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Original Article

STUDY OF MOLECULAR RELATIONS BETWEEN THREE LINES OF *CAVIA PORCELLUS* (GUINEA PIGS) PERU, ANDINA AND INTI OF EL PRADO FARM, UNIVERSITY OF THE ARMED FORCES–ESPE, ECUADOR

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ABSTRACT

Objective: The present study was undertaken to investigate the morphological and to establish molecular relations between three lines of guinea pigs, which are phenotypically selected and form the basis for a molecular marker-assisted breeding program for the species, in the breeding farm of El Prado.

Methods: Synthetic variable was used for qualitative and quantitative analysis of the study population. The DNA was extracted, amplified by PCR and then was sequenced the mitochondrial cytochrome-b gene of the lines Peru, Andina and Inti. The extracted sequences were compared with others of *Cavia porcellus,* and other associated genes, from neighboring countries, deposited in Genbank.

Results: Phenotypic analysis was prioritized desirable characteristics for the producer. The synthetic variable generated three groups of individuals. The best group comprises of guinea pig Andina line, two of Peru, and four of Inti. Molecular characterization showed that the specimens under study achieved an average of 1101.5 bp corresponding to 98.27% of the complete mitochondrial cytochrome-b gene (1120.8bp). On comparison with Genbank sequences we observed a similarity of 98% (identical pairs), and 1.92% of variability (transition and transversion). Site analysis found 85.5% of conserved sites and 14.5% of variable sites, of which 55.48% were parsimony informative.

Conclusion: These results suggest that the specimen I5 (Inti) was selected for its qualitative characteristics, good weight of reproductive age and greater intra-population genetic distance. The dendrogram for intraspecific phylogenetic inference of *Cavia porcellus* was robust, 100% resampling under a frequency analysis of 1000 replicates. These findings not only help in design effective breeding experiments but also help in selecting animals for experiment purposes.

Keywords: *Cavia porcellus,* Cytochrome-b, DNA, Phylogenetic analysis

INTRODUCTION

Guinea pig (*Cavia porcellus*) is a rodent mammal known worldwide, originally from the Andean countries of Bolivia, Ecuador, Peru and Colombia [1], where it was domesticated before the arrival of the Spaniards [2]. In Ecuador, guinea pig production is increasing, according to the III Agricultural Census [3], the population exceeded 5 million people and an annual consumption of 25,590 MT registered. Currently, the semi-technified breeding methodology was used by a selection of breeding based on phenotypic parameters of color and weight to increase production in order to meet domestic and international demand [4].

The hatchery farm El Prado has used this same methodology to potentiate productive parameters after getting poor results in classical breeding programs [5]. The use of modern techniques such as molecular marker-assisted selection, in order to achieve greater efficiency in the selection of breeding, is necessary to full fill the present day requirement [6]. After considering the above-mentioned facts, this study was established to evaluate the morphological and molecular relationships between three lines of guinea pigs: Peru, Andina and Inti, and the technique employed in this study forms the basis for a breeding program for the species, assisted by molecular markers in the hatchery of El Prado farm.

There have been a very few registered studies on the application of molecular markers for breeding programs of the species *C. porcellus*. However, the molecular-based approach has been well evaluated as an effective technique for estimating genetic variance between and within guinea pig populations [2].

MATERIALS AND METHODS

Population

Seven male specimens were evaluated for each line: Peru, Andina and Inti, of same reproductive age (three months) collected from hatchery farm, El Prado. Additionally, an animal of the Inka line of the same age was also evaluated.

Morphological characterization

The qualitative phenotypic characteristics such as hair color, eyes, ears, and a number of digits were analyzed [5]. The quantitative phenotypic traits like body weight (BW), total length (TL), head-body length (H-B), caudal tail rudiment (CT), ear length (EL), leg length (LL) [7], head length (HL), head width (HW) and hair length (HL) were also assessed in the present study [8]. Each variable qualitative and the quantitative phenotypic character were given a rating based on the level of acceptance from the producer.

Two points for characters with greater acceptance, a single point to moderately acceptable characters and zero points for less acceptable producer characters. The sum of the points assigned to variable phenotypes, in each instance, resulted in the synthetic variable. The mean of this variable was calculated, and three groups of individuals were established. Group 1 was made up of individuals whose values of the synthetic variable were above average, specimens of group 2 showed values around the mean and the units that formed the group 3 had values below the mean for the synthetic variable.

Molecular characterization

3-4 discs of ear samples were collected from each animal. The DNA from each sample was extracted following the instruction mentioned in the kit insert (Omega Bio-tek) From the isolated DNA the Cytochrome-b gene was amplified using forward primer (F78) 5'-TCCAATGTAGGAATTATGACCCACC-3' and Reverse primer (B149) 5'-TTTCCCATCTCTGGCTTACAAGAC-3' following PCR conditions: 94 °C for 2 min, 95 °C for 45 seconds, 54 °C for 30 seconds and 72 °C for 60 seconds for 30 cycles [9]. The amplified products were sequenced in the laboratory of Eurofins Operon (Alabama-USA) with only one primer (F78) and data analyzed using Mega 4.0 software. The complete cytochrome-b gene sequences were used for further characterization.

Phylogenetic inference

The evolutionary history of *C. porcellus* from El Prado farm was analyzed in comparison with other specimens from nearby countries, the Caviidae family (table 1) whose complete sequence was available in Genbank (www. ncbi. nlm. nih. gov/genbank/) [8].

Table 1: List of accessions used in phylogenetic inference of *C. porcellus* species

Species	Origin place	Name and number of collection	Accession number genbank	(pb)
Dolichotis patagonum	Argentina: Santa Cruz	LCM 3077	AY382787	1140 pb
Galea musteloides	Perú: Puno, desaguadero	LCM 2494	AY466603	1125 pb
Galea musteloides	Perú: Puno, Sillustani	LCM 2496	AY466604	1125 pb
Galea musteloides	Bolivia: La Paz	MSB 30655	AY466605	1125 pb
Microcavia niata	Chile: Colchane	LCM 2500	AY382788	1140 pb
Microcavia niata	Chile: Colchane	LCM 2501	AY382789	1140 pb
Cavia aperea	Bolivia: Santa Cruz	MSB 22547	AY382790	1140 pb
Cavia aperea	Paraguay: Concepción	MSB 22546	AY382791	1140 pb
Cavia tschudii	Perú: Cuzco	LCM 2562	AY382792	1140 pb
Cavia tschudii	Perú: Puno	LCM 2495	AY245099	1140 pb
Cavia porcellus	Argentina: San Luis. Pet shop	LCM D2441	AY228362	1140 pb
Cavia porcellus	Colombia: Mercado de Palmira	LCM D2347	AF490405	1140 pb
Cavia porcellus	Perú: Piura, mercado de la ciudad	LCM D2410	AY245094	1140 pb
Cavia porcellus	Perú: Trujillo, mercado de la ciudad	LCM D2414	AY245095	1140 pb
Cavia porcellus	Chile: Arica. Agromercado popular	LCM d2463	AY382793	1140 pb
Cavia porcellus	Chile: San Pedro, Atacama	LCM d2548	AY228361	1140 pb
Cavia porcellus	Perú: Arequipa. Mercado popular	LCM d2498	AY247008	1140 pb
Cavia porcellus	Perú: Cajamarca. Mercado popular	LCM d2412	AY245096	1140 pb
Cavia porcellus	Perú: Cuzco, Mercado Popular	LCM d2416	AY245097	1140 pb
Cavia porcellus	Perú: Tacna, mercado popular	LCM d2308	AY228363	1140 pb
Cavia porcellus	Perú: Puno, mercado popular	LCM d2497	AY245098	1140 pb

RESULTS AND DISCUSSION

Morphological characterization

Qualitative phenotypic variables

The phenotypic character like hair color character was variable in the lines evaluated. Each line showed its typical coloring: white color for the Andean guinea pigs, sorrel (brown) combined with white for Peru line, and fawn of Inti line guinea pigs. The variable hair coloration between breeds was already mentioned by Guzman [5] and Chauca [10]. On the contrary, 95.45% of the population showed black colored eye and the remaining 4.55% had red eyes. The characteristic black eye color is a positive attribute for the selection of individuals of different lines since apparently animal with red eyes (albinos) have difficulty seeing, limiting their ability to reach the food and causing negative effects on their profit weight [11].

Polydactyly comprising the presence of supernumerary fingers appeared in 18.18% of the population. This feature is highly heritable in *C. porcellus* therefore, one should pay special attention at the time of selection. Usually, symmetry in the number of fingers this symmetry corresponds to 4 fingers on each hand and 3 feet [12]. For the Inca line, the normal number of fingers varies from 5 to 7 in front and hind limbs [10]. Regarding the variable phenotypic colored ears are not registered studies, so it is unknown whether the presence of different colors of the same specimen is due to consanguinity or own skin pigmentation, however; in this assay, greater diversity of black and pink colors in 27.28% of the population was observed.

Quantitative phenotypic variables

According to Chauca *et al.* [10] from birth to 9 w of age animals weighed about 1100-1350 g. In our study, we recorded an average weight of 1237.62 g which was similar to the observations made by Moncayo [13] in animals of 10 to 12 w old. The other parameters studied for the selected animal lines are TL (total length), HBL (head-body length), LL (Long leg), EL (ear length) and LH (long hair) and the average values are 360.24 mm, 353.75 mm, 53.39 mm, 35.04 mm and 31.30 mm respectively, surpassing the findings of Guzman [5].

The correlation between BW and HBL variables was 54%, which shows a positive correlation between the two variables but according to Chauca [1], there was a 71% correlation between body length and weight in Bolivian guinea pigs. The CT variable (flow length rudiment), measured from the base to the tip of the rudiment [7], favors the animals with a shorter length. The presence of a rudiment flow too long detracts from the quality of the animal carcass. The recorded measurements for this variable in this study were between 7.7 mm and 5 mm, values match those mentioned by Guzman [5] animals of similar characteristics.

Long head variable registered an average of 82.16 mm in this study while Samaniego [9] recorded values ranging between 82.7 mm and 95.0 mm for Chimborazo province animals in Ecuador. Head height variable showed values between 35.9 mm and 45.4 mm in this trial. Chauca *et al.* [10] noted that the head comprises between 16 and 18% of the housing, a percentage that varies according to the age and size of the animal but usually is excluded from the housing for marketing.

Synthetic variable

According to the score obtained in the phenotypic evaluation, the synthetic variable generated 3 groups (table 2).

Group 1 contains 7 individuals with acceptable for producers and higher than the average score of 16.14 points for characters. The most prominent of this group for its excellent qualitative and quantitative phenotypic characteristics were A5 and I3 animals, the Andina and Inti lines, respectively. Group 2 was composed of 10 individuals of moderately acceptable to the producer and scores ranging between 15 and 17 points morphological characters. Representatives of the group 3 showed the lowest score and its phenotypic characters were less acceptable.

Molecular characterization

DNA extraction and amplification

A good quality of DNA was extracted from animal samples and the DNA concentration was in the range of 10 to 50 ng/µl. The size of mitochondrial cytochrome-b PCR product was different in different samples the larger product size of 1200bp were obtained in 10 specimens of which four Andean line, two Peru line and four Inti line. According to Spotorno *et al.* [9] the size of the mitochondrial cytochrome-b gene was between 1125 and 1140pb in *C. porcellus*. Guevara [14] also ensured that in rodents the length of this gene is about 1140 nucleotides.

In this study, we observed that the mitochondrial cytochrome-b gene amplified from different animals were in the range of 1170 and 1200pb.

Table 2: Classification of the synthetic variable to *C. porcellus* of El prado farm

		Qualitative phenotype	Quantitative phenotype	Total
Line	**Code**	**Subtotal 1**	**Subtotal 2**	**Score**
Andina	A1	6	7	13
	A2	8	3	11
	A3	8	8	16
	A4	8	7	15
	A5	8	14	22
	A6	5	6	11
	A7	8	6	14
Peru	P1	7	8	15
	P2	6	12	18
	P3	8	10	18
	P4	6	10	16
	P5	7	9	16
	P6	4	11	15
	P7	8	9	17
Inti	I1	8	10	18
	I2	8	7	15
	I3	8	14	22
	I4	8	8	16
	I5	8	9	17
	I6	5	13	18
	I7	8	10	18
Inka	K1	6	8	14
average	(X)	7.09	9.05	16.14
standard deviation	(S)	1.27	2.70	2.80

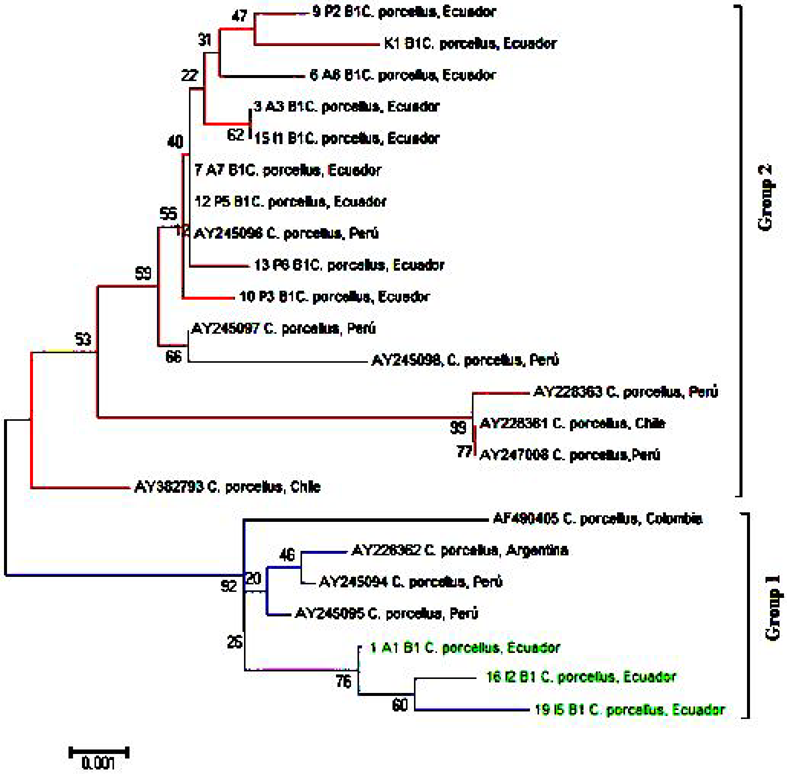


Fig. 1: Complete sequences dendrogram of cytochrome b gene of animals species *C. porcellus* based on the method of grouping neighbors. The evolutionary distances were calculated using the maximum likelihood method. Percentages support of re-sampling (1000 Bootstap test replicates) are located on each node

Mitochondrial cytochrome-b gene sequencing

The complete mitochondrial cytochrome-b sequence averaged 1101.5 bp, and average amino acids transcribed 331.9 which correspond to 98.27% of the entire gene. We assessed the complete sequences of the cytochrome-b gene of 25 individuals, 11 from Genbank and 12 from hatchery El Prado farm. In sequence analysis, 1082.8 intraspecific nucleotide pairs were found in *C. porcellus* and 1084.6 intra-generic nucleotides pair product contrast the species *C. porcellus* with *Dolichotis patagonium* external group. Concomitantly, the Intra-specific analysis showed 98.08% similarity and 1.92% divergence. A total of 1228 bp of the complete sequences of specimens *C. porcellus* were analyzed. Around 85.50% (1050 sites) were conserved and 14.50% (155 sites) were variables. Of the 55.48% variable sites (86 sites) were informative parsimonious. The low percentage of variability between sequences of the same species demonstrates the presence of highly heritable traits [14].

Phylogenetic analysis of the species cavys

The dendrogram generated for specimens of entire sequences was subdivided into two clusters (fig. 1). The smallest was the most distant cluster, it consists of four sequences from the Genbank, and three in the El Prado farm; of which two correspond to the Inti (I2_B1, I5_B1) line and one Andina (A1_B1).

The second cluster is larger; it is less divergent and consists of seven sequences from Genbank and nine copies of El Prado farm. Studies by Spotorno *et al.* [9] suggest that the molecular diversity of the species *C. porcellus* is higher in individuals "crossbred" in South America and lower in individuals known as "improved."

A1_B1, I2_B1 and I5_B1 sequences showed greater variability and genetic distances, while A7_B1, P5_B1 and I1_B1, samples were most homologous. The most distant of the group farm animals, the I5_B1 sequence, was the one with the largest number of dissimilarities along its sequence. The most representative sites and transitional transversional divergence for I5_B1 sample were placed in 364 positions (fig. 2) and 805 (fig. 3), respectively.

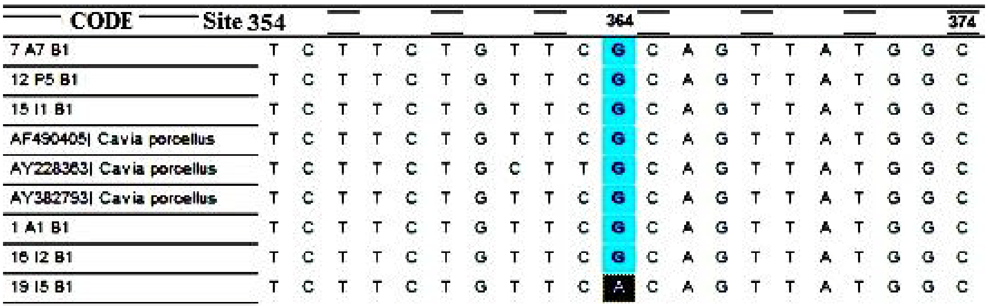
Selection of breeding animals

The El Prado farm animals showed greater variability as compared to Inti line I2 and I5. Both specimens showed genotypic distances between 0.0021 and 0.0139 centi Morgans (cM) relative to other *C. porcellus* animals. Both samples were analyzed through BLAST web interface (http://blast. ncbi. nlm. nih. gov/Blast. cgi) that revealed a high similarity of 97% provided by the identity of 1025bp to 1053bp that constituted these sequences [15]. According to qualitative and quantitative phenotypic Inti line animals showed good grades and were placed in group 2 by favorable characters like yield, head length, live weight sample of the animal. Overall experimental data points out the Inti line I5 animal, can be ideal for breeding program as it has showed excellent qualitative phenotypic traits, to achieve good reproductive age live weight (1320g at 3 mo) and at increased intra-population genetic variability; ensuring greatly the possibility of keeping the most acceptable phenotypic characteristics, improve those moderately acceptable, and reduce the level of consanguinity of the population in their offspring.

Phylogenetic inference

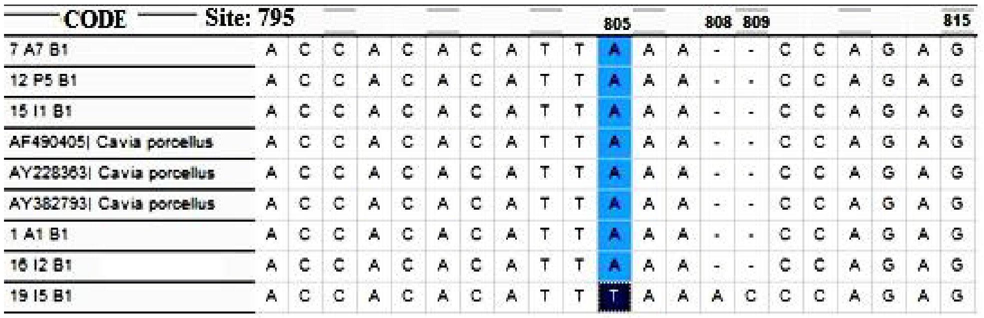
The topologies obtained for the mitochondrial cytochrome-b gene sequences located within a cluster of individuals of the genera Cavia and Microcavia, and the second cluster to less divergent copies of the out-group *Dolichotis patagonum* (AY382787).

The sequences of the genera Cavia and Microcaviase are located into two monophyletic subgroups, such that the dendrogram is divided into three clusters (fig. 4).



CODE Site 354										364										374
7 A7 B1	T	C	T	T	C	T	G	T	T	C	G	C	A	G	T	T	A	T	G	G	C
12 P5 B1	T	C	T	T	C	T	G	T	T	C	G	C	A	G	T	T	A	T	G	G	C
15 I1 B1	T	C	T	T	C	T	G	T	T	C	G	C	A	G	T	T	A	T	G	G	C
AF490405| Cavia porcellus	T	C	T	T	C	T	G	T	T	C	G	C	A	G	T	T	A	T	G	G	C
AY228363| Cavia porcellus	T	C	T	T	C	T	G	C	T	T	G	C	A	G	T	T	A	T	G	G	C
AY382793| Cavia porcellus	T	C	T	T	C	T	G	T	T	C	G	C	A	G	T	T	A	T	G	G	C
1 A1 B1	T	C	T	T	C	T	G	T	T	C	G	C	A	G	T	T	A	T	G	G	C
16 I2 B1	T	C	T	T	C	T	G	T	T	C	G	C	A	G	T	T	A	T	G	G	C
19 I5 B1	T	C	T	T	C	T	G	T	T	C	A	C	A	G	T	T	A	T	G	G	C

Fig. 2: Divergence in transitional position 364 of the sequence of Inti I5_B1 line, A = adenine; G = guanine



CODE Site: 795										805			808	809						815
7 A7 B1	A	C	C	A	C	A	C	A	T	T	A	A	A	-	-	C	C	A	G	A	G
12 P5 B1	A	C	C	A	C	A	C	A	T	T	A	A	A	-	-	C	C	A	G	A	G
15 I1 B1	A	C	C	A	C	A	C	A	T	T	A	A	A	-	-	C	C	A	G	A	G
AF490405| Cavia porcellus	A	C	C	A	C	A	C	A	T	T	A	A	A	-	-	C	C	A	G	A	G
AY228363| Cavia porcellus	A	C	C	A	C	A	C	A	T	T	A	A	A	-	-	C	C	A	G	A	G
AY382793| Cavia porcellus	A	C	C	A	C	A	C	A	T	T	A	A	A	-	-	C	C	A	G	A	G
1 A1 B1	A	C	C	A	C	A	C	A	T	T	A	A	A	-	-	C	C	A	G	A	G
16 I2 B1	A	C	C	A	C	A	C	A	T	T	A	A	A	-	-	C	C	A	G	A	G
19 I5 B1	A	C	C	A	C	A	C	A	T	T	T	A	A	A	C	C	C	A	G	A	G

Fig. 3: Transitional divergence in position 805 and nucleotide sequence insertions of Inti I5_B1 line. A = adenine; G = guanine

By comparing the sequences of cytochrome b gene of *Cavia porcellus* from the El prado farm and from Genbank, we observed that all belong to the same monophyletic group and shared, to a lesser extent, certain inherited characteristics with the group para phyletic of animals *C. tschudii* and *C. aperea.* I5_B1 and I2_B1 animals share the same hierarchical sublevel; and these, in turn, have a high degree of similarity with the counterpart from Colombia AF490405, consistent with 0.0087 cM of nucleotide divergence. In turn, these sequences form a similar group with AY228363 and AY382793, coming from Peru and Chile respectively, and both groups are closely related to AY245099 *Cavia tschudii*. Animals AY382791 *C. aperea* and AY382792 *C.*

tschudii share a subgroup with AY382790 of *C. aperea* species. Both species are sharing inherited characters with *C. porcellus* animals, so it follows that intervening in the evolutionary history of this species, as confirmed Spotorno *et al.* [9], through analysis of maximum parsimony and maximum likelihood where copies grouped *C. porcellus* with *C. tschudii* for its similarity in the sequences of the mitochondrial cytochrome b gene pointing as the species most closely related to *C. porcellus*. The detailed phenotypic characters and gene sequences of Peru, Andina and Inti lines are shown in fig. 5, 6 and 7.

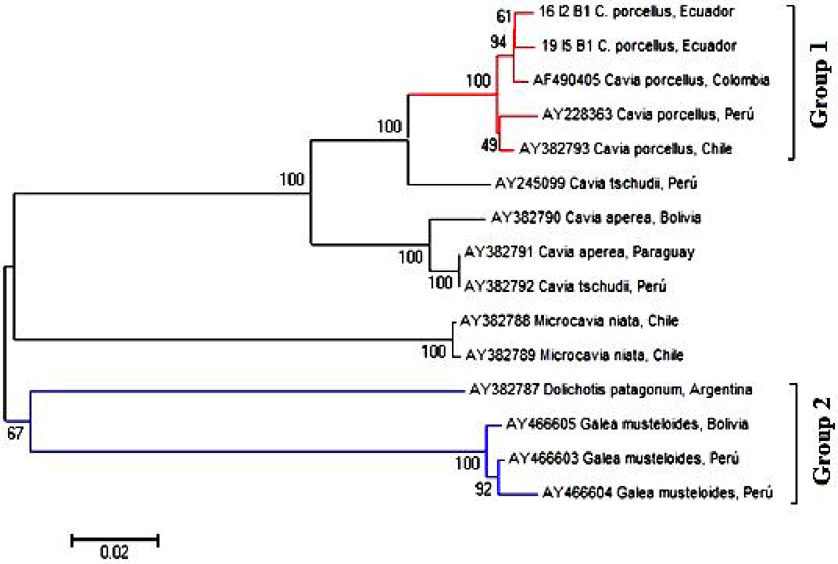
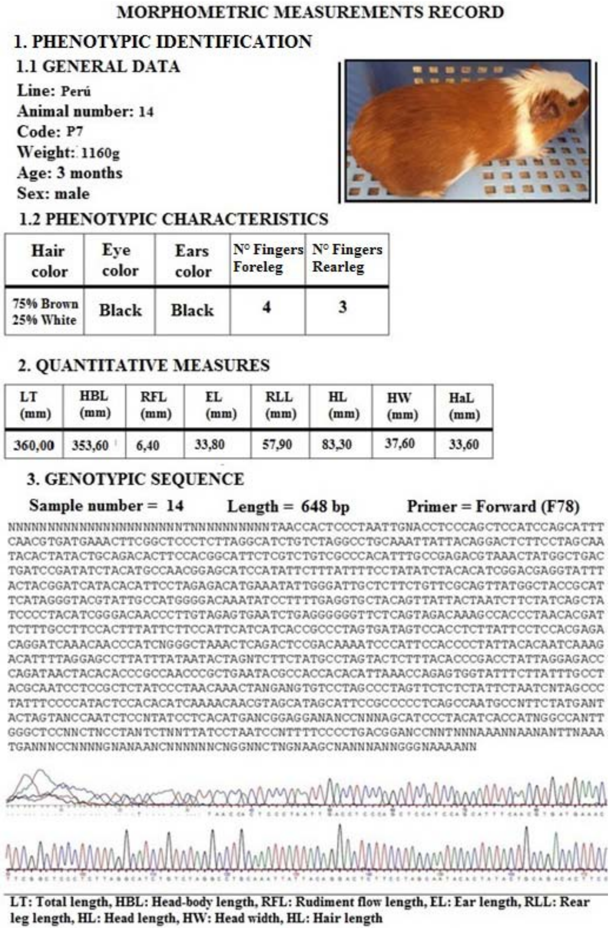


Fig. 4: The phylogenetic analysis of mitochondrial cytochrome b gene sequences of family cavidae species based on the method of grouping neighbors. The evolutionary distances were calculated using the maximum likelihood method. Percentages support of resampling (1000 Bootstap test replicates) are located on each node



MORPHOMETRIC MEASUREMENTS RECORD

1. PHENOTYPIC IDENTIFICATION

1.1 GENERAL DATA

Line: Perú
Animal number: 14
Code: P7
Weight: 1160g
Age: 3 months
Sex: male

1.2 PHENOTYPIC CHARACTERISTICS

Hair color	Eye color	Ears color	N° Fingers Foreleg	N° Fingers Rearleg
75% Brown 25% White	Black	Black	4	3

2. QUANTITATIVE MEASURES

LT (mm)	HBL (mm)	RFL (mm)	EL (mm)	RLL (mm)	HL (mm)	HW (mm)	HaL (mm)
360,00	353,60	6,40	33,80	57,90	83,30	37,60	33,60

3. GENOTYPIC SEQUENCE

Sample number = 14 Length = 648 bp Primer = Forward (F78)

```
NNNNNNNNNNNNNNNNNNNNNNTNNNNNNNNNNTAACCACTCCCTAATTGNACCTCCCAGCTCCATCCAGCATTT
CAACGTGATGAAACTTCGGCTCCCTCTTAGGCATCTGTCTAGGCCTGCAAATTATTACAGGACTCTTCCTAGCAA
TACACTATACTGCAGACACTTCCACGGCATTCTCGTCTGTCGCCCACATTTGCCGAGACGTAAACTATGGCTGAC
TGATCCGATATCTACATGCCAACGGAGCATCCATATTCTTTATTTTCCTATATCTACACATCGGACGAGGTATTT
ACTACGGATCATACACATTCCTAGAGACATGAAATATTGGGATTGCTCTTCTGTTCGCAGTTATGGCTACCGCAT
TCATAGGGTACGTATTGCCATGGGGACAAATATCCTTTTGAGGTGCTACAGTTATTACTAATCTTCTATCAGCTA
TCCCCTACATCGGGACAACCCTTGTAGAGTGAATCTGAGGGGGGTTCTCAGTAGACAAAGCCACCCTAACACGAT
TCTTTGCCTTCCACTTTATTCTTCCATTCATCATCACCGCCCTAGTGATAGTCCACCTCTTATTCCTCCACGAGA
CAGGATCAAACAACCCATCNGGGCTAAACTCAGACTCCGACAAAATCCCATTCCACCCCTATTACACAATCAAAG
ACATTTTAGGAGCCTTATTTATAATACTAGNTCTTCTATGCCTAGTACTCTTTACACCCGACCTATTAGGAGACC
CAGATAACTACACACCCGCCAACCCGCTGAATACGCCACCACACATTAAACCAGAGTGGTATTTCTTATTTGCCT
ACGCAATCCTCCGCTCTATCCCTAACAAACTANGANGTGTCCTAGCCCTAGTTCTCTCTATTCTAATCNTAGCCC
TATTTCCCCATACTCCACACATCAAAACAACGTAGCATAGCATTCCGCCCCCTCAGCCAATGCCNTTCTATGANT
ACTAGTANCCAATCTCCNTATCCTCACATGANCGGAGGANANCCNNNAGCATCCCTACATCACCATNGGCCANTT
GGGCTCCNNCTNCCTANTCTNNTTATCCTAATCCNTTTTCCCCTGACGGANCCNNTNNNAAANNAANANTTNAAA
TGANNNCCNNNNGNANAANCNNNNNNCNGGNNCTNGNAAGCNANNNANNGGGNAAAANN
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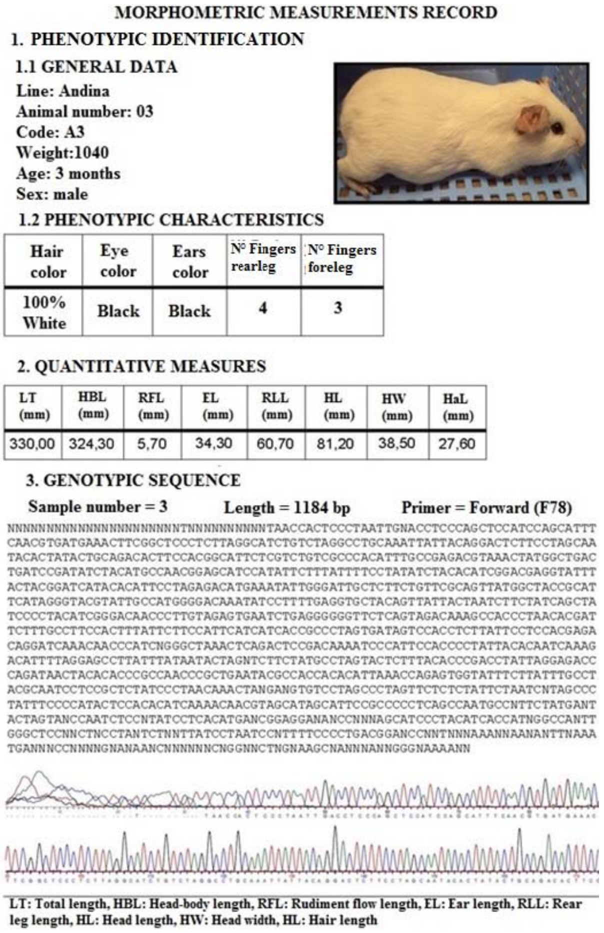
LT: Total length, HBL: Head-body length, RFL: Rudiment flow length, EL: Ear length, RLL: Rear leg length, HL: Head length, HW: Head width, HL: Hair length

Fig. 5: The detailed phenotypic characters and gene sequences of Peru line



MORPHOMETRIC MEASUREMENTS RECORD

1. PHENOTYPIC IDENTIFICATION

1.1 GENERAL DATA

Line: Andina
Animal number: 03
Code: A3
Weight:1040
Age: 3 months
Sex: male

1.2 PHENOTYPIC CHARACTERISTICS

Hair color	Eye color	Ears color	N° Fingers rearleg	N° Fingers foreleg
100% White	Black	Black	4	3

2. QUANTITATIVE MEASURES

LT (mm)	HBL (mm)	RFL (mm)	EL (mm)	RLL (mm)	HL (mm)	HW (mm)	HaL (mm)
330,00	324,30	5,70	34,30	60,70	81,20	38,50	27,60

3. GENOTYPIC SEQUENCE

Sample number = 3 Length = 1184 bp Primer = Forward (F78)

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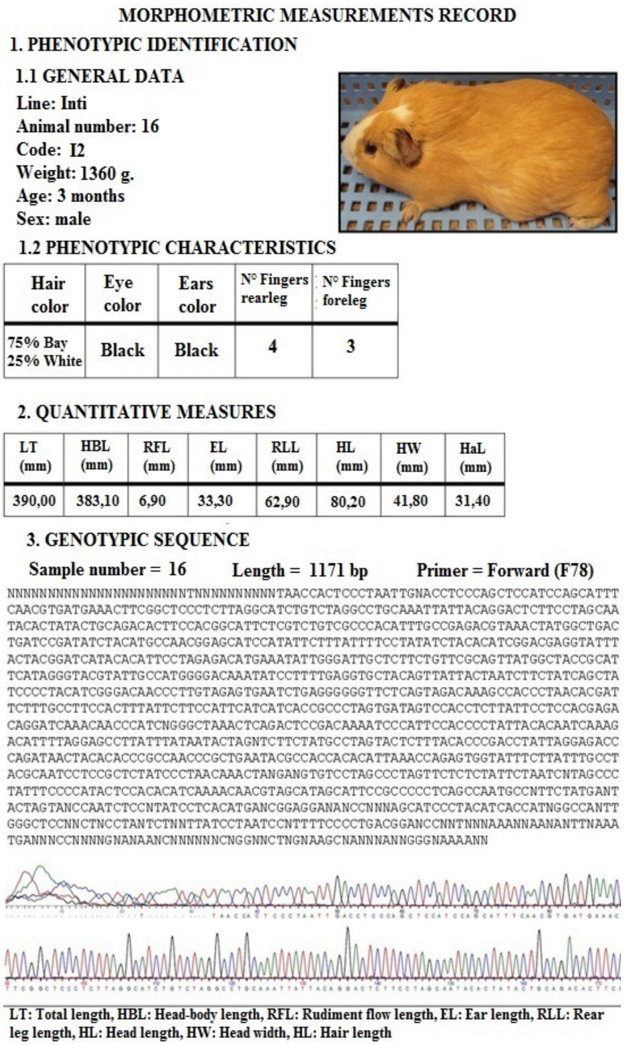
NNNNNNNNNNNNNNNNNNNNNNTNNNNNNNNNNTAACCACTCCCTAATTGNACCTCCCAGCTCCATCCAGCATTT
CAACGTGATGAAACTTCGGCTCCCTCTTAGGCATCTGTCTAGGCCTGCAAATTATTACAGGACTCTTCCTAGCAA
TACACTATACTGCAGACACTTCCACGGCATTCTCGTCTGTCGCCCACATTTGCCGAGACGTAAACTATGGCTGAC
TGATCCGATATCTACATGCCAACGGAGCATCCATATTCTTTATTTTCCTATATCTACACATCGGACGAGGTATTT
ACTACGGATCATACACATTCCTAGAGACATGAAATATTGGGATTGCTCTTCTGTTCGCAGTTATGGCTACCGCAT
TCATAGGGTACGTATTGCCATGGGGACAAATATCCTTTTGAGGTGCTACAGTTATTACTAATCTTCTATCAGCTA
TCCCCTACATCGGGACAACCCTTGTAGAGTGAATCTGAGGGGGGTTCTCAGTAGACAAAGCCACCCTAACACGAT
TCTTTGCCTTCCACTTTATTCTTCCATTCATCATCACCGCCCTAGTGATAGTCCACCTCTTATTCCTCCACGAGA
CAGGATCAAACAACCCATCNGGGCTAAACTCAGACTCCGACAAAATCCCATTCCACCCCTATTACACAATCAAAG
ACATTTTAGGAGCCTTATTTATAATACTAGNTCTTCTATGCCTAGTACTCTTTACACCCGACCTATTAGGAGACC
CAGATAACTACACACCCGCCAACCCGCTGAATACGCCACCACACATTAAACCAGAGTGGTATTTCTTATTTGCCT
ACGCAATCCTCCGCTCTATCCCTAACAAACTANGANGTGTCCTAGCCCTAGTTCTCTCTATTCTAATCNTAGCCC
TATTTCCCCATACTCCACACATCAAAACAACGTAGCATAGCATTCCGCCCCCTCAGCCAATGCCNTTCTATGANT
ACTAGTANCCAATCTCCNTATCCTCACATGANCGGAGGANANCCNNNAGCATCCCTACATCACCATNGGCCANTT
GGGCTCCNNCTNCCTANTCTNNTTATCCTAATCCNTTTTCCCCTGACGGANCCNNTNNNAAANNAANANTTNAAA
TGANNNCCNNNNGNANAANCNNNNNNCNGGNNCTNGNAAGCNANNNANNGGGNAAAANN
```

LT: Total length, HBL: Head-body length, RFL: Rudiment flow length, EL: Ear length, RLL: Rear leg length, HL: Head length, HW: Head width, HL: Hair length

Fig. 6: The detailed phenotypic characters and gene sequences of Andina line



MORPHOMETRIC MEASUREMENTS RECORD

1. PHENOTYPIC IDENTIFICATION

1.1 GENERAL DATA

Line: Inti
Animal number: 16
Code: I2
Weight: 1360 g.
Age: 3 months
Sex: male

1.2 PHENOTYPIC CHARACTERISTICS

Hair color	Eye color	Ears color	N° Fingers rearleg	N° Fingers foreleg
75% Bay 25% White	Black	Black	4	3

2. QUANTITATIVE MEASURES

LT (mm)	HBL (mm)	RFL (mm)	EL (mm)	RLL (mm)	HL (mm)	HW (mm)	HaL (mm)
390,00	383,10	6,90	33,30	62,90	80,20	41,80	31,40

3. GENOTYPIC SEQUENCE

Sample number = 16 Length = 1171 bp Primer = Forward (F78)

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NNNNNNNNNNNNNNNNNNNNNTNNNNNNNNNNNTAACCACTCCCTAATTGNACCTCCCAGCTCCATCCAGCATTT
CAACGTGATGAAACTTCGGCTCCCTCTTAGGCATCTGTCTAGGCCTGCAAATTATTACAGGACTCTTCCTAGCAA
TACACTATACTGCAGACACTTCCACGGCATTCTCGTCTGTCGCCCACATTTGCCGAGACGTAAACTATGGCTGAC
TGATCCGATATCTACATGCCAACGGAGCATCCATATTCTTTATTTTCCTATATCTACACATCGGACGAGGTATTT
ACTACGGATCATACACATTCCTAGAGACATGAAATATTGGGATTGCTCTTCTGTTCGCAGTTATGGCTACCGCAT
TCATAGGGTACGTATTGCCATGGGGACAAATATCCTTTTGAGGTGCTACAGTTATTACTAATCTTCTATCAGCTA
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GGGCTCCNNCTNCCTANTCTNNTTATCCTAATCCNTTTTCCCCTGACGGANCCNNTNNNAAANNAANANTTNAAA
TGANNNCCNNNNGNANAANCNNNNNNCNGGNNCTNGNAAGCNANNNANNGGGNAAAANN
```

LT: Total length, HBL: Head-body length, RFL: Rudiment flow length, EL: Ear length, RLL: Rear leg length, HL: Head length, HW: Head width, HL: Hair length

Fig. 7: The detailed phenotypic characters and gene sequences of Inti line

CONCLUSION

The use of molecular techniques and phenotypic morphometry allowed developing a proper selection process of improved breeding lines of Peru, Andina and Inti. The molecular characterization of a mitochondrial cytochrome-b gene of 1101 bp for specimens of the species Cavys helped to determine the genetic diversity of evaluated population and selected specimens having greater divergence (15_B1). Phylogenetic inference determined that *Cavia porcellus* species share hereditary characteristics with para phyletic group *Cavia tschudii* and *Cavia aperea* animals confirming *Cavia porcellus* offspring from *Cavia tschudii*. These findings not only help in design effective breeding experiments but also help in selecting animals for experiment purposes.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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