DNA BARCODING OF DOMESTIC SWINE BREEDS AND CROSSBREEDS (*Sus scrofa*) IN THE PHILIPPINES

Orville L. Bondoc, Jorge Michael D. Dominguez and Francisco F. Peñalba

ABSTRACT

DNA barcodes (*i.e.* cytochrome c oxidase subunit I or COI in the mitochondrial genome) obtained from eight domestic pig breeds and crossbreeds (*Sus scrofa*) in the Philippines and five swine breeds retrieved from GenBank were analyzed using Neighbour-Joining method based on Kimura 2-parameter model in MEGA5. Based on 617 COI positions, overall genetic diversity of domestic swine breeds and crossbreeds was 36.3%. Average genetic distance was highest among commercial purebred pigs (d=0.291), followed by crossbred pigs (d=0.289), native pigs (d=0.202) and smallest among GenBank-accessed breeds (d=0.008). The results indicate that DNA barcodes can be effective in differentiating between breeds sampled in the Philippines, but not among swine breeds whose COI sequences were derived from GenBank. DNA barcodes can distinguish purebred pigs sampled in the Philippines from their counterpart breed listed in GenBank. Wide genetic distances of COI sequences imply greater diversity of native genetic resources that are distinctly different from pig breeds raised locally and abroad. Genetic distances between a crossbred pig and its dam's breed are not small. However, more COI sequences should be determined from distinct crossbred populations to improve reliability of DNA barcoding to discriminate them from their dam's breed and to confirm breed origin of pigs.

Keywords: DNA barcodes, domestic swine breeds and crossbreeds, evolutionary analysis, genetic diversity

INTRODUCTION

The swine industry in the Philippines valued in 2011 at 172.57 billion pesos is the largest contributor to the total value of livestock and poultry production. Total pig inventory in 2011 was 11.86 million head of which 67.28% are raised in backyard (smallholder) farms, while per capita utilization of pork in 2009 was 14.87 kg/year (BAS, 2012). Imported breeding stocks have long been used to upgrade the performance of native and indigenous stocks (Peñalba, 1993). Recently, however, many swine breeding farms are increasingly becoming dependent on imported purebred boars and gilts for local multiplication and production of commercial hybrids.

Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines Los Baños, Laguna, Philippines (email: orville_bondoc@yahoo.com).

The Philippine native pigs originated from the wild species *Sus celebensis philippinensis* Nehring in Luzon, *Sus celebensis mindanensis* Major of Mindanao, *Sus celebensis* Sanborn of Negros Island, and *Sus barbatus ahoenobarus* Huet of Palawan (Eusebio, 1969). Since the introduction of pigs in the Philippines by early Chinese traders and Spaniards during the Spanish colonization era from the 16th to 19th century, various commercial breeds of swine (*e.g.*, Berkshire, Chester White, Poland China, Duroc Jersey, Hampshire, Lacombe, Large White, Landrace and Pietrain) have been imported mostly from the United States and Europe (Bondoc, 1998; Bondoc, 2008).

Genetic polymorphisms and distances between pig breeds in the Philippines have been determined in the past using various techniques such as blood typing (Nozawa *et al.*, 1978), karyotyping (Navarra *et al.,* 1997), electrophoresis methods (Nozawa *et al*., 1978; Francisco, 1992; Navarra *et al*., 1997), mitochondrial DNA analysis (Unpublished report), and Amplified Fragment Length Polymorphism (Sookmanee, 1998). In other countries, several mitochondrial DNA studies have shown that domestication of modern pigs occurred independently from wild boar subspecies in Europe and Asia (Giuffra *et al*., 2000; Kijas and Andersson, 2001; Larson *et al*., 2005; Wu *et al*., 2007). Molecular evidence for the introgression of Asian pigs into Europe during the 18th and early 19th centuries also indicated a hybrid origin of some major "European" pig breeds (Giuffra *et al*., 2000). Likewise, analysis of mitochondrial DNA data suggested human-mediated translocations, dispersals and exchanges of domestic pigs across Eurasia, as well as Neolithic expansion in South East Asia and Oceania that links mainland East Asian pigs to western Micronesia, Taiwan and the Philippines (Larson *et al*., 2007).

In this preliminary study, DNA barcodes (*i.e.* cytochrome c oxidase subunit I or COI gene of the mitochondrial genome) initially proposed as a standard for rapid species identification (Hebert *et al*., 2003), were used to analyze evolutionary relationships, genetic diversity and distances among domestic pig breeds — purebred exotic pigs, native pigs and crossbred pigs in the Philippines. DNA barcodes were also compared between swine breeds sampled in the Philippines and their counterpart breeds whose COI sequences were derived from GenBank. Practical applications of the unique identifications based on DNA barcodes to local swine improvement and conservation programs are likewise presented.

MATERIALS AND METHODS

The taxonomic classification of domesticated swine is as follows: Kingdom - Animalia, Phylum - Chordata, Class - Mammalia, Order - Artiodactyla, Family - Suidae, Genus - *Sus*, Species/subspecies - *S. scrofa domesticus* or *S. domesticus*.

Based on a survey of 144 countries, FAO (2007) reported 541 local pig breeds in the world, of which 229 are found in Asia, 165 in Europe and the Caucasus, 67 in Latin America and the Carribean and 49 in Africa. One hundred and forty nine (149) swine breeds have gone into extinction and 63 pig breeds are in the endangered list.

Field sampling

Materials used for the present study were obtained from the National Swine and Poultry Research and Development Center (NSPRDC), BAI-DA at Tiaong, Quezon and a privately-owned pig breeding farm in the Philippines which provided authoritative animal records and identifications. Table 1 shows the classification of eight domestic pig breeds used in the study based on their breed group, country of origin and farm location.

Table 1. Classification of domestic pig breeds, strains and crossbreeds used in swine production in the Philippines.

Name of breed, strain, or crossbreed	Country of Origin	Farm location
Purebred:		
Duroc	United States of America	BAI-DA, Tiaong, Quezon
Landrace	Denmark (Europe*)	BAI-DA, Tiaong, Quezon
Large White	England, UK (Europe*)	BAI-DA, Tiaong, Quezon
Pietrain	Belgium (Europe*)	Biñan, Laguna
Native strains:		
Kalinga native pig	Philippines	BAI-DA, Tiaong, Quezon
Quezon native pig	Philippines	BAI-DA, Tiaong, Quezon
Crossbred pigs		
F_1 "50% Landrace x 50% Large White"	Philippines	Biñan, Laguna
F_2 "25% Duroc x 25% Pietrain x 25% Landrace x 25% Large White"	Philippines	Biñan, Laguna

*Europe includes the United Kingdom, Netherlands, Belgium, Denmark, Sweden and Germany.

One specimen representing a breed or crossbreed was examined to ascertain COI sequence divergences within the domestic swine species. Close relatives of the same breed were expected to have the same COI sequences. Demographic information (*e.g.*, name of breed, purpose or type, sex, ID number and date of sampling) and morphological data (*e.g.*, live weight, height, body length, heart girth, midriff girth, flank girth, length of leg, tail, snout and ears) including digital images (pictures and videos) were likewise taken for each animal specimen and recorded in the local DNA barcode library.

Laboratory analysis

Most analytic methods followed those described by Hebert *et al*. (2004). DNA

sources for this study included blood samples extracted from the jugular or ear vein in live specimens without harming them using gauge 20 or 22 hypodermic needle, in accordance with institutional, local and national guidelines regarding animal care and use in experimentation. Fresh blood samples (~1-2 ml) were placed in NucleoSave blood storage cards (Machery-Nagel, USA) and allowed to dry for three days under room temperature. Laboratory protocols for DNA extraction, purification, elution and amplification for mammalian specimens were developed at the Animal Biotechnology Laboratory, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines Los Baños.

DNA extraction. Using a Harris 1.2 mm micropunch, at least 30 discs per dried NucleoSave card or sample were collected and placed in labeled microcentrifuge tubes.

DNA purification. Sample discs were washed with 200 µl of FTA Purification Reagent (Whatman Inc., USA) for 4 to 5 times and rinsed with 200 µl sterile molecular biology grade water. Sample discs were then dried in a laminar hood overnight.

DNA elution. Six dried sample discs were transferred in a sterile PCR tube and added with 55 µl sterile nanopure water. DNA was eluted by incubation at high temperature specifically at 90ºC for 10 min using Veriti 96 Well Thermal Cycler (Applied Biosystems). Eluted DNA was stored at -20ºC for further use.

DNA amplification. The COI gene was amplified using primers LCO1490 (5' GGTCAACAAATCATAAAGATATTGG 3') and HCO2198 (5' TAAACTTCAGGGTGACCAAAAAATCA 3') from Hebert *et al*. (2004). The 20-µl PCR reaction mix included 13.44 µl sterile ultrapure water, 2.0 µl of 10x buffer, 1.0 µl of MgCl2, 0.8 units of Taq DNA polymerase, 0.4 µl (0.2 mM) of each forward and reverse primer and 2.0 µl of DNA template. The optimized PCR amplification program was composed of three min at 94ºC followed by five cycles of 40 sec at 94ºC, 30 sec at 52ºC and 45 sec at 72ºC, followed by another 30 cycles of 40 sec at 94ºC, 30 sec at 54ºC, and 45 sec at 72ºC, and finally seven min at 72ºC.

PCR products were visualized in a 1.0% agarose gel with ethidium bromide. Post stained gels were viewed using Molecular Imager® Gel DocTM XR System (Bio-Rad, USA). PCR products were purified using GF-1 PCR Clean Up Kit (Vivantis, Malaysia). In cases where multiple bands occurred (*e.g.*, pseudogenes or short DNA sequences less than 200 bp), gels were excised and purified using GF-1 Gel DNA Recovery Kit (Vivantis, Malaysia). The DNA amplification regime was repeated four times for each sample specimen. The final PCR product for each sample specimen (about 30-50 µl final volume) was obtained from pooled amplicons of all four PCR reactions (replicates).

DNA sequencing. PCR products were sent to Macrogen Inc., Seoul, Korea for unidirectional sequencing using appropriate forward primer and analyzed using 3730L DNA analyzer (AB, USA) and BigDye (AB, USA).

COI sequence analysis

Evolutionary analyses were conducted in MEGA5 (Tamura *et al*., 2011). The COI sequences were aligned using ClustalW (Thompson *et al*., 1994). The evolutionary distance between a pair of sequences was measured by the number of

nucleotide substitutions (*i.e.* transition and/or transversion) or differences occurring between them.

Diversity analysis. Diversity analysis involved the calculation of sequence divergence using the Kimura 2-parameter or K2P model (Kimura, 1980) which corrected for multiple hits, taking into account transitional and transversional substitution rates, while assuming that the nucleotide frequencies were the same and that the rates of substitution do not vary among sites. Standard error estimates were obtained by a bootstrap procedure (1000 replicates) according to Nei and Kumar (2000).

Distance analysis. To estimate genetic distances among different pig breeds, the evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) with their variances estimated by a bootstrap approach. The average distance between sequence pairs were in the units of number of base substitutions per site (*i.e.* d units). All positions containing gaps and missing data were eliminated. Within (or between) group mean distance was estimated as the average evolutionary divergence over sequence pairs within (or between) groups.

Phylogeny analysis. The Neighbour-Joining (NJ) method was used to infer the evolutionary history (Saitou and Nei, 1987). The nearest-neighbour distance, the minimum genetic distance between a pig breed and its closest relative was examined to test the discriminatory power of COI barcodes. A bootstrap consensus NJ tree of K2P distances was inferred from 1000 replicates (Felsenstein, 1985).

DNA sequences from the mitochondrial genome of domestic swine breeds (Table 2) were retrieved from GenBank of the National Center for Biotechnology Information and included in the analysis to check for appropriate breed positions in the NJ tree of the Suidae family. All new DNA barcodes from eight pig breeds and crossbreeds were different and have been deposited in GenBank under Accession Numbers JX218082 - JX218087 and JX280483.

Table 2. Swine breeds with mitochondrial DNA sequences retrieved from GenBank, NCBI.

No.	Name of breed	GenBank accession number	Author(s)	Place and year of sampling
1	Chinese Meishan	AF304200	Kijas and Andersson (2001)	Uppsala, Sweden (2000)
2	Berkshire	AY574045	Cho *et al.* (2004) - Unpublished	Jeju, South Korea (2003)
3	Duroc	AF486858	Yang *et al.* (2003)	Hubei, China (2002)
4	Landrace	AF304202	Kijas and Andersson (2001)	Uppsala, Sweden (2000)
5	Large White	AF486874	Yang *et al.* (2003)	Hubei, China (2002)

RESULTS AND DISCUSSION

In the present study, the evolutionary divergence over COI sequence pairs was estimated between domestic pig groups, *i.e.* 4 commercial pure (exotic) breeds, 2 native breeds or strains obtained from two provinces in the island of Luzon, and 2 crossbreeds (*i.e.* 2-breed cross and 4-breed cross), and 5 swine breeds from China, South Korea and Sweden whose COI sequences were derived from GenBank.

The rooted Neighbour-Joining tree representing DNA barcodes (Figure) showed divergence into two distinct evolutionary clades. One clade included exotic pure breeds sampled in the Philippines (*i.e.* Duroc, Landrace, and Large White) and native pigs from the Kalinga and Quezon provinces. The GenBank-accessed COI sequences of five other swine breeds were clustered with Pietrain and two

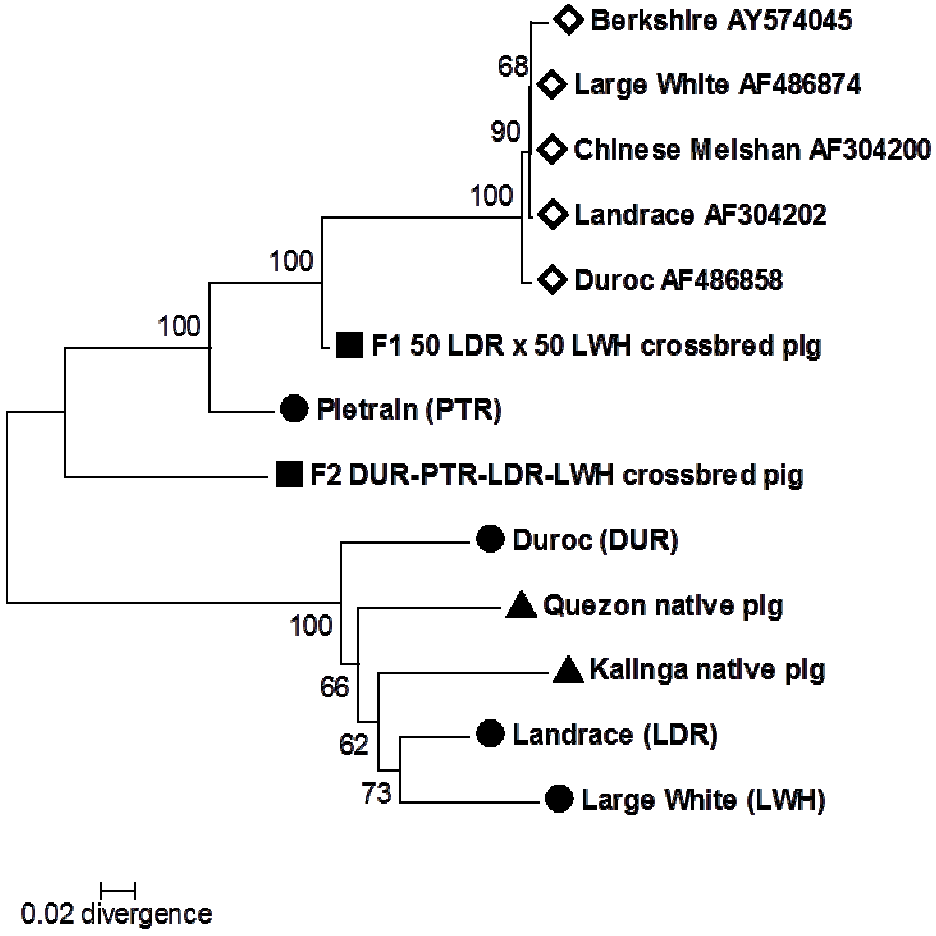

Figure. Neighbour-Joining tree with bootstrap support showing the evolutionary relationships of purebred exotic breeds (●), native pigs (▲) and crossbred pigs (■) sampled in the Philippines, and swine breeds derived from Genbank (◊), (N=13 COI sequences; 617 positions).

crossbred pigs in another clade. Similarly, Watanabe *et al*. (1986) reported that based on mitochondrial DNA lineages, modern pig breeds such as the Large White must have been derived from two different maternal origins, European and Asian wild boars. Giuffra *et al*. (2000) later confirmed the existence of three major clades namely, European Clade I (*i.e.* European wild boars, Israeli wild boars, most European domestic pigs - Duroc, Hampshire, Landrace, Large White), European Clade II (*i.e.* wild boars fro Southern Europe - Italy), and Asian Clade (*i.e.* Japanese wild boars, Chinese Meishan pigs and some European domestic pigs). The fact that some domestic pigs are closely related to European wild boar sequences, whereas others cluster with Asian wild boar sequence, provides conclusive evidence for independent domestication of pigs in Europe and Asia. Recently, Lucchini *et al*. (2005) who used molecular and morphometric techniques, also suggested the existence of two main evolutionary clades that are likely to have diverged during the Pliocene in Southeast Asia: one including wild pig populations distributed in the Philippines (*S. cebifrons*) and Sulawesi (*S. celebensis*); the other including Indonesian and Malaysian bearded pigs (*S. barbatus*), and the Eurasian wild boar (*S. scrofa*).

Based on 617 COI positions, the overall genetic diversity of domestic swine breeds and crossbreeds was about 36.3% (Table 3). Coefficient of differentiation which estimates the proportion of interpopulational diversity out of the combined pig samples was 45.5%. Average pair-wise distances was highest among commercial purebred pigs (d = 0.291 ± 0.019), followed by crossbred pigs (d = 0.289 ± 0.025), native pigs (d = 0.202 ± 0.020) and smallest among GenBank-accessed breeds (d = 0.008 ± 0.002). Following Hebert *et al*. (2003), a genetic diversity within the taxa of 2% may justify the effectiveness of COI barcodes as an identification tool to discriminate among members of the taxa. Similarly, a genetic distance value (d) less than 0.020 is considered low. DNA barcodes will, therefore, be effective in differentiating between breeds sampled in the Philippines, but not among swine breeds whose COI sequences were derived from GenBank.

Table 3. Mean diversity between breeds and within domestic pig groups of the Suidae family.

Diversity measures	No. of nucleotide sequences	N positions	Diversity (%)	
			Mean	Standard error
Within population	13	617	19.75	1.10
Interpopulation			16.52	1.47
Entire population			36.27	2.18
Coefficient of differentiation			45.54	2.34

Comparisons of COI sequences between pig breeds (within pig groups)

Comparisons among commercial purebred pigs. The genetic distances between commercial purebred pigs sampled in the Philippines was 0.291 units and ranged from d = 0.123 to 0.457 (Table 4). Large White was found to be genetically closer to Landrace (d = 0.123) than Duroc (d = 0.208). The average genetic distance between Duroc and Landrace was 0.144 units. The Large White and Landrace commercial breeds originating from Northwest and Central Europe are two of the most widely distributed international breeds found in 117 and 91 countries, respectively. Large White and Landrace are popularly used in crossbreeding schemes as dam lines because of their high reproductive potentials. Duroc, on the other hand, found in 93 countries actually originated from the United States (FAO, 2007). Pietrain which is native to the village of Piétrain in Wallonia, Belgium (Briggs, 1983) and now available in 35 countries had more distant genetic relationship with Duroc (d = 0.403), Landrace (d = 0.409) and Large White (d = 0.457). Duroc and Pietrain are commonly used as sire lines and noted for high growth performance and carcass quality.

Table 4. Pair-wise distances (d units) between commercial pure (exotic) breeds, native pigs, and crossbred pigs sampled in the Philippines and swine breeds retrieved from GenBank.

	Commercial pure (exotic) breeds				Native pigs		Crossbreeds		GenBank-derived breeds			
	1	2	3	4	5	6	7	8	9	10	11	12
2	0.144											
3	0.208	0.123										
4	0.403	0.409	0.457									
5	0.193	0.158	0.191	0.480								
6	0.170	0.152	0.175	0.434	0.202							
7	0.449	0.449	0.500	0.106	0.514	0.469						
8	0.460	0.416	0.427	0.263	0.399	0.430	0.289					
9	0.583	0.592	0.634	0.227	0.642	0.610	0.127	0.389				
10	0.601	0.610	0.653	0.238	0.661	0.628	0.137	0.403	0.010			
11	0.582	0.587	0.634	0.229	0.637	0.601	0.129	0.387	0.010	0.020		
12	0.586	0.595	0.638	0.227	0.638	0.607	0.127	0.389	0.002	0.011	0.011	
13	0.583	0.592	0.634	0.227	0.642	0.610	0.127	0.389	0.000	0.010	0.010	0.002

Legend: 1 = Duroc, 2 = Landrace, 3 = Large White, 4 = Pietrain, 5 = Kalinga native pig, 6 = Quezon native pig, 7 = F_1 “50% Landrace x 50% Large White” crossbred pig, 8 = F_2 “25% Duroc x 25% Pietrain x 25% Landrace x 25% Large White” crossbred pig, 9 = Chinese Meishan AF304200, 10 = Berkshire AY574045, 11 = Duroc AF486858, 12 = Landrace AF304202, 13 = Large White AF486874.

Comparisons between native pigs. The genetic distance between the Kalinga and Quezon native pigs was 0.202 ± 0.020 units. The genetic distances between native pigs and commercial purebreds (except Pietrain) sampled in the Philippines ranged from d = 0.416 to 0.500. Pietrain was related more closely to Kalinga native pig (d = 0.106) than to Quezon (d = 0.263). Wide genetic distances of native pigs compared to crossbred pigs and swine breeds accessed from GenBank were also found and ranged from d = 0.399 to 0.514 and d = 0.601 to 0.661, respectively. The generally wide genetic distances imply greater diversity of COI sequences of unique native pig genetic resources that are distinctly different from domestic pig breeds raised locally and abroad.

Comparisons between crossbred pigs. The genetic distance between two-breed and four-breed crosses was 0.289 ± 0.025 units. The F_1 "50% Landrace x 50% Large White" crossbred pig seemed to be more genetically distant to Large White (d = 0.500) than Landrace (d = 0.449). On the other hand, the F_2 "25% Duroc x 25% Pietrain x 25% Landrace x 25% Large White" crossbred pig was closer to the Pietrain (d = 0.263) than Duroc (d = 0.460), Landrace (d = 0.416), or Large White (d = 0.427). Interestingly, the genetic distances between the two-breed cross and Genbank-derived COI sequences was relatively smaller, ranging from d = 0.127 to 0.137. While a COI barcode will often assign F_1 hybrids to the breed or species of their female parent because mitochondrial DNA is maternally inherited (Hebert *et al*., 2004), the present results indicate that genetic distances between a crossbred pig and its dam are not small.

Comparisons between swine breeds whose COI sequences were derived from GenBank. Small average genetic distances were found among swine breeds whose COI sequences were derived from GenBank, ranging from d = 0.000 to 0.020. Genetic distances between breeds were too small to identify introgressions and conclusively determine their origins and diversification. However, the close genetic distances may be attributed to early introgression of various pig breeds into commercial herds that had been more frequent and widespread worldwide.

Comparisons among pig breeds sampled locally and those retrieved from GenBank. The average pair-wise distances between pig breeds (excluding Pietrain) sampled in the Philippines and their counterpart breeds accessed from GenBank were 0.582, 0.595 and 0.653 units for Large White, Landrace, and Duroc, respectively. This implies that (unrelated) pigs of the same breed do not have the same COI sequences and that DNA barcodes may be used to distinguish purebred pigs sampled in the Philippines from their counterpart breed listed in GenBank.

CONCLUSION

The present results indicate that COI sequences can be effective in differentiating between pig breeds sampled in the Philippines, but not between breeds whose COI sequences were derived from GenBank. More COI sequences, however, should be determined from native pigs and crossbred populations to improve reliability of using DNA barcodes to distinguish them from their dam's breed and to confirm their breed origin.

Potential applications of DNA barcodes to the local swine industry are mainly

towards the conservation of native pig diversity and further use of these populations in creating genetic stocks with improved adaptability and productivity in smallholder or commercial organic production systems. For example, DNA barcoding may be used to confirm the breed origin of a candidate pig (and its wild relatives) for conservation, provided that a reference data set has been defined for various pig breeds sampled from different geographic populations. DNA barcodes may also be used to identify poached animals that are unlawfully sold as common pork in the domestic and international food markets. Consequently, DNA barcoding can help detect and reduce illegal trade of rare or endangered wild pigs found in the country and nearby islands of Southeast Asia.

Moreover, DNA barcoding can be used for breed definition and traceability of imported boars and gilts/sows. DNA barcodes, in addition to breed performance data and pedigree records, may, therefore, be required to authenticate and certify their classification as a distinct breed or crossbreed utilized in accredited nucleus and multiplier breeding farms, in collaboration with local pig producers associations, government and university research centers/institutes. DNA barcodes later can be used to monitor the increased use of pig breeding materials from abroad.

ACKNOWLEDGEMENT

Funding for this study was provided by a research grant from the Biotechnology Implementation Program, Department of Agriculture - Philippines. The authors express their appreciation to Dr. Rene Santiago and staff of the National Swine and Poultry Research Center, Bureau of Animal Industry - Department of Agriculture at Tiaong, Quezon and Mr. Antonio Tamayo and Mr. Elmer Baure of TJ (Nena-ling) Farms, Biñan, Laguna for allowing access to the pig breeds or strains used in the study and providing assistance in the collection of samples. The authors also thank Walter Israel and Nestor Ebuenga, Jr. for preparing specimens and assistance with molecular work in the biotechnology laboratory.

REFERENCES

Bondoc OL. 1998. *Biodiversity of Livestock and Poultry Genetic Resources in the Philippines*. Los Baños: Institute of Animal Science, College of Agriculture, University of the Philippines Los Baños and PCARRD / Department of Science and Technology.

Bondoc OL. 2008. *Animal Breeding: Principles and Practice in the Philippine Context*. Diliman, Quezon City: University of the Philippines Press.

Briggs HM. 1983. *International Pig Breed Encyclopaedia*. Indianapolis, Indiana, USA: Elanco Products Company.

Bureau of Agricultural Statistics (BAS). 2012. *Swine Industry Performance Report*. Bureau of Agricultural Statistics. Department of Agriculture. Quezon City, Philippines. Accessed 2 July 2012. http://www.bas.gov.ph

Eusebio EJ. 1969. *The Science and Practice of Swine Production*. Los Baños,

Laguna: University of the Philippines College of Agriculture.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783-791.

Food and Agriculture Organization (FAO). 2007. *The State of the World's Animal Genetic Resources for Food and Agriculture*, (eds. Rischkowsky, B., Pilling, D.). FAO: Rome.

Francisco CC. 1992. Farmers' management practices and the morphological and genetic characteristics of native pigs in six Southern Luzon provinces. *Master of Science Thesis*. University of the Philippines Los Baños.

Giuffra E, Kijas JM, Amarger V, Carlborg O, Jeon JT and Andersson L. 2000. The origin of the domestic pig: independent domestication and subsequent introgression. *Genetics* 154: 1785-1791.

Hebert PDN, Cywinska A, Ball SL and DeWaard JR. 2003. Biological identifications through DNA barcodes. *Proc R Soc Lond B Biol Sci* 270: 313-321.

Hebert PDN, Stoeckle MY, Zemlak TS and Francis CM. 2004. Identification of birds through DNA barcodes. *PLoS Biol* 2 (10): 1657-1663.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 111-120.

Kijas JM and Andersson L. 2001. A phylogenetic study of the origin of the domestic pig estimated from the near-complete mtDNA genome. *J Mol Evol 52*: 302-308.

Larson G, Dobney K, Albarella U, Fang M, Matisoo-Smith E, Robins J, Lowden S, Finlayson H, Brand T, Willerslev E, Rowley-Conwy P, Andersson L and Cooper A. 2005. Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science* 307: 1618-1621.

Larson G, Cucchi T, Fujita M, Matisoo-Smith E, Robins J, Anderson A, Rolett B, Spriggs M, Dolman G, Kim TH, Thuy NTD, Randi E, Doherty M, Due RA, Bollt R, Djubiantono T, Griffin B, Intoh M, Keane E, Kirch P, Li K-T, Morwood M, Pedrina LM, Piper LPJ, Rabett RJ, Shooter P, van den Bergh G, West E, Wickler S, Yuan J, Cooper A and Dobney K. 2007. Phylogeny and ancient DNA of Sus provides insights into neolithic expansion in Island Southeast Asia and Oceania. *Proc Natl Acad Sci USA*. 104: 4834-4839.

Lucchini V, Meijaard E, Diong CH, Groves CP and Randi E. 2005. New phylogenetic perspectives among species of South-east Asian wild pig (*Sus* sp.) based on mtDNA sequences and morphometric data. *J Zool* 266: 25-35.

Nei M and Kumar S. 2000. *Molecular Evolution and Phylogenetics*. New York: Oxford University Press.

Navarra CF, Peñalba FF, Lambio AL, Laude RP and Barrion AA. 1997. Karyotype and blood protein polymorphism of the Philippine native pig (*Sus scrofa* L.). *Proc 34th Annual Convention Philippine Society of Animal Science*, Manila Philippines, pp. 346-358.

Nozawa K, Amano T, Hashiguchi T, Masangkay JS, Namikawa T, Nishida T, Otsuka J, Sugiura S, Tanaka K and Watanabe S. 1978. *Report of the Society for Researches on Native Livestock*. No. 8. Tokyo, Japan. pp. 122-140.

Saitou N and Nei M. 1987. The neighbour-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406-425.

Sookmanee N. 1998. Molecular markers in swine. *Ph.D. Dissertation*. University of the Philippines Los Baños.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol Biol Evol* 28: 2731-2739.

Thompson JD, Higgins DG and Gibson TJ. 1994. ClustalW - improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673-4680.

Watanabe T, Hayashi Y, Kimura J, Yasuda Y, Saitou N, Tomita T and Ogasawara N. 1986. Pig mitochondrial DNA: polymorphism, restriction map orientation and sequence data. *Biochem Genet* 24: 385-396.

Wu G-S, Yao Y-G, Qu K-X, Ding Z-L, Li H, Palanichamy MG, Duan Z-Y, Li N, Chen Y-S and Zhang Y-P. 2007. Population phylogenomic analysis of mitochondrial DNA in wild boars and domestic pigs revealed multiple domestication events in East Asia. *Genome Biol* 8 (11): R245.

Yang J, Wang J, Kijas J, Liu B, Han H, Yu M, Yang H, Zhao S and Li K. 2003. Genetic diversity present within the near-complete mtDNA genome of 17 breeds of indigenous Chinese pigs. *J Hered* 94 (5): 381-385.