactions for sampling from K=2 to K=8. The Structure results in graphic representations were obtained with the program Distruct® 1.1 (Rosenberg, 2004). The proportion of each individual genotype in each cluster or breed (q) and the probability of ancestry in other breeds were estimated.

RESULTS AND DISCUSSION

Microsatellite markers

Over the past 40 years, guinea pigs have experimented an increase in their population size from the introduction of improved lines, but the reduction of native animals might be relevant for the future sustainable utilization and conservation of this important "mini livestock" species. All the 20 microsatellite markers used in this study were successfully amplified in all the populations. A total of 216 alleles, with a mean value of 10.80 ± 3.49, were found for the 20 analysed microsatellites loci. All the microsatellites were highly polymorphic with a minimum of 6 alleles (CUY06) and a maximum of 19 (Cavy12). To evaluate the present situation, we have genetically characterized the South American guinea pig population with the efficiency of microsatellite panel has been demonstrated by the large number of alleles detected for the whole population (10.8 ± 3.40) (Avilés *et al.,* 2015), which was higher than the values found for Ivory Coast alleles, 5.98 ± 0.37, in creole guinea pigs by Kouakuo *et al.* (2015); for Colombian alleles, 6.8 ± 1.64, in domestic cavies (native line and unspecified commercial lines) by Burgos-Paz *et al.* (2011), and 7.4 alleles were found for Brazilian wild cavies, by Kanitz *et al.* (2009) and 10 Uruguayan alleles by Asher *et al.* (2008).

Breed diversity

The mean number of alleles for all the eight populations was high (8.67±2.65), ranging from a low 4.85 (SPAIN) to a high 11.15 (INTI). Overall genetic diversity was high (H_e = 0.733 ± 0.025). F_{IS} values were significantly different from zero and ranged between 0.072 and 0.327. All the breeds showed a significant heterozygosity deficit (0.153±0.091) as shown in Table 2. The diversity ratios, represented by heterozygosity, were high in all the South American populations. The SPAIN population obtained the lowest diversity (0.504). Kouakuo *et al.* (2015) in Ivory Coast, and Burgos-Paz *et al.* (2011) in Colombia showed a lower diversity than our study. Heterozygotes deficit was found in all the populations (F_{IS} = 0.153). Kouakuo *et al.* (2015) and Burgos-Paz *et al.* (2011) showed a high heterozygotes deficit

(0.225 and 0.323) respectively. These indexes indicate high levels of genetic variability in South American population's guinea pigs.

Genetic differentiation and population structure

The values of F_{ST} (Table 3, above diagonal) corresponding to the value of genetic differentiation by pairs of breeds, ranged from 0.006 (LPR vs INTI) to 0.2829 (BOL vs SPAIN). Reynolds' pairwise genetic distance (Table 3, below diagonal) ranged from 0.0012 (LPR vs INTI) to 0.3392 (BOL vs SPAIN). Reynolds' pairwise genetic distance (Table 2, below diagonal) ranged from 0.0012 (LPR vs INTI) to 0.3392 (BOL vs SPAIN). The SPAIN population accounted for the greatest distance from all the guinea pig populations in this study. The F_{ST} value (0.029) by Wright and G_{ST} value (0,064) by Nei shows that genetic differentiation between South America populations is very small (Table 4).

Table 2. Summary of the statistics for the eight populations' genetic parameters

Pop	N	MNA	H_e	H_e Ds	H_o	H_o Ds	F_{is}	CI 95%	HWE
AND	94	10.80	0.792	0.017	0.735	0.010	0.072	0.031 - 0.107	5*
INTI	94	11.15	0.787	0.020	0.700	0.011	0.112	0.060 - 0.153	6*
LPR	94	10.90	0.789	0.019	0.709	0.011	0.103	0.056 - 0.142	8*
NTVE	94	10.90	0.797	0.019	0.697	0.011	0.127	0.072 - 0.178	9*
PERU	41	8.50	0.761	0.020	0.707	0.016	0.072	0.005 - 0.114	4*
BOL	13	5.45	0.694	0.032	0.474	0.031	0.327	0.098 - 0.457	10*
COL	17	6.80	0.736	0.020	0.556	0.027	0.250	0.057- 0.371	8*
SPAIN	29	4.85	0.504	0.051	0.424	0.021	0.162	0.035 - 0.261	4*
Mean	59.5	8.67	0.733	0.025	0.625	0.017	0.153	0.052-0.233	6.75

The following estimates were obtained through averaging across the 20 microsatellites: sample size (N), mean number of alleles (MNA), expected (He) and observed (Ho) heterozygosity, within-breed deficit in heterozygosity (F_{IS}) and the confidence interval, and the number of loci deviated from HWE proportions (HWE). Populations abbreviations: AND: commercial line Andean, INTI: commercial line Inti, LPR: commercial line Peru, NTVE: Native Ecuadorean, PERU: Native Peruvian, BOL; Native Bolivian, COL: Native Colombian, SPAIN: out-group from Spain.

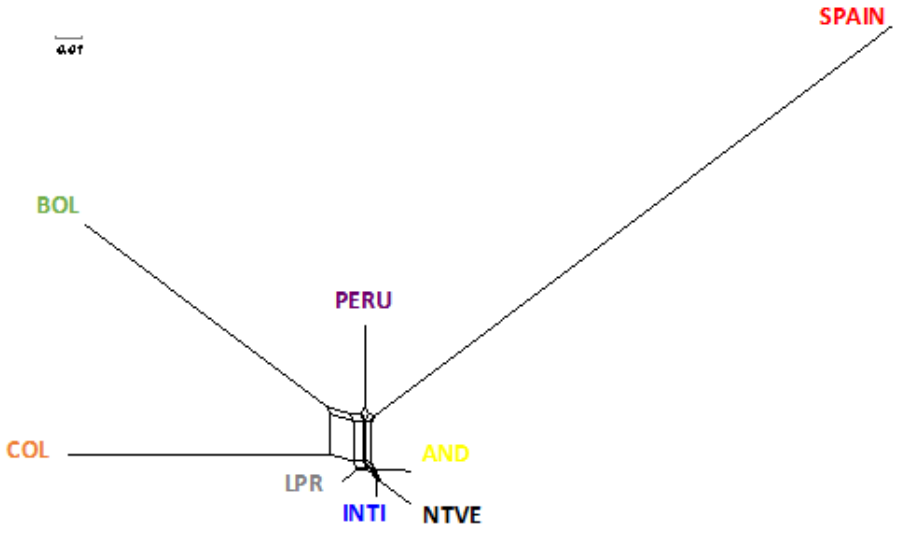

Figure 2. Neighbor-Net dendogram representing the Reynolds genetic distances between the eight studied populations.

These particular differentiations can be based on the great interchange of male and females without control and registration between Andean country markets. This open reproduction system among the South American populations favours their migration (Figure 2).

Table 3. Estimated pairwise F_{ST} as a measure of genetic differentiation (above diagonal) and Reynolds genetic distances (below diagonal).

Pop	AND	INTI	LPR	NTVE	PERU	BOL	COL	SPAIN
AND	0	0.0028	0.0013	0.0044	0.0182	0.0409	0.0212	0.1828
INTI	0.0033	0	0.0006	0.0023	0.0191	0.03196	0.0298	0.1822
LPR	0.0019	0.0012	0	0.0047	0.0155	0.0320	0.0236	0.1839
NTVE	0.0050	0.0030	0.0054	0	0.0264	0.0476	0.0223	0.1825
PERU	0.0190	0.0202	0.0165	0.0278	0	0.0379	0.0425	0.1979
BOL	0.0440	0.0355	0.0340	0.0521	0.0420	0	0.0594	0.2829
COL	0.0236	0.0330	0.0265	0.0255	0.0465	0.0718	0	0.2530
SPAIN	0.2029	0.2026	0.2046	0.2032	0.2224	0.3392	0.2966	0

AND: commercial line Andina, INTI: commercial line Inti, PLR: commercial line Peru, NTVE: Ecuador's native, PERU: Peru's native, BOL: Bolivia's native, COL: Colombia's native and SPAIN out-group from Spain.

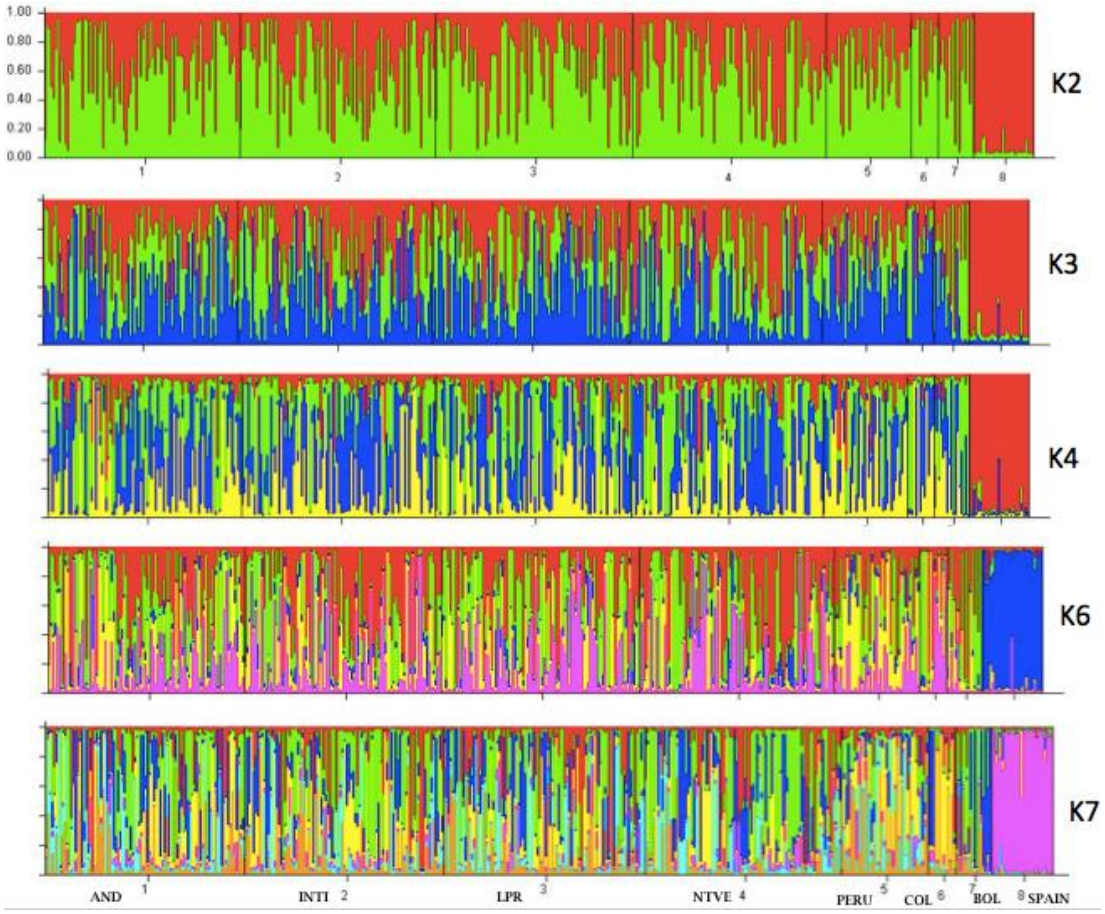

Figure 3. Graphical representation of the genetic structure of the 8 populations analysed.

The same way we can appreciate that between Andean populations we cannot find a clear population structure (Figure 3). The interesting trade started from the first settlers of South America which began to venture into new territories creating a trade route that began on the coasts of Ecuador and Peru with the trade of Spondilus or Mullu shell (*Spondilus calcyfer*) extended by the Pacific Ocean to Michoacan in Mexico to the north, crossing Central America, Colombia, Ecuador, Peru, and Bolivia (Hocquenghem, 2009), during these trips according to Sthal and Norton (1984) guinea pigs and ducks were transported to feed the crew and exchange with the pre-Columbian people. Thus, the inter-trade route, which persists to this day, was established. In the works of Burgos- Paz *et al.* (2011) and Kouakuo *et al.* (2015) neither observed genetic structure in the populations studied

The Neighbor-Net dendogram is presented in Figure 2. The tree shows the populations from Ecuador (AND, INTI, LPR and NTVE) clustered in the same branch; COL, PERU and BOL appeared in separate clusters, while the out-group from Spain showed the greatest distance and the longer branch when comparing it to the studied South America populations. This Neighbor-Net dendogram shown the guinea pigs in the Andean population seem to have had a common origin in one single branch as showed in the studies realized by Spotorno (2004, 2006, 2007).

Table 4. Values of the coefficient of genetic variation (G_{ST}) and F statistics (F_{IS}, F_{IT} y F_{ST}).

Locus	NA	G_{ST}	F_{IS}	F_{IT}	F_{ST}
Cavy02	9	0.083	-0.002	0.040	0.041
Cavy03	13	0.066	0.171	0.196	0.031
Cavy11	17	0.032	0.103	0.116	0.014
Cavy12	18	0.030	0.328	0.336	0.013
CUY01	8	0.110	0.019	0.057	0.039
CUY02	7	0.107	0.113	0.163	0.055
CUY03	11	0.111	0.104	0.145	0.047
CUY04	9	0.081	0.094	0.128	0.037
CUY05	12	0.047	0.059	0.077	0.019
CUY06	6	0.126	0.087	0.134	0.050
CUY07	7	0.017	0.442	0.446	0.007
CUY08	17	0.085	0.075	0.110	0.037
CUY09	7	0.023	0.101	0.110	0.009
CUY10	11	0.066	0.110	0.134	0.027
CUY12	9	0.020	0.068	0.085	0.018
CUY16	11	0.040	-0.028	-0.013	0.015
CUY17	10	0.043	0.106	0.130	0.026
CUY18	10	0.063	0.163	0.185	0.026
CUY20	14	0.044	0.068	0.084	0.016
CUY22	10	0.096	0.046	0.088	0.044
Mean		0.064	0.111	0.138	0.029
Ds		0.073	0.107	0.101	0.015

AMOVA results (Table 5) indicated the differentiation between breeds was significant (16.67%), when all the South American population (or group one) and the out-group (SPAIN) (or group two) were considered. AMOVA and Structure analysis confirmed the general features observed in the Neighbor-Net dendogram. The results indicate that, the Spain population represented the highest differentiation (16.67%) and showed the population structure. This differentiation began with the discovery of America; the colonists took guinea pigs to Europe, where they quickly became popular as exotic pets among the upper classes and royalty, including Queen Elizabeth I (Morales, 1995). In

Europe and USA, guinea pigs are considered pets, so that, Cavy clubs and associations devoted to showing and breeding guinea pigs have been established worldwide. Data Bayesian analysis through Structure program (Pritchard *et al.*, 2000) revealed no clear population structure in South American guinea pigs, while the out-group from Spain presented a clear separation as shown in Figure 3.

Table 5. Genetic variation of domestic guinea pigs between the populations of South America and Spain.

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	
Among groups	1	179.712	1.527	Va	16.67
Among populations within groups	6	110.989	0.091	Vb	0.99
Within populations	944	7117.24	7.539	Vc	82.34
Total	951	7407.941	9.157		
Fixation Indices					
F_{ST}: 0.1766					
F_{SC}: 0.0119					
F_{CT}: 0.1667					
Significance test (1023 permutations)					
Vc and F_{ST}	P(rand. val < obs. val)		0.0000*		
	P(rand. val = obs. val)		0.0000*		
	P(rand. val <= obs. val)		0.0000+- 0.0000*		
Vb and F_{SC}	P(rand. val > obs. val)		0.0000*		
	P(rand. val = obs. val)		0.0000*		
	P(rand. val >= obs. val)		0.0000+-0.0000*		
Va and F_{CT}	P(rand. val > obs. val)		0.0000*		
	P(rand. val = obs. val)		0.13294		
	P(rand. val >= obs. val)		0.13294+-0.01139		

*p<0.05. Gruop1: South America's guinea pig (AND, INTI, LPR, NTVE, COL, PERU and BOL)
Gruop2: Out-group (SPAIN)

CONCLUSION

This study has shown the guinea pigs in the Andean population seem to have had a common origin in one single branch. Over all, the results indicate the population of Latin American guinea pigs has a high genetic variability and poor population structure. The results suggest that it is necessary to take urgent measures to prevent the further genetic erosion of native guinea pigs from Andean countries. We should design and implement recovery and conservation plans for native andean guinea pigs to prevent the loss of this autochthonous genetic resource from South America. On the other hand, the comercial lines do not seem to have a clear population structure, needing to improve their marketing channel by genetically defining these popuations, without affecting the native ones.

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