



## Iberian origin of Brazilian local pig breeds based on *Cytochrome b* (*MT-CYB*) sequence

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### Summary

The aim of this work was to investigate the possible origin of local Brazilian pig breeds through *Cytochrome b* (*MT-CYB*) mitochondrial analyses. The results indicated that the main local pig breeds descended from two different European maternal lineages, both Iberian varieties. The haplotype relationship analysis showed that Monteiro, Nilo, Piau and Tatu breeds share haplotypes only with Iberian varieties, while the Moura breed presented a different maternal lineage. The Moura appears to share a high frequency of haplotypes with the Black Hairy Iberian variety and Hungarian Mangalica breed.

**Keywords** conservation genetics, farm animal genetic resources, livestock evolution, mtDNA, *Sus scrofa*.

Brazilian local pig breeds have originated from breeds introduced by the Portuguese settlers in the XVI century. During the following centuries, the animals introduced from different sources were bred all over Brazilian territory under different environments, causing animals to display diverse characteristics such as rusticity and adaptability. Brazilian local breeds were raised for centuries as an important food source all over the country. However, a number of breeds have been facing a dramatic reduction in numbers and diversity (and are therefore under threat of extinction) because of recent advances in pig genetic improvement followed by breed enhancement using imported exotic breeds (Mariante *et al.* 2003). In some regions, it is difficult to find local breeds without exotic breed admixture.

A proper census and evaluation of livestock genetic resources is of fundamental importance for conservation and utilization purposes. In this context, the aim of this work was to investigate the possible origin of the main local Brazilian pig breeds, based on *MT-CYB* sequence of mtDNA, to provide information relevant for the conservation of local pig breeds in Brazil.

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Accepted for publication 25 February 2009

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A total of 107 DNA samples of 16 genetic groups were extracted from blood, muscle tissue or hair including nine local breeds (Canastrão, Caruncho, Moura, Monteiro, Nilo, Piau, Pirapetinga, Rabo-de-peixe, Tatu), five European-derived Brazilian commercial lines (Landrace, Large White, Duroc, Pietrain and the composite MS60, developed by Embrapa Swine and Poultry) and local samples from Mule foot phenotype pigs and a group of crossbred animals (Table 1).

The *MT-CYB* gene (1140 bp) was amplified using primers described by Alves *et al.* (2003). The purified PCR products were sequenced with the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) using the same PCR primers. The raw data were edited and aligned using the software SEQSCAPE version 2.1 (Applied Biosystems), and the haplotypes were defined based on the reference sequence, accession number AJ002189 (Ursing & Arnason 1998). The Genbank accession numbers for the sequences described in this work are EU586516–EU586549.

A total of 979 bp of the 1140 bp from the *MT-CYB* Brazilian haplotypes were aligned and trimmed with 104 sequences of European and Asian breeds available on NCBI database (accession numbers: AF136541–AF136554, AF163099, AF163100, AY237484–AY237534, AY830159–AY830172, AF486855–AF486874, AF304200–AF304203, AY692029–AY692032, AY634180–AY634188,

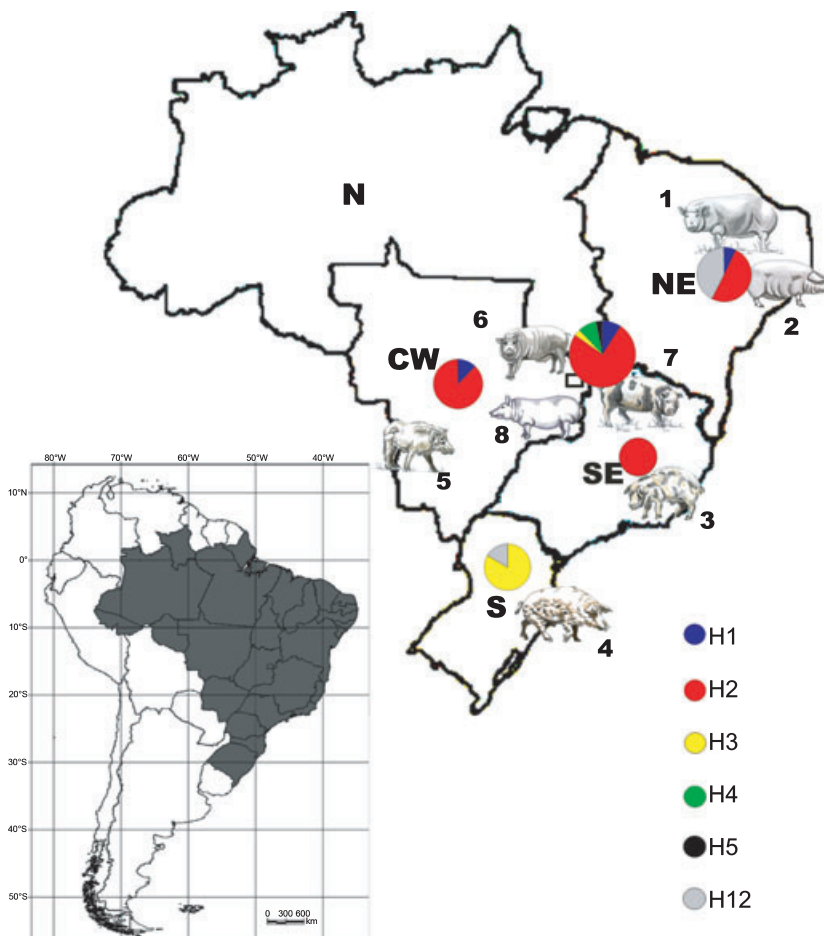
Genetic group	<i>n</i>	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
Canastrão	1	1											
Caruncho	2				2								
Mule foot	3		2	1									
Nilo	8		7			1							
Monteiro	12	1	11										
Moura	12		1	9									2
Piau	14	3	9										2
Pirapetinga	3		2		1								
Rabo-de-Peixe	2		2										
Tatu	8		6										2
Crossbreed	4	1	1										2
Duroc	3	3											
Landrace	14	5					1	1			3		4
Large White	3								1			2	
Pietrain	3	1	2										
MS60	15	7		1						1		3	3

**Table 1** Distribution of *MT-CYB* haplotypes among Brazilian populations.

*n*, no. samples collected.

DQ534707, DQ518915, DQ466081, DQ334860, DQ334861, AY337045, AF034253, AY334492, AY574045–AY574048, NC\_000845), using the software MEGA (Kumar *et al.* 1994). Hierarchical analyses were obtained by AMOVA, using

ARLEQUIN 3.01 (Excoffier *et al.* 2005) as well as the pairwise distance matrix and significance of the fixation indices. A median-joining network was constructed using the software package NETWORK 4.1 (Bandelt *et al.* 1999).



**Figure 1** Current geographical distribution of haplotypes and Brazilian Local breeds in Central-West, North, North-East, South-East Brazilian territories: (1) Tatu; (2) Canastrão; (3) Piau; (4) Moura; (5) Monteiro; (6) Nilo; (7) Caruncho; (8) Pirapetinga. The square in the centre of the map is the Federal District. Blue, Haplotype 1 (H1); Red, Haplotype 2 (H2); Yellow, Haplotype 3 (H3); Green, Haplotype 4 (H4); Black, Haplotype 5 (H5); Grey, Haplotype 12 (H12).

Six of the 12 haplotypes identified in the Brazilian populations were not yet described in literature (Table S1). Two of the six (H4 and H5) were identified only in local breeds, and the other four (H6, H7, H8 and H9) in commercial lines Landrace, Large White and MS60 (Table 1). The geographical distribution of haplotypes found on Brazilian local groups is represented in Fig. 1.

Clop *et al.* (2004) characterized two distinct European (E1 and E2) and Asian (A1 and A2) clades based on a fragment of pig *MT-CYB* sequence. In this work, nine haplotypes from European origin have been found (Fig. S1), all of the E1 clade and three (H7, H11 and H12) of Asian origin were all located in the A1 clade. Although some animals of the Tatu, Moura, Piau, Landrace and Large White breeds, as well as from the composite MS60, have also shown Asian haplotypes, most local and commercial Brazilian genetic groups presented European haplotypes (Table 1).

The Brazilian pig populations sampled, both local and commercial lines, presented a mean diversity ( $\pi$ ) value of  $0.0545 \pm 0.0264$  ( $n = 107$ ), and European haplotypes alone (GenBank data) showed a mean diversity value of  $0.0097 \pm 0.0067$  ( $n = 86$ ). Alves *et al.* (2003) found values of  $0.00255 \pm 0.00063$  among the 29 haplotypes in European domestic pig. Mean diversity of Asian-type mtDNA of Brazilian sampled populations was  $0.00476 \pm 0.00430$  ( $n = 21$ ), which is in good agreement with the result found by Alves *et al.* (2003). The genetic distance between European and Asian mtDNA-type Brazilian populations was  $0.015653 \pm 0.003708$ .

Amongst the most representative Brazilian local breeds (Moura, Nilo, Monteiro and Piau), the variation was of 32.44% ( $P < 0.0001$ ). Nevertheless, when the Moura breed was excluded from the structure analysis, the variation between the Nilo, Monteiro, Piau and Tatu local breeds was non-significant ( $P < 0.01$ ). In addition, the difference between these breeds and the Iberian breeds was also non-significant (Table S2). The Nilo, Monteiro, Piau and Tatu local breeds share haplotypes with the Black Hairless varieties from Guardiana Valley, La Serena and Black Hairless pigs found in the Torbiscal line as well as Black Hairy and Portuguese Red varieties (Alves *et al.* 2003), suggesting a possible common maternal ancestry.

The Moura breed diverged significantly from all breeds and groups tested (Table S2), which reflect a founder effect and different stock origins. However, the differentiation could be explained by the high proportion of H3 haplotypes found in the Moura breed, which have already been found in the Black Hairy Iberia varieties (Alves *et al.* 2003) and the Hungarian Mangalica breed (Giuffra *et al.* 2000). It is probable that one of these pig varieties might be the closest to the maternal lineage of Moura pigs.

The results presented here are the first reported for the study of mitochondrial *MT-CYB* diversity to ascertain the

origin of Brazilian local pigs. These results could be the basis for the creation of a conservation and utilization programme that will emphasize the haplotype diversity of the strains.

## Acknowledgements

This work was supported by CNPq, Embrapa (Brazil) and Agencia Española de Cooperación Iberoamericana (Spain) grant AECI/5267/06. We thank Embrapa Swine and Poultry (Mônica Corrêa Ledur, Giovani Bertani), Embrapa Pantanal (Ubiratan Piovezan, Sandra Aparecida Santos, Paulo Sérgio C. Moura) and EMATER-DF (José Lopes Germano) for biological material donation. We also wish to thank Dr Miguel Perez-Enciso and Dr Marcel Amills for useful advices and Dr Dário Grattapaglia for allowing utilization of ABI Machine.

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## Supporting information

Additional supporting information may be found in the online version of this article.

**Figure S1** Reduced median network of *CYTB* sequences from Brazilian populations and European and Asian haplotypes. Circles represent haplotypes and have a size proportional to their frequency. Line distances are proportional to the number of mutations.

**Table S1** Variable sequences of *MT-CYB* in mtDNA from Brazilian pigs of local and commercial genetic groups. Nucleotide positions are numbered according to the reference sequence GenBank AJ002189 (Ursing & Arnason 1998).

**Table S2** Pairwise distance between local and commercial genetic groups from Brazilian populations and European

haplotypes already described. *Fst* index values are under the diagonal, and respective *P*-values are above the diagonal.

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