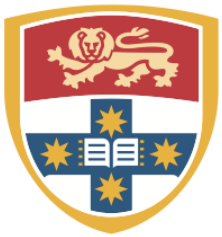
Genetic Structure and Biodiversity of Pigs *(Sus scrofa)* in South Asia and Papua New Guinea

KARMA NIDUP



THE UNIVERSITY OF SYDNEY

A thesis submitted to the Faculty of Veterinary Science, The University of Sydney, in fulfilment of the requirements for the Degree of
Doctor of Philosophy
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DEDICATION

I dedicate this Doctoral Thesis to the
most precious people in my life:

my wife - Tshering Lham
AND
two lovely children -
Phuntsho Tshering Nidup & Gyeltshen Tshering Nidup

"You don't choose your family. They are God's gift to you, as you are to them"

- Desmond Tutu

DECLARATION

I declare that the work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and that the material has not been submitted, either in whole or in part, for a degree at this or any other university.

(Karma Nidup)

12th August 2011

CONTRIBUTION OF CO-AUTHORS

CHAPTER 1 – Part II

Nidup K. & Moran C. (2011) Genetic diversity of domestic pigs as revealed by microsatellites: a mini review. *Genomics and Quantitative Genetics* **2**, 5-18.

- Professor Moran supervised this work, contributed to discussions, and edited the manuscript

CHAPTER 3 – Part I

Nidup K., Tshering D., Wangdi S., Gyeltshen C., Phuntsho T. & Moran C. (2011) Farming and Biodiversity of Pigs in Bhutan. *Animal Genetic Resources* **48**, 47-61.

- Mr Tshering, Wangdi, Gyeltshen and Phuntsho were involved in generating and compiling on-station research data
- Professor Moran supervised this work, provided useful suggestions, and edited the manuscript

CHAPTER 3 – Part II

Nidup K., Joshi D.D., Gongora J. & Moran C. (2010) Farming and Biodiversity of Indigenous Pigs in Nepal. *Biodiversity* **11**, 26-33.

- Dr Joshi has guided the first author in the field and provided useful input on the socio-cultural aspect of pigs for some ethnic communities in Nepal
- Dr Gongora provided useful analytical feedback and some editing on the manuscript
- Professor Moran supervised this work, provided useful suggestions, and edited the manuscript

CHAPTER 4

Nidup K., Silva G.L.L.P., Joshi D.D., Pem R., Gongora J. & Moran C. (*Submitted*) Genetic structure and diversity of South Asian pigs *(Sus scrofa)* as determined by microsatellites. *Animal Genetics*.

- Dr Silva collected blood samples from Sri Lankan village pigs and wild boar, extracted DNA, and provided useful feedback on the draft manuscript
- Dr Joshi collected most blood samples in Nepal, arranged further field work for first author, and provided laboratory facilities for DNA extraction
- Dr Pem provided extensive assistance during the field work and DNA extraction in Bhutan
- Dr Gongora offered technical and analytical advice

- Professor Moran supervised the project, contributed to extensive discussions concerning data analyses and interpretation, and performed editing of the manuscript

CHAPTER 5

Nidup K., Larson G., Gongora J., Joshi D.D., Silva G.L.L.P. & Moran C. (*Manuscript drafted*) Wild boar mitochondrial phylogeography, introgression, and dispersal in South Asia.

- Dr Larson contributed museum and ancient specimens of wild boar and domestic pig from South Asia. He also assisted in data analyses and provided extensive feedback on the first and second draft of the manuscript
- Dr Gongora offered technical and analytical advice, and extensive feedback on the first draft
- Dr Joshi collected most blood samples in Nepal, arranged further field work for first author, and provided laboratory facilities for DNA extraction
- Dr Silva collected blood samples from Sri Lankan village pigs and wild boar, extracted DNA, and provided useful feedback on the draft manuscript
- Professor Moran supervised the project, contributed to extensive discussions concerning data analyses and interpretation, and performed editing of the manuscript

CHAPTER 6 - Part I

Nidup K., Ayalew W., Danbaro G., Besari F., Gongora J. & Moran C. (*Manuscript drafted*) Genetic status of indigenous domestic pigs of Papua New Guinea.

- Together with the first author, Dr Ayelew and Dr Danbaro conceptualised the genetic characterization project on PNG pigs
- Dr Ayelew and Dr Danbaro conducted field work and extracted DNA
- Mr Besari has provided extensive assistance while collecting samples from domestic pigs of PNG and was also involved in DNA extraction
- Dr Gongora provided analytical advice and offered useful suggestions on the draft manuscript
- Professor Moran supervised the project, contributed to extensive discussions concerning data analyses and interpretation, and performed editing of the manuscript

CHAPTER 6 - Part II

Ayalew W., Danbaro G., Dom M., Amben S., Besari F., Moran C. & **Nidup K.** (2011) Genetic and cultural significance of indigenous pigs in Papua New Guinea and their phenotypic characteristics. *Animal Genetic Resources* **48**, 37-46.

My role as a Senior Author

- Conceptualised the field survey in order to complement genetic study
- Designed the survey questionnaire (Appendix 2)
- Made significant contribution while drafting the manuscript
- Submitted the manuscript to the journal and addressed reviewers' feedback

Contribution of Other Authors

- Dr Ayalew directed and supervised the field work, and drafted the manuscript
- Dr Danbaro, Mr Dom, Mr Amben, Mr Besari conducted field survey and assisted in sample collection for genetic study. Dr Danbaro also assisted with manuscript preparation
- Professor Moran contributed to discussions and performed extensive manuscript editing

I certify that the above statement about my contribution to the research work in this PhD thesis is true and accurate, and give **Karma Nidup** full permission to submit this work as part of his PhD thesis.

[redacted] (C. Moran) **Date:** 12th August 2011

[redacted] (J. Gongora) **Date:** 12th August 2011

[redacted] (D. D. Joshi) **Date:** 9th August 2011

[redacted]

(G.L.L.P. Silva) **Date:** 8th August 2011

[redacted] [redacted] Date: 08/08/2011

(G. Larson) (R. Pem)

Date: 8th August 2011

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(S. Wangdi)

Date: 11th August 2011 11th August 2011 11th August 2011 11/08/2011

1. W. Ayalew- Date: 08 August 2011.
2. G. Danbaro- Date: 09 August 2011
3. M. Dom -- Date: 10 August 2011
4. S. Amben -- Date: 09 AUGUST 2011
5. F. Besari -- Date: 10 AUGUST 2011

[redacted]

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PUBLICATIONS

MANUSCRIPTS PUBLISHED

1. **Nidup K** & Moran C. (2011). Genetic diversity of domestic pigs as revealed by microsatellites: a mini review. *Genomics and Quantitative Genetics.* **2:** 5-18.

2. **Nidup, K.** Tshering, D., Wangdi, S., Choedup, G., Phuntsho, T. & Moran, C. (2011). Farming and biodiversity of pigs in Bhutan. *Animal Genetic Resources.* **48:** 47-61.

3. Ayalew, W., Danbaro, G., Dom, M., Amben, S., Besari, F., Moran, C. & **Nidup, K.** (2011). Genetic and cultural significance of indigenous pigs in Papua New Guinea and their phenotypic features. *Animal Genetic Resources.* **48:** 37-46.

4. **Nidup, K.** Joshi, D.D., Gongora, J. & Moran, C. (2010). Farming and biodiversity of indigenous pigs in Nepal. *Biodiversity*. **11 (3 & 4):** 26-33.

5. **Nidup, K.** & Moran, C. (2010). Biodiversity and rapid loss of indigenous pig population in the Himalayas. *11th Association for Pacific Rim of Universities - Doctoral Student Conference*. **Full paper.** 12-16 July 2010. Universitas Indonesia, Jakarta, Indonesia.

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6. **Nidup, K.,** Silva G.L.L.P. Joshi, D.D. Pem, R., Gongora, J., Moran, C. ***(Submitted, peer reviewed, and reviewers' feedback incorporated)***. Genetic structure and diversity of South Asian pigs (*Sus scrofa*) as determined by microsatellites. *Animal Genetics*.

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7. **Nidup, K.,** Larson, G., Gongora, J., Joshi, D.D., Silva G.L.L.P., Moran, C. **(*Drafted*)**. Wild boar mitochondrial phylogeography, introgression, and dispersal in South Asia.

8. **Nidup, K.,** Ayalew, W., Danbaro, Besari, F., Gongora, J. & Moran, C. **(*Drafted*)**. Genetic status of indigenous pigs of Papua New Guinea as evealed by microsatellites and mitochondrial DNA.

ABSTRACTS PUBLISHED AND ORAL PRESENTATIONS

(**Note:** *Seminars conducted in the faculty and for the journal clubs are not included*)

1. **Nidup, K.,** Ayalew, W., Danbaro, G., Gongora, J. & Moran (2010). Genetic structure and diversity of indigenous pigs of Papua New Guinea. *Annual Postgraduate Conference.* 1-2 November 2010. Camperdown Campus, Faculty of Veterinary Science, University of Sydney, Australia; abstract P53.

2. **Nidup, K.** & Moran, C. (2010). Biodiversity and rapid loss of indigenous pig population in the Himalayas. *11th APRU Doctoral Student Conference*. 12-16 July 2010, Universitas Indonesia. Jakarta, Indonesia; abstract P68-69.

3. **Nidup, K.,** Silva G.L.L.P. Joshi, D.D. Pem, R., Gongora, J., Moran, C. (2010). Population structure and genetic diversity of Himalayan and Sri Lankan pigs (*Sus scrofa*). *32nd International Society for Animal Genetics Conference*. 26-30 July 2010. Edinburgh, Scotland (UK); abstract P3034, p74.

4. **Nidup, K.** (2009). Analysis of microsatellite and mtDNA data from South Asian pigs. *Phylogenetics Workshop*. Sydney Bioinformatics. University of Sydney. 28-29 September 2009, Australia.

5. **Nidup, K.** (2009). Current status of Domestic Animal Diversity Information System. *Asia-Pacific Regional Workshop on: Sustainable Management of Animal Genetic Resources: Development of Priorities, Policies and National Action Plans*. 17-20 November 2009. Beijing, China.

6. **Nidup, K.** (2009). Population genetic analysis of the South Asian pigs. Analytical methods for population genetics: *A National Workshop for Graduate Students and Scientists*. 6-9th October 2009. Research School of Biology, Australian National University, Canberra, Australia.

7. **Nidup, K.,** Gongora, J. & Moran (2009). Population genetics of South Asian indigenous pigs. *Annual Postgraduate Conference.* 2 November 2009. Camden campus, Faculty of Veterinary Science, University of Sydney, Australia; abstract P37.

8. **Nidup, K.,** Silva G.L.L.P. Joshi, D.D. Pem, R., Gongora, J., Moran, C. (2009). Population structure of South Asian Indigenous pigs determined by microsatellite markers. *Genetic Society of AustralAsia*. 7-10 July 2009. Brisbane, Australia; abstract P63.

9. **Nidup, K.,** Silva, G.L.L.P., Joshi, D.D., Gongora, J., Moran, C. (2009). Genetic characterization of South Asian pigs. *Sustainable Management of Animal Genetic Resources in South Asia: ILRI-SLU Workshop*. 16-18 February 2009. Kathmandu. Nepal.

10. **Nidup, K.** (2008). Genetic characterization of Himalayan pigs. A case study presented to the *First Globaldiv Summer School*. 8-12 September 2008. Piacenza, Italy. http://www.globaldiv.eu/SummerSchool/Home.html.

11. **Nidup, K.** (2008). Need for molecular genetic characterization of indigenous and wild pigs in the Hindu Kush Himalayan region. *National Zoonoses and Food Hygiene Research Centre*. 16-20 March 2008. Kathmandu, Nepal.

12. **Nidup, K.,** Gongora, J. & Moran (2008). Genetic diversity of Bhutanese indigenous pigs. *Annual Postgraduate Conference.* 3-4 November 2008. Camden campus, Faculty of Veterinary Science, University of Sydney, Australia; abstract P49.

13. **Nidup, K.** (2007). Genetic structure of wild and indigenous pig breeds in the Hindu Kush Himalayan Region. *Annual Postgraduate Conference.* 29-30 October 2007. Camperdown campus, Faculty of Veterinary Science, University of Sydney, Australia; abstract P58.

14. **Nidup, K.** (2007). Genetic structure of wild and indigenous pig breeds in the Hindu Kush Himalayan region. *Summer Symposium in Bioinformatics*. 10-11 December 2007. Australian National University. Canberra, Australia; abstract P25.

15. **Nidup, K.** (2007). Phylogenetic study of wild and indigenous pig breeds in the Hindu Kush Himalayan region. CONNECT 2007. *SUPRA Postgrad Conference*. 26-28 September 2007. University of Sydney, Australia; abstract P14.

POSTERS PRESENTED

1. **Nidup, K.,** Silva G.L.L.P. Joshi, D.D. Pem, R., Gongora, J., Moran, C. (2010). Genetic diversity of pigs (*Sus scrofa*) in the Himalayas and Sri Lanka. 26-30 July 2010. Edinburgh, Scotland, UK.

2. **Nidup, K.,** Silva G.L.L.P. Joshi, D.D. Pem, R., Gongora, J., Moran, C. (2009). Population structure of South Asian indigenous pigs determined by microsatellite markers. *Genetics Society of AustralAsia*. 7-10 July 2009. Brisbane, Australia, abstract 63.

3. **Nidup, K.,** Gongora, J. & Moran (2009). Population genetics of South Asian indigenous pigs. *Annual Postgraduate Conference.* 2 November 2009. Camden, Faculty of Veterinary Science, University of Sydney, Australia, abstract P27.

ABSTRACT

Biodiversity of livestock resources is critically important for achieving food security and alleviating poverty for the rapidly growing human population. Indigenous pigs (*Sus scrofa*) are an important part of these resources and have significant socio-economic and cultural importance to the livelihood of several hundreds of ethnic rural communities in South Asia and Papua New Guinea (PNG). However, very little attention has been given to research and development, and conservation of indigenous pigs, which are becoming increasingly marginalised by the introduction of exotic breeds. The objectives of this research project were to investigate the genetic biodiversity of indigenous pigs, and to document their physical characteristics, population trends, farming practices, and their socio-cultural and economic importance to the livelihood of rural communities living in South Asia and PNG.

Findings from the field surveys have indicated that pig improvement programmes in South Asia and PNG have mainly focused on introduction of exotic germplasm, whose influence is increasing. Indigenous pigs, which are hardy, resistant to many diseases, and adaptable to harsh rural environments with low inputs, are increasingly marginalised by introduction of commercial pigs of European origin. From the population trend, it was estimated that Bhutanese indigenous pigs will become extinct within the next decade, while Nepal, Sri Lanka and PNG will face dire shortage of their native genetic resources in the future unless appropriate measures are taken to prevent this genetic erosion. Once lost, these important resources are largely irreplaceable. Therefore, to protect and conserve indigenous pigs for breeding and sustainable utilization, it is imperative to understand their genetic structure and diversity. These were determined using microsatellite markers and mitochondrial DNA (mtDNA) sequences. The microsatellites were used for their abundance, even distribution in the genome, and high polymorphism while haploidy and uniparental inheritance properties have made mtDNA a powerful tool to examine relatedness of the populations and track the matrilineal component of historic genetic diversity and migration routes.

Using 21 microsatellite markers that were recommended by the Food and Agriculture Organization (FAO) of the United Nations and the International Society for Animal Genetics (ISAG), we have investigated the genetic structure and diversity of 313 domestic and wild boar from South Asia. Our analyses revealed four domestic and one wild boar populations in Bhutan, two domestic pig populations in Nepal, and clearly segregated populations of village pigs and wild boar in Sri Lanka. All populations showed equal or higher expected heterozygosities than Australian commercial pigs of composite breed. There was negligible genetic differentiation between one Bhutanese and one Nepalese population. When compared to Sri Lanka populations, the Himalayan pig populations from Bhutan and Nepal were closely related, not unexpected given their close geographical distribution. Surprisingly, the Sri Lankan village pigs clustered with Australian commercial pigs implying substantial genetic contamination by European pigs.

In addition to the microsatellite analyses, mtDNA control region sequences (652bp) were generated from 242 animals, both domestic pigs and wild boar, from South Asia. This included 11 wild boar museum specimens and even one ancient domestic pig sample. The sequences of seventy-three haplotypes detected in South Asia were combined with Genbank sequences representing almost 1800 wild boar and domestic pigs from all over the world. Our analyses, which are currently the most comprehensive porcine mtDNA sequence analyses ever performed, revealed very complex clustering patterns of porcine haplotypes but with clear phylogeographic signals. The segregation of European and Asian pigs was consistent with independent domestication of pigs in Europe and Asia. We observed three major mitochondrial porcine clades, which are unique to the Indian sub-continent. The shared haplotypes between domestic pigs of Bhutan, Northeast India, and Nepal with wild boar, possibly belonging to *Sus scrofa cristatus* from Northern India (Kashmir, West Bengal, and Chattishgarh) within Mixed Clade 1 (MC1) provides support for an independent centre of "cryptic domestication" in the foothills of the Himalayas and the Indian sub-continent. However, in the absence of corroborating archaeological or fossil evidence, this could also have resulted from an introgression of maternal genes from MC1 wild boar to domestic pigs. We also confirm the presence of two additional novel wild boar clades, the Northern South Asia (NSA) and Southern South Asia (SSA), which could possibly belong to two different wild boar subspecies (*S. s. davidi* and *S. s. affinis*) in Northern South Asia and Sri Lanka

respectively. Both NSA and SSA have not been detected in any domestic pigs. In addition to these, the W17 and W12 haplotypes detected in Bhutanese wild boar have not been detected in domestic pigs both in current and previous studies. The South Asian domestic pigs have also been influenced by widely distributed east Asian and European pigs. The shared haplotypes within and between domestic pigs of South Asia indicate some ancestral genetic signatures or movement of domestic pigs between countries, presumably mediated by humans.

Similarly, genotype data from sixty seven individual pigs were used to determine the genetic structure and diversity of indigenous domestic pigs of PNG. Despite using a relatively small number of individuals for population genetic analysis, we observed five inferred populations within indigenous domestic pigs. These inferred populations, which had low to moderate genetic differentiation, correlated well with sampling localities in PNG. They showed higher expected heterozygosities than Australian commercial pigs. To complement this, mtDNA analyses on 24 haplotypes (1044bp) from 70 domestic pigs and 3 haplotypes from 5 Australian commercial pigs were combined with 186 major porcine haplotypes retrieved from Genbank. We observed that the mitochondria of indigenous domestic pigs of PNG have been mainly influenced by Pacific clade (D6) or Oceania haplotypes followed by General Asian (D2) and European (D1). The shared haplotypes between wild and indigenous domestic pigs within D6 suggested that the latter have been derived from wild or feral pigs in the region and within PNG. The D2 haplotypes were also quite common within PNG domestic pigs. It is possible that pigs with D2 haplotypes may have been introduced along with pigs with D6 haplotypes during the expansion of the Lapita and Polynesian culture in Oceania. It is also likely that D2 haplotypes have been the result of introduction of European pigs carrying D2 haplotypes, which are reasonably common within Australian commercial pigs of European origin. Our extensive analyses of both domestic and wild boar haplotypes suggest the presence of a genuine wild boar mtDNA signature (D5) of Southeast Asian origin, within some Australian feral pigs and one domestic pig of PNG.

This thesis concludes that both South Asian and PNG pigs retain reasonably high levels of genetic structure and biodiversity and can thus continue to provide valuable information and resources for future agriculture that may no longer be retained in the

vast majority of intensively selected commercial pigs. Our findings, which meet the requirements of FAO's *Global Plan of Action for Animal Genetic Resources,* provides useful baseline scientific information on which any policy or holistic conservation decision related to pigs in the regions should be based.

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ABBREVIATIONS

µl	Microlitre
A	Adenine
AC	Australian commercial
AGRF	Australian Genome Resource Facility
AMOVA	Analysis of Molecular Variance
AnGR	Animal Genetic Resources
AQIS	Australian Quarantine and Inspection Service
BAFRA	Bhutan Agriculture Food Regulatory Authority
BhuVP	Bhutan village pig
BLASTn	Basic Local Alignment Search Tool – Nucleotide
bp	Base Pair
BP	Before Present
C	Cytosine
CBD	Convention on Biological Diversity
CC	Central China Type
cM	centiMorgans
CNR	College of Natural Resources
CR	Control Region
Cyt *b*	Cytochrome *b*
DAD-IS	Domestic Animal Diversity Information System
DNA	Deoxribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
DoL	Department of Livestock
DVH	District Veterinary Hospital
EDTA	Ethylenediamine Tetraacetic Acid
EECP4	East and East-central Population4
FAO	Food and Agriculture Organization
G	Guanine
GAC	General Asian Cluster/Clade
gm	Gram
GPA	Global Plan of Action
HD	Heterozygote Deficiency
He	Expected Heterozygosity
Ho	Observed Heterozygosity
H-strand	Heavy strand
HTS	High-Throughout Sequencing
HWE	Hardy-Weinberg Equilibrium
IBBH	Indo-Burma Biodiversity Hotspot
INRA	Institut National de la Recherche Agronomique
ISAG	International Society for Animal Genetics
LCR	Lower Changjiang River Basin Type
LD	Linkage Disequilibrium
L-strand	Light strand
M	Molar

masl	Metres Above Sea Level
MBF	Morobe Boana Finschhafen
MCMC	Markov Chain Monte Carlo
mg	Milligram
MGK	Morobe Garaina Kandep
MHGB	Morobe Huon Gulf Bulolo
MJN	Median-Joining Network
ml	Millilitre
mM	Millimolar
MMP1	Machay Madhuri Population1
MN	Morobe Nawaeb
MoAF	Ministry of Agriculture and Forests
mtDNA	Mitochondrial DNA
MTSEA	Mitochondrial DNA Haplotypes of South East Asia
mv	Medium Vector
ng	Nanogram
NGS	Next Generation Sequencing
NJ	Neighbour-Joining
NKD	Kalo Dharane Sunggur breed of Nepal
NKH	Hurrah breed of Nepal
NKVP	Nepal village pig
NSA	Northern South Asia
PCA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
PNG	Papua New Guinea
RGoB	Royal Government of Bhutan
RGP2	Rinchengang Population2
rpm	Revolutions Per Minute
S. s.	*Sus scrofa*
SLVP	Sri Lanka village pig
SLWB	Sri Lanka wild boar
SNP	Single Nucleotide Polymorphism
SSA	Southern South Asia
T	Thymine
TAE	Tris-acetate EDTA buffer
TBE	Tris-borate EDTA buffer
TE	Tris-EDTA buffer
Tris-HCl	Tris Hydrochloride
UN	United Nations
UNDP	United Nations Development Programme
UV	Ultraviolet
WB	Wild Boar
WHP	Western Highland Province
WHPT	Western Highland Province Tambul
WWCP	West and West-central Population3

CHAPTER 1

PART I: Introduction and Literature Review

1.1 INTRODUCTION

The family Suidae, commonly known as boars, hogs, pigs, or suids, comprises 18 species categorised into six genera (Wilson & Reeder 2005; Gongora *et al.* 2011) based on their morphological characteristics and geographical distribution. The genera are *Porcula* (pygmy hog) from the Indian sub-continent, *Babyrousa* (babirusa) from Southeast Asia, *Potamochoerus* (bushpig and red river hog), *Phacochoerus* (common and desert warthog), *Hylochoerus* (forest hog) from sub-Saharan Africa, and *Sus* (wild and domestic pigs) from Eurasia (Table 1.1; Figure 1.1).

Sus scrofa or the Eurasian wild boar (Figure 1.1 g-i) and its domestic derivatives is the most widely distributed species of the Old World Suidae (Jones 1998). It is found all throughout the European mainland and most parts of Asia including Southeast Asia, South Asia, Central Asia, and Far East Asia. It was extinct in the British Isles and Scandinavia but was reintroduced into England, South Finland and South Sweden (Erkinaro *et al.* 1982; Leaper *et al.* 1999; O' Connell 2008). European wild boar have been introduced to the Americas (Barrett 1978; Grossi *et al.* 2006; Mayer & Brisbin Jr, 2008). Feral pigs (domestic pigs that have escaped or been released into the wild) that belong to the same species exist in Australia, New Zealand, and many Pacific islands (Gongora *et al.* 2004; Fan *et al.* 2005; Larson *et al.* 2005; Spencer *et al.* 2006). Based on geographical distribution (Asia, Europe, and North Africa) and morphological characteristics, there are 21 subspecies (Table 1.2).

Table 1.1: Wild pigs (except *Sus scrofa*) of Asia and Africa identified based on their physical characteristics, geographical location, molecular, and morphometric data.

Common Name	Scientific Name	Description	Distribution	References
Pygmy hog	*Porcula salvania; also known as Sus salvanius*	Skin is dark brownish black and the hairs are dark; head is sharply tapered with slight crest of hair on the forehead and on the back of the neck; adult males have the upper canines visible on the sides of the mouth; weigh 6 to 11 kg; live up to 8 years; sexually mature at 1-2 years; feed on roots, tubers, insects, rodents, and small reptiles.	Previously spread across India, Nepal, and Bhutan but now only found in Assam.	Narayan (2006) ; Funk *et al.* (2007); (Oliver 1980)
Babirusa	*Babyrousa babyrussa; B. celebensis*	babi-rusa (Indonesian) = "Pig-deer"; endangered; some nearly bald (e.g. Sulawesi and Togian babirusa) while others are covered with dense golden hair (e.g. Buru babirusa); male has highly conspicuous tusks formed from the canine teeth; female has one pair of teats; tropical forest; mainly herbivore.	Tropical forest of Wallacea: Indonesian Islands of Sulawesi, Togian, Sula, and Buru	Clayton *et al.* (2000); Meijaard & Groves (2002); Milner-Gulland & Clayton (2002); Albarella *et al.* (2007); IUCN (2010)
Red river hog	*Potamochoerus porcus*	Red fur, black legs, white stripe along the spine; males have humps-like on both sides of the snout; small and sharp tusks. Mostly herbivorous but also eats insects, molluscs, small vertebrates and carrion.	Rainforests and wet-dense Savannas of Africa: Congo, Gambia	Dosimont (2004) ; Grubb (2005); Querouil & Leus (2008)
Bushpig	*Potamochoerus larvatus*	Resembles domestic pig; reddish-brown to dark brown colour which becomes darker with age; blunt and muscular snout; tufted ears; eats grasses, roots, crops, carrion, small vertebrates.	Africa: Ethiopia, DR Congo, South Africa, Somalia, Sudan, Madagascar, Comoros.	Bosma *et al.* (1991a); van Rensburg (1993); Grubb (2005)
Common warthog	*Phacochoerus africanus*	Two pairs of tusks; four large wart-like protrusions on the head; live in burrows head facing the opening; Diet consists of roots, fruits, bark, fungi, eggs, dead animals, and even small mammals, reptiles and birds.	Sub-Saharan African	Randi *et al.* (2002); Muwanika *et al.* (2003); D'Huart & Grubb (2005); Grubb (2005)
Desert or Cape warthog	*Phacochoerus aethiopicus.* Also called Cape or Somali warthog (*Phacochoerus aethiopicus delamerei*)		Nigeria, Kenya, Somalia, Ethiopia, South African Cape region	Randi *et al.* (2002); D'Huart & Grubb (2005); Hoffman & Sales (2007); de Jong *et al.* (2009); Kozubska-Sobocinska *et al.* (2009)
Giant forest hog	*Hylochoerus meinertzhageni*	Largest wild pig; tusks smaller than warthog but bigger than bush pig; large pointed ears; dense black hair; mainly herbivorous	Tropical and temperate forest of west and central Africa: mainly Ethiopia, Kenya	Cotton (1936); Dhuart (1980);; Klingel (1997); Klingel & Klingel (2004); Grubb (2005); Fimpel (2006)
Visayan warty pig	*Sus cebifrons*	Critically endangered species; three pairs of fleshy "warts" present on the visage of the boar; mainly herbivorous	Endemic to two Visayan islands in the Philippines	Cox (1987); Oliver (2004); Grubb (2005); De Leon *et al.* (2008)
Celebes or Sulawesi warty pig	*Sus celebensis*	Mainly herbivorous but small vertebrates and carrion are also eaten;	Indonesia: mainly Sulawesi Islands but also found in Buton, Muna, Kabeana, Peleng, Lembeh and on some of the Togian Islands	Bosma *et al.* (1991b); MacDonald *et al.* (1996); Grubb (2005); Lucchini *et al.* (2005); Burton & Macdonald (2006)
Bearded pigs	*Sus barbatus*	Has prominent beard; mainly herbivorous; can reproduce from the age of 18 months; inhabits rains and mangrove forests	Indonesian and Malaysian: Sumatra, Borneo, and Malay Peninsula	Mudar (1986); Hancock *et al.* (2005); Lucchini *et al.* (2005); Grubb (2005); Wu *et al.* (2006) ; Grubb (2005)

Table 1.2: Subspecies of wild boar grouped based on geographical distribution and morphological characteristics.

Races	Wild Boar Type	Subspecies	Distribution/Remarks	References
	European	*S. s. scrofa*	Western, Central and parts of Southern Europe, Britain, European Russian; Introduced in Sweden, Norway, Italy, USA, Canada	Gross *et al.* (2006); Fang & Andersson (2006); Fang *et al.* (2006); Goulding *et al.* (2010)
	Iberian	*S. s. baeticus*	A small subspecies present in the Iberian Peninsula	Scheggi (1999); Groves (2008)
	Castillian	*S. s. castilianus*	Northern Spain; Larger than *S. s. baeticus*	Scheggi (1999); Groves (2008)
	Sardinia	*S. s. meridionalis*	A small subspecies present in Sardinia	Beaux & Festa (1927); Randi *et al.* (Randi *et al.* 1996)
	Italian	*S. s. majori*	Central and Southern Italy. Has higher and wilder skull but smaller than *Sus scrofa* with which it has hybridised freely since 1950's.	Beaux & Festa (1927); Randi *et al.* (Randi *et al.* 1996) Vernesi *et al.* (2003)
		S. s. attila	East Europe, northern slope of Caucasus, parts of Western Siberia, Central and Western Asia. It was thought that boars present in Ukraine, Asia Minor and Iran are part of this subspecies.	Vernesi *et al.* (2003); Groves (2008)
	Barbary	*S. s. algira*	North-west Africa	Grossi *et al.* (2006); Groves (2008)
		S. s. lybica	Asia Minor, Middle East, southern part of Eastern Europe	Randi *et al.* (1996); Grossi *et al.*(2006); Groves (2008)
		S. s. nigripes	Southern Siberia, Central Asia	Grossi *et al.*(2006); Groves (2008)
Indian		*S. s. cristatus*	Northern India, Myanmar and Thailand, Western part of Indochina Lighter than European boar, larger and more pointed head; Smaller and pointed ears.	Sterndale (2006); Groves (2008)
		S. s. affinis	Southern India and Sri Lanka; smaller than *S. s. cristatus*	Sterndale (2006); Groves (2008)
		S. s. davidi	Western India, Iran	Sterndale (2006); Groves (2008)
		S. s. bucculentus	Laos. Previously thought to be another species. Control region sequences clustered with *Sus scrofa* from Thailand and Myanmar	Robins *et al.* (2006); Groves (2008)
Eastern	Manchurian	*S. s. ussuricus*	Largest sub-species of *Sus scrofa*. Manchuria (Northeast China), Russian Far East, Korea	Groves (2008); Zhang *et al.* (2008); Zhang (1986)
	Japanese	*S. s. leucomystax*	Main islands of Japan: Honshu, Shikoku, Kyushu	Watanobe *et al.* (1999); Hoongo *et al.* (2002); Watanobe *et al.* (2003); Watanobe *et al.* (2004)
	Ryuku	*S. s. riukiuanus*	Ryuku islands of Japan	
	Formosan	*S. s. taivanus*	Small, black wild pig from Taiwan	Wilson & Reeder (2005)
		S. s. moupinensis	North, Central and South China, Vietnam	Zhang (1986); Groves (2008)
	China	*S. s. chirodontus*	South China	Zhang (1986)
	Siberian	*S. s. sibiricus*	Eastern Siberia, Mongolia	Grossi *et al.*(2006); Groves (2008)
Indonesia	Banded	*S. s. vittatus*	Malay Peninsula, Sumatra, Java, Bali, and neighbouring islands Might be a separate species	Grossi *et al.*(2006); Groves (2008)

Figure 1.1: Wild pigs of Africa: *(a)* Bushpig (Seydack 2008); *(b)* Red River Hog (Querouil & Leus 2008); *(c)* Giant forest hog (Zappa 2008); *(d)* Warthog (Shears 2007). Wild pigs of Asia: *(e)* Babirusas (Clayton *et al.* 2010); *(f)* Bearded pig (Israel 2009); *(g)* European wild boar (Thompson 2010); *(h)* Italian wild boar or *S. s. attila* (Marian 2009)*; (i)* Bhutanese wild boar (MoA 2004); *(j)* Ryukyu wild or boar *S. s. riukiuanus* of Ryuku Islands, Japan (Kanpira-so 2001); *(k)* Thai wild boar or *S. s. jubatus* of Thailand (Shenglin 2007; Tanomtong *et al.* 2007); *(l)* Australian feral pigs (Salleh 2007).

1.2 DOMESTICATION OF PIGS

The domestic pig (*Sus scrofa domesticus*) was domesticated from *Sus scrofa,* the wild boar (Giuffra *et al.* 2000; Larson *et al.* 2005). The adaptable nature, omnivorous diet and uniquely flavoured meat led early agriculturists to domesticate wild boars. Early humans have used pigs' hides for shields, bones as tools and weapons, and bristles for brushes. Most civilizations, except Jews and Muslims, have included pork in their diets since the domestication of the pig. Pig farming flourished in Greece for thousands of years (Aristotle 350 B.C.E). Exact dating of domestication events of pigs has been difficult, although new archaeological and genetic information is constantly improving the understanding of the origin of *Sus scrofa*.

1.2.1 Archaeological Evidence

Archaeological evidence suggests that pigs were domesticated as early as 13,000 years Before Present (BP) (Hesse & Wapnish 1998). Morphological analysis and direct carbon dating of bones suggest that pigs, which are believed to have been introduced from the adjacent mainland, have been living on Cyprus for 11,400 – 11,700 years (Vigne *et al.* 2009), suggesting earlier domestication on the adjacent mainland. Pig bones and paintings, which have been discovered in various distant Neolithic sites, suggested that pigs have been domesticated in China for over 7000 years (Yuan & Flad 2002). However, the general acceptance based on zooarchaeological records is that domestication began about 9000 years BP.

1.2.2 Genetic Evidence

Mitochondrial DNA (mtDNA), which is haploid, maternally inherited and with rapid rate of evolution (Hartl & Clark 1997), has been a powerful tool to discern the origin of domestic pigs. Domestication has occurred independently in several locations after wild boar have initially dispersed from Island Southeast Asia (ISEA) throughout Eurasia (Larson *et al.* 2005). Giuffra *et al.* (2000) established the first clear evidence for domestication to have occurred, independently, from wild boar subspecies in Europe and Asia. This was followed by Larson *et al.* (2005), who suggested that at least six independent domestications of pigs across Europe (2), Asia (2), India (1), and Southeast Asia (1) have occurred in the past 9000 years. Wu *et al.* (2007) suggested that the

Mekong region and the middle and downstream regions of the Yangtze River may have been the places where pig domestications could have occurred. Tanaka *et al.* (2008) suggested another independent domestication event in the mountain areas of mainland Southeast Asia and foothills of the Himalaya. Some populations of pigs, which were domesticated in Southeast Asia, were dispersed into Oceania including mainland New Guinea during the migration of early agriculturists, and expansion of the Lapita and Polynesian people (Larson *et al.* 2005; Lum *et al.* 2006; Larson *et al.* 2007). Except for the Chinese case of domestication, which is supported by both genetic and archaeological evidence, Larson *et al.* (2010) have referred to some of these instances as "cryptic domestications", due to lack of corroborating archaeological evidence. However, some of these "cryptic domestications" events could be cases of genetic introgression, whereby maternal genes from wild boar have integrated into domestic populations.

1.2.3 Post Domestication

Several centuries after domestications, Europe and China became the two major pig-breeding centres in the Old World (Amills *et al.* 2010), developing a large number of varieties or local breeds or types of pigs adapted to the given environment. These local pigs were selected for various traits for centuries and provided strong foundations for the creation of modern breeds. For instance, Chinese pigs were used to improve European pig breeds during the 18th and early 19th centuries (Darwin 1868; Jones 1998; Fang & Anderson 2006). Chinese and European pigs have been rapidly dispersed around the globe. This was facilitated by ever-increasing commercial networks, exploratory routes, and colonization of several countries in America, Africa, and Oceania by the Europeans. Today, there are several hundred pig breeds around the world and many of them are in danger of extinction (FAO 2006, 2010). To rationalise conservation and breeding programmes, several genetic diversity studies have been conducted over the past decade using microsatellite genetic markers on many pig breeds and populations. These studies have been reviewed briefly and discussed in Part II of this Chapter.

However, very little attention has been given to research and development, and conservation of indigenous pigs, which have significant socio-economic and cultural importance to the livelihood of several hundreds of ethnic rural communities in South

Asia and Papua New Guinea (PNG). Indigenous pigs in these regions are becoming increasingly marginalised by the introduction of exotic breeds. They have not been adequately characterized (Nidup 2006). Therefore, this research project was conducted with various objectives, which are outlined in Section 1.3.

1.3 RESEARCH GOAL AND OBJECTIVES

i) Review existing studies of genetic diversity of domestic pig populations around the world as revealed by microsatellites.

ii) Record phenotypic characteristics of indigenous pigs, their farming practices, population trends, and their socio-cultural and economic importance to the livelihood of hundreds of rural communities living in South Asia and PNG.

iii) Conduct genetic characterization of pigs in South Asia and PNG pigs

iv) Investigate genetic relationship between domestic and wild pigs to determine gene flow.

v) Assess evidence for cryptic domestication or genetic introgression from wild boar into domestic pigs in South Asia and PNG.

vi) It is expected that information generated from this research project will be useful for conservation scientists and policy makers in devising relevant strategy for conservation of biodiversity and sustainable use of swine genetic resources in South Asia and PNG.

1.4 REFERENCES

Amills M., Clop A., Ramirez O. & Perez-Enciso M. (2010) Origin and genetic diversity of pig breeds. In: *Encyclopedia of Life Sciences (ELS)* (John Wiley & Sons, Ltd., Chichester. DOI: 10.1002/9780470015902.a0022884.

Aristotle (350 B.C.E) The History of Animals: Translated by D'Arcy Wentworth Thompson. URL http://classics.mit.edu/Aristotle/history_anim.8.viii.html.

Barrett R.H. (1978) The feral hog on Dye Creek Ranch, California. *Hilgardia*, 281-355.

Bosma A.A., de Haan N.A. & Macdonald A.A. (1991a) The current status of cytogenetics of the Suida: A review. . *Bongo Berlin* **18**, 258-72.

Bosma A.A., Dehaan N.A., Blouch R.A. & Macdonald A.A. (1991b) Comparative cytogenetics studies in S*us verrucosus*, *Sus celebensis* and *Sus scrofa-vittatus* (Suidae, Mammalia). *Genetica* **83**, 189-94.

Burton J. & Macdonald A.A. (2006) The Sulawesi warty pig (*Sus celebensis*), a status review. *Suiform Soundings* **6**, 5-13.

Clayton, Keeling & Gulland M. (2010) Bringing Home the Bacon: A Spatial Model of Wild Pig Harvesting in Sulawesi, Indonesia, (1997). *Ecological Applications* **7**, 642-52.

Clayton L.M., Milner-Gulland E.J., Sinaga D.W. & Mustari A.H. (2000) Effects of a proposed ex situ conservation program on in situ conservation of the Babirusa, an endangered suid. *Conservation Biology* **14**, 382-5.

Cotton W.B. (1936) Note on the Giant Forest Hog (*Hylochoerus meinertzhogeni*). *Proc. zool. Soc. London* **1936**, pp. 687-8.

Cox R. (1987) The Philippine spotted deer and the Visayan warty pig. *Oryx* **21**, 37-42.

D'Huart J.-P. & Grubb P. (2005) A photographic guide to the differences between the common warthog (*Phacochoerus africanus*) and the desert warthog (Ph. aethiopicus). *Suiform Soundings* **5**, 4-8.

Darwin C. (1868) *The variation of animals and plants under domestication*. Volume II, p. 95, John Murray, London.

de Jong Y.A., Culverwell J. & Butynski T.M. (2009) Desert warthog *Phacochoerus aethiopicus* found in Tsavo east National Park and Tsavo west National Park, southern Kenya. *Suiform Soundings* **8**, 4-6.

De Leon M., Gonzalez J.C.T. & Hedreyda C.T. (2008) Complete DNA sequence analysis of the mitochondrial 16s rRNA and cytochrome b genes of the Visayan warty pig (Sus cebifrons). *Asia Life Sciences* **17**, 295-308.

Dhuart J.P. (1980) Age-determination by counting biannual layers of growth in the dental-tisues - a new method of preparation in giant forest hog. *Mammalia* **44**, 129-&.

Dosimont O. (2004) Red river hog (*Potamochoerus porcus*) capture and breeding in the equatorial forest of Gabon. *Game & Wildlife Science* **21**, 375-85.

Erkinaro E., Heikura K., Lindgren E., Pullianen E. & Sulkava S. (1982) Occurrence and spread of the wild boar (*Sus scrofa*) in eastern Fennoscandia. *Memoranda Flora and Fauna Fennoscandia* **58**, 39-47.

Fan B., Gongora J., Chen Y., Garkavenko O., Li K. & Moran C. (2005) Population genetic variability and origin of Auckland Island feral pigs. *Journal of the Royal Society of New Zealand* **35**, 279-85.

Fang M. & Anderson L. (2006) Mitochondrial diversity in European and Chinese pigs is consistent with population expansions that occurred prior to domestication. *Proceedings of the Royal Society* **273**, 1803-10.

FAO (2007) *TheState of World's Animal Genetic Resources for Food and Agriculture*. (First edn, edited by Barbara Rischkowsky & Dafydd Pilling). FAO, Rome

FAO (2010) Domestic Animal Diversity Information System (DAD-IS) of the Food and Agriculture Organization. URL http://www.fao.org/dad-is.

Fimpel S. (2006) Aspects of the ecology and social behaviour of wild groups of the giant forest hog (*Hylochoerus meinertzhageni)* in the Queen Elizabeth Park, Uganda. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin N F* **44**, 41-9.

Funk S.M., Verma S.K., Larson G., Prasad K., Singh L., Narayan G. & Fa J.E. (2007) The pygmy hog is a unique genus: 19th century taxonomists got it right first time round. *Molecular Phylogenetics and Evolution* **45**, 427-36.

Giuffra E., Kijas J.M., Amarger V., Carlborg O., Jeon J.T. & Andersson L. (2000) The origin of the domestic pig: independent domestication and subsequent introgression. *Genetics* **154**, 1785-91.

Gongora J., Cuddahee R.E., do Nascimento F.F., Palgrave C.J., Lowden S., Ho S.Y.W., Simond D., Damayanti C.S., White D.J., Tay W.T., Randi E., Klingel H., Rodrigues-Zarate C.J., Allen K., Moran C. & Larson G. (2011) Rethinking the evolution of extant sub-Saharan African suids (Suidae, Artiodactyla). *Zoologica Scripta* **40**, 327-35.

Gongora J., Fleming P., Spencer P.B.S., Mason R., Garkavenko O., Meyer J.N., Droegemueller C., Lee J.H. & Moran C. (2004) Phylogenetic relationships of Australian and New Zealand feral pigs assessed by mitochondrial control region sequence and nuclear GPIP genotype. *Molecular Phylogenetics and Evolution* **33**, 339-48.

Grossi S.F., Lui J.F., Garcia J.E. & Meirelles F.V. (2006) Genetic diversity in wild (*Sus scrofa*) and domestic (*Sus scrofa domestica*) pigs and their hybrids based on polymorphism of a fragment of the D-loop region in the mitochondrial DNA *Genetics and Molecular Research* **5**, 564-8.

Groves C. (2007) *Current views on taxonomy and zoogeography of the genus Sus*. First edn. Oxford University Press, Oxford (UK).

Groves C. (2008) Current views on taxonomy and zoogeography of the genus *Sus*. In: *Pigs and humans: 10,000 years of interaction* (eds. by Albarella U, Dobney K & Ervynck A), pp. 15-29. Oxford University Press, Oxford, UK.

Grubb P. (2005) *Order Artiodactyla*. Third edn. Johns Hopkins University Press, USA.

Hancock, Gulland M. & Keeling (2005) An individual based model of bearded pig abundance. *Ecological Modelling*, 123-37.

Hartl D.L. & Clark A.G. (1997) *Principles of Population Genetics.* Sinauer Associates, Inc., USA (Sunderland).

Hesse B. & Wapnish P. (1998) Pig Use and Abuse in the Ancient Levant: Ethnoreligious Boundary-building with Swine. In: *Ancestors for the Pigs: Pigs in prehistory* (ed. by Nelson SM), pp. 123-35. University of Pennsylvania Press, Pennsylvania.

Hoffman L.C. & Sales J. (2007) Physical and chemical quality characteristics of warthog (*Phacochoerus aethiopicus*) meat. *Livestock Research for Rural Development* **19**, 153.

Israel S. (2009) Planet of the Tobys: Bako National Park URL http://www.travelblog.org/Asia/Malaysia/Sarawak/Bako-National-Park/blog-425227.html.

IUCN (2010) IUCN Red List of Threatened Species. Version 2010.1. URL http://www.iucnredlist.org/.

Jones G.F. (1998) Genetic aspects of domestication, common breeds and their origin. In: *The Genetics of the Pig* (eds. by Rothschild MF & Ruvinsky A), pp. 17-50. CAB International, Wallingford, Oxon, UK.

Kanpira-so (2001) Outline of Iriomote Island. URL http://www.kanpira.com/english/outline.htm.

Klingel H. (1997) Social organisation of the giant forest hog *Hylochoerus meinertzhageni*. *Advances in Ethology; Contributions to the XXV International Ethological Conference*, 259.

Klingel H. & Klingel U. (2004) Giant forest hog *Hylochoerus meinertzhageni* in Queen Elizabeth National Park, Uganda. *Suiform Soundings* **4**, 24-5.

Kozubska-Sobocinska A., Rejduch B., Oczkowicz M. & Babicz M. (2009) Genetic Conservation of Microsatellite Sequences in Suidae. *Annals of Animal Science* **9**, 243-8.

Larson G., Cucchi T., Fujita M., Matisoo-Smith E., Robins J., Anderson A., Rolett B., Spriggs M., Dolman G., Kim T.H., Thuy N.T.D., Randi E., Doherty M., Due R.A., Bollt R., Djubiantono T., Griffin B., Intoh M., Keane E., Kirch P., Li K.T., Morwood M., Pedrina L.M., Piper P.J., Rabett R.J., Shooter P., Van den Bergh G., West E., Wickler S., Yuan J., Cooper A. & Dobney K. (2007) Phylogeny and ancient DNA of Sus provides insights into neolithic expansion in island southeast Asia and Oceania. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 4834-9.

Larson G., Dobney K., Albarella U., Fang M.Y., Matisoo-Smith E., Robins J., Lowden S., Finlayson H., Brand T., Willerslev E., Rowley-Conwy P., Andersson L. & Cooper A. (2005) Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science* **307**, 1618-21.

Leaper R., Massei G., Gorman M.L. & Aspinall R. (1999) The feasibility of reintroducing Wild Boar (*Sus scrofa*) to Scotland. *Mammal Rev.* **29**, 239-59.

Lucchini V., Meijaard E., Diong C.H., Groves C.P. & Randi E. (2005) New phylogenetic perspectives among species of South-east Asian wild pig (*Sus* sp.) based on mtDNA sequences and morphometric data. *Journal of Zoological Society of London* **266**, 25-35.

Lum J.K., McIntyre J.K., Greger D.L., Huffman K.W. & Vilar M.G. (2006) Recent Southeast Asian domestication and Lapita dispersal of sacred male pseudohermaphroditic "tuskers" and hairless pigs of Vanuatu. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 17190-5.

MacDonald A.A., Leus K., Florence A., Clare J. & Patry M. (1996) Notes on the behaviour of Sulawesi Warty pigs *Sus celebensis* in North Sulawesi, Indonesia. *Malayan Nature Journal* **50**, 47-53.

Marian H. (2009) *Sus scrofa Attila*: A photograph taken by Huc Marian, Location: Sibiu Zoo, Romania. URL http://www.biolib.cz/en/taxonimage/id40818/?taxonid=163421.

Mayer J.J. & Brisbin Jr I.L. (1991) *Wild pigs in the United States: The history, comparative morphology, and current status*. The University of Georgia press, Athens, Georgia.

Meijaard E. & Groves C. (2002) Proposal for taxonomic changes within the genus *Babyrousa*. *Asian Wild Pig News* **2**, 9-10.

Milner-Gulland E.J. & Clayton L. (2002) The trade in babirusas and wild pigs in North Sulawesi, Indonesia. *Ecological Economics* **42**, 165-83.

MoAF (2004) Project completion report on wild pig conservation and management project. NCD, Ministry of Agriculture and Forests, Royal Government of Bhutan, Thimphu.

Mudar K.M. (1986) *A morphometric analysis of the five subspecies of Sus barbatus, the bearded pig*. Michigan State University, Department of Zoology, Michigan.

Muwanika V.B., Nyakaana S., Siegismund H.R. & Arctander P. (2003) Phylogeography and population structure of the common warthog (*Phacochoerus africanus*) inferred from variation in mitochondrial DNA sequences and microsatellite loci. *Heredity* **91**, 361-72.

Narayan G. (2006) Pygmy Hog Conservation Programme-an update. . In: *Suiform Soundings, PPHSG Newsletter*, pp. 14-5.

Nidup K. (2006) Swine resources in the Hindu Kush Himalayan region: a need for molecular genetic characterization. *SAARC Journal of Agriculture* **4**, 113-21.

O' Connell S. (2008) Back from dead: Could wolves and wild boar roam Britain again? In: *The Independent*. UK, UK.

Oliver W. (2004) [First (and last?) breeding loan export of Visayan (Negros) warty pigs to Europe.]. *ZGAP Mitteilungen* **20**, 23.

Oliver W.L.R. (1980) *The Pygmy Hog: the Biology and Conservation of the Pygmy Hog, Sus (Porcula) salvanius, and the Hispid Hare, Caprolagus hispidus.* Jersey Wildlife Preservation Trust, Jersey, USA.

Querouil S. & Leus K. (2008) *Potamochoerus porcus*. In: IUCN 2009. IUCN Red List of Threatened Species. Version 2009.1. URL http://www.iucnredlist.org/apps/redlist/details/41771/0; http://www.puguhills.com/forests/conservation/mammals.htm#Bush%20Pig.

Randi E., Huart J.P.D., Lucchini V. & Aman R. (2002) Evidence of two genetically deeply divergent species of warthog, *Phacochoerus africanus* and *P. aethiopicus* (Artiodactyla: Suiformes) in East Africa. *Mammalian Biology* **67**, 91-6.

Randi E., Lucchini V. & Diong C.H. (1996) Evolutionary genetics of the suiformes as reconstructed using mtDNA sequencing. *Journal of Mammalian Evolution* **3**, 163-94.

Robins J.H., Ross H.A., Allen M.S. & Matisoo-Smith E. (2006) Sus bucculentus revisited. *Nature* **440**, E7.

Salleh A. (2007) Pig shooters helping to spread disease. URL http://www.abc.net.au/science/news/stories/2007/2064449.htm.

Scheggi M. (1999) *Black Beast: Hunting Boar between Myth, History and current affairs (English translation)*. Olimpia, Florence.

Seydack A. (2008) Potamochoerus larvatus. In: IUCN 2008. IUCN Red List of Threatened Species. URL http://www.iucnredlist.org/.

Shears T.M. (2007) Warthog: *Phacochoerus africanus*. URL http://commons.wikimedia.org/wiki/File:Warthog_Face_001.jpg.

Shenglin Y. (2007) Genetic diversity of Thai indigenous pigs, wild boars and Chinese Qianbei black pigs based on microsatellite DNA and sequence polymorphism of mitochondrial DNA. In: *Institute of Agriculture Technology*, p. 161. Suranaree University of Technology, Nakhon Ratchasima

Spencer P.B.S., Hampton J., Lapidge S.J., Mitchell J., Lee J. & J.R. P. (2006) An assessment of the genetic diversity and structure within and among populations

of wild pigs (*Sus scrofa*) from Australia and Papua New Guinea. *Journal of Genetics* **85**, 63-6.

Tanaka K., Iwaki Y., Takizawa T., Dorji T., Tshering G., Kurosawa Y., Maeda Y., Mannen H., Nomura K., Dang V.B., Chhum-Phith L., Bouahom B., Yamamoto Y., Daing T. & Namikawa T. (2008) Mitochondrial diversity of native pigs in the mainland South and South-east Asian countries and its relationships between local wild boars. *Animal Science Journal* **79**, 417-34.

Tanomtong A., Supanuam P., PSiripiyasing P. & Bunjonrat R. (2007) A comparative chromosome analysis of Thai wild boar (*Sus scrofa jubatus*) and relationship to domestic pig (*S. s. domestica)* by conventional staining, G-banding and high-resolution technique. *Songklanakarin J. Sci. Technol.* **29** 1-13.

Thompson L. (2010) A Photograph of British Wild Boar taken at the Forest of Dean, Gloucestershire, UK. URL http://www.britishwildboar.org.uk/profile.html; http://www.ltimages.co.uk/

van Rensburg P.J.J. (1993) *Physical capture of the bushpig Potamochoerus larvatus*. Wildlife Decision Support Services and The South African Veterinary Foundation.

Vernesi C., Crestanello B., Pecchioli E., Tartari D., Caramelli D., Hauffe H. & Bertorelle G. (2003) The genetic impact of demographic decline and reintroduction in the wild boar (Sus scrofa): A microsatellite analysis. *Molecular Ecology* **12**, 585-95.

Vigne J.D., Zazzo A., Saliege J.F., Poplin F., Guilaine J. & Simmons A. (2009) Pre-Neolithic wild boar management and introduction to Cyprus more than 11,400 years ago. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 16135-8.

Wilson D.E. & Reeder D.M. (2005) *Mammal species of the world: A Taxonomic and Geographic References*. Johns Hopkins University Press, USA.

Wu G.-s., Pang J.-f. & Zhang Y.-p. (2006) Molecular phylogeny and phylogeography of Suidae. *Zoological Research* **27**, 197-201.

Yuan J. & Flad R.K. (2002) Pig domestication in ancient China. *Antiquity* **76**, 7624-732.

Zappa M. (2008) Photograph taken by Michell Zappa and provided by Uganda Wildlife Education Centre to the online Wikipedia. URL http://en.wikipedia.org/wiki/Giant_forest_hog.

Zhang B., Zhang C., Chen J., Ding D., Li C., Zhou K. & Chang Q. (2008) Genetic structure and subspecies divergence of wild boars *Sus scrofa* in mainland China based on the microsatellite variation analysis. *Acta Zoologica Sinica* **54**, 753-61.

Zhang Z. (1986) *Pig Breeds in China*. Shanghai Scientific and Technical Publishers, Shanghai.

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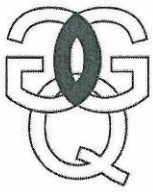

Genetic diversity of domestic pigs as revealed by microsatellites: a mini review

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ABSTRACT

Of the several hundred breeds of pigs in the world, many are in danger of extinction and others are threatened by inefficient use or loss due to cross breeding. Special efforts are required to conserve these genetic resources for food security and rural development but it is not possible to conserve all breeds. Microsatellites, which are short tandem nucleotide repeats found scattered throughout the genome of eukaryotes, have been used to evaluate genetic diversity present within livestock populations to assist in rationalising breed conservation programmes and ensure the greatest possible conservation of diversity. This review provides insights into the use of microsatellite markers to reveal origin, genetic structure and diversity within and across various domestic pig breeds around the world. However, in future, microsatellites may be replaced by panels of single nucleotide polymorphism (SNP) markers for genome-wide population genetic analysis. Meanwhile, microsatellites are still widely employed and for some species may never be replaced by SNP.

Key words: conservation, genetic resources, molecular markers, swine breeds.

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INTRODUCTION

At present there are more than 730 breeds or lines of pigs throughout the world and more than two thirds are found in China and Europe (FAO 2006). Many of these (possibly more than 270 breeds) are now in danger of extinction and others are threatened by inefficient use or loss due to cross breeding (FAO 2006). To meet future challenges in the agricultural and food industries, special efforts are required to conserve genetic resources but it is not possible to conserve all breeds. To evaluate genetic uniqueness and breed diversity of pigs and assist in rationalising breed conservation

programmes, microsatellites have been and remain efficient markers.

Microsatellites are short (2-6 bp) tandem nucleotide repeats, highly polymorphic, and found scattered throughout the genome of eukaryotes (Ellegren 2004). Because of the nature of nucleotide repeats (mono, di, tri, penta and hexa), microsatellites are also called short tandem repeats (STR) or simple sequence repeats (SSR). They have been used to measure genetic diversity in various farm animal species (Kemp *et al.,* 1995; MacHugh *et al.,* 1998; Handley *et al.,* 2007; Granevitze *et al.,* 2007), many endangered animals (Akst *et al.,* 2002; Vidya *et al.,* 2005; Bhagavatula & Singh 2006; Gaur *et al.,* 2006), plants (Yu *et al.,* 2009) and human studies (Ghosh *et al.,* 2003). In pigs, microsatellites have often been used in genetic diversity studies to address the biodiversity and conservation in commercial, indigenous, and rare breeds.

PIG GENETIC DIVERSITY STUDIES

European Pig Project

Europe has a large number of local pig breeds but the vast majority of animals in production systems belong to few intensively selected breeds. For instance, Large White alone represented one third of the gene pool of slaughter pigs in the European Union (EU) (Laval *et al.,* 2000). To facilitate and rationalise the maintenance of pig diversity in Europe, the EU initiated three major projects over the period of 1991 to 2000. The genetic diversity of European pigs was evaluated during the second phase of the Pig Gene Mapping Project (PiGMap) over the period of 1991-1996 (Archibald *et al.,* 1995; Laval *et al.,* 2000). Having gained experiences from PiGMaP, another project entitled "Characterisation of genetic variation in the European pig to facilitate the maintenance and exploitation of biodiversity (PigBioDiv1)" was executed from 1998 to 2000 (Ollivier *et al.,* 2003). The separate project "European gene banking project for pig genetic resources (RESGEN)", which was implemented over 1996-1998, also had a component for evaluating genetic diversity of pigs in Europe (Ollivier 2009). The PigBioDiv2 project was built on the results generated from PigBioDiv1 and included 50 Chinese pig breeds (Blott *et al.,* 2003; Megens *et al.,* 2008).

During these projects, genotyping facilities and associated databases were established. Many new polymorphic markers were developed and evaluated (Groenen *et al.,* 2003; Ollivier 2009). Based on these evaluations, the Food and Agriculture Organization of the United Nations (FAO) and the International Society for Animal Genetics have recommended specific set of markers for assessing biodiversity of pigs (FAO 2004; Hoffmann *et al.,* 2009). There are several publications arising from the projects (Table 1) but the raw genotype data are still not available from the database, which is maintained at the Roslin Institute, UK (Russell *et al.,* 2003).

Diversity of European Pigs

The genetic diversity of eleven pig breeds from six European countries along with a small sample of wild pigs was evaluated by Laval *et al.* (2000) using 18 microsatellite markers (Table 2). Significant breed differentiation (F_{ST} = 0.27) and moderate diversity (H_e= 0.35 to 0.60) was observed amongst the eleven breeds with the French Basque found to be the most genetically distinct. During the PigBioDiv I project (Ollivier *et al.,* 2005; Ollivier 2009), 58 European populations including 29 local breeds, 18 national varieties of international breeds, 21 commercial lines and the Chinese Meishan breed, used as an out-group were genotyped for 50 microsatellite markers. Data from 11 breeds generated during the PiGMap project (Laval *et al.,* 2000) were also included in the analysis. These data showed that the individual breed contributions to between breed diversity ranged from 0.04% to 3.94% of the total European between breed-diversity, and that local breeds accounted for 56% of the total, followed by commercial lines and international breeds. Conversely, the international breeds contributed mostly to within breed diversity followed by commercial

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Table 1 Studies on European and Latin American pigs using microsatellite markers.

Country	Pig breeds or populations	Number of individuals	Mean heterozygosity		Mean number of alleles		Number of markers	Reference
			Observed	Expected	Observed	Effective		
Belgium	Pietrain	50	0.60	0.59	5.33	2.44	18	Laval *et al.* (2000)
Denmark	Sortbroget	59	0.53	0.55	5.17	2.22	18	
France	Basque	47	0.35	0.35	3.22	1.54	18	
	Gascon	56	0.47	0.50	4.05	2.00	18	
	Limousin	56	0.43	0.44	3.70	1.78	18	
	Normand	52	0.50	0.50	4.28	2.00	18	
Germany	German Landrace	50	0.54	0.62	5.61	2.63	18	
	Schwabisch Hallisches	45	0.53	0.66	5.72	2.94	18	
Netherlands	Great Yorkshire	32	0.51	0.50	4.11	2.00	18	
Sweden	Swedish Landrance	24	0.57	0.57	4.78	2.32	18	
	European wild boar	12	0.58	0.59	4.55	2.44	18	
Spain	Retino Extremeno	30	0.56 ± 0.05		5.44 ± 1.54		25	Martinez *et al.* (2000)
	Retinto Portugues	14	0.57 ± 0.05		3.64 ± 1.17		25	
	Silvela	14	0.64 ± 0.05		3.64 ± 1.07		25	
	Mamellado	30	0.58 ± 0.06		3.64 ± 1.29		25	
	Torbiscal	29	0.56 ± 0.05		4.24 ± 1.49		25	
	Entrepelado	41	0.58 ± 0.05		5.84 ± 1.70		25	
	Lampino	30	0.58 ± 0.05		4.84 ± 1.93		25	
	Dorado	7	0.57 ± 0.06		3.44 ± 1.21		25	
	Manchado de Jabugo	30	0.46 ± 0.05		4.04 ± 1.31		25	
	Duroc	20	0.61 ± 0.04		5.00 ± 1.44		25	

Table 1 (cont.) Studies on European and Latin American pigs using microsatellite markers.

Country	Pig breeds or populations	Number of individuals	Mean heterozygosity		Mean number of alleles		Number of markers	Reference
			Observed	Expected	Observed	Effective		
Mexico	Hairless Creole	117	0.48 ± 0.04	0.71 ± 0.05			10	Lemus-Flores *et al.* (2001)
Europe	58 breeds	2737		0.56	4.5		50	SanCristobal *et al.* (2006)
Spain	Iberian	173	0.58	0.70		7.2	36	Fabuel *et al.* (2004)
	Duroc	40	0.55	0.65		5.4	36	
Portugal	Alentejano	23	0.55 ± 0.02	0.63 ± 0.45		3.19 ± 1.24	22	Vicente *et al.* (2008)
	Bisaro	32	0.57 ± 0.02	0.69 ± 0.03		3.70 ± 1.48	22	
	Duroc	31	0.47 ± 0.02	0.49 ± 0.05		2.26 ± 0.84	22	
	Landrace	40	0.65 ± 0.02	0.67 ± 0.04		3.47 ± 1.18	22	
	Large White	33	0.52 ± 0.02	0.56 ± 0.05		2.68 ± 1.05	22	
	Malhado de Alcobaca	50	0.58 ± 0.02	0.57 ± 0.03		2.62 ± 0.95	22	
	Pietrain	40	0.56 ± 0.02	0.59 ±0.04		2.85 ± 1.10	22	
Brazil	Monteiro	35	0.52	0.57		2.34	28	Sollero *et al.* (2009)
	Moura	37	0.60	0.57		2.32	28	
	Piau	31	0.58	0.66		2.94	28	

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Table 2 Studies on Asian pigs using microsatellite markers.

Country	Pig breeds or populations [1]	Number of individuals	Mean heterozygosity		Mean number of alleles		Number of markers	Reference
			Observed	Expected	Observed	Effective		
China	Erhualian	23		0.74 ± 0.09		4.32 ± 1.63	27	Li *et al.* (2000)
	Tongcheng	16		0.75 ± 0.09		4.52 ± 1.68	27	
	Qingping	11		0.70 ± 0.12		3.74 ± 1.11	27	
	Wannanhua	11		0.69 ± 0.12		3.70 ± 1.43	27	
Australia	Commercial line	30		0.64 ± 0.13		3.10 ± 1.10	27	
Thailand	North	22	0.45	0.60			15	Chaiwatanasin *et al.* (2002)
	Northeast	27	0.56	0.77			15	
Thailand	South Thai	22		0.84	9.92	6.30	12	Shenglin (2007)
	Northeast	50		0.86	10.75	7.09	12	
China	Taihu	65	0.52	0.62	5.74	2.92	27	Fan *et al.* (2002)
	Taihu/ Middle Meishan	61	0.49	0.60	5.11	2.90	27	
	Taihu/ Small Meishan	67	0.33	0.43	3.70	2.05	27	
	Taihu/ Mizhu	48	0.50	0.62	5.33	2.90	27	
	Taihu/ Shautou	16	0.52	0.61	4.33	2.91	27	
	Jiangquhai	63	0.50	0.68	5.78	3.45	27	
	Dongchuan	60	0.47	0.58	4.44	2.64	27	
India	Desi/North Indian	25	0.71 ± 0.14	0.80 ± 0.06	7.74 ± 2.13	5.00 ± 1.35	23	Behl *et al.* (2002)
	Gahuri/North-East Indian	25	0.68 ± 0.12	0.79 ± 0.07	7.00 ± 2.02	5.33 ± 1.41	23	
China	Tibetan miniature/Linzhi	31	0.52	0.75	6.11	4.98	37	Fan *et al.* (2003)
	Miniature/Guizhou – XING	30	0.41	0.60	5.11	3.61	37	
	Miniature/ Hainan – WZSN	30	0.50	0.71	5.70	4.29	37	
	Miniature/Yunnan – DNSE	30	0.35	0.64	5.24	3.61	37	

[1] Commercial breeds used in various studies were not indicated.

Table 2 (cont.) Studies on Asian pigs using microsatellite markers.

Country	Pig breeds or populations [1]	Number of individuals	Mean heterozygosity		Mean number of alleles		Number of markers	Reference
			Observed	Expected	Observed	Effective		
China	Penzhou Mountain	57	0.65	0.86	14.19	8.24	26	Yang *et al.* (2003)
	Taoyuan	60	0.69	0.86	13.42	7.38	26	
	Ningziang	60	0.68	0.85	14.23	7.76	26	
	Daweizi	56	0.67	0.87	14.46	8.25	26	
	Shaziling	51	0.56	0.85	13.15	7.73	26	
	Hetao Large-Ear	51	0.67	0.87	13.38	7.77	26	
	Rongchang	59	0.61	0.86	14.15	7.63	26	
	Neijiang	60	0.63	0.84	13.31	6.96	26	
	Chenghua	60	0.51	0.83	12.77	6.65	26	
	Kele	60	0.56	0.86	14.23	8.41	26	
	Fuyuandahe	60	0.59	0.86	14.27	7.78	26	
	Min	60	0.46	0.83	12.92	7.27	26	
	Xiang	60	0.63	0.78	12.54	6.30	26	
	Diannan Small-Ear	60	0.57	0.82	13.46	6.95	26	
	Guanling	33	0.63	0.88	11.85	7.73	26	
	Hanjiang Black	60	0.70	0.86	13.50	7.84	26	
	Mashen	60	0.50	0.70	10.54	5.28	26	
	Tibet	34	0.65	0.87	13.23	8.00	26	
China	56 Indigenous Breeds			0.44 – 0.87		2.12 – 9.03	27	Zhang *et al.* (2003)
China	Dong Shan	32	0.48	0.63	7.60	3.99	20	Li *et al.* (2004)
	Gan Xi Two-Ends Black	34	0.50	0.63	7.50	3.98	20	
	Jin Hua	33	0.52	0.57	6.25	2.98	20	
	Jian Li	29	0.62	0.71	7.60	4.16	20	
	Jia Xing Black	40	0.46	0.67	9.20	4.22	20	

[1] Commercial breeds used in various studies were not indicated.

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Table 2 (cont.) Studies on Asian pigs using microsatellite markers.

Country	Pig breeds or populations [1]	Number of individuals	Mean heterozygosity		Mean number of alleles		Number of markers	Reference
			Observed	Expected	Observed	Effective		
China	Huai Nan Black	46	0.48	0.65	7.75	4.17	20	Li *et al.* (2004), cont.
	Hai Nan	49	0.49	0.77	11.05	5.62	20	
	Nan Yang Black	53	0.52	0.75	11.55	5.12	20	
	Sheng Xian Spotted	30	0.66	0.75	9.54	5.21	20	
	Shazi Ling	33	0.49	0.68	9.50	4.51	20	
Korea	Korean native	32	0.494	0.49			16	Kim *et al.* (2005)
China	Min pig	12	0.735	0.70			16	
	Wuzhishan pig	22	0.515	0.50			16	
	Xiang pig	28	0.599	0.62			16	
China	Wuzhishan pig	13 families		0.56		13.66	32	Huang *et al.* (2005)
India	Ankamali/Kerala	26	0.74 ± 0.09	0.83 ± 0.03	7.43 ± 1.41	5.34 ± 0.77	23	Behl *et al.* (2006)
Vietnam	Muong Khuong	32	0.71 ± 0.02	0.79 ± 0.02		5.14 ± 1.84	20	Thuy *et al.* (2006)
	Co	31	0.71 ± 0.03	0.77 ± 0.02		4.70 ± 1.71	20	
	Meo	32	0.74 ± 0.03	0.79 ± 0.02		4.98 ± 1.59	20	
	Tap Na	25	0.69 ± 0.03	0.77 ± 0.03		4.86 ± 1.78	20	
	Mong Cai	32	0.63 ± 0.04	0.63 ± 0.03		3.03 ± 1.18	20	
China and Europe	46 Chinese, 52 European						39	Megens *et al.* (2008)
Taiwan	Lanyu	44	0.38	0.56		2.39	19	Chang *et al.* (2009)

[1] Commercial breeds used in various studies were not indicated.

Table 2 (cont.) Studies on Asian pigs using microsatellite markers.

Country	Pig breeds or populations [1]	Number of individuals	Mean heterozygosity		Mean number of alleles		Number of markers	Reference
			Observed	Expected	Observed	Effective		
China (village pigs)	Dajie	33		0.83 ± 0.01		5.758	19	Fang *et al.* (2009)
	Guan Xian	53		0.81 ± 0.02		5.260	19	
	Huan Jin	39		0.82 ± 0.01		5.635	19	
	Hong Miao	42		0.82 ± 0.02		5.669	19	
	Jin Tan	127		0.84 ± 0.02		6.141	19	
	Liang Tang	242		0.85 ± 0.01		6.826	19	
	Long Wang	28		0.81 ± 0.02		5.159	19	
	Shan Dong	21		0.83 ± 0.02		5.897	19	
	Xie Dian	79		0.81 ± 0.02		5.300	19	
	Zhang Ji	153		0.84 ± 0.01		6.247	19	

[1] Commercial breeds used in various studies were not indicated.

lines and local breeds. In another analysis on the same data set, SanCristobal *et al.* (2006) showed genetic structuring among the European pig breeds (F_{ST} = 0.21) and this was consistent with Laval *et al.* (2000). The PigBioDiv2 has also included 50 Chinese breeds and the first results were published (Megens *et al.,* 2008). All these data provided baseline information for establishing pig conservation policies within the European countries.

Martinez *et al.* (2000) showed a high level of genetic diversity within Iberian pigs, which are distinct pig population of the Mediterranean region. Their findings are consistent with Fabuel *et al.* (2004) who observed division of Iberian pigs into strains and varieties with high level of genetic diversity, higher than those reported in other European breeds (Laval *et al.,* 2000). These studies have been used to define priorities and tactics for conservation and sustainable use of pig genetic resources in the region (Fabuel *et al.,* 2004; Alves *et al.,* 2006). In Portugal, all breeds with the exception of Malhado de Alcobaca, showed a significant deficit in heterozygosity, most notably in the Bisaro and Alentejano, suggesting inbreeding as a major concern within Portuguese native pigs (Vicente *et al.,* 2008). Appropriate conservation strategies to avoid further loss of genetic diversity in Portuguese native pigs have been proposed.

Latin American Pigs

The main Creole pig in Mexico is hairless and endangered (Sierra *et al.,* 2005). This breed has never been improved or conserved but shows a high level of genetic diversity (Lemus-Flores *et al.* (2001), suggesting that Creole pigs may be a potential reservoir of genetic resource for the improvement of commercial pigs. Three distinctively naturalized pig breeds that are potential genetic resources for future have been found in Brazil (Sollero *et al.,* 2009). Latin American pig breeds are currently being characterized and studied through the Latin American International Pig Biodiversity project (Revidatti *et al.,* 2010).

Diversity of Domestic Pigs in Asia

Chinese Pigs. Asian countries, particularly China, have a huge pool of highly diverse indigenous pigs, many with special characteristics such as high prolificacy and good meat quality. China has the largest number of pig breeds accounting for almost one-third of all breeds in the world (FAO, 2006; Zang, 1986). According to widely accepted categorisation, there are 48 Chinese indigenous breeds divided traditionally into six types based on geographical origin, distribution, body conformation, and coat colour (Zang, 1986). These six categories of Chinese indigenous pigs are: North China Type (NC), Central China Type (CC), South China type (SC), Southwest Type (SW), Plateau Types (PC), and Lower Changjiang River Basin Type (LCR). However, many commercial breeds introduced into China have contributed to the genetic pool of most indigenous breeds (Yang *et al.,* 2003). The population size of many breeds is relatively small with some on the verge of extinction or even effectively lost (Li *et al.,* 2000).

In one of the largest studies, Zhang *et al.* (2003) analysed genetic diversity (H_e = 0.44 - 0.87) and clustering of fifty six Chinese indigenous breeds, finding twelve groups, which aligned with the traditional classification system (Zhang 1986). Group I, II and III correspond to NC type, group IV with LCR type, group V, VI, VII, VIII and IX groups with NC types, Group X and XI group largely to SC type, and XII is equal to SW type. Similarly, Yang *et al.* (2003) found abundant genetic diversity (H_e = 0.70 - 0.88) between 18 Chinese indigenous breeds (n=1001) representing five of the six traditionally classified types (NC, CC, SC, SW, and TC type). Most variation was observed within breeds with little variation among populations. The genetic structure and variability of the sixth category known as LCR type and constituting five types of Taihu pigs (Erhualian, Middle Meishan, Small Meishan, Mizhu, and Shawutou), Jiangquhai and Donchauan pigs of China were investigated by Fan *et al.* (2002). Among the Taihu populations, Erthualian and Mizhu clustered together, while Meishan, Small Meishan and

Shawutou were grouped. The Jiangquhai pig was the most variable while Small Meishan had the lowest genetic variability.

The most comprehensive analysis of Chinese pig breeds by Fang *et al.* (2005) showed only partial agreement with the traditional classification (Zhang 1986) of Chinese pigs. Four breeds (Erhualian, Tongcheng, Qingping, and Wannanhua) from the preservation farms in Southern and Central China were studied but no concrete conclusions could be drawn (Li *et al.,* 2000) due to small sample size (n=62). Another attempt to evaluate the status of conservation farms were made by Li *et al.* (2004) who found 78% of the genetic variation within-population and 22% across 10 local breeds sampled from various provinces in China. Based on these findings, it was suggested that the number of conservation farms could be reduced in China.

As a part of PigBioDiv2 project, the biodiversity and possible domestication processes of 46 Chinese and 52 European pig populations were assessed by Megens *et al.* (2008). Genetic distances revealed strong geographic structure within Chinese breeds while there was little evidence for such structure in European populations. Among the Chinese breeds, those from the North were quite similar to the European breeds. Chinese breeds showed a higher average heterozygosity when compared to European ones. Between breed diversity was even more pronounced and was highest in the Central Chinese pigs. A study on Chinese miniature pig breeds (Xiang, Wuzhishan, Diannan Small-Ear, and Tibetan pig), which are mainly found in the mountain areas of South and Southwest China, showed that the highest level of genetic variability was in outdoor Tibetan pigs, followed by intensively selected State-owned pigs of Guizhou, Hainan, and Yunnan provinces (Fan *et al.,* 2003).

However, despite many studies on indigenous pigs, the village pigs of China are poorly studied. Fang *et al.* (2009) investigated genetic characteristics of ten Chinese village pig populations along with indigenous and commercial breeds using 19 microsatellite markers. The village pigs had relatively high average heterozygosity when compared with other populations. The genetic distance tree clearly segregated village pigs as separate populations from other indigenous Chinese and Euro-American pig breeds. There was relatively little variation between village pig populations with 96.14% of the variation within populations. Because of their genetic uniqueness, village pigs are vital for future pig production in China (Fang *et al.,* 2009).

Other Asian Pigs. The Korean native pig is believed to have originated approximately 2000 years ago from a black coated North China pig breed (Kim and Choi 2002). The number of Korean native pigs decreased dramatically until the 1980s and the breed faced extinction due to interbreeding with Western commercial breeds. Kim and Choi (2002) used six microsatellite loci on 67 individuals to characterize genetic variability and structure of Korean native black pigs. Korean native pigs are more closely related to Chinese Yanbian pigs than to Japanese Kagoshima pigs. Kim *et al.* (2005) subsequently used 16 microsatellite markers to investigate the genetic structure of both native and wild Korean pigs along with Western (Berkshire, Duroc, Landrace, and Yorkshire) and three Chinese breeds (Min, Xiang, Wuzhishan). They found clear genetic differentiation among the Korean wild boar, Xiang pig and Wuzhishan pigs.

Chang *et al.* (2009) analysed the Lanyu pig, an indigenous miniature pig breed from Lanyu Islet, Southeast Taiwan using 19 microsatellite loci. A low level of heterozygosity was observed, possibly due to inbreeding caused by small population size on the conservation farm but the Lanyu breed was found to be genetically distinct without influence from European or other Asian domestic pigs (Chang *et al.,* 2009). This is consistent with mtDNA sequences (Larson *et al.,* 2010).

Vietnam has more than 29 million pigs, the second largest number of on-farm pigs in Far East Asia after China, which holds 84% of all on-farm pigs (FAOSTAT 2006). However,

Vietnamese pigs are much less studied than Chinese pigs. Thuy *et al.* (2006) analysed five Vietnamese indigenous breeds (Muong Khuong, Co, Meo, Tap Na, and Mong Cai) and showed a higher degree of polymorphism, allelic diversity, and heterozygosity than in the commercial pigs of European origin. Similarly, there are limited studies on the indigenous pigs of Thailand. Chaiwatanasin *et al.* (2002) showed that the genetic diversity of indigenous pigs from Northeast was higher than the North Thailand. The Northeast and Southern pigs are genetically distinct from Thai wild boar, but closer to South and Southwest Chinese Qianbei black pigs, suggesting an origin from Chinese domestic pigs rather than from the local wild boar (Shenglin, 2007).

India has a total domestic pig population of slightly over 13 million of which more than 78% are indigenous pigs (LCI 2007). However, very few of these have been studied. The Desi (North India) and Gahuri (Northeast India) Indian pigs were found to be genetically identical (Behl *et al.,* 2002, but distinct from South Indian Ankamali, the native pigs of Kerala (Behl *et al.,* 2006). The preliminary findings from the microsatellite data of the indigenous pigs from Bhutan, Nepal, Sri Lanka, and Papua New Guinea, which have been recently reported (Nidup *et al.,*2010a; Nidup *et al.,* 2010b) suggest rich biodiversity of indigenous pigs resource in South Asia and Papua New Guinea.

Despite some limitations of sampling methods, numbers of markers used and type of analyses, all these microsatellite studies indicate the existence of biodiversity, potential conservation, and sustainable utilization of swine genetic resources, particularly the indigenous breeds, in European and Asian countries.

DISCUSSION

Genetic diversity is important for food security and rural development. The use of microsatellites has revealed high levels of genetic diversity amongst the huge pool of genetic resources of pigs around the world, particularly in Asia. One of the purposes of genetic diversity studies, if appropriate, is to formulate recommendations for genetic management and conservation and disseminate these to breeding organizations and relevant government agencies. To check if genetic diversity studies have influenced the design an implementation of conservation programmes in pigs, an online discussion was initiated in two popular discussion forums namely; Domestic Animal Diversity Network (http://dgroups.org/Community.aspx?c=66ada01b-ae15-4793-8552-32cc4b7c4061) of the FAO and ANGENMAP of Animal Gene Mappers (http://www.animalgenome.org/community/discuss.html) on 20 September 2010. Responses gathered indicate that the information from molecular characterization studies was explicitly utilized in two countries. Based on molecular information, 2 goat, 1 sheep, and 1 cattle breeds have been included in the national conservation programme in Austria. In USA, it has guided some of the actions of the American Livestock Breeds Conservancy, an NGO that works with pigs, cattle, sheep, goats, and horses. Most respondents described valuable information gained from such studies but did not specifically indicate how the information was to be used.

Today, large numbers of single nucleotide polymorphism (SNP) markers, which are likely to replace microsatellites, are now available. SNP have been successfully used to study population structure and diversity of some species (McKay *et al.,* 2008; Decker *et al.,* 2009; Kijas *et al.,* 2009). Various pig breeds, including wild pigs, are currently being genotyped using the Illumina porcine 60K iSelect Beadchip (Groenen *et al.,* 2010). However, there are still some limitations with SNP for biodiversity studies. The problems arise from ignoring all SNP loci with minor allele frequency lower than 5% and from the choice of populations from which SNP are discovered. SNP genotyping can be very expensive considering the large number of individuals required in biodiversity studies. With SNP, there are far more markers per

individual but far fewer individuals per marker. As a result, there are high chances that population dimension is lost in many population structure and diversity studies. In addition, there are limitation with existing genetic programs and computer applications to be able to process the huge amounts of data generated in genome wide SNP studies (Decker *et al.,* 2009). Therefore, while microsatellites may be considered an outdated technology, some laboratories are still reluctant to switch on to next generation sequencing and genotyping technologies.

Finally, genetic diversity measured with neutral markers should never be the sole criterion in conservation. There should be a holistic approach to conservation which takes account of the phenotype properties also. Decisions on choice of breeds should also take into account traits of economic value, specific adaptive features, distinct phenotypes, role of breed in local production systems, population size, level of endangerment, and availability of resources and infrastructure in the region where a breed is located.

REFERENCES

Akst, EP, PD Boersma, and RC Fleischer (2002) A comparison of genetic diversity between the Galapagos Penguin and the Magellanic Penguin. *Conservation Genetics* **3**: 375-83.

Alves, E, Al Fernandez, C Barragan, C Ovilo, C Rodriguez, and L. Silio (2006) Inference of hidden population substructure of the Iberian pig breed using multilocus microsatellite data. *Spanish Journal of Agricultural Research* **4**: 37-46.

Archibald, AL, CS Haley, JF Brown, S Couperwhite, HA McQueen, D Nicholson, W Coppieters, A Weghe, A Stratil, AK Winterø *et al.* (1995) The PigMaP consortium linkage map of the pig (*Sus scrofa*). *Mammalian Genome* **6**: 157-175

Behl, R, R Kaul, N Sheoran, J Behl, MS Tantia, and RK Vijh (2002) Genetic identity of two Indian pig types using microsatellite markers. *Animal Genetics* **33**: 158-159.

Behl, R, N Sheoran, J Behl, and RK Vijh (2006) Genetic analysis of Ankamali pigs of India using microsatellite markers and their comparison with other domesticated Indian pig types. *Journal of Animal Breeding and Genetics* **123**: 131-135.

Bhagavatula, J, and L Singh L (2006) Genotyping faecal samples of Bengal tiger Panthera tigris tigris for population estimation: A pilot study. *BMC Genetics* **7**: 48

Blott, S, L Andersson, M, Groenen, M SanCristobal, C, Chevalet, R, Cardellino, N Li, L Huang, K Li, G Plastow, and & C Haley (2003) Characterisation of genetic variation in the pig breeds of China and Europe - The PigBioDiv2 Project. *Archivos de Zootecnia* **52**: 207-17.

Chaiwatanasin, W. S Chantsavang, S Chomchai, N Sookmanee, and S Tabchareon (2002) Genetic diversity of native pig in Thailand using microsatellite analysis. *Kasetsart J. (Nat. Sci.)* **36**: 133-137.

Chang, WH., HP Chu,YN Jiang, SH Li, Y Wang, CH Chen, KJ Chen, CY Lin, and YT Ju (2009) Genetic variation and phylogenetics of Lanyu and exotic pig breeds in Taiwan analyzed by nineteen microsatellite markers. *Journal of Animal Science* **87**: 1-8.

Decker, JE, JC Pires, GC Conant, SD McKay, MP Heaton, KF Chen, A Cooper, J Vilkki, CM Seabury, AR Caetano, GS Johnson, RA Brenneman, O Hanotte, LS Eggert, P Wiener, JJ Kim, KS Kim, TS Sonstegard, CP VanTassell, HL Neibergs, JC McEwan, R Brauning, LL Coutinho, ME Babar, GA Wilson, MC McClure, MM Rolf, J Kim, RD Schnabel, and JF Taylor (2009) Resolving the evolution of extant and extinct ruminants with high-throughput phylogenomics. *Proceedings of the National Academy of Sciences* **106**: 18644-18649.

Ellegren, H. (2004) Mircrosatellites: Simple sequences with complex evolution. *Nature Reviews Genetics* **5**: 435-445.

Fabuel, E. C Barragan, L Silio, MC Rodriguez and MA Toro (2004) Analysis of genetic diversity and conservation priorities in Iberian pigs based on microsatellite markers. *Heredity* **93**: 104-113.

Fan, B. SL Yang, B Lie, M Yu, SH Zhao, and K Li (2003) Characterization of the genetic diversity on natural populations of Chinese miniature pig breeds. *Animal Genetics* **34**: 465-476.

Fang M. X Hu, T Jiang, M Braunschweig, L Hu, Z Du, J Feng, Q Zhang, C Wu, and N Li (2005) The phylogeny of Chinese indigenous pig breeds inferred from microsatellite markers. *Animal Genetics* **36**: 7-13.

Fang, M, X Hu, W Jin, N Li, and C Wu (2009) Genetic uniqueness of Chinese village pig populations inferred from microsatellite markers. *Journal of Animal Science* **87**: 3445-3450.

FAO (2004) New microsatellite markers set – Recommendations of joints ISAG/FAO standing committee. In: *Secondary Guidelines for development of national farm animal genetic resources management plans: Measurement of domestic animal diversity (MoDAD) recommended microsatellite markers*. FAO: Rome.

FAO (2006) *The State of World's Animal Genetic Resources for Food and Agriculture*. FAO: Rome.

FAOSTAT (2006) Live Animals: Pig. URL http://faostat.fao.org/default.aspx. Accessed on 2 October 2010.

Gaur, A, K Shailaja, A Singh, V Arunabala, B Satyarebala, and L Singh (2006) Twenty polymorphic microsatellite markers in the Asiatic lion (Panthera leo persica). *Conservation Genetics* **7**: 1005-1008.

Ghosh, A, B Das, and M Seshadri (2003) Population genetic analysis among five Indian population groups using six microsatellite markers. *Human Biology* **75**, 189-203.

Granevitze, Z, J Hillel, GH Chen, NTK Cuc, M Feldman, H Eding & S Weigend (2007) Genetic diversity within chicken populations from different continents and management histories. *Animal Genetics* **38:** 576-583.

Groenen, MAM, R Joosten, MY Boscher, Y Amigues, A Rattink, B Harlizius, JJ Poel, and Croojimans (2003) The use of microsatellite genoypeing for population studies in the pig with individual and pooled DNA samples. *Arch. Zootec* **52:** 145-155.

Handley, LLJ, KB Santucci, S Townsend, M Taylor, MW Bruford, and GM Hewitt (2007) Genetic structure of European sheep breeds. *Heredity* **99:** 620-631.

Hoffmann, I, PA Marsan, JSF Baker, G Cothrane, O Hanotte, JA Enstra , D Milan, S Weigend, and H Simianer (2009) New MoDAD marker sets to be used in diversity studies for the major farm animal species: recommendations of a joint ISAG/FAO working group. FAO, Rome, Italy.

Huang, L, X Wang, J Ou, C Guo, X Li, F Wang, and X Zhen (2005) Genetic analysis of 32 microsatellite loci in 13 families of Wuzhishan pig by multiplex PCR and gene scanning technique. *Hereditas (Beijing)* **27:** 70-74.

Kemp, S, O Hishida, J Wambugu, A Rink, ML Longeri, and RZ Ma, *et al.* (1995) A panel of polymorphic bovine, ovine, and caprine microsatellite markers. *Animal Genetics* **26:** 299-306.

Kim, KS, and CB Choi (2002) Genetic structure of Korean native pig using microsatellite markers. *Korean Journal of Genetics* **24:** 1-7.

Kim, TH, KS Kim, BH Choi, DH Yoon, GW Jang, KT Lee, HY Chung, HY Lee, HS Park, and JW Lee (2005) Genetic structure of pig breeds from Korea and China using microsatellite loci analysis. *Journal of Animal Science* **83:** 2255-2263.

Laval, G, N Iannuccelli, C Legault, D Milan, MAM Groenen, E Giuffra, L Andersson, PH Nissen, CB Jargensen, P Beeckmann, H Geldermann, J-L Foulley, C Chevalet, and Ollivier, L. (2000) Genetic diversity of eleven European pig breeds. *Genetic Selection and Evolution* **32**, 187-203.

Laval, G, N Iannuccelli, C Legault, and *et al.* (2000) Genetic diversity of eleven European pig breeds. *Genetic Selection and Evolution* **32**: 187-203.

Larson, G, RR Liu, XB Zhao, J Yuan, D Fuller, L Barton, K Dobney, QP Fan, ZL Gu, XH Liu, YB Luo, P Lv, L Andersson, and Li, N. (2010). Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. *Proceedings of the National Academy of Sciences of the United States of America* **107:** 7686-7691.

LCI (2007) Livestock census of India. URL http://www.indiaagristat.com. Accessed on 23 April 2010.

Lemus-Flores, C, R Ulloa-Arvizu, M Ramos-Kuri, FJ Estrada, and RA Alonso (2001) Genetic analysis of Mexican hairless pig populations. *Journal of Animal Science* **79:** 3021-3026.

Li, K, Y Chen, C Moran, B Fan, S Zhao, and Z Peng (2000) Analysis of diversity and genetic relationships between four Chinese indigenous pig breeds and one Australian commercial pig breed. *Animal Genetics* **31:** 322-325

Li, SJ, SL Yang, SH Yang, SH Zhao, B Fan, M Yu, SH Wang, MM Li, B Liu, TA Xiong and K Li (2004) Genetic diversity analyses of 10 indigenous Chinese pig populations based on 20 microsatellites. *J Anim Sci* **82:** 368-74.

Luetkemeier, ES, M Sodhi, SL Schook, and RS Malhi (2010) Multiple Asian pig origins revealed through genomic analyses. *Mol Phylogenet Evol* **54:** 680-686.

MacHugh, D, RT Loftus, P Cunningham, and DG Bradley (1998) Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers. *Animal Genetics* **29:** 333-340.

Martinez, AM, JV Delgado, A Rodero, and JL Vega-Pla (2000) Genetic structure of the Iberian pig breed using microsatellites. *Animal Genetics* **31:** 295-301

McKay SD, RD Schnabel, BM Murdoch, LK Matukumalli, J Aerts, W Coppieters, D Crews, E Neto, CA Gill, C Gao, H Mannen, Z Wang, CPV Tassell, JL Williams, JF Taylor, and SS Moore (2008) An assessment of population structure in eight breeds of cattle using a whole genome SNP panel. *BMC Genetics* **9:** 1-9

Megens, HJ, R Crooijmans, MS Cristobal, X Hui, N Li, and MAM Groenen (2008) Biodiversity of pig breeds from China and Europe estimated from pooled DNA samples: differences in microsatellite variation between two areas of domestication. *Genetics Selection Evolution* **40:** 103-128.

Nidup, K, W Ayalew, G Danbaro, J Gongora and C Moran (2010a). Genetic structure and diversity of indigenous pigs of Papua New Guinea. *Annual Postgraduate Conference.* P53, 1-2 November. Camperdown Campus, Faculty of Veterinary Science, University of Sydney, Australia.

Nidup, K, GLLP Silva, DD Joshi, R Pem, J Gongora, and C Moran (2010b). Genetic Diversity of Pigs (*Sus scrofa*) in the Himalayas and Sri Lanka. *32nd International Society for Animal Genetics Conference*, Edinburgh.

Ollivier, L. (2009) European pig genetic diversity: a minireview. *Animal* **3:** 915-924.

Ollivier, L, L Alderson, GC Gandini, JL Foulley, CS Haley, *et al.* (2005) An assessment of European pig diversity using molecular markers: Partitioning of diversity among breeds. *Conservation Genetics* **6:** 729-741.

Ollivier, L, Y Amigues, and MY Boscher (2003) An EC-funded project on characterization of genetic variation in the European pig. Objectives, organization, breed sampling, DNA preparation, and circulation. *Arch. Zootec* **52:** 137-144.

Paszek, AA, GH Flickinger, L Fontanesi, CW Beattie, GA Rohrer, L Alexander, and LB Schook (1998) Evaluating evolutionary divergence with microsatellites. *Journal of Molecular Evolution.* **46:** 121-126.

Russell, GA, AL Archibald, CS Haley, and AS Law (2003) The pig genetic diversity database and the WWW. *Archivos de Zootecnia* **52:** 165-172.

Saitbekova, N, C Gaillard, RG Obexer, and G Dolf (1999) Genetic diversity in Swiss goat breeds based on microsatellite analysis. *Animal Genetics* **30:** 36-41.

SanCristobal, M, C Chevalet, CS Haley, *et al.* (2006) Genetic diversity within and between European pig breeds using microsatellite markers. *Animal Genetics* **37:** 189-198.

Shenglin, Y. (2007) Genetic diversity of Thai indigenous pigs, wild boars and Chinese Qianbei black pigs based on microsatellite DNA and sequence polymorphism of mitochondrial DNA. In: *Institute of Agriculture Technology*, p. 161. Suranaree University of Technology, Nakhon Ratchasima

Sierra, AC, TB Poot, AL Diaz, AH Cordero, and JV Delgado (2005) The Mexican hair-less pig, an endangered breed. *Archivos de Zootecnia* **54:** 165-170.

Sollero, BP, SR Paiva, DA Faria, SEF Guimaraes, STR Castro, AA Egito, MSM Albuquerque, U Piovezan, GR Bertani, and AD Mariante (2009) Genetic diversity of Brazilian pig breeds evidenced by microsatellite markers. *Livestock Science* **123**: 8-15.

Thuy, NTD, WE Melchinger, AW Kuss, NV Cuong, H Bartenschlager, and H Geldermann(2006) Comparison of Vietnamese and European pig breeds using microsatellites. *Journal of Animal Science* **84:** 2601-2608.

Vicente, AA, MI Carolino, MCO Sousa, C Ginja, FS Silva, AM Martinez, JL Vega-Pla, N Carolino & LT Gama (2008) Genetic diversity in native and commercial breeds of pigs in Portugal assessed by microsatellites. *Journal of Animal Science* **86:** 2496-2507.

Vidya, TNC, P Fernando, DJ Melnick, and R Sukumar (2005) Population genetic structure and conservation of Asian elephants (Elephas maximus) across India. *Animal Conservation* **8:** 377-388.

Yang, SL, ZG Wang, B Liu, GX Zhang, SH Zhao, M Yu, B Fan, MH Li, TA Xiong, and K Li (2003) Genetic variation and relationships of eighteen Chinese indigenous pig breeds. *Genet Sel Evol* **35:** 657-671.

Yu, JW, A Dixit, KH Ma, JW Chung, and YJ Park (2009) A study on relative abundance, composition and length variation of microsatellites in 18 underutilized crop species. *Genetic Resources and Crop Evolution* **56**: 237-246.

Zhang, GX, ZG Wang, FZ Sun, WS Chen, GY Yang, SJ Guo, YJ Li, XL Zhao, Y Zhang, J Sun, B Fan, SL Yang, and K Li (2003) Genetic diversity of microsatellite loci in fifty six Chinese native pig breeds. *Yi Chuan Xue Bao* **30:** 225-233.

Zhang, Z. (1986) *Pig Breeds in China*. Shanghai Scientific and Technical Publishers, Shanghai.

CHAPTER 2

2.0 Field Work

2.1 SAMPLING IN BHUTAN

2.1.1 Sampling of Domestic Pigs

Using the 2006 and 2007 pig population records from the Bhutanese Department of Livestock as a guide (DoL 2007, 2008), 32 sub-districts across 13 districts were chosen using appropriate sampling methods (Denzin & Lincoln, 2000) to cover as much as possible of the geographical distribution of indigenous pigs. The samples collected from these sub-districts, which lie in altitudes ranging from 200 to 5400 metres above sea level (masl) covering various agro-ecological zones (Table 2.1) in the country are shown in Table 4.S1. Villages within each sub-district with a record of pigs (DoL 2007, 2008) were randomly chosen. An opportunistic or non-strategic sampling method was employed in remote villages when pigs with special features were spotted.

One sample was taken from a pig in the quarantine centre of the Bhutan Agriculture Food and Regulatory Authority (BAFRA). It was apparently confiscated from a person trying to smuggle it into Gelephu (Bhutan) from Datgari (Assam, India). More than twenty Bhutanese pig samples were obtained from Dr Tanaka Kazuaki, School of Veterinary Medicine, Asabi University, Japan, through the Council for Renewable Natural Resources Research of Bhutan, Jakarta, as a part of Bhutan-Japan livestock research collaboration. However, some of these DNA samples were degraded and others had DNA concentrations too low for genotyping.

Despite the rugged terrain and the remote and inaccessible nature of much of the country (Figure 2.1), every effort was made to ensure that the samples represented a wide cross-section of the entire pig population in the country. However, 6 of 20 districts in Bhutan were not included in the study. The Gaza district in the west-central region is situated at a very high altitude (1500-4500 masl) and there is no record of indigenous

pigs; only a very few exotic pigs. Bumthang and Trongsa in the East-central region have no pigs. Bumthang, which is the most devout religious district, was the first to prohibit pig farming due to strong religious (Buddhist) sentiments against slaughtering. Trongsa, a historically important district, which shares its border with Bumthang, is similarly influenced by the largest monastic body in the country. For the same reason, pig farming is also decreasing in the Eastern region. Sampling was not conducted in three additional districts, namely Pemagatshel, Samdrup Jongkhar and Lhuntse, due to smaller populations of indigenous pigs and the strong influence of a large number of religious communities.

2.1.2 Sampling of Wild Boar

Hair and serum samples of wild boar were collected from six districts (Wangdue, Trongsa, Samdrup Jhongkhar, Chukha, Punkha, and Sarpang), consisting of all the four developmental regions (Eastern, East-central, Western, and West-central) (Table 2.2). Hair samples were from dead wild boar, while the serum was obtained from wild boar shot during the Nature Conservation Divison's wild boar project (MoAF, 2004).

Table 2.1: Samples collected from 32 sub-districts of the 13 districts in Bhutan and one sample from Assam in India.

Sl no	Dzongkhag (District)	Geog (sub-district)	No. of villages	Geog Altitude (masl)	Area (Sq km)	No. of pigs sampled
1	Chukha	Bongo	14	400 -3400	396.78	12
		Darla	18	600 – 2100	144.54	22
		Gelay	7	1000 – 3300	218.70	2
		Pasakha	17	600 – 1400	73.58	6
						42
2	Dagana	Drujegang	3	600 – 1400	57.32	5
		Goshing	5	800 – 1000	20.36	11
		Lhamoizingkha	7	800 – 1400	106.32	10
		Tseza	8	2200 – 4200	592.30	6
						32
3	Haa	Eusu	13	3400 – 4100	64.74	5
		Katsho	11	3400 – 3800	42.37	5
						10
4	Paro	Dogar	10	2600 – 3800	106.23	8
		Dop-shari	7	2000 – 2600	36.71	7
		Naja	17	3000 – 3800	139.18	4
						19
5	Punakha	Chubu	6	2200 – 5400	90.13	6
		Dzomi	6	1800 – 2000	22.01	4
						10
6	Samtse	Samtse	23	600 – 1400	117.19	3

Sl no	Dzongkhag (District)	Geog (sub-district)	No. of villages	Geog Altitude (masl)	Area (Sq km)	No. of pigs sampled
						3
7	Sarpang	Dekiling	16	600 -1800	115.72	3
		Gelephu	5	200 – 600	54.12	10
		Shompongkha	8	200 – 600	22.42	4
		Umling	8	600 -1800	122.5	9
						29
8	Trashigang	Uzurong	8	1000 – 1800	1059570	2
9	Trashiyangtse	Khamdang	30	1800 – 2200	44.49	10
		Ramjar	30	1000 – 1600	21.81	9
						21
10	Tsirang	Rangthaling	8	600 – 1600	24.50	4
		Tsholingkhor	6	500 – 1400	13.77	6
						10
11	Thimphu	Chang	30	1800 -2200	160.17	2
						2
12	Wangdue	Phangyuel	7	1800 – 2600	32.75	3
		Kazhi	5	2200 – 4600	622.54	3
		Phobjikha	8	3000 – 3800	145.71	7
		Thoetsho	7	1400 – 3800	20.87	17
						30
13	Zhemgang	Bardo	8	600 – 2900	209.96	4
		Trong	13	800 – 2600	358.38	5
						9
14	Assam	Datgari	-	200		**1**
						1
					TOTAL	**215**

Table 2.2: Details of wild boar samples collected in Bhutan.

Sample Type	No. of Samples	Sampling Location	Sample Collected By
Hair	3	Phangyul-Kazhi, Wangdue district, West-central region	Livestock extension agent
Hair	1	Jigme Singye Wangchuk National Park (JSWNP), Trongsa area, Trongsa district, East-central region	Park manager
Hair	1	Langthel, Trongsa district, East-central region	Livestock extension agent
Faecal	1	Shingkhar-Lauri, Samdrup Jhongkhar district, Eastern region	Livestock extension agent
Hair	1	Shingkhar-Lauri, Samdrup Jhongkhar district, Eastern region	Livestock extension agent
Hair	1	Bjacho, Chukha district, Western region	Forestry extension agent
Serum	2	Wild boar from Thinlaygang, Punakha district, West-central region	Deputy Chief Veterinarian, NCAH, Serbithang
Hair	2	Bhur, Sarpang, East-central region	Regional Veterinary Lab, Gelephu

Figure 2.1: Aspects of field work in Bhutan. (a) Collecting samples in Bhutan required treks on foot to remote and relatively inaccessible farms in mountainous areas; (b) Typical Bhutanese farm house showing pig sty in the Pakshiga village, Bongo sub-district, Chukha district; (c) Typical pig sty in Kazhi sub-district, Wangdue district; (d) Restraining pig using an animal control pole; (e) Livestock extension staff assisting during blood collection at Darla sub-district, Chukha district; (f) Blood samples were taken from the jugular vein in smaller pigs; (g) Gelephu Regional Veterinary Laboratory (RVL) staff involved in blood collection at Lhamoizingkha sub-district, Dagana district; (h) Farmer collecting hair sample from his Agouti indigenous pig in Langdarbi village, Bardo sub-district, Shemgang district; (i) Farmer‘s highly prized pig in a village called Purano Busty, Gelelphu sub-district, Sarpang district.

2.1.3 Sampling Period in Bhutan

The fieldwork in Bhutan was carried out in two phases, from May 2007 to July 2007 and from February 2008 to March 2008. In total, 5 months were required for sample collection including planning, travel, sampling and DNA extraction.

2.2 SAMPLING IN NEPAL

Nepal has 14 zones and 75 districts grouped into five development regions, namely Eastern, Central, Western, Mid-Western and Far-Western. Pigs are concentrated mainly in the Eastern development region with the least number of pigs in the far-Western development region (Nidup *et al.* 2010). Samples were collected from four districts, namely Saptari (Eastern region), Chitwan (Central region), Laitpur (Central region), and Gorkha (Western region) (Figure 4.1b). A total of 50 blood samples were obtained constituting 21 from Kalo Dharane Sunggur, 4 Chwanche crossbred, and 25 samples from Hurrah pigs (Table 4.S2).

Blood sampling and DNA extraction were conducted between November 2007 and March 2008 with the help of Dr D.D. Joshi, National Zoonosis and Food Hygiene Research Centre, Chagal, Kathmandu, Nepal.

2.3 SAMPLING IN SRI LANKA

Sampling in Sri Lanka was conducted by Dr Pradeepa Silva, Department of Animal Science, University of Peradeniya, Kandy, Sri Lanka. Samples of village pigs were collected from several farms in four districts, namely Kalutara, Kurunagala, Puttalam and Chilaw (Figure 4.1c). These districts were found to have an abundance of village pigs. Wild boar samples were obtained from Batticaloa, Polonnaruwa, Anuradhadapura, Kandy and Kurunagala districts (Figure 4.1c).

A total of 27 village pigs were sampled. Since the village pigs were reared mostly under extensive conditions without any records of matings, geographic separation was considered as a strategy to avoid sampling of closely related individuals. An opportunistic sampling method was used to collect samples from wild boars. Contacts with farmers, forest officials, wild life veterinarians and veterinary clinics were

maintained to collect 21 blood samples from dead, slaughtered, or captured wild boars. Samples were collected in 2008 during a period of about six months.

2.4 SAMPLING IN PAPUA NEW GUINEA

Sampling in Papua New Guinea (PNG) was conducted by the collaborating partners, Dr Workneh Ayew of Papua New Guinea's (PNG) National Agriculture Research Institute (NARI) and Dr Danbaro Gariba PNG University of Technology. Blood and hair samples were collected from presumed unrelated indigenous pigs in three provinces (Western Highlands Province, Morobe, Enga; Figure 6.1) comprising eight districts (Tambul, Nawaeb, Finschaffen, Garaina, Huon Gulf, Kandeep, Bulolo, and Boana; Table 6.S1), which were considered less affected by the introduction of exotic pigs and their crossbreeding with indigenous stocks. Based on existing information and with the help of extension agents, villages and households raising indigenous pigs were identified.

(a) (b) (c)

Figure 2.2: Nepalese indigenous pigs photographed during the field work in Nepal. (a) Hurrah pigs in Nepal ready to be slaughtered and marketed; (b) Scavenging Chwanche sow with her piglets in Nepal; (c) Farmed wild boar (Badel) crossed indigenous pig in Nepal.

(a) (b) (c)

Figure 2.3: Sri Lankan village and wild pigs. (a) Tethered Sri Lanka indigenous pig; (b) Free-range scavenging indigenous pigs in Sri Lanka; (c) Taking samples from wild pig in Sri Lanka. (**Photo courtesy:** Dr G.L.L.P. Silva, University of Peradeniya, Sri Lanka).

(a) (b) (c) (d)

Figure 2.4: PNG village (a, b) and wild (c, d) pigs of PNG. (**Photo courtesy:** Dr Gariba Danbaro, PNG University of Technology; Dr Workneh Ayalew, Livestock Research Programme, National Agricultural Research Institute of PNG)

2.5 PIG RESTRAINT AND SAMPLE COLLECTION

2.5.1 Pig Restraint

To collect blood, proper and adequate restraint of pigs is important. Pigs were restrained using an animal control pole (JORGEN KRUUSE A/S, Marslev Byvej 35 5290 Marslev, Denmark), which has a light weight Aluminium body with durable grip, an automatic locking mechanism, a quick release knob, and a braided and coated wire rope cable that will not harm the animals. In use, the rope cable loop is slowly placed around the snout, behind the canine teeth so that the cable loop does not slip off. The cable at the end of the pole is pulled making sure that the loop at the other end tightens on the snout. It is then slightly twitched so as to keep the pigs in a calm position. Once the samples are collected, the small knob in the middle of the pole is pulled outward to release the loop around the snout. Smaller animals are easier to restrain, with both hind and forelimbs tightly held with the help of at least two persons.

2.5.2 Blood Collection and Storage

Blood samples were collected mainly from ears in adult pigs, while jugular veins were used in smaller pigs (Figure 2.1 e & f). To avoid any possibility of cross contamination, fresh sterile needles were used for every animal sampled. In the case of slaughtered pigs, blood samples were collected from blood vessels close to the liver.

2.5.2.1 Ear veins

Ear veins are branches of the caudal auricular and the superficial cervical veins. Pigs have three prominent marginal ear veins. The lateral or central vein is usually the largest of these. Although they are visible on pigs of any size, they are more prominently seen in large healthy pigs, particularly pigs of European origin, as compared to indigenous pigs. To collect blood, the area around the marginal veins was wiped with 70% alcohol. Blood was taken using a 5 ml syringe and 18-22 gauge needles, which were inserted into the peripheral part of the vein (Figure 2.1e). If it was unsuccessful due to movement of the pig or clotting of the blood, a second attempt was made nearer to the head. After blood was drawn, an iodine-soaked cotton compress was used to stem any bleeding. The tube containing approximately 15 mg EDTA as anticoagulant was mixed

immediately by inversion to avoid coagulation. In cold weather, it was very difficult to locate and extract blood from ear veins of pigs.

2.5.2.2 Jugular veins

It was often difficult to locate veins in the ears of indigenous piglets, weaners and growers. Blood from this group of animals was collected from the jugular vein with an 18 gauge needle into a 10 ml plastic EDTA Vacutainer (Becton Dickinson) containing 15 mg EDTA as anticoagulant. The blood was collected caudally and more medially in the jugular groove, nearer the manubrium. It is difficult to say with certainty which vessel was punctured: blood was drawn from either the cephalic, external or internal jugular veins depending on the puncture site, angle and depth of the needle penetration. The syringe and needle were held firmly once the blood started flowing into the needle. The tubes were immediately mixed by inversion to avoid coagulation.

2.5.2.3 Blood Storage

An ice box filled with ice packs was used to store blood immediately after collection in the field. It was later transferred to a refrigerator at 4°for a few days up to a few weeks before the extraction of DNA. Some blood samples were stored at -20°C for a period of up to 8 months (July 2007- February 2008) before the DNA could be extracted.

2.5.3 Hair Sample Collection

Coarse hair with large follicles can be obtained from the switch of the tail and the top of the neck. To collect hair roots, 10-15 hairs are grasped close to the skin as recommended by Rourke (2005). The hairs are wrapped around a finger for leverage and pulled smoothly but with enough force to extract the hairs. This was repeated three to five times to obtain 50-70 hairs with intact follicles. The follicles plus 3-4 inches of hair were retained. After ensuring that hair roots were not covered with faecal matter or dirt, samples were placed in a labelled envelope and immediately sealed to minimise contamination. To reduce the risk of cross-contamination, hands were washed before collecting from the next animal.

2.6 SAMPLES OF OUTGROUP ANIMALS

DNA samples from 15 Australian commercial pigs of mixed ancestry breed (Large White and Landrace) of European origin from QAF Meat Industries Corowa, NSW, were used to provide an outgroup for analyses (Aldenhoven 2006; Cowled *et al.* 2008).

2.7 DNA EXTRACTION

2.7.1 DNA Extraction from Blood

Blood is a convenient and commonly used source of DNA but the predominant red cells in mammals are not nucleated and thus lack DNA. Therefore, DNA was extracted from whole blood using QIAamp® DNA Blood Mini Kit supplied by QIAGEN, Australia. The Blood and Body Fluid Spin Protocol from the QIAGEN handbook (QIAGEN 2003) was followed to extract DNA from both fresh and defrosted blood.

2.7.2 DNA Extraction from Blood using the Salting Method

DNA samples from Sri Lankan pigs were extracted using the salting method (Miller *et al.* 1988) by Dr Pradeepa Silva, Department of Animal Science, the University of Peradeniya, Sri Lanka.

2.7.3 DNA Extraction from Other Sources

2.7.3.1 Serum Samples

Serum samples of two wild boar that were shot at Thinleygang area, Punakha district, during the MoAF wild pig project (MoA 2005) were obtained from the National Centre for Animal Health of Bhutan (NCAH). Using the Blood and Body Fluid Spin Protocol (QIAGEN 2003), a QIAamp® DNA Blood Mini Kit was used to extract DNA from these samples.

2.7.3.2 Hair and Faecal Samples

The Blood and Body Fluid Spin Protocol (QIAGEN 2003) was used to extract DNA from hair samples. DNA from both dried and fresh faecal samples from wild pigs were extracted using the QIAamp DNA Stool Mini kit.

2.7.4 Determination of DNA Concentration

The concentration of DNA, which ranged from 25-200ng/µl, was determined using an ethidium bromide fluorescent staining as described by Sambrook *et al.* (1989). The concentration of DNA was also determined using a NanoPhotometer (18 VDC, 50VA; Serial 1196, IMPLEN, UK) but due to concerns about the reliability of consecutive concentration readings for the same sample, the gel method was preferentially used for determining the DNA concentration from all the samples. This also allowed assessment of DNA sample quality.

2.7.5 Importing Purified DNA Samples

Purified DNA samples from Bhutan were imported to Australia with approval of the Australian Quarantine Inspection Service [AQIS Permit # IP07011173, *Quarantine Act 1908, Section 13 (2AA),* Department of Agriculture, Fisheries and Forestry], the Government of Bhutan's Quarantine Permit (BAFRA/MoAF/1-2/115), and a Material Transfer Agreement (MTA) signed (16/7/2007) between the University of Sydney, the Royal University of Bhutan and the Ministry of Agriculture and Forests (Bhutan). The same AQIS permit was used for importing purified DNA samples from Nepal and Sri Lanka. In addition to the permit, a letter from the University of Sydney was sent to the collaborators in Sri Lanka and Nepal stating that samples would be used only for agreed research. The samples from PNG were imported using AQIS Permit IP09010579 and the MTA signed between the University of Sydney, PNG University of Technology, and National Agricultural Research Institute of PNG.

2.8 FIELD SURVEY

2.8.1 Papua New Guinea

A semi-structured questionnaire was designed to document sex and age of the pigs, their origin, purpose of raising, herd size and structure, the traits preferred by the farmers, body measurements (heart girth, height at withers and body length) and the common pig husbandry practices observed in the rural areas of PNG. The survey included information from 82 households from six districts of three provinces. These sites were chosen because of their abundance of indigenous domestic pig population, which were

presumed to be less affected by the introduction of exotic pigs. The data were compiled, analysed, and the findings have been recently published (Ayalew *et al.* 2011). See Chapter 7, Part II, for details.

2.8.2 South Asia

During the field visit and sample collection, observations on the physical characteristics of Bhutanese indigenous pigs, which are generally described as "non-descript", were recorded across to their distribution in four developmental regions (Eastern, East-central, Western, and West-central regions). In addition, the original phenotypic data (national average) from a previous study (Timsina & Sherpa 2005) on age at sexual maturity, litter size at birth and weaning, farrowing index, body measurement including live-weight of indigenous pigs, were obtained and re-analysed appropriately. Similarly, the original research data (unpublished) of the on-station performance of indigenous and exotic pigs were obtained from the National Pig Breeding Centre in Bhutan. Data ranging from 1986 to 2008 were obtained from the Department of Livestock (DoL), Bhutan were obtained to check the population trend of indigenous pigs. All these data were compiled and analysed and documented accordingly. The findings from this exercise have been published recently (Nidup *et al.* 2011). Details are provided in Chapter 3, Part I.

A similar exercise was carried out on the indigenous pigs of Nepal. Observations during the field visit were recorded, unanalysed population data and data from previous reports were retrieved, and other relevant information including pig farming practices were documented appropriately (Nidup *et al.* 2010). A publication arising from this mini-study is presented in Chapter 3, Part II. Our collaborators in Sri Lanka have carried out a parallel case-study and their findings have also been published (Subalini *et al.* 2010).

All these field studies have provided useful background information, which has supported microsatellite and mtDNA studies on indigenous pigs from South Asia (Chapter 4 & 5) and Papua New Guinea (Chapter 6).

2.9 REFERENCES

Aldenhoven J.T. (2006) Discovery and use of molecular markers in pigs. PhD thesis. *Faculty of Veterinary Science*, p. 255. University of Sydney, Sydney.

Ayalew W., Danbaro G., Dom M., Amben S., Besari F., Moran C. & Nidup K. (2011) Genetic and cultural significance of indigenous pigs in Papua New Guinea and their phenotypic characteristics. *Animal Genetic Resources* **48**, 37-46.

Cowled B.D., Aldenhoven J., Odeh I.O.A., Garrett T., Moran C. & Lapidge S.J. (2008) Feral pig population structuring in the rangelands of eastern Australia: applications for designing adaptive management units. *Conservation Genetics* **9**, 211-24.

Denzin N.K. & Lincoln Y.S. (2000) *Handbook of qualitative research*. Sage Publications, London.

DoL (2007) Livestock population and production bulletin: Data pertaining to year 2005 and earlier periods. (ed. by Department of Livestock MoAF, Royal Government of Bhutan). Department of Livestock, MoAF, Thimphu, Thimphu, Bhutan.

DoL (2008) Livestock Statistics 2007. (ed. by Department of Livestock MoAF). Ministry of Agriculture and Forests, Thimphu, Bhutan.

Miller S.A., Dykes D.D. & Polesky H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* **16**, 1215.

MoAF (2005) Project on Conservation and Management of Wild Pig. Nature Conservation Division, Department of Forest, MoAF, Thimphu, Bhutan.

Nidup K., Joshi D.D., Gongora J. & Moran C. (2010) Farming and Biodiversity of Indigenous Pigs in Nepal. *Biodiversity* **11**, 26-33.

Nidup K., Tshering D., Wangdi S., Gyeltshen C., Phuntsho T. & Moran C. (2011) Farming and Biodiversity of Pigs in Bhutan. *Animal Genetic Resources* **48**, 47-61.

QIAGEN (2003) *QIAamp$^{(R)}$ DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook*. QIAGEN.

Rourke B.O. (2005) Collection of hair samples for DNA testing. (ed. by Industries NDoP), p. 3. Australian Government, NSW.

Sambrook J., Fritsch E.F. & Maniatis T. (1989) *Molecular cloning: A laboratory manual*. Cold Spring Harbour Laboratory, New York.

Subalini E., Silva G.L.L.P. & Demetawewa C.M.B. (2010) Phenotypic Characterization and Production Performance of Village Pigs in Sri Lanka. *Tropical Agricultural Research* **21**, 198-208.

Timsina M.P. & Sherpa D.L. (2005) Characterisation of Bhutan's indigenous pig genetic resources and production systems. Renewable Natural Resources Research Centre, Council of RNR Research of Bhutan, The Ministry of Agriculture and Forests, Bajo.

CHAPTER 3

Part I: Farming and Biodiversity of Pigs in Bhutan

Publication

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Farming and biodiversity of pigs in Bhutan

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Summary
Pigs have socio-economic and cultural importance to the livelihood of many Bhutanese rural communities. While there is evidence of increased religious disapproval of pig raising, the consumption of pork, which is mainly met from imports, is increasing every year. Pig development activities are mainly focused on introduction of exotic germplasm. There is an evidence of a slow but steady increase in the population of improved pigs in the country. On the other hand, indigenous pigs still comprise 68 percent of the total pig population but their numbers are rapidly declining. If this trend continues, indigenous pigs will become extinct within the next 10 years. Once lost, this important genetic resource is largely irreplaceable. Therefore, Government of Bhutan must make an effort to protect, promote and utilize indigenous pig resources in a sustainable manner. In addition to the current *ex situ* conservation programme based on cryopreservation of semen, which needs strengthening, *in situ* conservation and a nucleus farm is required to combat the enormous decline of the population of indigenous pigs and to ensure a sustainable source of swine genetic resources in the country.

Keywords: *Bhutan, biodiversity, conservation, exotic breeds, farming, indigenous pigs*

Résumé
Les porcs ont une importance socio-économique et culturelle pour les moyens d'existence de nombreuses communautés rurales du Bhoutan. Bien qu'il existe des preuves de la désapprobation croissante de la religion pour ce qui est de l'élevage des porcs, la consommation de leur viande, principalement satisfaite par l'importation, augmente chaque année. Les activités de mise en valeur des porcs sont surtout concentrées sur l'introduction de matériel génétique exotique. Certaines indications montrent un accroissement lent mais régulier de la population de porcs améliorés dans le pays. D'autre part, les porcs indigènes représentent encore 68 pour cent du total de la population porcine, mais ils sont en baisse rapide. Si cette tendance se poursuit, les porcs indigènes seront disparus d'ici dix ans. Une fois perdue, cette ressource génétique importante est en grande partie irremplaçable. Par conséquent, le Gouvernement du Bhoutan doit faire des efforts pour protéger, promouvoir et utiliser de façon durable les ressources des porcs indigènes. Pour lutter contre la baisse considérable de la population de porcs indigènes et pour assurer dans le pays une source durable de ressources génétiques porcines, il est nécessaire d'organiser, en plus du renforcement du programme en cours de conservation *ex situ* basé sur la cryoconservation du sperme, la conservation *in situ* et une exploitation de base.

Mots-clés: *biodiversité, Bhoutan, conservation, élevage, porcs indigènes, races exotiques*

Resumen
Los cerdos tienen importancia socio-económica y cultural para el sustento de muchas comunidades rurales de Bután. Si bien existen evidencias de que ha aumentado la desaprobación religiosa de la cría de cerdos, el consumo de su carne, que es principalmente conocido por las importaciones, crece cada año. El desarrollo de actividades relacionadas con el cerdo se centra principalmente en la introducción de germoplasma exótico. Se ha constatado un aumento lento pero constante de la población de cerdos mejorados en el país. Por otra parte, los cerdos autóctonos siguen constituyendo el 68 percent de la población porcina total, pero su número está disminuyendo rápidamente. Si esta tendencia continúa, los cerdos pertenecientes a poblaciones locales se extinguirán en los próximos diez años. Una vez perdido, este importante recurso genético es en gran parte insustituible. Por lo tanto, el Gobierno de Bután debe hacer un esfuerzo para proteger, promover y utilizar los recursos porcinos autóctonos de manera sostenible. Además del actual programa de conservación ex situ, basado en la crioconservación de semen que es preciso reforzar la conservación in situ y el establecimiento de un núcleo de producción, necesario para combatir la enorme disminución de la población de cerdos autóctonos y para asegurar una fuente sostenible de recursos genéticos en la especie porcina en el país.

Palabras clave: *biodiversidad, Bután, cerdos autóctonos, conservación, crianza, razas exóticas*

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Introduction

Bhutan is a small kingdom situated in the Eastern part of the Himalayan range between latitudes 26°45′ N and 28° 10′ N, and longitudes 88°45′ E and 92°10′ E. It is a land-locked country bordered by Tibet (autonomous region of China) in the north, the Indian states of Bengal and Assam in the south, Arunachal Pradesh in the east and Darjeeling and Sikkim in the west.

Bhutan has an area of 38 394 km^2 (14 824 sq mi) with a population of 634 982 (RGoB, 2009). It has 20 districts, which are broadly divided into four developmental regions namely (Figure 1a): eastern region, east-central region, western region and west-central region.

In addition to a rich flora and fauna that make Bhutan one of the ten global biodiversity hotspots, the country is also endowed with diverse domestic animal species including yaks, cattle, horses, sheep, goats, buffaloes, poultry and pigs. Domestic animals are found in almost all the six agro-ecological zones ranging from the subtropical to the alpine region (Figure 1b).

Bhutanese pigs have been an important contributor to human welfare in the past, and may possess characteristics that will be needed again to meet new or re-emerging needs. The loss of these genetic resources would be catastrophic to the livelihood of many poor rural communities. Therefore, it is urgent to draw up an inventory and understand the nation's pool of swine resources for promotion and sustainable utilization as envisaged in Bhutan 2020 vision (PCS, 1999).

Objectives

To document the socio-economic and cultural importance of pigs to the Bhutanese people, review current state of rural pig farming and its development initiatives, and assess the biodiversity and population trends of both improved and indigenous pigs. This paper will provide baseline information for future studies.

Importance of pigs in Bhutan

Bhutan has both indigenous and imported exotic breeds of pigs. The former are also called native or local to distinguish them from exotic breeds. The exotic breeds are frequently crossed with indigenous animals to generate composite breeds, commonly called "improved breeds" that are considered an upgraded form of the indigenous breed with a good blend of "superior quality" exotic germplasm. To be consistent with the terminology used within the country, both exotic and composite breeds are hereafter referred to as "improved breeds".

Pigs are found throughout Bhutan, despite the strong Buddhist sentiment against rearing and slaughtering of pigs. The Southern Bhutanese, who are mainly Hindus, consist of multiple ethnicities with a caste system and pigs are reared by certain ethnic groups only (Rai, Limbu, Magar, Tamang, Sherpa, Tharu and Biswakarma). Unlike the Buddhist, there is little religious disapproval of raising and slaughtering of pigs among Hindus. Irrespective of ethnicities, pig raising has been economically beneficial, particularly to the rural poor and socially disadvantaged people (Timsina and Sherpa, 2005).

Bhutanese rear pigs for many purposes, including social, cultural and economic reasons. Traditionally, pig ownership and slaughtering conveyed status, wealth and informal power. In the early Bhutanese cashless society, pigs were a very important medium by which social significance was measured. Meals served with pork promoted group cohesion and identity, and facilitated civic and private celebrations. Even today, pork is one of the vital components of Bhutanese cuisines, be it during marriages, festivals or New Year celebrations. Penjor (2008) provides an account of the important roles of pigs during marriages in the lower Kheng of the Zhemgang district. In remote villages, people still carry pigs from one place to another either as a gift or in exchange for other commodities.

Pigs also serve as sacrificial animals, as votive offerings to local deities (*Yul-Lha* or *Naep*). Bonism and Shamanism (native religion of Tibet) rituals, which still prevail in many parts of Bhutan, require the sacrifice of pigs to the local deities for bountiful crops, to reduce the risk of natural calamities, to improve the health of an ailing person, and for peace, happiness and prosperity of the community. Such practices in the Bongo village of the Chukha district and in the Trashi Tokha village of Wangdue district have been documented by Wangchuk (2005) and Dorji (2004), respectively. Animal sacrifice provides a good source of protein for those involved in the rituals, which in some cases involve distribution of meat immediately after the sacrifice.

Consumption of pork is well imbedded in Bhutanese gastronomic tradition and continues to rise (DoL, 2007), despite increasing prices per kg of pork. The current cost of a kg of pork sold with bones intact is about Nu. 100 (~US$2.00). The consumption of pork is increasing every year (Figure 2) while domestic production remains static.

Over the last five years, pork importation has increased significantly, more than threefold, in contrast to a negligible rise in domestic production. This averages Nu. 73.48 million (US$1.63 m) per year (DoL, 2007) contributing significantly to the trade deficit of the national economy.

Origin of indigenous pigs

It is not recorded when domestic pigs were introduced to Bhutan or who introduced them nor is there archaeological evidence. Linguistic evidence shows that there are not many words for pig in Bhutan despite several dialects.

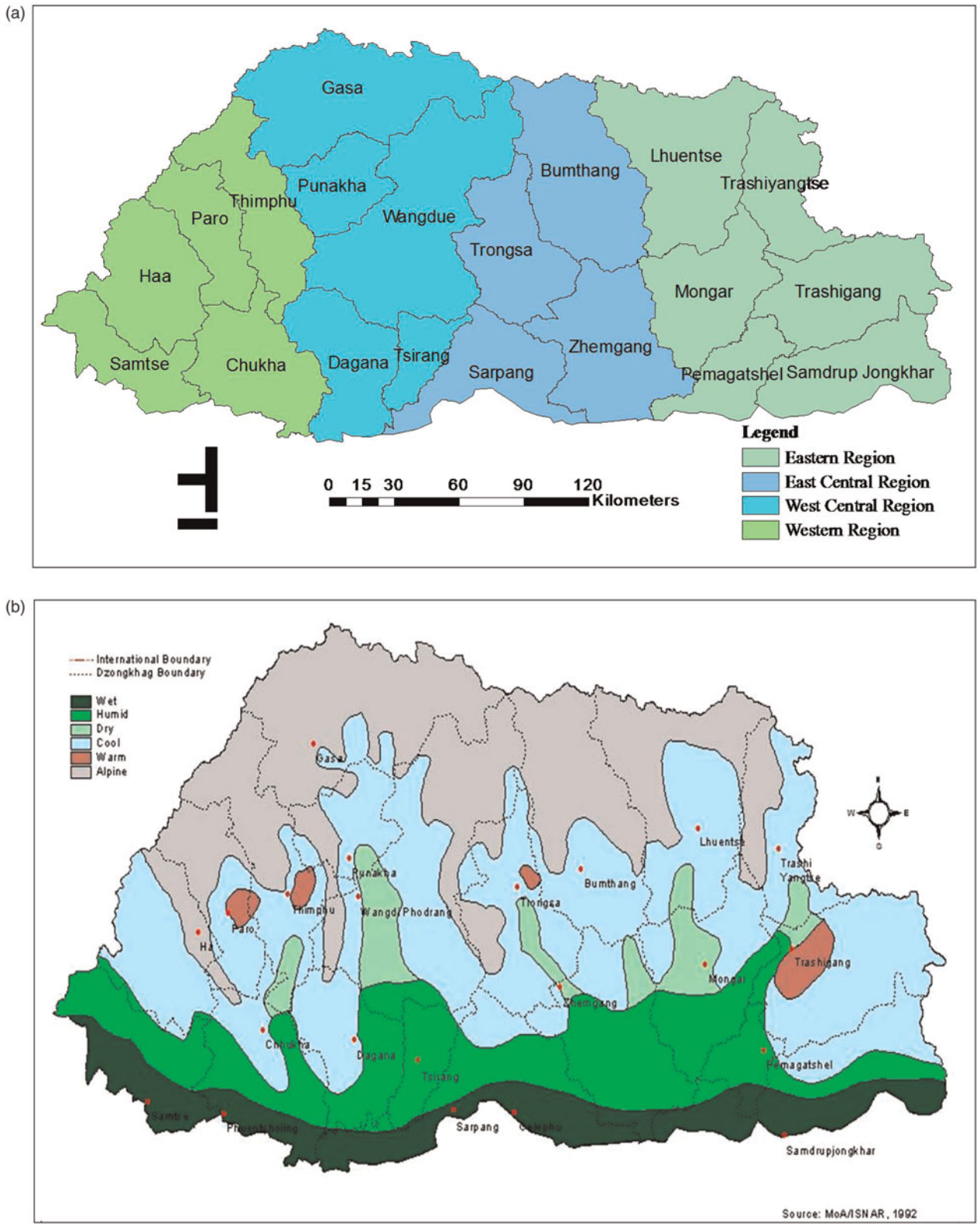

Figure 1. (a) Twenty districts of Bhutan according to regions (Courtesy: Dorji, 2010a); (b) agro-ecological zones in Bhutan (MoA/ISNAR, 1992).

This suggests that pigs could have been introduced from one particular region and spread slowly towards other parts of the country relatively recently. Timsina and Sherpa (2005) suggest that indigenous domestic and wild boar (*Sus scrofa*) could be considered to share a common genetic pool due to mating between village sows and wild boars. Feral pigs that are domestic pigs, which have escaped captivity, have not been reported in Bhutan.

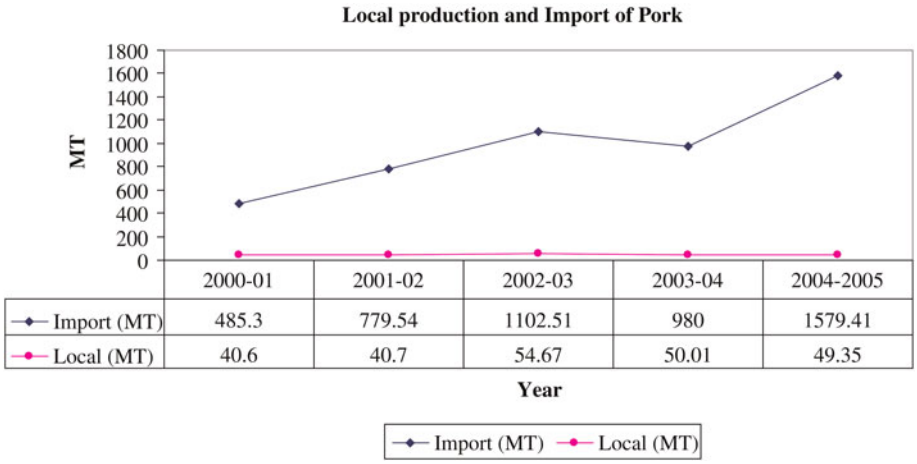

Figure 2. Trend in pork consumption.
Source: Unpublished data from Department of Livestock (DoL, 2007).

A recent study of mitochondrial DNA sequences from 30 domestic and 3 wild pigs suggest three origins for Bhutanese pigs. (i) East Asia probably Tibet or China, (ii) Southeast Asia and (iii) East Indian wild boars (Tanaka *et al.*, 2008). The East Asian type was found to be distributed widely in Bhutan, whereas the Southeast Asian types were found only in the Mongar district. The native pigs in the southwest part of Bhutan were found to have experienced gene flow from East Indian wild boars. Because the sample size used in the study (Tanaka *et al.*, 2008) was small, further investigation is required to provide more comprehensive information on the origin of the indigenous pigs of Bhutan.

Rural pig farming in Bhutan

Pig farming in Bhutan is typically divided into two systems: the backyard pig farming seen in the villages and modern intensive farming seen in state operated farms. The village farming is normally characterized by small numbers of pigs reared by the subsistence farmers, either in a small confined pigsty constructed usually with locally available materials (stones, mud, wood, bamboo thatch) or pigs are tethered near the house or in a paddock. While farmers are required to enclose their pigs to comply with national health regulation, some still allow free-range scavenging for various reasons ranging from scarcity of feeds to the ease of management. More than 13percent of Bhutanese farmers rear pigs as free-range scavenging pigs (Timsina and Sherpa, 2005). Feeds consist of mainly brewery wastes, kitchen wastes (leftover foods, vegetable peels), bran (maize, millet and rice), wild weeds, nettle leaves, pumpkins, yams and taro. Oil cakes, flour and maize grain supplements are used to fatten pigs.

The indigenous pigs are hardy, resistant to many diseases and can adapt to harsh rural environment under low inputs (Timsina and Sherpa, 2005). Under scavenging, they have better mothering ability and increased survival of litters per farrowing than exotics (Timsina and Sherpa, 2005).

Exotic pigs and development programmes

Realizing the importance of pig farming in the livelihood of rural poor, the Royal Government of Bhutan (RGoB) initiated development programmes to improve pig production. Several exotic breeds of European origin have been introduced to the country since the early 1960s. The main objective was to generate lines of improved piglets or F1 (exotics vs local). The purebred progeny or F1 were hoped to have better production than pureline indigenous stock. The overall goal of the programme was to improve nutritional status of the rural population, increase income and alleviate poverty through increased meat and protein production.

The first exotic breed, Wessex Saddleback, was introduced to Bhutan during the First Five-year Plan (1961–1965) and reared in Samtse and Wangchutaba livestock breeding farm. Subsequently, Large White (Yorkshire) was introduced towards the end of the First Five-year Plan. This was followed by introduction of Landrace, which was imported from India. The focus on the white breeds aimed to exploit their relatively large litter size, higher growth rate and earlier sexual maturity than the indigenous pigs or most coloured exotic breeds.

The RGoB formulated another phase of the piggery development programme in 1981. Through a (United Nations Development Programme) UNDP/FAO (Food and Agricultural Organization)-funded project, 44 Duroc Jersey pigs were imported from the Philippines in 1981. With further assistance, Bhutan imported 30 head of Large Black from Australia in 1985, followed by 24 more Large White and Duroc Jersey from Bangkok, Thailand. These high productive breeds of pigs were reared in the government central farms at the National Pig Breeding Centre (NPBC), at Serbithang, and the Regional Pig and Poultry Breeding Centre (RPPBC) at Lingmithang (Mongar) and Gelephu. Various crosses were produced and the piglets were sold to the farmers at a government subsidized rate of Ngultrum 672.00 (~US$15.00) for a piglet weaned at 35–42 days.

While there were reports of difficulty in management of exotic piglets at the village level, the major problem was the colour of the Large White and the Landrance pigs. Many white pigs suffered severe sunburn, with inflammation followed by scabbing and necrosis. The white parts of the body became reddened, oedematous and irritable, and the animals appeared to be in pain. The presence of reddening blistering and peeling of skin on the dorsal surface and flanks is an indication of exposure to sunlight and poor sanitation. In adults, exposure to such extreme environment would reduce fertility and prevent mating. Most white pigs with skin diseases had low production and some died in severe cases. Consequently, white pigs are unpopular among farmers in Bhutan. In practice, white pigs should be given good feed, shade, plenty of water and access to wallow. Figure 3a shows poor body conditioning and skin problems with government supplied white pig of Landrace origin. Hybrids (Figure 3b) thrive better than exotics whereas pure indigenous are the best suited under harsh rural environment.

Figure 3. Body conditioning of three different breeds of pigs reared in similar housing condition in Rinchengang village. Government supplied white pig of Landrace origin (a); hybrid of Saddleback origin (b); and pure indigenous with agouti coat (c).

Considering these problems, the RGoB changed its approach by supplying coloured animals of the Large Black, Saddleback and Duroc breeds to the farmers. In 2000, a review was conducted on the status of this exotic pig germplasm in the country. It was found there were no proper records maintained on this pureline exotic germplasm in the country. Subsequently, coloured pureline breeds, namely Large Black, Saddleback and Duroc, were imported from the United Kingdom in 2003 with the assistance of FAO. Today, these pureline breeds (Figure 4) are carefully bred in the nucleus farm at Gelephu.

Biodiversity of pigs in Bhutan

At least four types of indigenous pigs (Dempha, Dromfak, Sofak and Jitu) have been reported in FAO's Domestic Animal Diversity Information System (DAD-IS) (FAO, 2010). However, caution must be taken as there are no evidences or adequate rational to this form of categorization. During the nationwide blood sampling of indigenous pigs for genetic study (Nidup *et al.*, 2009, 2010), it was observed that Bhutanese indigenous pigs were generally non-descript. Their physical characteristics are described briefly based on distribution across four developmental regions in the country.

Eastern region pigs

Eastern Bhutan constitutes six districts namely Mongar, Lhuntse, Tashigang, Pemagatshel, Samdrup Jhongkhar and Tashiyangtse (Figure 1). Most of the pigs found in Eastern Bhutan (Figure 5) have long dense hair, whereas some have sparse hair, medium-sized body, bristles along the dorsal line, medium snout, medium-sized prick ears and curly to straight tail. Some of the indigenous pigs in Tashiyangtse are found to have white forehead and coat around their shoulders (Figure 5b).

East-central region pigs

The east-central region constitutes four districts, namely Sarpang, Zhemgang, Trongsa and Bumthang (Figure 1). Almost no pigs are present in Bumthang and very few pigs are found in Trongsa. Pigs from Bardo (Figure 6a) in the Zhemgang district have medium-sized body, sparse

to medium hair density, medium-sized prick ears, straight snout, mature females have a sagging belly and most have a long straight tail.

Most pigs in the Sarpang district (Figures 6c and d) are not indigenous but illegally imported across the Bhutan-Assam (India) border. These smuggled pigs are called "Machay Sunggur" after one of the tribes of the Indian state of Assam.

Similarly, pigs found in Darla and Sampheling in the Chukha district are called "Madhuri", which is another phenotypically similar pigs smuggled through Bhutan West Bengal (India) border. For simplicity, these similar looking smuggled pigs will be termed "Machay madhuri" in Bhutan. Machay madhuri are also becoming increasingly popular in other parts of the country particularly in west, west-central and east-central regions. Machay madhuri have similar phenotypic characteristics to Pakhribas and Kalo Dharane Sunggur of Nepal (Nidup *et al.*, unpublished). Machay madhuri with shorter snouts look similar to Pakhribas and the longer snout to that of Kalo

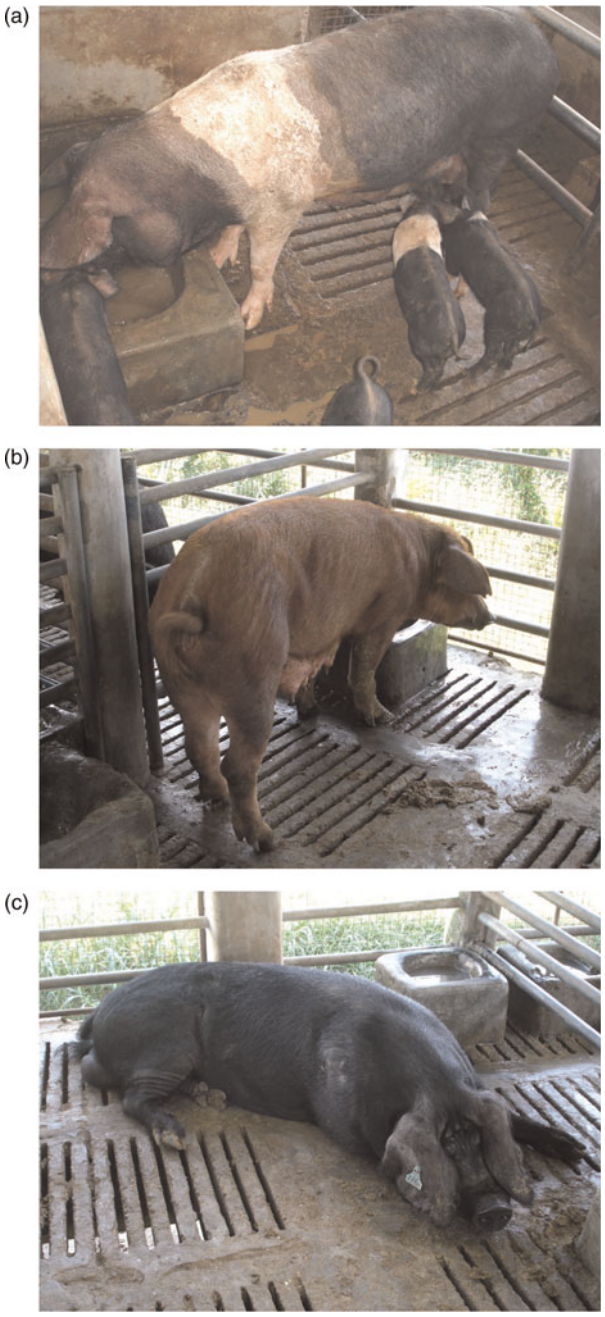

Figure 4. Saddleback (a) and Duroc (b) and Large Black (c) in Gelephu nucleus farm (Courtesy: Dorji, 2010b).

Figure 5. Eastern region pigs: indigenous pigs in Ramjhar, Tashiyangtse (a, b); and indigenous grower in Uzorong, Tashigang (c).

Figure 6. East-central region pigs: (a)indigenous pigs found in Bardo, Zhemgang. Machay madhuri pigs found in Trong, Zhemgang (b), Sarpang (c) and Dekiling, Sarpang (d).

Dharane Sunggur. The most common characteristics of Machay madhuri are wrinkled and diamond-shaped face, large floppy ears and firm body.

Western region pigs

The western region constitutes five districts namely Chukha, Thimphu, Haa, Paro and Samtse (Figure 1). The most common characteristics of pigs (Figure 7) in this region are straight hair ranging from sparse to dense, short to medium with some cylindrical-shaped snout and most with short to medium-sized prick ears.

There is not much difference between the pigs found in Chukha, Haa and Paro districts. Paro and Haa pigs had slender body length. Machay madhuri pigs were also found in Chukha and Samtse districts.

West-central region pigs

The five districts in the west-central region are Gasa, Punakha, Wangdue, Dagana and Tsirang (Figure 1). There are virtually no indigenous pigs in Gasa, only a few head of exotic pigs supplied from the central farm. Some differences between indigenous pigs in Rinchengang and Phagyul-Kazi in the Wangdue district were seen. Rinchengang pigs (Figures 8a and b) have bristles along the dorsal line, broad rectangular-shaped body with females having a slightly sagging belly, small-to-medium-sized prick ears, medium snout and dense hair. On the other hand, Phangyul-Kazi pigs (Figures 8c and d) have longer bodies bristles along the dorsal line, medium to slightly large ears, most with prick ears but some with slightly droopy ear, a somewhat cylindrical snout and long straight tail.

The Dagana district has diverse pigs. For instance, pigs from Lhamoizingkha (Figures 8f, g and h) have sparse hair, prominent prick ears and pointed head, whereas pigs from Drujegang (Figure 8e) are slightly smaller with dense hair over their entire bodies. Some live piglets from Drujegang are usually sold at a weekly open market in Tsirang. For this reason, there are similarities between Drujegang and Tsirang pigs.

Characteristics of indigenous pigs

The official nationwide survey on the characterization of indigenous pigs (Timsina and Sherpa, 2005) concluded that there was only one type of indigenous pigs in Bhutan. It was based on the phenotypic similarities of indigenous pigs across the country. The phenotypic data that were merged to obtain a national average shows indigenous pigs attain sexual maturity at nine months of age. The litter size at birth and weaning is 6.0 and 5.0, respectively, with 2.0 farrowing index. In general, males have longer snouts and ears than their female counterparts (Table 1).

The live weight of indigenous pigs was estimated based on body length and heart girth measurements. The males are bigger and heavier than females. The live weight of indigenous pigs in various age groups is given in Table 2.

However, caution should be taken with the above findings because of the method used for compilation and analysis of

Figure 7. Pigs found in Western Bhutan. Pigs of Bongo (a, b, c, d) and Darla (e, f, g, h) both in the Chukha district; Dogar (i, j) and Naja (k) both in the Paro district; and Katsho in the Haa district (j).

the data. In consistent geographical pattern of morphological variation does not imply lack of variation. For instance, European sheep breeds are readily distinguishable phenotypically but they do not possess that much genetic variation (Peter *et al.*, 2007). On the other hand, sheep breeds in the Middle-East are all of the "generic type" phenotypically and not easy to tell apart, but they display much more genetic variation than European breeds (Peter *et al.*, 2007). Similarly, Bhutanese indigenous pigs may retain high levels of genetic variation and potentially variation in productive ability regardless of the fact that there is no obvious portioning into breeds. Therefore, a nationwide survey to record on-farm production and phenotypic characterization of indigenous pigs across the country would be required once again.

On-station performance

A breeding trial was conducted at NPBC (MoA, 1999) using exotics boars and indigenous female lines (Table 3). The performance of indigenous sows (mated with exotic boars) was better than exotics in terms of average litter size and piglets weaned per sow. The average daily weight gains of the piglets were more similar. However, piglet and maternal mortality was caused by a high incidence of dystocia since large exotic boars were mated with smaller indigenous sows. On the other hand, there was no piglet mortality seen with pureline indigenous (Table 3) whereas high percentage of piglet mortality (22 percent, Table 3) was observed with pureline exotic breed. The trial suggests that the overall performance of indigenous pigs under good management (feeding,

Figure 8. West-central pigs. Indigenous pigs found in Rinchengang (a, b) and Phayul-kazi in the Wangdue district; Drujegang (e) and Lhamoizingkha (f, g, h) in the Dagana district; crossbreds (i, j, k, l) found in various parts of west-central region.

housing and sanitation) is reasonably comparable with the exotic breeds. In spite of this, the RGoB did not make an attempt to improve indigenous pigs. Instead, it constantly pursued its policy of importation and introduction of exotic livestock into the country.

Population of pigs in Bhutan

Overall population

The populations of exotic and composite breeds have been merged as "improved breeds". The overall pig population recorded in 1986 was 87 987 and this reduced to 27 501 in 2008.

The western part of the country has the highest number of pigs followed by west-central and eastern regions (Table 4). Today, Chukha district has the highest overall pig population whereas Gasa has recorded the least number of pigs (Figure 9). As expected, east-central region, particularly Bumthang and Trongsa districts, recorded the least number of pigs because of increasing Buddhist sentiments against raising and slaughtering of pigs.

Bumthang, which is the most religious centre, was the first district to prohibit pig farming. Trongsa, a historically important district, which shares its border with Bumthang, is influenced by the largest monk body in the country. Similarly, pig farming is becoming increasingly unpopular in the eastern region (Table 4) due to the influence of

Table 1. Body measurements of indigenous pigs according to age and sex groups.

	<1 year female		<1 year male		1–2 years female		1–2 years male	
Measurements	***N***	**Mean**	***N***	**Mean**	***N***	**Mean**	***N***	**Mean**
Ear length (cm)	13	7.31 ± 0.75	12	7.63 ± 0.55	17	9.18 ± 0.49	10	9.55 ± 0.73
No. of teats	13	10.31 ± 0.21			17	9.53 ± 0.21		
Tail length (cm)	13	14.23 ± 1.73	12	17.58 ± 1.20	17	20.29 ± 1.43	10	17.7 ± 1.92
Body height (cm)	13	60.69 ± 3.13	12	64.42 ± 3.64	17	74.35 ± 5.38	10	86.9 ± 6.53
Heart girth (cm)	13	52.46 ± 2.93	12	56.67 ± 3.27	17	72.06 ± 4.51	10	84.7 ± 7.93
Shoulder height (cm)	13	39.92 ± 3.88	12	37.5 ± 2.29	17	49.88 ± 1.84	10	51.4 ± 3.25
Face length (cm)	13	19.31 ± 0.91	12	20.17 ± 0.94	17	24.97 ± 1.05	10	25.1 ± 1.34
Measurements	>2-year female	> 2-year male						
Ear length (cm)	8	9.5 ± 0.46	3	12.33 ± 1.20				
No. of teats	8	10 ± 0.0						
Tail length (cm)	8	19.75 ± 1.06	3	26.33 ± 2.19				
Body height (cm)	8	84.75 ± 2.58	3	107.33 ± 6.69				
Heart girth (cm)	8	82.25 ± 2.09	3	95.67 ± 9.94				
Shoulder height (cm)	8	51.63 ± 4.93	3	62.67 ± 5.21				
Face length (cm)	8	26 ± 1.74	3	28.67 ± 2.19				

Source: Generated from the original data (national average) obtained from Timsina and Sherpa (2005).

religious communities. However, there is no sign of reduction of pork consumption in these regions.

Looking at the national trend, the pig population in the country declined sharply from 1986 to 1995 (Figure 10) with slight increase between 1993 and 1996. After 1996, the population declined steadily but seems to have plateaued since 2006.

The possible reasons for this decrease in population are increased influence of religion on animal slaughter and growing social stigma against pig farming. Other reasons include shortage of feeds and increased purchasing power of the people coupled with availability of freshly imported pork. While overall pig population is decreasing, the population of improved breeds is slowly increasing (Figure 11).

Increasing improved pig population

In spite of a declining overall pig population in the country, improved breeds of pigs are slowly increasing in numbers (Figures 11 and 12). Today, improved breeds constitute about 38percent of the total pig population with 5 383 males and 5 159 females when compared with a total of 2 055 heads in 1986. There was gradual increase in the population from 1986 to 1992 which picked up between 1992 and 2008 (Figure 12).

Table 2. Live weight of indigenous pigs according to age and sex.

Age group (years)	**Sex**	***N***	**Mean**
<1	Female	13	12.64 ± 2.05
<1	Male	13	14.40 ± 2.37
1–2	Female	17	30.38 ± 4.99
1–2	Male	10	51.10 ± 11.20
>2	Female	8	40.07 ± 2.72
>2	Male	3	71.80 ± 17.00

Calculated based on body length and heart girth measurement (Table 1).

Records from the three government breeding farms indicate distribution of approximately 20 000 improved piglets in the last six years alone (Table 5).

Today, Chukha has the highest number of improved pigs followed by Mongar, Thimpu, Wangdue and Sarpang (Figure 13). The high record of pig population in Mongar, Sarpang and Thimphu is due to the presence of government farms where exotic or improved breeds are reared.

Despite of the intensity of introduction of exotic breeds since 1964, the current number of improved pigs in Bhutan is relatively low but increasing steadily. There are several factors to this slow pace of growth. First, because of the prolific growth rate of exotic pigs, the government supplied piglets were often fattened and slaughtered instead of being used for crossbreeding. Exotic breeds can attain market weight of 100 kg in less than a year. This fetches up to Nu. 10 000 (=~US$220.00), which is more than average annual rural income (Nu. 7 488 = ~US$166.40) of the Bhutanese farmers. Second, increasing incidence of "dystocia" or farrowing difficulty has been observed when crossbreeding exotic boars with indigenous sows and gilts leading to high piglet and maternal mortality. Finally, exotic breeds require good housing, sanitation and relatively good feed, and are more vulnerable to diseases when compared with local pigs. The mortality rate of exotic breeds is higher than indigenous pigs. In spite of these bottlenecks and slow population growth, the increase in numbers of improved pigs is seen as a threat to the survival of indigenous pigs.

Alarming loss of indigenous pig population

The status of indigenous pigs is alarming. More than 85 932 indigenous pigs were recorded in 1985 and this reduced to 16 959 in 2008 comprising 9 863 males and

Table 3. Performance evaluation of indigenous sows (mated with various exotic boars), pureline indigenous and exotic breeds at NPBC, Serbithang (NPBC, unpublished data)

Parameters	DU ♂ X native ♀	LB ♂ X native ♀	LW ♂ X native ♀	SB ♂ X native ♀	Native ♂ X native ♀	Exotic ♂ X exotic ♀
No. of sow farrowed	23.00	4.00	8.00	11.00	1.00	5
Average litter size at birth	6.87	7.75	8.25	9.00	8.00	7.6
Average birth weight (kg)	0.93	1.09	1.03	0.98	0.70	1.06
Average piglets weaned	6.35	6.75	7.38	8.18	8.00	6.07
Piglet mortality (%)	7.57	12.90	10.55	9.11	0.00	20.13
Average weaning age (days)	54.94	45.75	40.50	46.90	45.00	42
Average weaning weight (kg)	6.53	7.03	6.52	6.48	5.00	7.06
Average daily weight gain (kg)	0.10	0.13	0.14	0.12	0.10	0.14

Note: DU = Duroc; LB = Large Black; LW = Large White; SB = Saddleback.

Table 4. 2008 Pig populations according to the regions.

Regions	Indigenous		Improved		Total
	Male	Female	Male	Female	
Eastern	1 873	1 746	1 414	1 506	6 539
East-central	919	648	633	900	3 100
Western	4 519	2 540	2 375	1 689	11 123
West-central	2 552	2 162	961	1 064	6 739
Overall Pig Population					27 501

Source: Unpublished data from DoL (2009).

7 096 females (Figures 14 and 16); a fivefold loss in the last two decades alone (Figure 14).

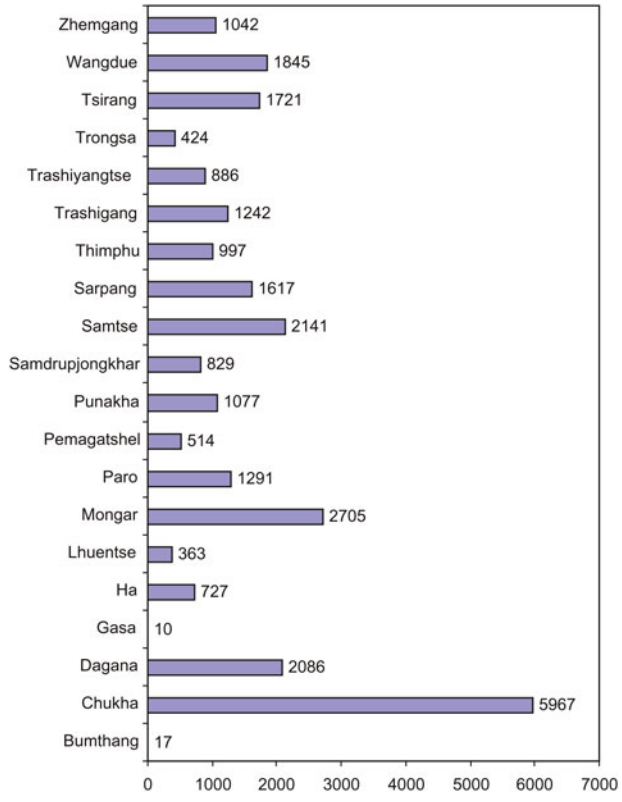

Figure 9. Current population of pigs across all 20 districts.
Source: Unpublished data from DoL (2009).

There were similar observations from the farmers in the field. In a mini survey ($n = 55$) conducted by Nidup (unpublished) in Haa, Wangdue, Samtse and Tashiyangtse districts, more than 56 percent of the farmers felt that the population of indigenous pigs is decreasing.

Today, of 20 districts in Bhutan, only 7 have equivalent to or more than 1 000 indigenous pigs. Chukha district has the highest number of indigenous pigs followed by Samtse, Dagana, Tsirang, Wangdue and Mongar. All other districts have less than 1 000 animals (Figure 15). Indigenous pigs in the Bumthang district are completely extinct.

There are more males than female (Figure 16) in almost all the districts in Bhutan because male pigs (*Pho pha*) are usually used as sacrificial animals. In addition, most males are castrated, fattened and slaughtered for various purposes. Since there is limited information on the number of breeding males, it is difficult to determine the risk status of indigenous pigs in Bhutan.

The rapid decline of indigenous pigs coupled with steady increase in the number of exotic pigs (Figures 11, 12 and 14) is a clear evidence of marginalization of indigenous population by the exotics. Such widespread practice that threatens the indigenous populations, particularly the pigs, is also evident in Sri Lanka and Nepal (Nidup and Moran, 2010, Subalini *et al.*, 2010). Considering the current population trend (Figure 14), indigenous pigs in Bhutan are likely to become extinct within the next decade, unless a serious effort is made in conservation,

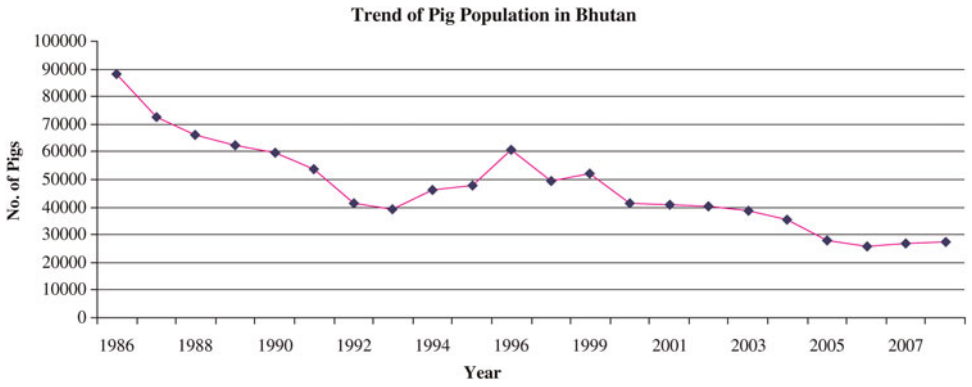

Figure 10. Pig population trend in Bhutan.
Source: Unpublished data from DoL (2007, 2008a, 2008b, 2009).

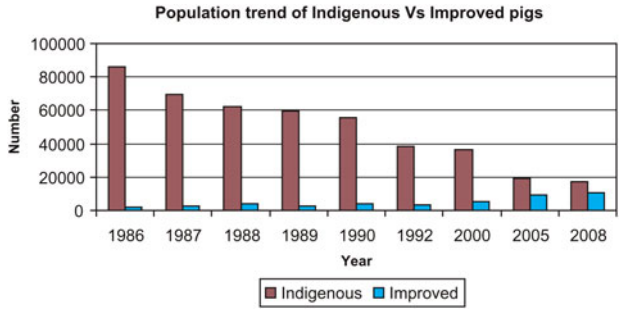

Figure 11. Population trend of indigenous and improved pig breeds.
Source: Unpublished data from DoL (2007, 2008a).

promotion and sustainable utilization of these important genetic resources.

Conclusion and recommendation

Pigs have socio-economic and cultural importance to many Bhutanese people, particularly the rural poor communities. Domestic pork production has remained constant, whereas imports have increased several folds contributing to a large trade deficit for the country. Government initiatives to improve pig farming are mainly focused on introduction of exotic breeds to crossbreed with indigenous pigs. Despite several bottlenecks and slow pace of population growth rate of exotic pigs, their presence in the country is now a threat to the survival of indigenous pigs. This is a clear evidence of growing marginalization of the indigenous pigs mainly driven by the introduction of exotic breeds. In addition to this, religious disapproval of pig breeding and slaughter, increasing purchasing power of the people and readily available imported pork are factors accelerating the reduction in the population of indigenous pigs in the country. Bhutan has lost more than 60 486 heads of pigs since 1986.

Bhutan can draw a lesson from a bird flu outbreak in the bordering Indian states, during which import of poultry and related products from India and other countries were banned (Nidup and Tshering, 2007). The cost of poultry-related products increased dramatically due to limited production within the country. As a result, many people including middle-income earning could not afford to buy eggs (Nidup, 2008), let alone chicken meat which were virtually unobtainable. Similarly, a ban on live pigs and pork imports because of swine flu or other related

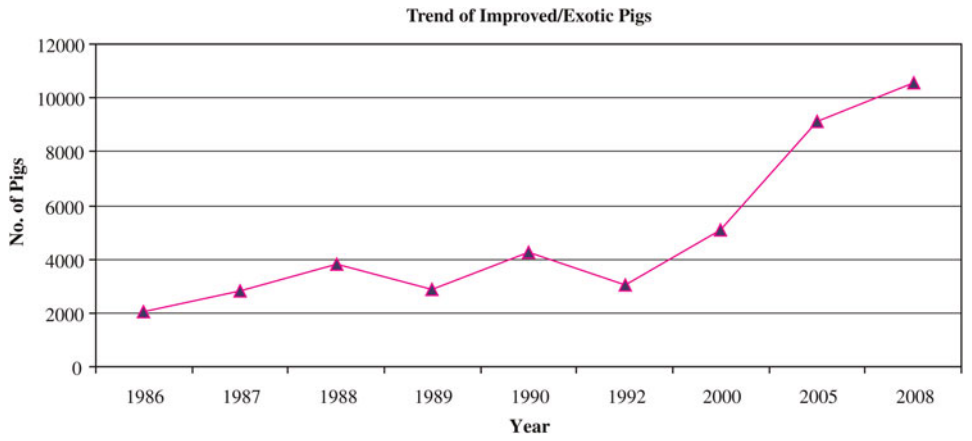

Figure 12. Increasing trend of improved breeds of pigs in Bhutan.
Source: Unpublished data from DoL (2007, 2008a).

Table 5. Record of piglets supply from government breeding farms (unpublished data)

Year	[1]NPPBC Thimphu	[2]RPPB Lingmithang	[2]RPPBC Gelephu
2003–2004	1 465	Not available	662
2004–2005	1 446	30	670
2005–2006	1 032	112	696
2006–2007	1 089	6 350	848
2007–2008	984	623	796
2008–2009	1 038	1 070	1 063
Total	7 054	8 185	4 735
Overall total piglets supplied			19 974

[1]National Pig Breeding Centre.
[2]Regional Pig and Poultry Breeding Centre.

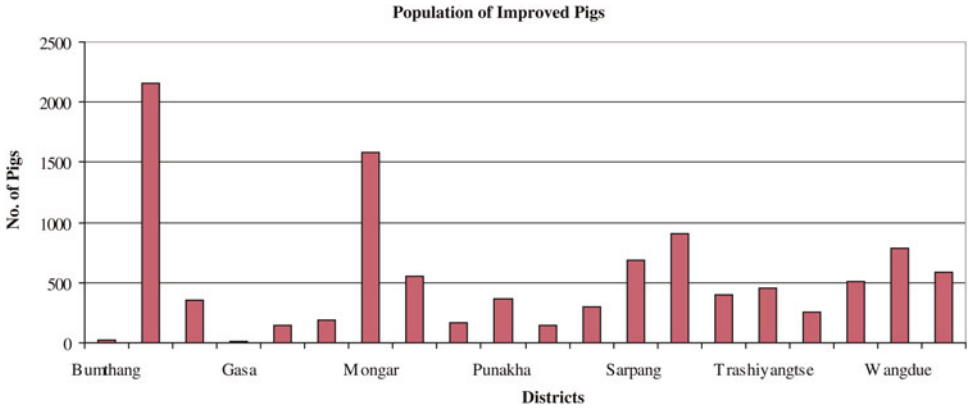

Figure 13. Distribution of improved pigs across 20 districts in the country. *Source:* Unpublished data from DoL (2009).

outbreak of diseases in the neighbouring countries, and coupled with already depleted indigenous pig genetic resources, would endanger the food security of the country. Therefore, better understanding of the diversity of indigenous pig resources, their value and the environment in which they are reared is crucial so that government and other stakeholders will be able to fully appreciate this biodiversity and make strategic decision for its conservation and sustainable use.

In an effort to implement the *Global Plan of Action* (FAO, 2007a, 2007b), indigenous pigs should be protected, promoted and utilized in a sustainable manner. They have been genotyped using FAO and International Society for Animal Genetics recommended microsatellite markers (Nidup *et al.*, 2009, 2010) and their mitochondrial DNA sequences are currently being analysed. These will provide baseline for both *in situ* and *ex situ* conservation. The *in situ* conservation includes protected areas or conservation farms, and payment of other support (e.g. subsidy) for those who keep rare breeds within their production environment. Cryopreservation or *ex situ* conservation of genetic material can provide a valuable complement to *in situ* approaches.

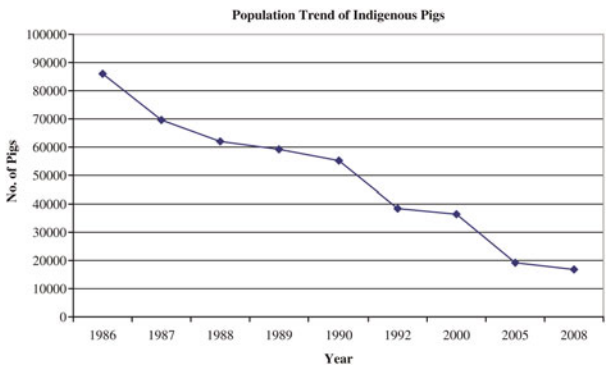

Figure 14. Population trend of indigenous pigs of Bhutan. *Source:* Unpublished data from DoL (2009).

Efforts should be made to coordinate conservation activities, such as the participation of local communities, government institutions and NGOs. For instance, *in situ* conservation through community-based approach can be one of the viable options. Such strategy has been proven successful in maintaining indigenous Pelong pigs and Creole breed in rural Mexico (Pattison, 2002; Pattison *et al.*, 2007). The National Biodiversity Centre (NBC) of the Ministry of Agriculture (MoA) has national mandate on the conservation of biological resources in the country. They should work together with the Department of Livestock (DoL), MoA, to establish conservation and nucleus farm so as to combat the dramatic decline of population of indigenous pigs in the country. The current *ex situ*

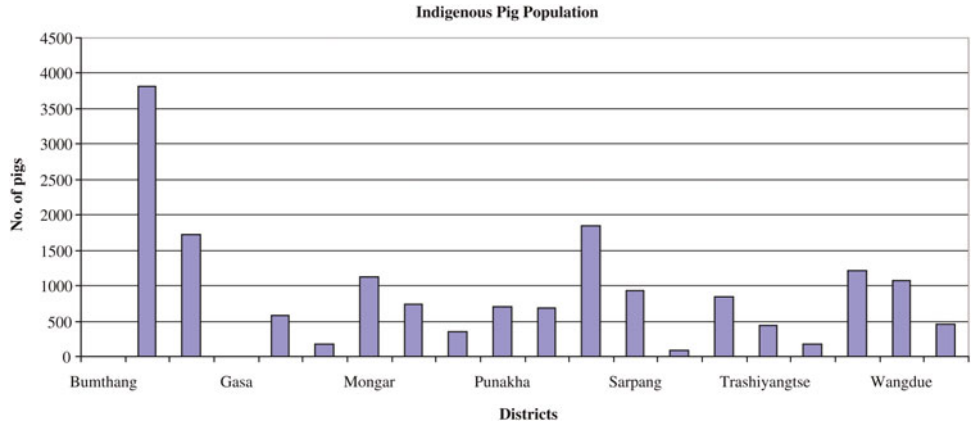

Figure 15. Distribution of indigenous pigs in 20 districts of Bhutan.
Source: Unpublished data from DoL (2009).

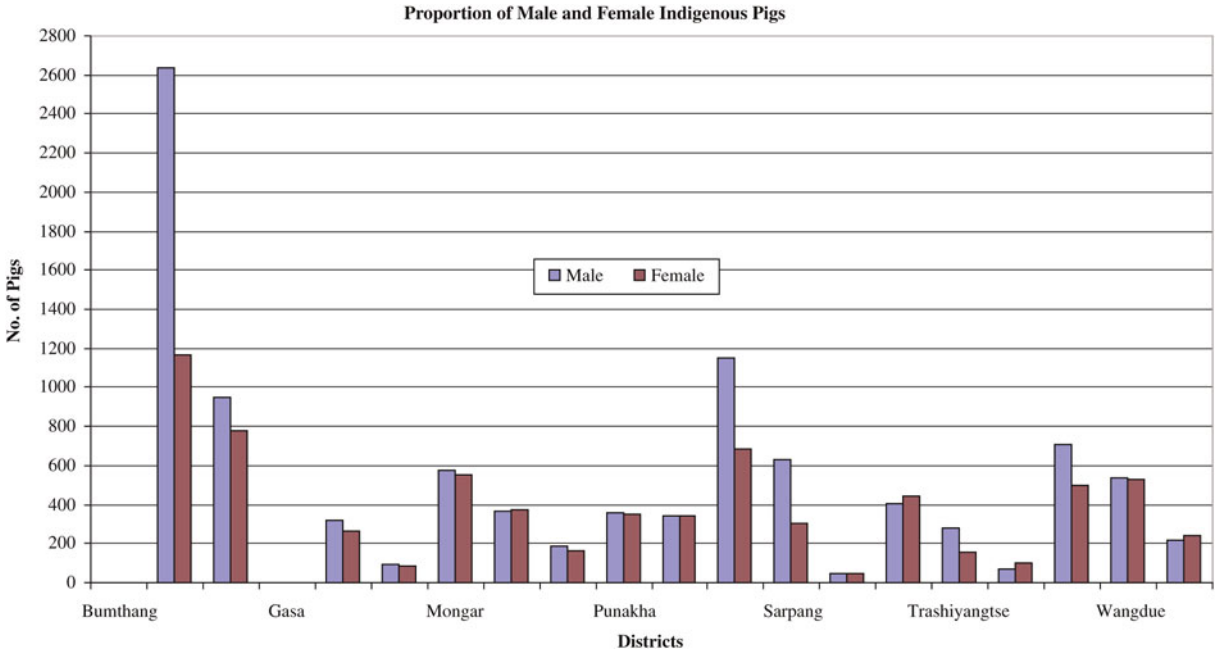

Figure 16. Proportion of male and female indigenous pigs across 20 districts of Bhutan.
Source: Unpublished data from DoL (2009).

conservation or cryopreservation facility at NBC needs to be strengthened with appropriate facilities including human resources. In addition, the Department of Livestock should start the recording number of breeding males and females in the Annual Livestock Census record so that risk status of indigenous pigs could be easily determined.

On the other hand, the livestock research institution should make an attempt to develop a synthetic breed with better litter size, growth rate, resistance to diseases and ability to cope with the harsh rural environment. While the Pakhribas breed in Nepal (Nidup *et al.*, unpublished) is a good example, synthetic breeds containing a reasonable level of indigenous genes could provide viable source of parent stocks for meeting the consumption requirements of Bhutan, although this would do little to conserve biodiversity.

Finally, the role of local and indigenous communities and farmers as custodians of much of the country's agricultural biodiversity should be cherished and strengthened further. It must be noted that preservation of AnGR is linked with the promotion of historical, economical, social and cultural importance, and they are important components of Bhutan's development philosophy of Gross National Happiness.

Acknowledgements

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References

DoL. 2007. Livestock population and production bulletin: data pertaining to year 2005 and earlier periods. Thimphu, Bhutan, Department of Livestock, MoA.

DoL. 2008a. Brief history of exotic pig pig introduction in Bhutan and livestock trend from 1987–2007. Thimphu, Bhutan, Information Management System, DoL, MoA.

DoL. 2008b. Livestock statistics 2007. Thimphu, Bhutan, Ministry of Agriculture.

DoL. 2009. Livestock statistics 2008. Thimphu, Bhutan, Ministry of Agriculture, Royal Government of Bhutan.

Dorji, T. 2004. The spider and the piglet. In *Proc. First Int. Seminar on Bhutan Studies*. TThimphu, Bhutan, Centre for Bhutan Studies.

Dorji, T. 2010a. Maps of Bhutan. Created using ArcGIS 9.3. Gelephu, Bhutan, Regional Veterinary Laboratory, Ministry of Agriculture, RGoB.

Dorji, T. 2010b. *Images of exotic breeds of pigs in Gelephu nuclelus farm*. Gelephu, Bhutan, Regional Veterinary Laboratory, Ministry of Agriculture, RGoB.

FAO. 2007a. *Global plan of action for animal genetic resources and the Interlaken Declaration*. Rome, Italy, Food and Agriculture Organization of the United Nations (available at ftp://ftp.fao.org/docrep/fao/010/a1404e/a1404e00.pdf)

FAO. 2007b. *The global strategy for the management of farm animal genetic resources*. Rome, Italy, Food and Agriculture Organization of the United Nations (available at ftp://ftp.fao.org/ag/cgrfa/cgrfa11/r11w9e.pdf)

FAO. 2010. *Domestic animal diversity information system (DAD-IS).* Food and Agriculture Organization (available at http://www.fao.org/dad-is), accessed on 28 April 2010.

MoA/ISNAR. 1992. *National research programme.*, Bhutan, Ministry of Agriculture, Royal Government of Bhutan.

MoA. 1999. *A study report on crossbreeding of native pigs*. Thimphu, Bhutan, National Pig Breeding Centre, Serbithang.

Nidup, K. 2008. Poultry can alleviate poverty: must be protected from bird flu. *RNR J. Bhutan*, 4(1): 175–178.

Nidup, K. & Tshering, P. 2007. Status of the family poultry production and HPAI in Bhutan. In *Proc. 8th Asian Pacific Poultry Conf. of the World's Poultry Science Association*, March 2007, Bangkok, Thailand, pp. 78–83.

Nidup, K. & Moran, C. 2010. Biodiversity and rapid loss of indigenous pig population in the Himalayas. In *11th APRU Doctoral Student Conf*., 12–16 July 2010. Jakarta, Indonesia, Universitas Indonesia.

Nidup, K., Silva, G.L.L.P., Joshi, D.D., Pem, R., Gongora, J. & Moran, C. 2009. Population structure of South Asian Indigenous pigs determined by microsatellite markers. In *Proc. Genetic Society of AustralAsia*, 7–10th July 2009, Brisbane, Australia, p. 63.

Nidup, K., Silva, G.L.L.P., Joshi, D.D., Pem, R., Gongora, J. & Moran, C. 2010. Genetic diversity of pigs (Sus scrofa) in the Himalayas and Sri Lanka. *32nd Conference for the International Society for Animal Genetics*. Edinburgh, Scotland (UK), 26–30 July 2010.

Pattison, J. 2002. Characterizing backyard pig keeping households of rural Mexico and their willingness to accept compensation for maintaining the indigenous Creole breed: a study of incentive measures and conservation options. London, University of London.

Pattison, J., Drucker, A.G. & Anderson, S. 2007. The cost of conserving livestock diversity? Incentive measures and conservation options for maintaining indigenous Pelon pigs in Yucatan, Mexico. *Trop. Anim. Health Prod.*, 39: 339–353.

PCS. 1999. *Bhutan 2020: a vision for peace, prosperity and happiness*. Thailand, Planning Commission, RGoB; Thimphu, Keen Publishing Co., Ltd.

Penjor. 2008. *The marriage system in lower Kheng*. Bhutan: Ways of knowing. London, IAP.

Peter, C., Bruford, M., Perez, T., Dalamitra, S., Hewitt, G., Erhardt, G., & the ECONOGENE Consortium. 2007. Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. *Anim. Genet.*, 38(1): 37–44.

RGoB. 2009. Bhutan Portal (available at http://www.bhutan.gov.bt).

Subalini, E., Silva, G.L.L.P. & Demetawewa, C.M.B. 2010. Phenotypic characterization and production performance of village pigs in Sri Lanka. *Trop. Agric. Res.*, 21: 198–208.

Tanaka, K., Iwaki, Y., Takizawa, T., Dorji, T., Tshering, G., Kurosawa, Y., Maeda, Y., Mannen, H., Nomura, K., Dang, V.B., Chhum-Phith, L., Bouahom, B., Yamamoto, Y., Daing, T. & Namikawa, T. 2008. Mitochondrial diversity of native pigs in the mainland South and South-east Asian countries and its relationships between local wild boars. *Anim. Sci. J.l*, 79: 417–434.

Timsina, M.P. & Sherpa, D.L. 2005. Characterization of Bhutan's indigenous pig genetic resources and production systems. Bajo, Bhutan, Renewable Natural Resources Research Centre, Council of RNR Research of Bhutan, The Ministry of Agriculture.

Wangchuk, R. 2005. In *Kuensel*, ed. *Bonism and Shamanism: an integral part of Bongop culture*. http://www.kuenselonline.com/modules.php?name=News&file=article&sid=5318, Kuensel Corporation Ltd. Thimphu, Bhutan. Accessed on 29 April 2009.

Part II: Farming and Biodiversity of Pigs in Nepal

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Farming and biodiversity of indigenous pigs in Nepal

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Abstract. Livestock biodiversity is critically important for achieving food security and alleviating poverty for the rapidly growing human population. Indigenous pigs are an important part of Nepal's rich biodiversity of livestock resources. It is imperative that an inventory is made and their properties characterized to avoid their genetic erosion. Once lost, these natural resources are largely irreplaceable. Therefore, the main objective of this paper is to provide comprehensive documentation on the biodiversity and characteristics of indigenous pigs, their population trends, and socio-economic and cultural importance to the livelihood of many rural communities in Nepal.

Nepal is a country of multiple ethnicities with a caste system. Pig farming in Nepal has socio-economic and cultural importance for some ethnic groups, while for others it is a social or religious taboo. Paradoxically, there are no taboos or restrictions in the slaughtering and consumption of wild pig, whose meat is considered a delicacy. While the natural population of wild pigs is declining, the overall population of domestic pigs and pork production in Nepal has increased over the years. Today, indigenous pigs constitute 58% of the total pig population while the remaining 42% are exotic or improved breeds. The physical and production characteristics of three main indigenous breeds namely Chwanche, Hurrah and Bampudke, are described in this paper. While the population of Chwanche is stable, the number of Hurrah is declining and Bampudke is under threat of extinction.

Pig improvement programmes have mainly focused on the introduction of exotic germplasm; little attention has been paid to conservation and sustainable use of indigenous pig resources. As a part of the implementation of the *Global Plan of Action for Animal Genetic Resources*, *in situ* and *ex situ* conservation for promotion and sustainable utilization of indigenous pig genetic resources is imperative.

Key Words. Nepal, indigenous pigs, exotic breeds, farming, biodiversity, conservation

INTRODUCTION

LIVESTOCK BIODIVERSITY

In line with the ratification of the UN Convention on Biological Diversity (UN 1992), the Food and Agriculture Organization (FAO) initiated a global strategy for the management of farm animal genetic resources in 1993 (FAO 2007b) to provide a framework for global efforts to achieve sustainable use, development and conservation of animal genetic resources for food and agriculture. The *State of the World Animal Genetic Resources for Food and Agriculture* was the first ever global assessment of livestock biodiversity aimed at ensuring that the world's livestock biodiversity is sustainably managed and provides options for the future (FAO 2007c). Around 20% of the 7,616 livestock breeds reported were classified as at risk. More than 62 breeds over a 6 year period were reported to have gone extinct, amounting to a loss of almost one breed per month. These figures presented only a partial picture of the genetic erosion of global livestock diversity as population data for 36% of the total breeds reported were unavailable.

Farm animal genetic diversity is critically important for achieving food security for the world's rapidly growing human population and in helping national economies to respond to structural changes. However, a number of threats to genetic diversity have been identified. Probably the most significant is the marginalization of traditional production systems and the associated local breeds, driven mainly by the rapid spread of intensive livestock production, often large-scale and utilizing a narrow range of breeds. Many countries consider their indigenous adapted species and breeds of farm livestock to be of low productivity and genetic potential. They are thus neglected and replaced by "improved" breeds. In this replacement policy, often no account is taken of the adaptive characteristics of local resources to drought, heat, cold, poor nutrition, disease stress, indifferent management or to the expressed or un expressed needs of the small-holder farmers, who in many developing countries own almost all of the domestic livestock. Improved breeds rarely possess these traits and demand a high level of input, often not available at an affordable cost, if they are to perform to their potential.

With a growing human population creating a rising demand for meat, milk and eggs, and the concurrent depletion of genetic diversity, strong policy measures are needed to minimize the potential loss of the global public goods embodied in animal genetic resource diversity. In September 2007, the international community adopted the *Global Plan of Action for Animal Genetic Resources* (FAO 2007a) to combat erosion of genetic diversity and promote sustainable management of animal genetic resources. At the same time, the international community endorsed the *Interlaken Declaration on Animal Genetic Resources*, by which they confirmed their common and individual responsibilities for the conservation, sustainable use and development of animal genetic resources for world food security, improving human nutritional status, and for rural development. The Government of Nepal is a signatory to the Convention on Biological Diversity (CBD 1992), The Millennium Declaration (UN 2000) and Interlaken Declaration (FAO 2007a). However, little has been done in Nepal to properly

characterize its wide range of farm animal genetic resources, while continuous attempts have been made to replace them. Once lost, these invaluable natural resources are largely irreplaceable.

OBJECTIVES

Comprehensive documentation of the biodiversity and characteristics of the indigenous pigs, their population trend and socio-cultural importance to the livelihood of the poor rural communities in Nepal, will provide a baseline for future studies. Pig farming practices and the initiatives taken by the government to develop and improve pig farming in Nepal will also be reviewed.

HISTORY

The Federal Democratic Republic of Nepal is a landlocked country in South Asia with Kathmandu as the nation's capital. It lies along the southern slopes of the Himalayan Mountains with India on three sides and China to the north. It has a total land area of 147,181 sq km and harbours eight of the world's ten highest mountains including Mount Everest. Today, Nepal has 14 zones and 75 districts grouped into five development regions namely; Eastern, Central, Western, Mid-Western and Far-Western (Figure 1).

Nepal has diverse agro-ecosystems in relation to its size. The climate varies from tropical in the Terai, through subtropical, warm and cool temperate at medium altitudes, to alpine and frigid at the highest elevations. It has more than 25 million people comprising various castes, religious and ethnic groups. More than 82% of the Nepalese depend on agriculture for their livelihood. At least 17 species of livestock have been reported (Wilson 1997). Diverse beliefs have had profound effects on the keeping and rearing of animals. Thirty-one percent of the agricultural gross domestic product is contributed by the livestock sector (MoAC 2004). Some 76% of holdings have cattle, 48% buffaloes, 76% goats, 3% sheep, 10% pigs, 51% fowls, 3% ducks, and 8% pigeons (Wilson 1997).

Pigs are predominantly reared by the ethnic groups such as Rai, Limbu, Magar, Tamang, Sherpa, Tharu and Biswakarma in certain restricted areas of the country. Both ethnicity and religious belief have imposed restrictions on pig farming which does not receive much attention from policy makers when compared to other livestock species (Gurung 1990; Dhaubhadel 1992).

IMPORTANCE OF PIGS IN NEPAL

Pig raising has been economically beneficial, particularly to the socially disadvantaged (Shakya 2008). Pigs in Nepal continue to have a multitude of functions. People rear pigs for meat, manure and to meet socio-cultural beliefs. Pork is used during festivals such as New Year and marriages, and in exchange for other commodities in poor rural communities. Black is the only acceptable colour for social and sacrificial purposes. Black pigs are used as sacrificial animals to implore

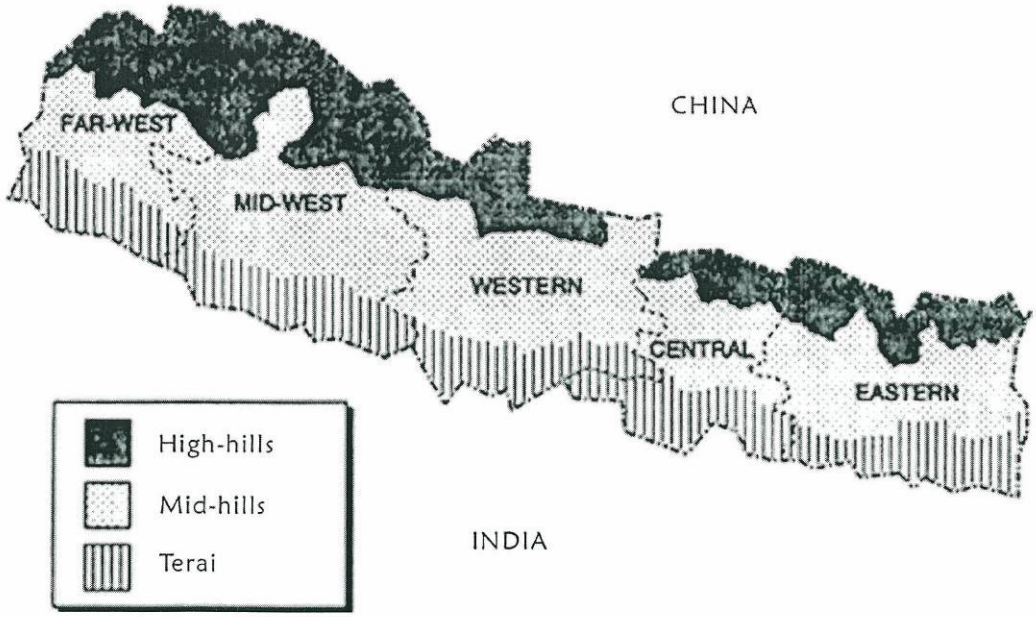
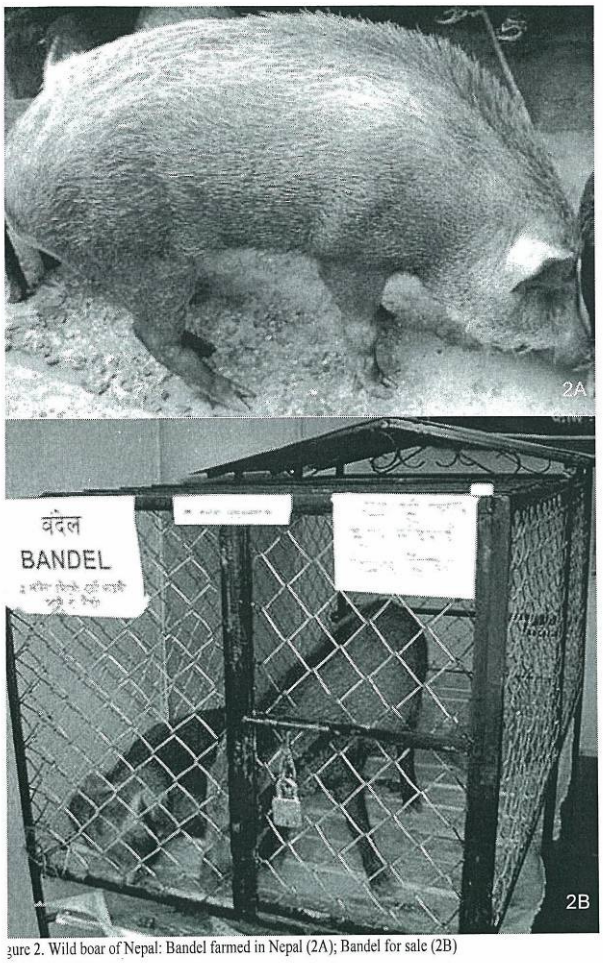


Figure 1. Shows agro-ecological zones and development regions of Nepal (Abbington 1992).



ɜure 2. Wild boar of Nepal: Bandel farmed in Nepal (2A); Bandel for sale (2B)

local deities (*deota*) for abundant crop yields, to improve the health of an ailing person, and for general good health and safety of the whole family. This might explain why indigenous pigs are predominantly black in colour. Males are preferred over females for such rituals.

Wild pigs (*Sus scrofa*) include both feral pigs (domestic pigs that have escaped captivity) and wild boars. To date, there are no reports of feral pigs in Nepal, therefore, wild pigs consist mainly of wild boars, called "Bandel" in Nepalese. Bandel (Figure 2) play a very important roles in the livelihood of poor rural communities. Paradoxically, there are no taboos and restrictions on the slaughtering and consumption of Bandel. In fact, meat of a Bandel is considered a delicacy, consumed by most castes, and is increasingly popular in star hotels and special ceremonies. Bandel sausage production has increased over the years and is now exported as a niche product.

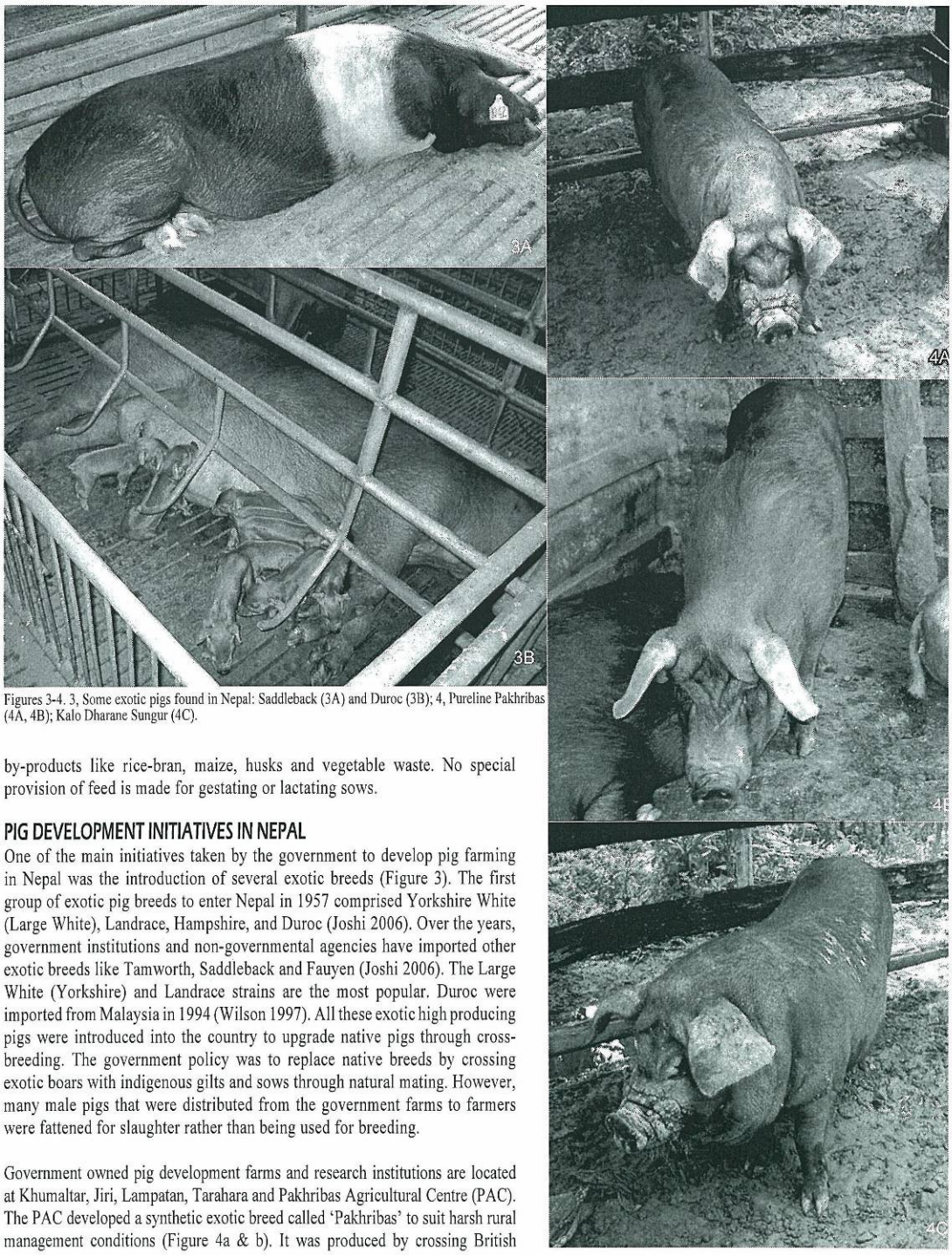
In addition to this, it is believed that there is much good "karma" to be gained by offering guests meat of a fattened Bandel on ceremonial and religious occasions. Such cultural practices and beliefs have led to severe hunting of wild pigs, which are also shot by farmers to protect their crops from damage, making the wild population vulnerable to extinction. To combat this, Nepal has launched wildlife farming, breeding and research policies to conserve their rich biodiversity (HMG 2003; Gajural 2004). According to the policy, individuals, groups, and institutions can farm wild pigs. The cost to obtain each seed animal is Nepalese Rupees (NRS) 10,000 (~ USD $150.00). While the policy underlines an effective mechanism to avoid negative impacts on the natural population, there is no report or evidence to suggest any re-introduction of wild pigs back to the wild, even though the natural population is believed to be under severe threat.

PIG FARMING PRACTICES IN NEPAL

Pig farming is seen as one of the potential tools to alleviate poverty, particularly amongst underprivileged communities (Joshi 2006). It is included in several rural development ventures including some projects for empowerment of women (DoS 2009). Pig farmer cooperatives have proven successful in many areas (HT 2009).

Farmers in rural areas keep two to three indigenous pigs. Most pigs are reared on a scavenging system with shelter provided during the night to protect them from theft and predation. The scavenging system increases the probability of internal parasitic infection in both animals and humans and thus is of public health concern in rural Nepal (Joshi *et al.* 2003; Joshi *et al.* 2005; Pant 2006). Some farmers have backyard sties constructed with locally available materials (bamboo, wood, thatch, mud, stones) to ensure minimum cost. Bedding in the form of straw and leaf litter is added if available. No separate provision is made for farrowing, or mating. Backyard sties normally have poor sanitation and limited floor spacing. In peri-urban areas, improved pigs are reared in sheds constructed with concrete with provision of run areas. Most improved pigs are intensively reared. Government farms keep pureline, high yielding, exotic breeds and their crosses, which receive concentrated feeds that very few farmers can afford to feed to their pigs.

Feed for the village or scavenging indigenous stock is comprised mainly of kitchen wastes, garbage, roots and green forages. Some supplement may be offered, depending upon the availability of rice bran, brewers' residue, distillers' residue and other crop by-products. For confined village pigs, the bulk of their diet will be cereal



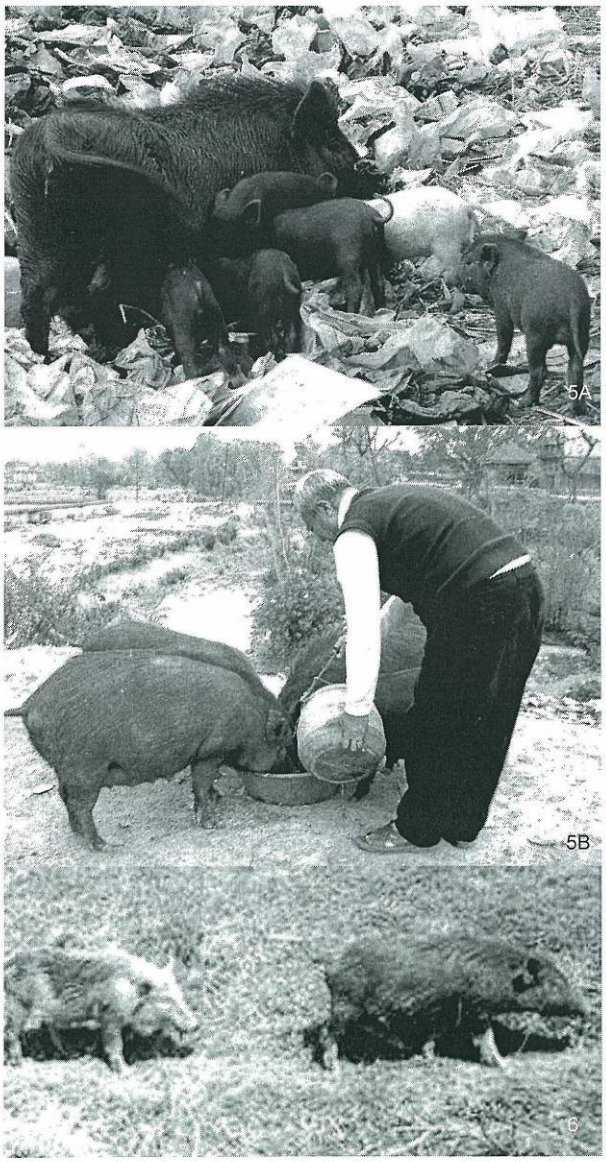
Figures 3-4. 3, Some exotic pigs found in Nepal: Saddleback (3A) and Duroc (3B); 4, Pureline Pakhribas (4A, 4B); Kalo Dharane Sungur (4C).

by-products like rice-bran, maize, husks and vegetable waste. No special provision of feed is made for gestating or lactating sows.

PIG DEVELOPMENT INITIATIVES IN NEPAL

One of the main initiatives taken by the government to develop pig farming in Nepal was the introduction of several exotic breeds (Figure 3). The first group of exotic pig breeds to enter Nepal in 1957 comprised Yorkshire White (Large White), Landrace, Hampshire, and Duroc (Joshi 2006). Over the years, government institutions and non-governmental agencies have imported other exotic breeds like Tamworth, Saddleback and Fauyen (Joshi 2006). The Large White (Yorkshire) and Landrace strains are the most popular. Duroc were imported from Malaysia in 1994 (Wilson 1997). All these exotic high producing pigs were introduced into the country to upgrade native pigs through cross-breeding. The government policy was to replace native breeds by crossing exotic boars with indigenous gilts and sows through natural mating. However, many male pigs that were distributed from the government farms to farmers were fattened for slaughter rather than being used for breeding.

Government owned pig development farms and research institutions are located at Khumaltar, Jiri, Lampatan, Tarahara and Pakhribas Agricultural Centre (PAC). The PAC developed a synthetic exotic breed called 'Pakhribas' to suit harsh rural management conditions (Figure 4a & b). It was produced by crossing British



;ure 5-6. 5, Chwanche pigs found in Kathmandu valley, Nepal. Chwanche sow with her piglets (5A) and mer feeding group of Chwanche pigs (5B); 6, Bampudke, the smallest indigenous pigs of Nepal

Saddleback, Chinese Fayeum, Tamworth, Large White, and Hampshire.

Pakhribas is characterized by a short snout, wrinkled face, large floppy ears, and firm body. It is becoming increasingly popular, with piglets exported to the north-east Indian States of Assam, West Bengal, Himachal Pradesh, Arunchal Pradesh, Sikkim, and Darjeeling. Some have even found their way to Bhutan through the Bhutan-India border. This breed is sometimes called Pakhribas Kalo Banggur.

Favourable characteristics are large litter size, good pre-weaning survival, relatively rapid growth, strong bristles and black colour, which is important in local religion and culture. Pakhribas are frequently cross-bred with the local Chwanche breed to produce Kalo Dharane Sunggur (Figure 4c), which looks very similar to Pakhribas but with broad and slighter longer snout.

Pakhribas and Kalo Dhrane Sunggur are becoming increasingly popular both within and outside the country. It is estimated that about 10,000 piglets are exported annually from the eastern region of Nepal alone (Joshi 2006) with some being illegally traded into Bhutan across Bhutan-India border (Nidup *et al.* 2009).

CHARACTERIZATION OF INDIGENOUS PIGS IN NEPAL

Generally, indigenous pigs are considered as "low producers". While the growth and meat production are lower compared to their exotic counterparts, indigenous pigs are hardy, resistant to many diseases, and can adapt to harsh rural conditions with low inputs. Under scavenging, they have good mothering ability and better survival of litters per farrowing than exotics.

The Domestic Animal Diversity Information System (DAD-IS) of the FAO (2010) reports 11 pig breeds in Nepal. Only three indigenous breeds have been reported. They are: Chwanche, Hurrah and Bampudke. The Hurrah and Chwanche have been karyotyped (Douge *et al.* 1989) and, as expected, found similar to exotic breeds (Large White, Landrace, and Hampshire). Nidup (2006) underlined the need for molecular genetic characterization of indigenous pigs in the Hindu Kush Himalayan region. In line with this, Hurrah and Kalo Dharane Sunggur have been genotyped using FAO recommended microsatellite markers (Nidup *et al.* 2009; Nidup *et al.* 2010) and their mitochondrial DNA sequences are currently being analyzed. To better interpret these molecular data, it is important to document and quantify the production characteristics of:

Chwanche: This is the most common indigenous pig, constituting 58% of the total pig population. It is a medium sized breed, intermediate between Hurrah and Bampudke. Females are heavier than males. Chwanche pigs (Figure 5a & b) are hardy, excellent in scavenging, and found in the low to mid hills region of Nepal.

Chwanche pigs are black in colour with a slightly sagging belly, relatively long slender head, thin snout, short prick ears, long neck, narrow shoulders, and razor back. The tail is long and straight. There is a dense coat of hard bristles

which form a mane on the back of the head, neck, and shoulders. According to the records compiled by Neopane & Kadel (2008) for the Nepal Agricultural Research Council (NARC), the average body weight of female Chwanche is around 32 kg at one year of age; 76 cm body length (head point to base of the tail); 86 cm heart girth; the litter size at birth and weaning is 7.0 and 6.0 respectively, with more than 7 months farrowing interval.

Bampudke: This is the smallest indigenous breed with adults weighing only 18-25 kg (Figure 6). Because of its size, it is also called "Sanu Bandel" meaning small wild boar. Epstein (1977) and Neopane & Kadel (2008) have referred to it as a "Pygmy hog" (*Porcula salvania*) which is altogether a different genus (Funk *et al.* 2007).

Bampudke pigs are black, and are hardy scavenging pigs. NARC records (Neopane & Kadel 2008) show that the average body weight of Bampudke is around 20 kg with a body length of 45 cm and 52 cm heart girth. They attain sexual maturity at 6 months, much earlier than other breeds. The litter size averages 5 piglets with 5 months farrowing interval. The Bampudke breed is reported to be on the verge of extinction.

Hurrah: This breed (Figure 7) constitutes 23% of the total pig population. Its body colour ranges from black to greyish black with some being rusty brown with patches of white. It has a drooping barrel shape, short neck, slightly curved back, loin and rump, and long legs with a small narrow body. The head and snout are not as long as the Chwanche pigs. It has long, stiff and dense bristles extending from the front of the head down the backline to the loins.

NARC records (Neopane & Kadel 2008) show that Hurrah pigs attain an average of 42 kg body weight at one year of age with a body length of 79 cm; 88 cm heart girth; 7.0 and 5.7 litter size at birth and weaning respectively, with less than 6 months farrowing interval. Hurrah pigs are the largest indigenous pigs and are raised mainly for meat by the under-privileged and poor communities of the Tarai region. The population of Hurrah is estimated to be declining.

ON-STATION PERFORMANCE OF INDIGENOUS PIGS

According to records maintained at Lampatan Livestock farm of Western Nepal (Wilson 1997), the performance of indigenous pigs is better on-station than under village conditions. Chwanche are much more prolific than Hurrah reproducing earlier, more frequently and with larger litter size with better survival. On the other hand, Hurrah has higher

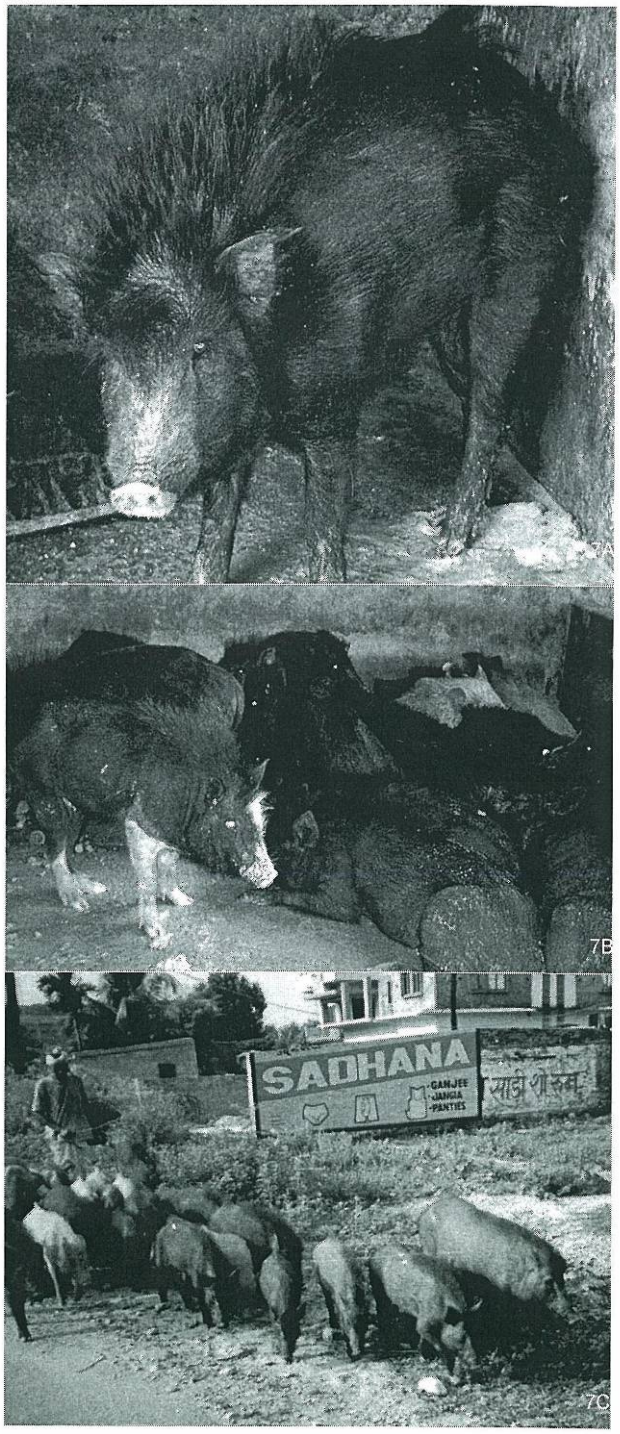


Figure 6. Hurrah pigs of Nepal. Typical Hurrah pig (6A); Group of Hurrah pigs ready to be slaughtered at Kathmandu slaughter house (6B). A herd of Hurrah being taken for grazing (6C).

birth, weaning, and adult weights. Compared to Landrance and locally developed Pakhribas, the indigenous breeds mature earlier with shorter breeding cycles but with lower overall reproductive performance, slower growth and higher pre-weaning mortality.

PIG POPULATION

The total pig population of Nepal (Figure 8) recorded in the 1992-1993 livestock population census was 604,902 head of animals. By 2006, this increased to 960,827 (MoAC 2006) of which 58% are indigenous pigs while the remaining 42% are exotic and composite pig breeds (Kayastha 2006). The annual growth rate of 5.2% recorded between 1992 to 2002 (FAO 2003) was the second highest growth rate amongst South Asian countries. This growth was mainly due to increased population of exotic pigs, increased marketability of pork and live pigs both within and outside the country. In addition, there has been an increase in consumption of pork in Nepal due to easing of cultural and social taboos associated with pigs.

Pigs are widely distributed across all the agro-ecological zones in Nepal. About 53% of the total pig population is found in mid-hills, followed by Terai region (36%), with the lowest numbers recorded in the high hills region (11%). Pigs are concentrated in the eastern development region, with the far-western development region having the least number of pigs. Similarly, pork production is highest in the mid-hill and eastern development zones. Pork production in Nepal has increased from 8,700 metric tons (MT) in 1988 (DFAMS 1989) to 15,389 MT in 2002 (DLS 2002) recording an annual growth rate of 2.72%.

CONCLUSIONS AND RECOMMENDATIONS

Indigenous pigs, including wild pigs, have socio-cultural and economic importance to many ethnic groups of Nepalese people. There is clear evidence of increased pork consumption and trading within and outside the country. The overall pig population in Nepal has increased over recent years even though there are social and religious taboos associated with pig farming. The little observational and experimental data available on the indigenous pigs indicates relatively early maturity and short breeding cycles, but poor overall reproductive performance, slow growth rates and very high early mortality. However, this is under very a low input system and a harsh rural environment. Indigenous pigs perform better under improved conditions at research stations implying that improved nutrition management at the village will increase the productivity of indigenous pigs. Given the low input system, the adaptedness of indigenous pigs makes them worthy of conservation.

Pig development in Nepal has mainly involved in introducing exotic breeds. Apart from creation of Pakhribas breed, cross-breeding using exotic boars supplied by the government farms have not made any major impact in rural pig farming. Beside their poor adaptation to harsh local conditions, breeding large exotic males with smaller native females is likely to cause "dystocia". Such birthing difficulty has been encountered in Bhutan when crossbreeding exotic boars with native sows and gilts (Nidup, Personal observation). Some success has been achieved in developing a synthetic breed that can withstand the harsh rural condition and meet the social-cultural

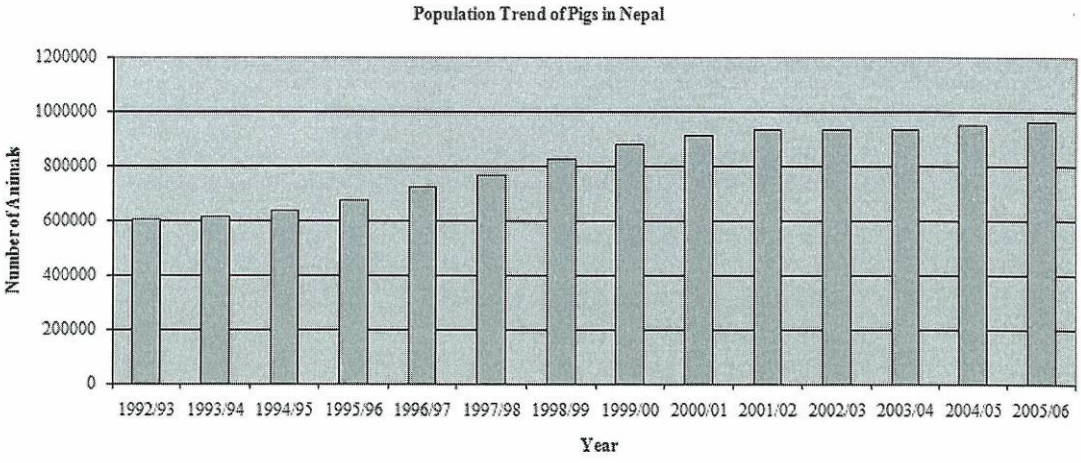


Figure 8. Shows the population trend of pigs in Nepal from 1992-2006 (MoAC 2006)

beliefs of the people with Pakhribas now comprising 15% of the village pig populations in the eastern hills. There is a need to improve pig population census. Recording the population data on breed, sex and regions is important to determine risk status of the animals.

Government policy must take account of the adaptedness of indigenous pigs. Wild pigs in Nepal also need special attention considering their importance to the livelihood of many rural folks and economy of the country. Bandel meat, which is a delicacy, must be promoted as a niche product. Government should make special efforts in conserving and maintaining the natural population of this important species from extinction.

Both indigenous domestic and wild pigs are an important genetic resource of a nation. As part of the implementation of the *Global Plan of Action* (FAO 2007a, c), further studies on morphological characteristics including precise recording of production traits are required. In addition to this, the current molecular characterization studies using microsatellite markers and mtDNA sequences on Hurrah and Kalo Dharane Sunggur (Nidup *et al.* 2009; Nidup *et al.* unpublished) must be extended to Badel, Chwanche, and Bampudke. At the same time, efforts must be made to protect, promote, and make sustainable utilization of both indigenous and wild pig biodiversity resources. For this, Government must consider both *in situ* and *ex situ* conservation. The *in situ* conservation options include dedicated conservation farms or protected areas, and payments or other support (e.g. subsidy) for those who keep rare breeds within their production environment. Cryopreservation or *ex situ* conservation of genetic material can provide a valuable complement to *in situ* approaches. The current cryopreservation programme for exotic pigs (Neopane & Kadel 2008) must be extended to indigenous breeds. Above all, a conservation and nucleus farm for the indigenous and wild pigs would be an ideal start to combat decreasing populations of Bampudke, Hurrah and the natural population of wild pigs.

REFERENCES

Abbington J.B. 1992. *Sustainable livestock production in the mountain agro-ecosystem of Nepal*. Food and Agriculture Organization, Rome.

DFAMS 1989. Pork consumption in Nepal. In: (ed. by Services DoFaAM). His Majesty's Government of Nepal, Kathmandu, Nepal.

Dhaubhadel T.S. 1992. *The role of monogastric and small stock*. Food and Agriculture Organization, Rome.

DLS 2002. Annual report of Department of Livestock Services (DLS). (ed. by (DLS) DoLS). Government of Nepal, Kathmandu, Nepal.

DoS U.S. 2009. USAID Celebrates Women's Empowerment. (ed. by State USDo). U.S. Department of State, Embassy of the United States, Kathmandu, Nepal.

Douge K., Y. Kurusawa, K. Tanaka, T. Nishida, S.M. Pradhan, & H.B. Rajbhandary. 1989. *A study on karyotype of the Indian wild pig and domestic native pig in Nepal*. Faculty of Agriculture, University of Tokyo, Japan.

Epstein H. 1977. *Domestic Animals of Nepal*. Meier Publisher Inc., London & New York.

FAO 2003. Selected Indicators of Food and Agriculture Development in Asia-Pacific Region 1992-2002. In: FAO Regional Office for Asian and the Pacific, Bangkok, Thailand.

FAO 2007a. Global Plan of Action for Animal Genetic Resources and the Interlaken Declaration. FAO, Rome.

FAO 2007b. The Global Strategy for the Management of Farm Animal Genetic Resources. FAO, Rome.

FAO 2007c. The state of the world's animal genetic resources for food and agriculture. FAO, Rome.

FAO 2010. Domestic Animal Diversity Information System (DAD-IS). http://www.fao.org/dad-is. Food and Agriculture Organization. Accessed on 22 November, 2010.

Funk S.M., S.K. Verma, G. Larson, K. Prasad, L. Singh, G. Narayan, &. E. J. Fa. 2007. The pygmy hog is a unique genus: 19th century taxonomists got it right first time round. *Molecular Phylogenetics and Evolution* 45: 427-36.

Gajural D. 2004. Nepal authorizes wildlife farming for conservation. In: *ENS*, Kathamandu.

Gurung B.B. 1990. Performance of native pig breeds of Western Region of Nepal. In: *Lampatan Livestock Farm Annual Report*, p. 106. Department of Livestock Services, Government of Nepal Kathmandu.

HMG 2003. Working policy on wildlife farming, breeding and research. (ed. by Conservation MoFaS). His Majesty's Government of Nepal, Kathmandu, Nepal.

HT 2009. Nepal pig farmers and their fat piggy banks. In: *The Himalayan Times*. The Himalayan Times, Kathmandu, Nepal.

Joshi D.D. 2006. Pig farming systems in Nepal: how can research and development enhance benefits to the poor farmers of Nepal. National Zoonoses and Food hygiene Research Centre, Nepal, Kathmandu.

Joshi D.D., M. Maharjan, M.V. Johansen, A.L. Willingham, & M. Sharma. 2003. Improving meat inspection and disease control in resource poor communities: the Nepal example. *Acta Tropica* 87: 119-27.

Joshi D.D., L.N. Moller, M. Maharjan, & C.M.O. Kapel. 2005. Serological evidence of trichinellosis in local pigs of Nepal. *Veterinary Parasitology* 132: 155-7.

Kayastha K.P. 2006. A scenario on pig production in Nepal: Present situation challenges in treatment and elimination of *Taeniasis/ Cysticerosis* in Nepal., pp. 47-54. National Zoonosis and Food Hygiene Research Centre (NZFHRC), Kathmandu, Nepal.

MoAC 2004. Statistical information on Nepalese agriculture. (ed. by Cooperatives. MoAa). MoAC, Kathmandu, Nepal.

MoAC 2006. 2005/06 Livestock Population of Nepal. (ed. by ABPSD MoAac). MoAC, Kathmandu, Nepal.

Neopane S.P. & R. Kadel. 2008. *Indigenous Pigs of Nepal*. National Animal Science Research Insititute & Nepal Agriculture Research Council, Khumaltar, Kathmandu.

Nidup K. 2006. Swine Resources in the Hindu Kush Himalayan Region: A Need for Molecular Genetic Characterization. *SAARC Journal of Agriculture* 4: 113-121.

Nidup K., G.L.L.P. Silva, D.D. Joshi, R. Pem, J. Gongora, & C. Moran. 2009. Population structure of South Asian Indigenous pigs determined by microsatellite markers. In: *Genetic Society of AustralAsia*, p. 63. Genetic Society of AustralAsia, Brisbane.

Nidup, K., G.L.L.P. Silva, D.D. Joshi, R. Pem, J. Gongora, C. Moran. 2010. Genetic Diversity of Pigs (*Sus scrofa*) in the Himalayas and Sri Lanka. *32nd International Society for Animal Genetics Conference*. 26-30 July 2010. Edinburgh, Scotland (UK).

Pant G.R. 2006. A Serological Survey of Pigs, Horses, and Ducks in Nepal for Evidence of Infection with Japanese Encephalitis Virus. *Annals of the New York Academy of Sciences* 1081: 124-9.

Shakya P. 2008. Social inclusion as a precondition of development in Nepal. In: *Leisa Magazine* Lalitpur, Nepal.

UN 1992. The Convention on Biological Diversity. URL http://www.cbd.int/convention/convention.shtml.

UN 2000. Resolution adopted by the General Assembly: United Nations Millennium Declaration.

Wilson R.T. 1997. Animal genetic resources and domestic animal diversity in Nepal. *Biodiversity and conservation* 6: 233-51.

CHAPTER 4

4.0 Genetic Structure and Diversity of South Asian Pigs *(Sus scrofa)* as Determined by Microsatellites

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4.1 ABSTRACT

Indigenous pigs are important to poor communities in many parts of the world including Bhutan, Nepal and Sri Lanka in South Asia. They are generally hardy, resistant to several diseases, thrive on low input rural management, and are highly adaptable to a broad range of environments. However, their rapidly declining populations and marginalization by the increasing number of exotic breeds is of major concern. To investigate their genetic structure and diversity, DNA from 322 indigenous pigs of South Asia and 15 Australian commercial pigs (outgroup) of European origin were genotyped using 21 microsatellite markers. The number of alleles, heterozygosities, genetic distances, and genetic variations within and among populations were estimated. Our analyses revealed four domestic and one wild boar populations in Bhutan, two domestic pig populations in Nepal, and clearly segregated populations of village pigs and wild boar in Sri Lanka. All populations showed equal or higher expected heterozygosities than the Australian composite commercial outgroup (He = 0.69 ± 0.03). The average F_{ST} across all loci among Bhutanese, Nepalese, and Sri Lankan pig populations were 0.09 (SE = 0.01), 0.07 (SE = 0.01), and 0.14 (SE = 0.02), respectively. There was negligible genetic differentiation between one Bhutanese and one Nepalese population, although overall Bhutanese and Nepalese domestic pigs are closely related. Surprisingly, the Sri Lankan village pigs clustered with outgroup animals thereby implying substantial introgression of European genes. Our findings, which confirm a rich biodiversity of pigs in South Asia, will be useful for conservation and sustainable utilization of porcine genetic resources in the region.

Keywords: *South Asia, indigenous pigs, population structure, genetic diversity, genetic variation, conservation, swine genetic resources*.

4.2 INTRODUCTION

Animal genetic diversity is important for food security and rural development (Hoffmann 2010). However, the genetic diversity of the world's livestock is rapidly declining both within and across breeds (FAO 2007b). The Food and Agriculture Organization (FAO) has recorded more than 730 breeds or lines of pigs throughout the world in its Domestic Animal Diversity Information System (FAO 2007b, 2010). Many of these are now in danger of extinction.

The three South Asian countries of Bhutan, Nepal, and Sri Lanka, which are the focus of this study, still harbour large numbers of pigs including indigenous populations. However, there are no planned breeding or conservation programmes initiated for indigenous pigs (Figure 4.S1; Figure 4.S2) which are rapidly marginalised by the increasing number of exotic breeds (Chandrasi 2002; Nidup *et al.* 2010; Nidup *et al.* 2011). In Bhutan, more than 85,932 indigenous pigs were recorded in 1985 and this reduced to 16,959 in 2008 recording a fivefold loss in the last two decades alone (Nidup *et al.* 2011). The exact official figure is not available but the reports suggest that the population of indigenous pigs in Sri Lanka is rapidly decreasing (Chandrasi 2002; Subalini *et al.* 2010). In Nepal, the population of the Hurrah breed is decreasing while Bampudke is almost extinct (Nidup *et al.* 2010).

Nevertheless, indigenous pigs still represent a valuable component of local genetic resources for many poor rural communities. Besides their socio-cultural (Dorji 2004; Wangchuk 2005; Joshi 2006) and economic importance (Joshi 2006; Subalini *et al.* 2010; Nidup *et al.* 2011) to the local people, indigenous pigs are hardy and resistant to many diseases (Reiner *et al.* 2002; Blacksell *et al.* 2006; Marufu *et al.* 2008) and highly adaptable to a broad range of environments with low inputs (Joshi 2006; Subalini *et al.* 2010; Nidup *et al.* 2011). Their potential to adapt to climate and environmental changes can compensate for their "low productivity" in terms of growth rate, litter size, and carcass yield. Therefore, to meet future agricultural and environmental challenges, special efforts are required to conserve indigenous genetic resources. Conservation is one of the four strategic priority areas of the Global Plan of Action for AnGR (FAO 2007a). However, as it is not possible, especially in the developing countries, to conserve all indigenous breeds,

prioritizing breeds for conservation is necessary. Molecular marker technologies, particularly microsatellite markers, have been used extensively to assist in rationalizing the breed conservation programmes of several domestic (Groeneveld *et al.* 2010) and wild species (Vidya *et al.* 2005; Gaur *et al.* 2006).

Nidup and Moran (2011) documented microsatellite use in many genetic diversity studies to address the biodiversity and conservation of commercial, indigenous, and rare breeds of pigs in Europe, China, and several Asian countries. Although there are some limitations, microsatellite studies have revealed substantial within and between breed genetic diversity and potential for conservation (Nidup & Moran 2011). Therefore, this study was conducted in South Asia with an objective of evaluating genetic structure and diversity of indigenous pigs of Bhutan, Nepal, and Sri Lanka. It aims to generate baseline genetic information to devise an appropriate strategy for conservation of biodiversity and sustainable utilization of swine genetic resources in the region.

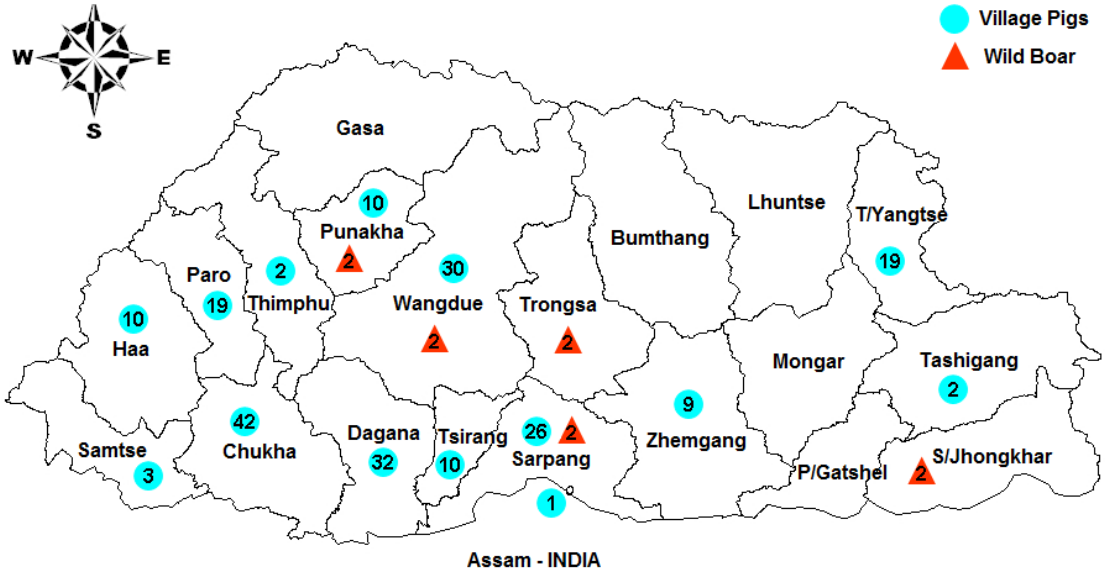
4.3 MATERIALS AND METHODS

4.3.1 Sampling and DNA Extraction

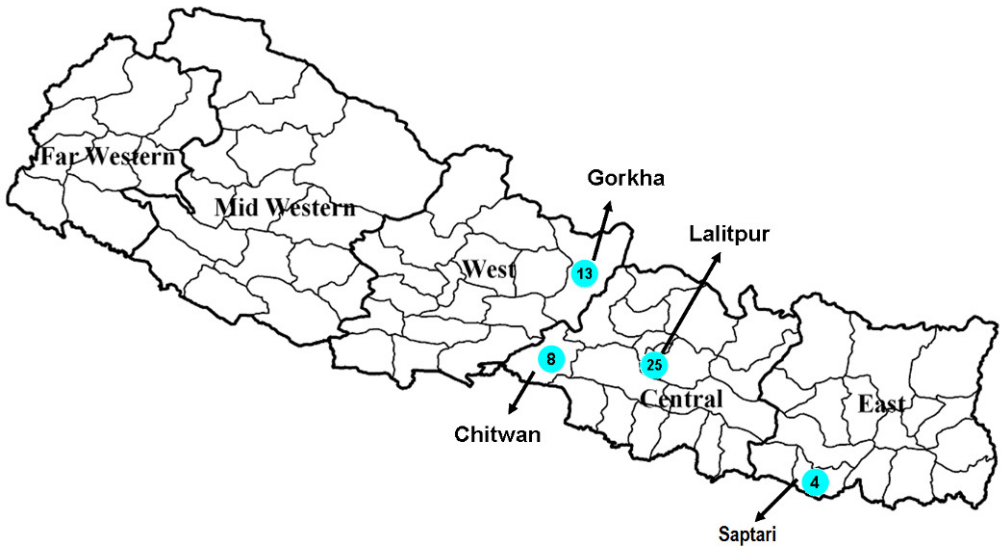
Bhutan: 215 blood samples were collected from indigenous pigs in 32 sub-districts of 13 districts (Figure 4.1a) across various agro-ecological zones (200-5400 metre above sea level) of Bhutan. Every effort was made to ensure that the samples represented a wide cross section of the entire pig population in the country. The wild boar samples, consisting of 8 hair and 2 serum samples, were obtained from five districts (Figure 4.1a; Table 4.S1). The physical characterization of indigenous pigs, husbandry practices, and their population trend has been well documented (Timsina & Sherpa 2005; Nidup *et al.* 2011).

Nepal: 50 samples were collected from Saptari (Eastern region), Gorkha (Western region), Chitwan and Lalitpur (Central region) districts, constituting 4 from Chwanche, 25 from Hurrah (NKH), and 21 from Kalo Dharane Sunggur (NKD) breeds (Table 4.S2). Nidup *et al.* (2010) has provided brief account of characterization of these breeds (Figure 4.S1 a-c), their population trend, and pig husbandry practices in Nepal.

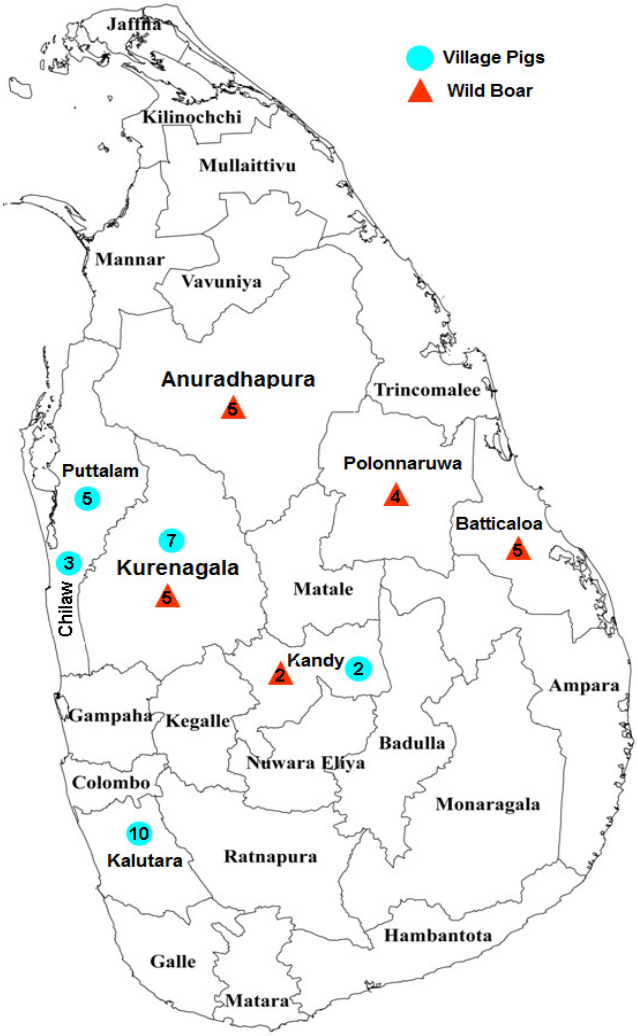
Sri Lanka: Blood samples from 27 unrelated village pigs (Table 4.S3) were collected from four districts of Kalutara, Kurunagala, Puttalam and Chilaw (Figure 4.1c) while 21 wild boar (Figure 4.S2f) samples were collected from five districts, namely Batticaloa, Polonnaruwa, Anuradhadapura, Kandy and Kurunagala (Figure 4.1c). Opportunistic sampling was used to collect samples from wild boar. Both indigenous village pigs and wild boar Sri Lanka have been recently characterised (Subalini *et al.* 2010; Subalini *et al.* in press).



(a)



(b)



(c)

Figure 4.1: Sampling areas in Bhutan *(a)*, Nepal *(b)*, and Sri Lanka *(c)*. Number within each circle indicates number of village pigs sampled from each district while number in triangle indicates number of samples of wild boar obtained in each district.

The brief account of characterization of indigenous pigs, their husbandry practices, and their population trend in Bhutan (Nidup *et al.* 2011), Nepal (Nidup *et al.* 2010), and Sri Lanka (Subalini *et al.* 2010) have been documented recently.

Outgroup: 15 Australian commercial (AC) pigs of mixed ancestry (Large White and Landrace composite) were sampled from a large commercial piggery at Corowa, New South Wales, Australia, and used as outgroup animals.

DNA Extraction: DNA was extracted from whole blood, serum, and hair samples using QIAamp® DNA Blood Mini Kit (QIAGEN) and a salting out method (Miller *et al.* 1988).

4.3.2 Microsatellite Loci

21 highly polymorphic microsatellite markers (Table 4.S4), without any indication of null alleles, were used. Markers had a minimum spacing of at least 30 centiMorgans to avoid linkage disequilibrium (Groenen *et al.* 2003). Except for *SW951* which has been used in several studies (Hampton *et al.* 2004; Cowled *et al.* 2008), all other markers are recommended by FAO and the International Society for Animal Genetics (FAO 2004; Hoffmann *et al.* 2009). All markers were detected using dye-labelled primers except *S0228*, *SW72* and *S0143*, which were CAG-tagged (5'-CAGTCGGGCGTCATCA-3') (Glenn 2006).

4.3.3 Polymerase Chain Reaction (PCR) and Genotyping

For dye-labelled primers, the PCR mixture contained 40ng of porcine genomic DNA, 80nM dye-labelled (HEX, 6-FAM, NED) forward primer, 80nM reverse primer, 10 x *Taq* DNA polymerase buffer, 1.5mM $MgCl_2$ and 100µM dNTPs in 15µl. For CAG-tagging, the mixture contained 40ng of porcine genomic DNA, 0.05 µM CAG tagged forward primer labelled with dye (VIC, NED, PET), 40nM reverse primer, 1.5mM $MgCl_2$, and 100µM dNTPs 15µl. Both mixtures were covered with two drops of paraffin oil, denatured at 95˚C for 10 minutes, followed by addition of 1U of *Taq* polymerase enzyme while holding the mixture at 80˚C. A touchdown program was used (44 cycles 95ºC 40 seconds, 62-55ºC 60 seconds, 72ºC 60 seconds, 1 cycle 72 ºC for 20 minutes) for all markers, using a PTC-100^{TM} Programmable Thermal Controller (MJ Research, Inc, Waltham, MA).

Amplified products were pooled, based on their size and dye types and analysed using an ABI PRISM 3730 DNA Analyser (Applied Biosystems, Warrington, UK) at the University of Sydney Prince Alfred Molecular Analysis Centre (SUPAMAC). GeneScan500 Liz (PN4322682, Applied Biosystems) was used as a size standard and genotypes were called using GeneMapper v3.7.

4.3.4 Data Analyses

All loci were tested for Hardy-Weinberg Equilibrium (HWE) using Genepop (Raymond & Rousset 1995) across all samples pooled within each country. Genetic structure of pig population within each country was investigated using a Bayesian clustering procedure implemented in STRUCTURE (Pritchard *et al.* 2000).

The number of clusters tested was 1 to 13 (K = 1-13), using a burn-in period of 50,000 iterations, with 10^6 iterations of Markov Chain Monte Carlo (MCMC) simulation, with five replicates for each estimate of K to check for consistency between runs, initially on the Bhutanese domestic pig data alone. *K* was plotted against mean log likelihood (Figure 4.2) and the number of inferred populations determined from the point where the mean log likelihood plateaued. The true value of *K* was verified following the method of Evanno *et al.* (2005). The second analysis involved testing the clustering of Bhutanese wild boar with their domestic counterparts. Subsequent analyses involved Nepalese followed by Sri Lankan pigs. Then, the overall analysis on all the sampled populations from South Asia along with the outgroup was performed. Most of the populations separated out clearly in an overall analysis but Sri Lankan village and outgroup pigs clustered together. Therefore, a separate test on Sri Lanka pigs and outgroup was performed to get a clear picture of structure between Sri Lankan village pigs and outgroup animals.

The average number of alleles per locus as well as observed and expected heterozygosities for inferred populations was determined using GenAlEX (Peakall & Smouse 2006). The consistency of output was tested using PopGene (Yeh *et al.* 1999) and Genepop (Raymond & Rousset 1995). Nei's genetic distance (Nei 1972, 1978) was calculated using PopGene and GenAlEx. Matrices of genetic distances between pairs of populations were summarised diagrammatically in a Neighbour-joining (NJ) tree (Saitou & Nei 1987). Bootstraps of 1000 replicates were performed in order to test the robustness of tree topology. The clustering of the populations was further tested by constructing a Neighbornet tree using SPLITSTREE 4.8 (Huson & Bryant 2006). F-statistics (Wright 1965, 1978) according to Weir and Cockerham (1984) were calculated using several different packages (Raymond & Rousset 1995; Peakall & Smouse 2006) to ensure computational accuracy and consistency of interpretation. Analysis of molecular variance (AMOVA) and Principal Coordinates Analysis (PCA) were calculated using GenAlEx.

4.4 RESULTS

4.4.1 Heterozygosity and Hardy-Weinberg Equilibrium

Missing genotype data for each loci is less than 5%. All loci in Bhutanese domestic pigs (n = 207) showed moderate to high allele numbers varying between 12 (*SW2008*) and 38 (*S0002*) with a mean of 22.57 ± 1.58 (Table 4.S5). The effective number of alleles ranged from 3.67 to 13.70. All the loci deviated significantly from Hardy-Weinberg Equilibrium (Table 4.S1). The expected heterozygosity ranged between 0.73 (*SW951*) and 0.93 (*SW122*) with a mean of 0.85 ± 0.01. The observed heterogygosity ranged between 0.29 (*SW936*) and 0.76 (*SW122 & SW240*) with a mean of 0.63 ± 0.03.

Similarly, the number of alleles in Nepalese pigs (n = 49) ranged between 5 (*SW951*) and 17 (*S0068*) with a mean of 12.10 ± 0.71 (Table 4.S5). The observed heterogygosity ranged between 0.44 (*SW122*) and 0.86 (*SW857*) with a mean of 0.69 ± 0.03. The mean expected heterozygosity was 0.81 ± 0.01. Except for three loci (*S0143, SW632, SW857*), all loci did not conform to HWE (Table 4.S6).

Considering the claimed frequent interbreeding (Subalini *et al.* 2010) with village pigs, the Sri Lankan wild boar were included in a combined analysis with domestic village pigs. The effective number of alleles ranged from 2.79 to 11.66 in SL pigs (Table 4.S7). The expected heterozygosity ranged between 0.70 (*SW951*) and 0.91 (*S0005*) with a mean of 0.83 ± 0.01. With the exception of loci *S0228* and *SW951*, all loci deviated significantly from HWE.

4.4.2 Population Structure and Genetic Diversity

STRUCTURE revealed four domestic and one wild boar populations in Bhutan, three domestic pig populations in Nepal, and clearly separated village pigs and wild boar in Sri Lanka (Figure 4.2; a-f). The five inferred populations of Bhutan were: Machay Madhuri (MMP1), Rinchengang (RGP2), West and West-central (WWCP3), East and East-central (EECP4), and Bhutanese wild boar. Thirteen animals were excluded from the analyses because of their low genetic inference (<50%) to the inferred populations.

MMP1 was found to have a consistently recognisable phenotype and mainly distributed across Southern Bhutan close to the border with the Indian States of Assam and West Bengal. Pigs from the Thoetsho sub-district in the Wangdue district belonged to RGP2. Some individuals from Haa and Paro districts were also allocated to the same cluster, since most individuals had resemblance to pigs from Rinchengang village of Thoetsho sub-district. The third inferred population consisted of pigs from the West and west-central regions and some from the Eastern region were clustered into WWCP3.

Pigs from the Bardo and Uzorong sub-districts of Zhemgang (East-central region) and Tashigang (Eastern region) districts belonged to the EECP4 cluster along with some individuals from Western Bhutan, particularly the Darla and Chubu sub-districts of Chukha and Punakha districts. Admixture individuals were evident in ECP4 and WWCP3. The fifth inferred population consisted only of wild boar without evidence of admixture with domestic pigs.

STRUCTURE confirmed genetic distinctness of the Hurrah (NKH), Kalo Dharane Sunggur (NKD), and Chwanche breeds in Nepal. However, since the Chwanche population was represented by only four individuals, it was omitted from further analyses. Similarly, inferred populations of village pigs (SLVP) and wild boar (SLWP) in Sri Lanka were clearly segregated. When STRUCTURE simulation was performed on all populations from South Asia, SLVP clustered with AC. However, clear segregation was observed when simulation was performed only with AC and Sri Lankan populations.

A quite high number of admixture hybrids were observed in Bhutan. The populations from Nepal and Sri Lanka did not contain admixed individuals except for two Sri Lankan wild boars (SL 31 & 32) which were found to have genes from village pigs.

The inferred Bhutanese pig populations; MMP1, RGP2, WWCP3, and EECP4 showed high levels of genetic diversity with mean expected heterozygosity of 0.79 ± 0.02, 0.72 ± 0.03, 0.78 ± 0.02, and 0.82 ± 0.02, respectively. The wild boar was the most diverse with mean expected heterozygosity of 0.83 ± 0.01. Similarly, the Nepalese pigs, NKH and NKD also showed a high level of genetic diversity with mean of 0.74 ± 0.03 and 0.75 ± 0.02 respectively. The Bhutanese domestic pigs were more heterozygous than the Nepalese or

Sri Lankan populations (Table 4.S8). Interestingly, the Sri Lankan wild boar was genetically less diverse (0.69 ± 0.04) than their domestic counterpart (0.77 ± 0.02), perhaps due to long term isolation in Sri Lanka. The expected heterozygosity in AC was 0.69 ± 0.03.

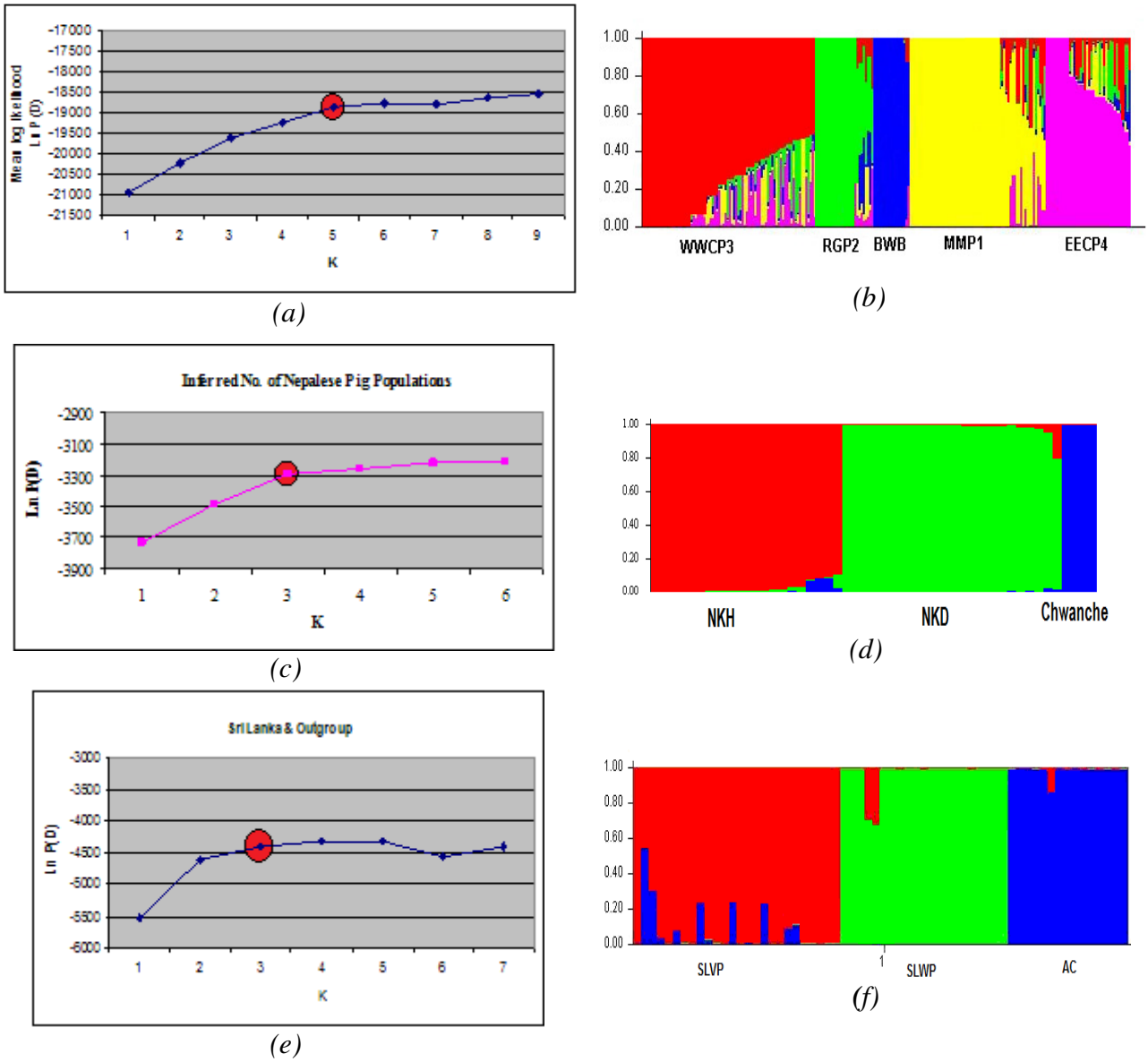


Figure 4.2: Number of inferred populations (*K*) of pigs in South Asia. Inference of Bhutanese (*a, b*), Nepalese *(c, d)* and Sri Lankan *(e, f)* pig populations.

4.4.3 Population Differentiation and Genetic Distance

When the "rule of thumb" for interpretation of F_{ST} values and genetic differentiation (Wright 1978) was applied, the pairwise F_{ST} estimates between the inferred populations showed varying levels of genetic differentiation (Table 4.S10). There was low level of differentiation between WWCP3 and EECP4 (F_{ST} = 0.03) and moderate differentiation between RGP2 and MMP1 (F_{ST} = 0.07). RGP2 was the most genetically differentiated population, particularly when compared to wild boar. Overall, there was low to moderate genetic differentiation among the Bhutanese domestic pigs. Bhutanese wild boar was moderately differentiated from domestic pigs.

The Nepalese NKH and NKD were moderately differentiated (F_{ST} = 0.06). Surprisingly, there was negligible genetic differentiation (F_{ST} = 0.03) between Nepalese NKD and Bhutanese MMP1, which was most divergent from other Bhutanese populations. In general, NKH was relatively more differentiated from all other domestic pigs except for other than Bhutanese WWCP3. Relatively less genetic differentiations were observed among Himalayan pigs when compared to Sri Lankan and AC. There was strong genetic differentiation (F_{ST} = 0.12) between SLVP and SLWP. The latter was the most divergent from other South Asian pigs.

Generally, genetic divergence of wild boar from domestic pigs was more pronounced; whereas genetic divergence amongst domestic pigs was smaller. The Bhutanese and Sri Lankan wild boars are strongly differentiated (F_{ST} = 0.12). These were consistent with Nei's D_A genetic distance between each pair of populations. Both NJ and Neighbornet dendograms (Figure 4.3 & Figure 4.S3) have clustered South Asian pig populations into three major clades. Despite low bootstrap support values, the dendogram indicates clear phylogenetic signature suggesting close relationship amongst Himalayan populations consisting of Bhutanese and Nepalese pigs when compared to Sri Lankan pigs. The MM1 have clustered with NKD (bootstrap value = 84%) and AC with Sri Lankan village pigs (bootstrap value = 60%).

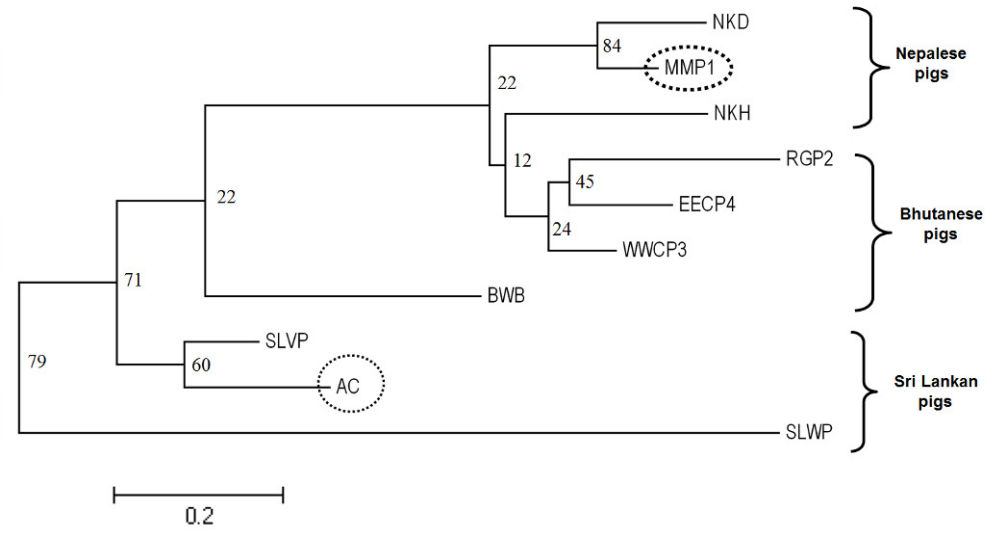


Figure 4.3: The NJ dendogram shows genetic relationships amongst South Asian pigs. Despite most bootstrap values (1000 replicates; given as percentage) being low, the dendogram indicates clear phylogeographic signature.

Hierarchical AMOVA revealed that the greatest variation was within population (83%) and little variation existed among populations. PCA shown in Figure 4.4 illustrates the relative positions of ten porcine populations. The Bhutanese and Nepalese domestic pigs clustered together while the most distant outliers were Sri Lankan wild boar followed by outgroup animals, Sri Lankan village pigs, and Bhutanese wild boar.

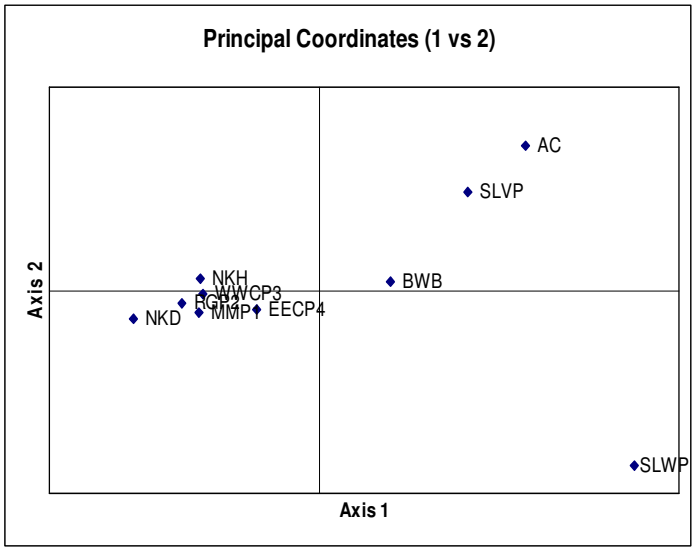


Figure 4.4: Principal Coordinate Analysis of the South Asian pig populations.

4.5 DISCUSSION

The country analyses showed heterozygote deficiencies indicative of genetic structure within Bhutan, Nepal and Sri Lanka. Generally, indigenous pigs are non-descript but upon closer observation, the populations inferred from STRUCTURE analyses of genetic markers are supported in some cases by phenotype and/or geographical distribution.

Phenotypically, the MMP1 pigs resemble Pakhribas and NKD of Nepal (Nidup *et al.* 2010). The fact they are restricted to Southern Bhutan is due to illegal dispersal of live pigs, of Pakhribas and NKD origins, into the country across the Bhutan-India border (Nidup *et al.* 2011). Some MMP1 pigs have been dispersed into inner Bhutan as illustrated by clustering of pigs from the Zhemgang district in central region with those from Sarpang district in the Southern Bhutan, an observation consistent with a recent report (Nidup *et al.* 2011).

The RGP2 was mostly restricted to Thoetsho sub-district of Wangdue district and found to be reared by small communities. The clustering of some individuals from Haa and Paro districts in RGP2 suggest the pigs may have been dispersed by humans into these places from Wangdue district. They were described as having dense bristles along the dorsal line, dense hair, and broad rectangular shaped body with females having moderately sagging belly, small to medium sized prick ears, and medium snout (Nidup *et al.* 2011). On the other hand, WWCP3 consisted of pigs from West and west-central regions and have been described as having longer body bristles along the dorsal line, medium to slightly large ears, most with prick ears but some with slightly droopy ears, a somewhat cylindrical snout, and long straight tail (Nidup *et al.* 2011). Similarly, EECP4 pigs have medium sized body, sparse to medium hair density, prick ears, straight snout, and mature females having slightly sagging belly (Nidup *et al.* 2011).

The Nepalese NKD and NKH populations inferred from genetic markers are morphologically distinct and occur in different parts of the country. The latter were sampled from Lalitpur district while the former from the Chitwan, Gorkha and Saptari districts of Nepal. Their physical characteristics have been well documented (Nidup *et al.* 2011).

Similarly, the two populations inferred for Sri Lanka clearly show that the village pigs and wild boar are distinct and thus rarely interbreed. Surprisingly two hybrids (SLWP31 and SLWP32) were detected among the wild boar samples, implying gene flow from village pigs to wild boar rather than in the expected opposite direction, given the apparent initiatives by local people to encourage matings of wild boar males with village pig sows. Since it seems unlikely that a domestic village boar would mate with a wild boar sow, this suggests either that a population of feral domestic pigs exists in the wild to mate with the wild boar or alternatively that the progeny of domestic sow by wild boar matings normally escape into the wild rather than remain in the villages.

4.5.1 Genetic Diversity

4.5.1.1 Bhutanese Pigs

Gene flow from wild to domestic pigs can occur as indicated by mitochondrial DNA sequences (Tanaka *et al.* 2008; Nidup *et al.* unpublished-b). However, it was not evident in the STRUCTURE analysis. This could be due to small sample size (n=10) and nature of sampling of the wild boar. Although the wild boar samples were not adequate to obtain accurate estimates of heterozygosity, they were sufficient to differentiate wild boar from domestic pigs during the STRUCTURE and clustering analysis.

The MMP1 are phenotypically distinct but the markers indicate they are less divergent from WWCP3 and EECP4. They may be experiencing introgression of alleles from other local pigs (RGP2, WWCP3, & EECP4). Conversely, RGP2 is more strongly differentiated by markers but is morphologically more similar to WWCP3 and EECP4. However, there was not necessarily a strong correlation between neutral genetic markers divergence and morphological variation. For instance, European sheep breeds are readily distinguishable phenotypically when compared to the Middle-east sheep breeds that are morphologically uniform but the latter display much more genetic marker divergence than European breeds (Peter *et al.* 2007). Inbreeding could be the possible reason for low genetic diversity in RGP2, as there was no record of any breeding boar being purchased outside the village (Nidup, unpublished). Additionally, pig owners did not keep any written breeding records,

so it is possible that samples from closely related animals may sometimes have been collected.

There was evidence of admixture hybrids within the Bhutanese indigenous domestic pigs but no such evidence was detected between indigenous and exotic. This is because every possible commercial-indigenous hybrid was avoided during sampling although several such hybrids, which resulted from introduction of exotic pigs (Nidup *et al.* 2011), were observed. Consequently, there is no clustering of Bhutanese pigs with AC.

4.5.1.2 Nepalese pigs

NKD were expected to display a higher level of genetic diversity than NKH since they are putatively a mixture of indigenous Chwanche and the synthetic Pakhribas, which is a blend of British Saddleback, Chinese Fayeum, Tamworth, Large White and Hampshire (Neopane & Kadel 2008). However, the difference is trivial. Further, NKD showed no tendency to cluster with AC, perhaps suggesting that the exotic contribution to this breed has been substantially diluted out by crosses to indigenous Chwanche. NKH are unique indigenous pigs raised for meat by the poor communities of Tarai region of Nepal (Nidup *et al.* 2010). Despite sampling from only one district, the high genetic diversity observed in NKH is possibly due to free-range and scavenging system of rearing which allows unselected mating. Although its population is declining (Joshi 2006), there is potential for conservation of Hurrah (NKH) pigs.

4.5.1.3 Sri Lankan pigs

The expected level of heterozygosity is higher in Sri Lankan village pigs (He = 0.77 ± 0.02) than wild boar (He = 0.69 ± 0.04). A similar situation was found in wild goats (Saitbekova *et al.* 1999) with substantially lower heterozygosity than their domestic counterparts. Compared to values from other studies (Zhang *et al.* 2008; Nikolov *et al.* 2009; Paule *et al.* 2009; Luetkemeier *et al.* 2010), the level of heterozygosity in Sri Lankan wild boar (He = 0.686 ± 0.043) is lower than Chinese (He = 0.86), Romanian (He = 0.77), Serbian (He = 0.77), Slovakian (He = 0.77), French (He = 0.76) and Japanese wild boar (He = 0.72) but higher than Italian (He = 0.66), Portuguese (He = 0.67 ± 0.10) Bulgarian (He = 0.63) and German wild boar (He = 0.55).

Crossing of indigenous village pigs with introduced exotics promoted by government policy (Subalini *et al.* 2010) is a likely reason for their high diversity (He = 0.77 ± 0.02) as well as their relatively close relatedness to AC (Figure 4.3). Although village sows are sometimes tethered in the jungle during oestrus to promote matings with wild boar, we found no evidence of a contribution from wild boar to the village pig gene pool (Figure 4.2).

4.5.2 Comparing South Asian Pigs with Other Breeds

The highest level of genetic diversity was observed within Bhutanese pigs followed by Sri Lankan village and Nepalese pigs (Table 4.S8). The close genetic relatedness between Bhutanese and Nepalese pigs may be strongly attributed to similar agro-ecological conditions, geographical proximity and human-mediated dispersal of pigs. The strong differentiation between Bhutanese and Sri Lankan wild boar may imply two different sub-species of wild boar.

The South Asian pigs were compared to the outgroup animals of composite breed, which were expected to have enhanced diversity. Instead, the result shows that South Asian pigs exhibited a greater level of genetic diversity. This is consistent with past studies (Laval *et al.* 2000; SanCristobal *et al.* 2006) which showed a lower level of diversity within most European pig breeds compared with indigenous breeds.

When compared to other Asian pigs (Table 4.S11), the level of genetic diversity within the South Asian pigs is comparable to Vietnamese pigs (Thuy *et al.* 2006) and Tibetan miniature pigs (Fan *et al.* 2003) but slightly lower than North and South Indian pigs (Behl *et al.* 2002; Behl *et al.* 2006), and most Chinese breeds including Tibetan plateau type (Yang *et al.* 2003) and Chinese village pigs (Fang *et al.* 2009).

This study further supports the fact that the developing countries harbour unique variation and therefore are potentially a rich source of genetic resources for the future improvement of animal agriculture and food security.

4.6 CONCLUSION

Microsatellite markers used in this study are well suited for evaluation of genetic relationships among closely related populations confirming the continuing utility of microsatellites for understanding the structure and diversity of pig populations. Although morphological uniformity and frequent human-mediated dispersal of pigs challenges recognition of breeds or varieties, the study confirms that South Asian pigs are a reservoir of rich biodiversity harboring a considerable amount of genetic diversity. Mitochondrial DNA sequences from the South Asian pigs are currently being analysed to complement the microsatellite based analysis.

Indigenous animal genetic resources will play a major role in future food security in the face of climate change (Hoffmann 2010). However, conserving all breeds or populations is financially not feasible. Some studies have indicated that those breeds or populations with higher overall genetic distance and clearly differentiated in the PCA should be obvious targets for conservation (Bruford *et al.* 2003; Handley *et al.* 2007). However, caution must be taken to interpret such genetic data for making conservation recommendations. It possible that genetically differentiated populations may have the lowest genetic diversity. Both levels of genetic variation within populations and between populations should be taken into account to identify not only the breeds that are a rich source of genetic diversity, but also those that are genetically vulnerable. The genetic result not be the sole criterion in conservation but must be combined with other properties such as phenotypes, traits of economic value, population size and level of endangerment.

Considering the current state of alarming loss of indigenous pigs in Bhutan (Nidup *et al.* 2011), it is recommended to conserve at least three populations with RGP2 receiving top priority. The indigenous pigs from East and East-central regions might best be treated as one population (EEC) while those from West and West-central regions might be considered as another (WWC) population. Conservation of these two populations (EEC and WWC) would minimise genetic loss and enhance the prospects for genetic improvement of Bhutanese indigenous pigs. There is no need to take special measures to conserve either MMP1 or NKD. Efforts must be directed to NKH, which is indigenous to Nepal and whose population is rapidly declining. Sri Lankan village pigs may be

confronting rapid marginalization by the exotic breeds. Both *ex* and *in situ* conservation programmes must be initiated in South Asia so as to implement the *Global Plan of Action* (FAO 2007a) for protecting and utilizing indigenous swine genetic resources in a sustainable manner.

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4.8 REFERENCES

Behl R., Kaul R., Sheoran N., Behl J., Tantia M.S. & Vijh R.K. (2002) Genetic identity of two Indian pig types using microsatellite markers. *Animal Genetics* **33**, 158-9.

Behl R., Sheoran N., Behl J. & Vijh R.K. (2006) Genetic analysis of Ankamali pigs of India using microsatellite markers and their comparison with other domesticated Indian pig types. *Journal of Animal Breeding and Genetics* **123**, 131-5.

Blacksell S.D., Khounsy S., Van Aken D., Gleeson L.J. & Westbury H.A. (2006) Comparative susceptibility of indigenous and improved pig breeds to classical swine fever virus infection: Practical and epidemiological implications in a subsistence-based, developing country setting. *Tropical Animal Health and Production* **38**, 467-74.

Bruford M.W., Bradley D.G. & Luikart G. (2003) DNA markers reveal the complexity of livestock domestication. *Nature Reviews Genetics* **4**, 900-10.

Chandrasi A.G.N. (2002) Country Report on the State of Animal Genetic Resources of Sri Lanka: Submitted to Food and Agriculture Organization of the United Nation. Veterinary Research Institute, Perideniya, Kandy, Sri Lanka.

Cowled B.D., Aldenhoven J., Odeh I.O.A., Garrett T., Moran C. & Lapidge S.J. (2008) Feral pig population structuring in the rangelands of eastern Australia: applications for designing adaptive management units. *Conservation Genetics* **9**, 211-24.

Dorji T. (2004) The Spider and the Piglet. In: *Proceedings of the First International Seminar on Bhutan Studies*. Centre for Bhutan Studies, Thimphu, Bhutan.

Fan B., Yang S.L., Lie B., Yu M., Zhao S.H. & Li K. (2003) Characterization of the genetic diversity on natural populations of Chinese miniature pig breeds. *Animal Genetics* **34**, 465-76.

Fang M., Hu X., Jin W., Li N. & Wu C. (2009) Genetic uniqueness of Chinese village pig populations inferred from microsatellite markers. *Journal of Animal Science* **87**, 3445-50.

FAO (2004) New microsatellite markers set - Recommendations of joints ISAG/FAO standing committee. In: *Secondary Guidelines for development of national farm animal genetic resources management plans: Measurement of domestic animal diversity (MoDAD) recommended microsatellite markers*. FAO.

FAO (2007a) Global Plan of Action for Animal Genetic Resources and the Interlaken Declaration. FAO, Rome.

FAO (2007b) *The State of the World's Animal Genetic Resources for Food and Agriculture*. FAO, Rome.

FAO (2010) Domestic Animal Diversity Information System (DAD-IS) of the Food and Agriculture Organization. URL http://www.fao.org/dad-is.

Gaur A., Shailaja K., Singh A., Arunabala V., Satyarebala B. & Singh L. (2006) Twenty polymorphic microsatellite markers in the Asiatic lion (Panthera leo persica). *Conservation Genetics* **7**, 1005-8.

Glenn T.C. (2006) 5' Primer Tags: A cheap way to fluorescently tag PCR products Savannah River Ecology Lab, Department of Biological Sciences, University of South Carolina, Columbia, USA

Groenen M.A.M., Joosten R., Boscher M.Y., Amigues Y., Rattink A., Harlizius B., Poel J.J. & Croojimans (2003) The use of microsatellite genoypeing for population studies in the pig with individual and pooled DNA samples. *Arch. Zootec* **52**, 145-55.

Groeneveld L.F., Lenstra J.A., Eding H., Toro M.A., Scherf B., Pilling D., Negrini R., Finlay E.K., Jianlin H., Groeneveld E., Weigend S. & Consortium G. (2010) Genetic diversity in farm animals - a review. *Animal Genetics* **41**, 6-31.

Hampton J.O., Spencer P.B.S., Alpers D.L., Twigg L.E., Woolnough A.P., Doust J., Higgs T. & Pluske J. (2004) Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. *Journal of Applied Ecology* **41**, 735-43.

Handley L.L.J., Santucci K.B., Townsend S., Taylor M., Bruford M.W. & Hewitt G.M. (2007) Genetic structure of European sheep breeds. *Heredity* **99**, 620-31.

Hanotte O., Tadelle D. & Kemp S. (2010) Time to tap Africa's livestock genomes. *Science* **328**, 1640-1.

Hoffmann I. (2010) Climate change and the characterization, breeding and conservation of animal genetic resources. *Animal Genetics* **41**, 32-46.

Hoffmann I., Marsan P.A., Baker J.S.F., Cothrane G., Hanotte O., Ensra J.A., Milan D., Weigend S. & Simianer H. (2009) New MoDAD marker sets to be used in diversity studies for the major farm animal species: recommendations of a joint ISAG/FAO working group. FAO, Rome, Italy.

Huson D.H. & Bryant D. (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* **23**, 254-67.

Joshi D.D. (2006) Pig farming systems in Nepal: how can research and development enhance benefits to the poor farmers of Nepal. National Zoonoses and Food hygiene Research Centre, Nepal, Kathmandu.

Laval G., Iannuccelli N., Legault C., Milan D., Groenen M.A.M., Giuffra E., Andersson L., Nissen P.H., Jargensen C.B., Beeckmann P., Geldermann H., Foulley J.-L., Chevalet C. & Ollivier L. (2000) Genetic diversity of eleven European pig breeds. *Genetic Selection and Evolution* **32**, 187-203.

Luetkemeier E.S., Sodhi M., Schook L.B. & Malhi R.S. (2010) Multiple Asian pig origins revealed through genomic analyses. *Mol Phylogenet Evol* **54**, 680-6.

Marufu M.C., Chanayiwa P., Chimonyo M. & Bhebhe E. (2008) Prevalence of gastrointestinal nematodes in Mukota pigs in a communal area of Zimbabwe. *African Journal of Agricultural Research* **3**, 091-5

Miller S.A., Dykes D.D. & Polesky H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* **16**, 1215.

Nei M. (1972) Genetic distance between populations. *American Naturalist* **106**, 283-393.

Nei M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583-90.

Neopane S.P. & Kadel R. (2008) *Indigenous pigs of Nepal*. National Animal Science Research Insititute & Nepal Agriculture Research Council, Khumaltar, Kathmandu.

Nidup K., Joshi D.D., Gongora J. & Moran C. (2010) Farming and Biodiversity of Indigenous Pigs in Nepal. *Biodiversity* **11**, 26-33.

Nidup K., Larson G., Gongora J., Joshi D.D., Silva G.L.L.P. & Moran C. (unpublished) Wild boar mitochondrial phylogeography, introgression, and dispersal in South Asia. *Manuscript under preparation*.

Nidup K. & Moran C. (2011) Genetic diversity of domestic pigs as revealed by microsatellites: a mini review. *Genomics and Quantitative Genetics* **2**, 5-18.

Nidup K., Tshering D., Wangdi S., Gyeltshen C., Phuntsho T. & Moran C. (2011) Farming and Biodiversity of Pigs in Bhutan. *Animal Genetic Resources* **48**, 47-61.

Nikolov I.S., Gum B., Markov G. & Kuehn R. (2009) Population genetic structure of wild boar *Sus scrofa* in Bulgaria as revealed by microsatellite analysis. *Acta Theriologica* **54**, 193-205.

Paule L., Romsakova I., Krajmerova D., Hell P. & Ionescu O. (2009) Genetic diversity and differentiation of wild boar (Sus scrofa) populations. In: *Lucrarile sesiunii stiintifice bienale cu participare internationala Padurea si Dezvoltarea Durabila Brasov, 17-18 octombrie 2008*, pp. 289-96.

Peakall R. & Smouse P. (2006) GENALEX 6: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research. *Molecular Ecology* **6**, 288-95.

Pritchard J.K., Stephens M. & Donnelly P. (2000) Inference of Population Structure Using Mulitlocus Genotype Data. *Genetics* **155**, 945-59.

Raymond M. & Rousset F. (1995) GENEPOP (Version 1.2): Population genetic software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-9.

Reiner G., Eckert J., Peischl T., Bochert S., Jäkel T., Mackenstedt U., Joachim A., Daugschie A. & Geldermann H. (2002) Variation in clinical and parasitological traits in Pietran and Meishan pigs infected with *Sarcocystis miescheriana*. *Veterinary Parasitology* **106**, 99-113.

Saitbekova N., Gaillard C., G O.-R. & Dolf G. (1999) Genetic diversity in Swiss goat breeds on microsatellite analysis. *Animal Genetics* **30**, 36-41.

Saitou N. & Nei M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406-25.

SanCristobal M., Chevalet C., Haley C.S., Joosten R., Rattink A.P., Harlizius B., Groenen M.A.M., Amigues Y., Boscher M.Y., Russell G., Law A., Davoli R., Russo V., Desautes C., Alderson L., Fimland E., Bagga M., Delgado J.V., Vega-Pla J.L., Martinez A.M., Ramos M., Glodek P., Meyer J.N., Gandini G.C., Matassino D., Plastow G.S., Siggens K.W., Laval G., Archibald A.L., Milan D., Hammond K. & Cardellino R. (2006) Genetic diversity within and between European pig breeds using microsatellite markers. *Animal Genetics* **37**, 189-98.

Subalini E., Silva G.L.L.P. & Demetawewa C.M.B. (2010) Phenotypic Characterization and Production Performance of Village Pigs in Sri Lanka. *Tropical Agricultural Research* **21**, 198-208.

Subalini E., Silva G.L.L.P. & Demetawewa C.M.B. (in press) Phenotypic variations in native pig types: village and wild pigs in Sri Lanka. *Tropical Agricultural Research* **22**.

Tamura K., Dudley J., Nei M. & Kumar S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596 - 9.

Tanaka K., Iwaki Y., Takizawa T., Dorji T., Tshering G., Kurosawa Y., Maeda Y., Mannen H., Nomura K., Dang V.B., Chhum-Phith L., Bouahom B., Yamamoto Y., Daing T. & Namikawa T. (2008) Mitochondrial diversity of native pigs in the mainland South and South-east Asian countries and its relationships between local wild boars. *Animal Science Journal* **79**, 417-34.

Thuy N.T.D., Melchinger-Wild E., Kuss A.W., Cuong N.V., Bartenschlager H. & Geldermann H. (2006) Comparison of Vietnamese and European pig breeds using microsatellites. *Journal of Animal Science* **84**, 2601-8.

Timsina M.P. & Sherpa D.L. (2005) Characterisation of Bhutan's indigenous pig genetic resources and production systems. Renewable Natural Resources Research Centre, Council of RNR Research of Bhutan, The Ministry of Agriculture, Bajo.

Vidya T.N.C., Fernando P., Melnick D.J. & Sukumar R. (2005) Population genetic structure and conservation of Asian elephants (Elephas maximus) across India. *Animal Conservation* **8**, 377-88.

Wangchuk R. (2005) Bonism and Shamanism: an integral part of Bongop culture. In: *Kuensel*. Kuensel Corporation Ltd., Thimphu.

Weir B.S. & Cockerham C.C. (1984) Estimating F-Statistics for the analysis of population structure. *Evolution* **38**, 1358-70.

Wright S. (1965) The interpretation of population structure by F-statistis with special regards to systems of mating. *Evol* **19**, 395-420.

Wright S. (1978) *Evolution and the genetics of populations. Variability within and among natural populations.* The University of Chicago Pess, Chicago.

Yang S.-L., Wang Z.-G., Liu B., Zhang G.-X., Zhao S.-H., Yu M., Fan B., Li M.-H., Xiong T.-A. & Li K. (2003) Genetic variation and relationships of eighteen Chinese indigenous pig breeds. *Genet Sel Evol* **35**, 657-71.

Yeh F., Yang R. & Boyle T. (1999) PopGene 2 (Version1.31): Microsoft windows-based freeware for population genetics analysis. University of Alberta and Centre for International Forestry Research.

Zhang B., Zhang C., Chen J., Ding D., Li C., Zhou K. & Chang Q. (2008) Genetic structure and subspecies divergence of wild boars *Sus scrofa* in mainland China based on the microsatellite variation analysis. *Acta Zoologica Sinica* **54**, 753-61.

4.9 SUPPLEMENTARY MATERIALS

Figure 4.S1: Bhutanese domestic pigs. Pigs from Eastern Bhutan *(a)* and central Bhutan *(b)*; Machay madhuri (MM), a Pakhribas type *(c)* Machay madhuri, a Kalo Dharane Sunggur type found in Southern Bhutan *(d)*; Indigenous pigs found in Western *(e, f)* and Western-central Bhutan *(g)*; Wild boar and striped wild boar piglets *(h, i)*.

(a) *(b)* *(c)*

(d) *(e)* *(f)*

Figure 4.S2: Nepalese pigs: Chwanche *(a)*, Hurrah (NKH) *(b)*, and Kalo Dhrane Sunggur (NKD) *(c)*; Sri Lankan pigs: Village pigs *(d,e)* and Wild boar *(f)*.

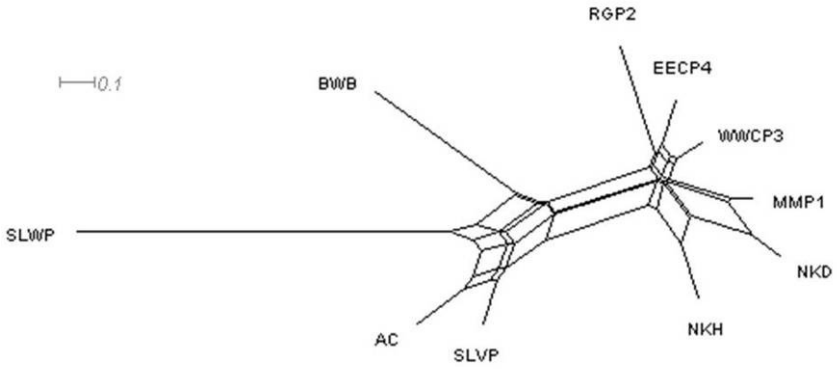

Figure 4.S3: Neighbornet tree of South Asian pigs.

Table 4.S1: Details of samples of indigenous domestic pigs and wild boar collected in Bhutan.

Sl.No	District	Sub-district	Sex	POP ID	Sample Type	Breed	Samples/ District
1	Assam	Datgari	f	Bhu-179	Blood	Village pig	1
2	Chukha	Bongo	f	Bhu-102	Blood	Village pig	42
3	Chukha	Bongo	f	Bhu-104	Blood	Village pig	
4	Chukha	Bongo	f	Bhu-105	Blood	Village pig	
5	Chukha	Bongo	f	Bhu-106	Blood	Village pig	
6	Chukha	Bongo	f	Bhu-128	Blood	Village pig	
7	Chukha	Bongo	m	Bhu-103	Blood	Village pig	
8	Chukha	Bongo	m	Bhu-107	Blood	Village pig	
9	Chukha	Bongo	m	Bhu-124	Blood	Village pig	
10	Chukha	Bongo	m	Bhu-125	Blood	Village pig	
11	Chukha	Bongo	m	Bhu-126	Blood	Village pig	
12	Chukha	Bongo	m	Bhu-127	Blood	Village pig	
13	Chukha	Bongo	m	Bhu-129	Blood	Village pig	
14	Chukha	Darla	f	Bhu-90	Blood	Village pig	
15	Chukha	Darla	f	Bhu-108	Blood	Village pig	
16	Chukha	Darla	f	Bhu-113	Blood	Village pig	
17	Chukha	Darla	f	Bhu-121	Blood	Village pig	
18	Chukha	Darla	f	Bhu-122	Blood	Village pig	
19	Chukha	Darla	f	Bhu-123	Blood	Village pig	
20	Chukha	Darla	m	Bhu43	Blood	Village pig	
21	Chukha	Darla	m	Bhu-89	Blood	Village pig	
22	Chukha	Darla	m	Bhu-91	Blood	Village pig	
23	Chukha	Darla	m	Bhu-92	Blood	Village pig	
24	Chukha	Darla	m	Bhu-93	Blood	Village pig	
25	Chukha	Darla	m	Bhu-109	Blood	Village pig	
26	Chukha	Darla	m	Bhu-110	Blood	Village pig	
27	Chukha	Darla	m	Bhu-111	Blood	Village pig	
28	Chukha	Darla	m	Bhu-112	Blood	Village pig	
29	Chukha	Darla	m	Bhu-114	Blood	Village pig	
30	Chukha	Darla	m	Bhu-115	Blood	Village pig	
31	Chukha	Darla	m	Bhu-116	Blood	Village pig	
32	Chukha	Darla	m	Bhu-117	Blood	Village pig	
33	Chukha	Darla	m	Bhu-118	Blood	Village pig	
34	Chukha	Darla	m	Bhu-119	Blood	Village pig	
35	Chukha	Darla	m	Bhu-120	Blood	Village pig	
36	Chukha	Geyling	f	Bhu44	Blood	Village pig	
37	Chukha	Geyling	m	Bhu-94	Blood	Village pig	
38	Chukha	Sampheling	f	Bhu-175	Blood	Village pig	
39	Chukha	Sampheling	f	Bhu-176	Blood	Village pig	
40	Chukha	Sampheling	f	Bhu-177	Blood	Village pig	
41	Chukha	Sampheling	m	Bhu-173	Blood	Village pig	
42	Chukha	Sampheling	m	Bhu-174	Blood	Village pig	
43	Chukha	Sampheling	m	Bhu-178	Blood	Village pig	

Sl.No	District	Sub-district	Sex	POP ID	Sample Type	Breed	Samples/ District
44	Dagana	Drujagang	m	Bhu-133	Blood	Village pig	32
45	Dagana	Drujegang	f	Bhu-3	Blood	Village pig	
46	Dagana	Drujegang	m	Bhu-2	Blood	Village pig	
47	Dagana	Drujegang	m	Bhu-134	Blood	Village pig	
48	Dagana	Drujegang	m	Bhu-138	Blood	Village pig	
49	Dagana	Goshi	f	Bhu-139	Blood	Village pig	
50	Dagana	Goshi	f	Bhu-141	Blood	Village pig	
51	Dagana	Goshi	f	Bhu-143	Blood	Village pig	
52	Dagana	Goshi	f	Bhu-144	Blood	Village pig	
53	Dagana	Goshi	f	Bhu-151	Blood	Village pig	
54	Dagana	Goshi	m	Bhu-140	Blood	Village pig	
55	Dagana	Goshi	m	Bhu-142	Blood	Village pig	
56	Dagana	Goshi	m	Bhu-145	Blood	Village pig	
57	Dagana	Goshi	m	Bhu-146	Blood	Village pig	
58	Dagana	Goshi	m	Bhu-152	Blood	Village pig	
59	Dagana	Goshi	m	Bhu-153	Blood	Village pig	
60	Dagana	Lhamoizingkha	f	Bhu-167	Blood	Village pig	
61	Dagana	Lhamoizingkha	f	Bhu-168	Blood	Village pig	
62	Dagana	Lhamoizingkha	f	Bhu-169	Blood	Village pig	
63	Dagana	Lhamoizingkha	m	Bhu-163	Blood	Village pig	
64	Dagana	Lhamoizingkha	m	Bhu-164	Blood	Village pig	
65	Dagana	Lhamoizingkha	m	Bhu-165	Blood	Village pig	
66	Dagana	Lhamoizingkha	m	Bhu-166	Blood	Village pig	
67	Dagana	Lhamoizingkha	m	Bhu-170	Blood	Village pig	
68	Dagana	Lhamoizingkha	m	Bhu-171	Blood	Village pig	
69	Dagana	Lhamoizingkha	m	Bhu-172	Blood	Village pig	
70	Dagana	Tseza	f	Bhu-131	Blood	Village pig	
71	Dagana	Tseza	m	Bhu-135	Blood	Village pig	
72	Dagana	Tseza	m	Bhu-137	Blood	Village pig	
73	Dagana	Tseza	f	Bhu-132	Blood	Village pig	
74	Dagana	Tseza	f	Bhu-136	Blood	Village pig	
75	Dagana	Tseza	m	Bhu-130	Blood	Village pig	
76	Haa	Eusu	f	Bhu-19	Blood	Village pig	10
77	Haa	Eusu	f	Bhu-39	Blood	Village pig	
78	Haa	Eusu	f	Bhu-45	Blood	Village pig	
79	Haa	Eusu	f	Bhu-46	Blood	Village pig	
80	Haa	Eusu	f	Bhu-47	Blood	Village pig	
81	Haa	Katsho	f	Bhu-14	Blood	Village pig	
82	Haa	Katsho	f	Bhu-48	Blood	Village pig	
83	Haa	Katsho	f	Bhu-49	Blood	Village pig	
84	Haa	Katsho	m	Bhu-29	Blood	Village pig	
85	Haa	Katsho	m	Bhu-30	Blood	Village pig	
86	Paro	Dogar	f	Bhu-4	Blood	Village pig	19
87	Paro	Dogar	f	Bhu-79	Blood	Village pig	
88	Paro	Dogar	f	Bhu-81	Blood	Village pig	
89	Paro	Dogar	f	Bhu-82	Blood	Village pig	
90	Paro	Dogar	m	Bhu-26	Blood	Village pig	

Sl.No	District	Sub-district	Sex	POP ID	Sample Type	Breed	Samples/ District
91	Paro	Dogar	m	Bhu-38	Blood	Village pig	
92	Paro	Dogar	m	Bhu-80	Blood	Village pig	
93	Paro	Dogar	m	Bhu-83	Blood	Village pig	
94	Paro	Dotay	f	Bhu-18	Blood	Village pig	
95	Paro	Dotay	f	Bhu-25	Blood	Village pig	
96	Paro	Dotey	f	Bhu-84	Blood	Village pig	
97	Paro	Dotey	f	Bhu-88	Blood	Village pig	
98	Paro	Dotey	m	Bhu-85	Blood	Village pig	
99	Paro	Dotey	m	Bhu-86	Blood	Village pig	
100	Paro	Dotey	m	Bhu-87	Blood	Village pig	
101	Paro	Naga	f	Bhu-147	Blood	Village pig	
102	Paro	Naga	f	Bhu-148	Blood	Village pig	
103	Paro	Naga	f	Bhu-149	Blood	Village pig	
104	Paro	Naga	m	Bhu-150	Blood	Village pig	
105	Punakha	Chubu	f	Bhu-23	Blood	Village pig	10
106	Punakha	Chubu	f	Bhu-64	Blood	Village pig	
107	Punakha	Chubu	f	Bhu-65	Blood	Village pig	
108	Punakha	Chubu	f	Bhu-67	Blood	Village pig	
109	Punakha	Chubu	f	Bhu-68	Blood	Village pig	
110	Punakha	Chubu	m	Bhu-66	Blood	Village pig	
111	Punakha	Dzomi	f	Bhu-28	Blood	Village pig	
112	Punakha	Dzomi	f	Bhu-62	Blood	Village pig	
113	Punakha	Dzomi	f	Bhu-63	Blood	Village pig	
114	Punakha	Dzomi	m	Bhu-37	Blood	Village pig	
115	Thimphu	Chang	f	Bhu-22	Blood	Village pig	2
116	Thimphu	Chang	m	Bhu-33	Blood	Village pig	
117	Samtse	Samtse	f	Bhu-40	Blood	Village pig	3
118	Samtse	Samtse	m	Bhu-24	Blood	Village pig	
119	Samtse	Samtse	m	Bhu-32	Blood	Village pig	
120	Sarpang	Dekiling	m	Bhu-69	Blood	Village pig	26
121	Sarpang	Dekiling	m	Bhu-70	Blood	Village pig	
122	Sarpang	Dekiling	m	Bhu-98	Blood	Village pig	
123	Sarpang	Gelephu	f	Bhu-16	Blood	Village pig	
124	Sarpang	Gelephu	f	Bhu-20	Blood	Village pig	
125	Sarpang	Gelephu	f	Bhu-71	Blood	Village pig	
126	Sarpang	Gelephu	f	Bhu-72	Blood	Village pig	
127	Sarpang	Gelephu	m	Bhu-1	Blood	Village pig	
128	Sarpang	Gelephu	m	Bhu-5	Blood	Village pig	
129	Sarpang	Gelephu	m	Bhu-73	Blood	Village pig	
130	Sarpang	Gelephu	m	Bhu-99	Blood	Village pig	
131	Sarpang	Gelephu	m	Bhu-100	Blood	Village pig	
132	Sarpang	Gelephu	m	Bhu-101	Blood	Village pig	
133	Sarpang	Shompongkha	m	Bhu-27	Blood	Village pig	
134	Sarpang	Shompongkha	m	Bhu-95	Blood	Village pig	
135	Sarpang	Shompongkha	m	Bhu-96	Blood	Village pig	
136	Sarpang	Shompongkha	m	Bhu-97	Blood	Village pig	
137	Sarpang	Umling	f	Bhu-154	Blood	Village pig	

Sl.No	District	Sub-district	Sex	POP ID	Sample Type	Breed	Samples/ District
138	Sarpang	Umling	f	Bhu-155	Blood	Village pig	
139	Sarpang	Umling	f	Bhu-156	Blood	Village pig	
140	Sarpang	Umling	f	Bhu-160	Blood	Village pig	
141	Sarpang	Umling	m	Bhu-157	Blood	Village pig	
142	Sarpang	Umling	m	Bhu-159	Blood	Village pig	
143	Sarpang	Umling	m	Bhu-161	Blood	Village pig	
144	Sarpang	Umling	m	Bhu-162	Blood	Village pig	
145	Sarpang	Umling		Bhu-158	Blood	Village pig	
146	Tashigang	Uzurong	f	Bhu41	Blood	Village pig	2
147	Tashigang	Uzurong	m	Bhu42	Blood	Village pig	
148	T/yangtse	Khamdang	f	Bhu-190	Blood	Village pig	19
149	T/yangtse	Khamdang	f	Bhu-191	Blood	Village pig	
150	T/yangtse	Khamdang	f	Bhu-192	Blood	Village pig	
151	T/yangtse	Khamdang	f	Bhu-193	Blood	Village pig	
152	T/yangtse	Khamdang	f	Bhu-198	Blood	Village pig	
153	T/yangtse	Khamdang	m	Bhu-194	Blood	Village pig	
154	T/yangtse	Khamdang	m	Bhu-195	Blood	Village pig	
155	T/yangtse	Khamdang	m	Bhu-196	Blood	Village pig	
156	T/yangtse	Khamdang	m	Bhu-197	Blood	Village pig	
157	T/yangtse	Khamdang	m	Bhu-199	Blood	Village pig	
158	T/yangtse	Ramjar	f	Bhu-202	Blood	Village pig	
159	T/yangtse	Ramjar	f	Bhu-203	Blood	Village pig	
160	T/yangtse	Ramjar	f	Bhu-204	Blood	Village pig	
161	T/yangtse	Ramjar	f	Bhu-205	Blood	Village pig	
162	T/yangtse	Ramjar	f	Bhu-206	Blood	Village pig	
163	T/yangtse	Ramjar	f	Bhu-207	Blood	Village pig	
164	T/yangtse	Ramjar	m	Bhu-200	Blood	Village pig	
165	T/yangtse	Ramjar	m	Bhu-201	Blood	Village pig	
166	T/yangtse	Ramjar	m	Bhu-208	Blood	Village pig	
167	Tsirang	Rangthangling	f	Bhu-182	Blood	Village pig	10
168	Tsirang	Rangthangling	f	Bhu-183	Blood	Village pig	
169	Tsirang	Rangthangling	m	Bhu-180	Blood	Village pig	
170	Tsirang	Rangthangling	m	Bhu-181	Blood	Village pig	
171	Tsirang	Tsholingkhor	f	Bhu-184	Blood	Village pig	
172	Tsirang	Tsholingkhor	f	Bhu-185	Blood	Village pig	
173	Tsirang	Tsholingkhor	f	Bhu-186	Blood	Village pig	
174	Tsirang	Tsholingkhor	f	Bhu-187	Blood	Village pig	
175	Tsirang	Tsholingkhor	f	Bhu-188	Blood	Village pig	
176	Tsirang	Tsholingkhor	f	Bhu-189	Blood	Village pig	
177	Wangdue	Phangyul-kazhi	f	Bhu-6	Blood	Village pig	30
178	Wangdue	Phangyul-kazhi	f	Bhu-9	Blood	Village pig	
179	Wangdue	Phangyul-kazhi	f	Bhu-35	Blood	Village pig	
180	Wangdue	Phangyul-kazhi	f	Bhu-61	Blood	Village pig	
181	Wangdue	Phangyul-kazhi	m	Bhu-59	Blood	Village pig	
182	Wangdue	Phangyul-kazhi	m	Bhu-60	Blood	Village pig	
183	Wangdue	Phobjikha	f	Bhu-211	Blood	Village pig	
184	Wangdue	Phobjikha	f	Bhu-213	Blood	Village pig	

Sl.No	District	Sub-district	Sex	POP ID	Sample Type	Breed	Samples/ District
185	Wangdue	Phobjikha	m	Bhu-209	Blood	Village pig	
186	Wangdue	Phobjikha	m	Bhu-210	Blood	Village pig	
187	Wangdue	Phobjikha	m	Bhu-212	Blood	Village pig	
188	Wangdue	Phobjikha	m	Bhu-214	Blood	Village pig	
189	Wangdue	Phobjikha	m	Bhu-215	Blood	Village pig	
190	Wangdue	Thoetsho	f	Bhu-7	Blood	Village pig	
191	Wangdue	Thoetsho	f	Bhu-8	Blood	Village pig	
192	Wangdue	Thoetsho	f	Bhu-11	Blood	Village pig	
193	Wangdue	Thoetsho	f	Bhu-13	Blood	Village pig	
194	Wangdue	Thoetsho	f	Bhu-15	Blood	Village pig	
195	Wangdue	Thoetsho	m	Bhu-10	Blood	Village pig	
196	Wangdue	Thoetsho	m	Bhu-21	Blood	Village pig	
197	Wangdue	Thoetsho	m	Bhu-36	Blood	Village pig	
198	Wangdue	Thoetso	f	Bhu-50	Blood	Village pig	
199	Wangdue	Thoetso	f	Bhu-52	Blood	Village pig	
200	Wangdue	Thoetso	f	Bhu-53	Blood	Village pig	
201	Wangdue	Thoetso	f	Bhu-54	Blood	Village pig	
202	Wangdue	Thoetso	f	Bhu-56	Blood	Village pig	
203	Wangdue	Thoetso	f	Bhu-58	Blood	Village pig	
204	Wangdue	Thoetso	m	Bhu-51	Blood	Village pig	
205	Wangdue	Thoetso	m	Bhu-55	Blood	Village pig	
206	Wangdue	Thoetso	m	Bhu-57	Blood	Village pig	
207	Zhemgang	Bardo	f	Bhu-17	Blood	Village pig	9
208	Zhemgang	Bardo	f	Bhu-31	Blood	Village pig	
209	Zhemgang	Bardo	m	Bhu-12	Blood	Village pig	
210	Zhemgang	Bardo	m	Bhu-78	Blood	Village pig	
211	Zhemgang	Trong	m	Bhu-34	Blood	Village pig	
212	Zhemgang	Trong	m	Bhu-74	Blood	Village pig	
213	Zhemgang	Trong	m	Bhu-75	Blood	Village pig	
214	Zhemgang	Trong	m	Bhu-76	Blood	Village pig	
215	Zhemgang	Trong	m	Bhu-77	Blood	Village pig	
216	Punakha	Thinlaygang	NR	WP-52	Serum	Wild boar	2
217	Punakha	Thinlaygang	NR	WP-53	Serum	Wild boar	
218	S/Jhongkhar	Shinkhar Lauri	NR	WP-5	Hair	Wild boar	2
219	S/Jhongkhar	Shinkhar Lauri	NR	WP-6	Hair	Wild boar	
220	Sarpang	Bhur	NR	WP-7	Hair	Wild boar	2
221	Sarpang	Bhur	NR	WP-8	Hair	Wild boar	
222	Trongsa	JSWN Park	NR	WP-3	Hair	Wild boar	2
223	Trongsa	Langthel	NR	WP-4	Hair	Wild boar	
224	Wangdue	Kazi	NR	WP-1	Hair	Wild boar	2
225	Wangdue	Kazi	NR	WP-2	Hair	Wild boar	
						Total	**225**

Note: NR = not recorded

Table 4.S2: Sampling of indigenous pigs in Nepal.

Sl.No	District	Sub-district	Sex	POP ID	Sample Type	Breed	
1	Chitawan	Naryananghat	m	NK-26	Blood	NKD	8
2	Chitawan	Naryananghat	m	NK-27	Blood	NKD	
3	Chitawan	Naryananghat	m	NK-28	Blood	NKD	
4	Chitawan	Naryananghat	m	NK-29	Blood	NKD	
5	Chitawan	Naryananghat	m	NK-30	Blood	NKD	
6	Chitawan	Naryananghat	m	NK-31	Blood	NKD	
7	Chitawan	Naryananghat	m	NK-32	Blood	NKD	
8	Chitawan	Naryananghat	m	NK-33	Blood	NKD	
9	Gorkha	Kurintar	f	NK-34	Blood	NKD	13
10	Gorkha	Kurintar	f	NK-35	Blood	NKD	
11	Gorkha	Kurintar	f	NK-36	Blood	NKD	
12	Gorkha	Kurintar	f	NK-37	Blood	NKD	
13	Gorkha	Kurintar	m	NK-38	Blood	NKD	
14	Gorkha	Kurintar	m	NK-39	Blood	NKD	
15	Gorkha	Kurintar	m	NK-40	Blood	NKD	
16	Gorkha	Kurintar	m	NK-41	Blood	NKD	
17	Gorkha	Kurintar	m	NK-42	Blood	NKD	
18	Gorkha	Kurintar	m	NK-45	Blood	Chwanche	
19	Gorkha	Kurintar	m	NK-46	Blood	Chwanche	
20	Gorkha	Kurintar	m	NK-47	Blood	Chwanche	
21	Gorkha	Kurintar	f	NK-48	Blood	Chwanche	
22	Lalitpur	Talchikhel	m	NK-1	Blood	NKH	25
23	Lalitpur	Talchikhel	f	NK-10	Blood	NKH	
24	Lalitpur	Talchikhel	m	NK-11	Blood	NKH	
25	Lalitpur	Talchikhel	f	NK-12	Blood	NKH	
26	Lalitpur	Talchikhel	m	NK-13	Blood	NKH	
27	Lalitpur	Talchikhel	m	NK-14	Blood	NKH	
28	Lalitpur	Talchikhel	m	NK-15	Blood	NKH	
29	Lalitpur	Talchikhel	f	NK-16	Blood	NKH	
30	Lalitpur	Talchikhel	f	NK-17	Blood	NKH	
31	Lalitpur	Talchikhel	f	NK-18	Blood	NKH	
32	Lalitpur	Talchikhel	f	NK-19	Blood	NKH	
33	Lalitpur	Talchikhel	m	NK-2	Blood	NKH	
34	Lalitpur	Talchikhel	m	NK-20	Blood	NKH	
35	Lalitpur	Talchikhel	m	NK-21	Blood	NKH	
36	Lalitpur	Talchikhel	m	NK-22	Blood	NKH	
37	Lalitpur	Talchikhel	m	NK-23	Blood	NKH	
38	Lalitpur	Talchikhel	m	NK-24	Blood	NKH	
39	Lalitpur	Talchikhel	m	NK-25	Blood	NKH	
40	Lalitpur	Talchikhel	m	NK-3	Blood	NKH	
41	Lalitpur	Talchikhel	f	NK-4	Blood	NKH	
42	Lalitpur	Talchikhel	m	NK-5	Blood	NKH	
43	Lalitpur	Talchikhel	m	NK-6	Blood	NKH	
44	Lalitpur	Talchikhel	m	NK-7	Blood	NKH	
45	Lalitpur	Talchikhel	m	NK-8	Blood	NKH	
46	Lalitpur	Talchikhel	f	NK-9	Blood	NKH	
47	Saptari	Lahan	m	NK-43	Blood	NKD	

Sl.No	District	Sub-district	Sex	POP ID	Sample Type	Breed	
48	Saptari	Lahan	f	NK-44	Blood	NKD	4
49	Saptari	Lahan	f	NK-49	Blood	NKD	
50	Saptari	Lahan	m	NK-50	Blood	NKD	
						Total	**50**

Note: NKH = Hurrah, NKD = Kalo Dharane Sunggur

Table 4.S3: Samples of village pigs and wild boar of Sri Lanka.

Sl. No	District	Village	Province	Sex	POP ID	Sample Type	Breed	Samp/ Distrt
1	Chilaw	Chilaw	North western	m	SLVP-6	Blood	Village pig	3
2	Chilaw	Chilaw	North western	f	SLVP-11	Blood	Village pig	
3	Chilaw	Chilaw	North western	f	SLVP-19	Blood	Village pig	
4	Kalutara	Beruwila	Western	m	SLVP-2	Blood	Village pig	10
5	Kalutara	Beruwila	Western	m	SLVP-3	Blood	Village pig	
6	Kalutara	Beruwila	Western	f	SLVP-5	Blood	Village pig	
7	Kalutara	Beruwila	Western	f	SLVP-7	Blood	Village pig	
8	Kalutara	Beruwila	Western	m	SLVP-20	Blood	Village pig	
9	Kalutara	Kalumulla	Western	f	SLVP-15	Blood	Village pig	
10	Kalutara	Kalumulla	Western	f	SLVP-16	Blood	Village pig	
11	Kalutara	Kalumulla	Western	f	SLVP-17	Blood	Village pig	
12	Kalutara	Kalumulla	Western	m	SLVP-23	Blood	Village pig	
13	Kalutara	Kalumulla	Western	f	SLVP-24	Blood	Village pig	
14	Kandy	Kandy	Central	NR	SLVP-27	Blood	Village pig	2
15	Kandy	Kandy	Central	NR	SLVP-26	Blood	Village pig	
16	Kurunagala	Kudumbuwa	North western	m	SLVP-4	Blood	Village pig	7
17	Kurunagala	Kudumbuwa	North western	f	SLVP-9	Blood	Village pig	
18	Kurunagala	Kudumbuwa	North western	f	SLVP-10	Blood	Village pig	
19	Kurunagala	Kudumbuwa	North western	f	SLVP-13	Blood	Village pig	
20	Kurunagala	Kudumbuwa	North western	m	SLVP-14	Blood	Village pig	
21	Kurunagala	Kudumbuwa	North western	f	SLVP-18	Blood	Village pig	
22	Kurunagala	Kudumbuwa	North western	m	SLVP-21	Blood	Village pig	
23	Puttalam	Marawila	North western	f	SLVP-1	Blood	Village pig	5
24	Puttalam	Marawila	North western	f	SLVP-8	Blood	Village pig	
25	Puttalam	Marawila	North western	f	SLVP-22	Blood	Village pig	
26	Puttalam	Marawila	North western	m	SLVP-25	Blood	Village pig	
27	Puttalam	Marawila	Western	f	SLVP-12	Blood	Village pig	
28	Anuradhapura	Puliyankulama	Northern Central	f	SLWB-28	Blood	Wild boar	5
29	Anuradhapura	Puliyankulama	Northern Central	f	SLWB-43	Blood	Wild boar	
30	Anuradhapura	Puliyankulama	Northern Central	m	SLWB-47	Blood	Wild boar	
31	Anuradhapura	Puliyankulama	Northern Central	f	SLWB-48	Blood	Wild boar	
32	Anuradhapura	Puliyankulama	Nothern central	f	SLWB-37	Blood	Wild boar	
33	Batticaloa	Chiththandy	Eastern	m	SLWB-31	Blood	Wild boar	5
34	Batticaloa	Chiththandy	Eastern	m	SLWB-40	Blood	Wild boar	
35	Batticaloa	Murakoddanchenai	Eastern	m	SLWB-30	Blood	Wild boar	
36	Batticaloa	Walikandha	Eastern	m	SLWB-41	Blood	Wild boar	
37	Batticaloa	Walikandha	Eastern	m	SLWB-42	Blood	Wild boar	
38	Kandy	Galaha	Central	f	SLWB-33	Blood	Wild boar	2
39	Kandy	Galaha	Central	f	SLWB-38	Blood	Wild boar	

Sl. No	District	Village	Province	Sex	POP ID	Sample Type	Breed	Samp/ Distrt
40	Kurunagala	Kudumbuwa	North western	f	SLWB-29	Blood	Wild boar	5
41	Kurunagala	Kudumbuwa	North western	f	SLWB-32	Blood	Wild boar	
42	Kurunagala	Kudumbuwa	North western	m	SLWB-34	Blood	Wild boar	
43	Kurunagala	Kudumbuwa	North western	m	SLWB-35	Blood	Wild boar	
44	Kurunagala	Kudumbuwa	North western	f	SLWB-46	Blood	Wild boar	
45	Polonnaruwa	Manampittiya	Northern Central	f	SLWB-36	Blood	Wild boar	4
46	Polonnaruwa	Manampittiya	Northern Central	m	SLWB-39	Blood	Wild boar	
47	Polonnaruwa	Manampittiya	Northern Central	f	SLWB-44	Blood	Wild boar	
48	Polonnaruwa	Manampittiya	Northern Central	f	SLWB-45	Blood	Wild boar	
NR = Not recorded; f = female; m = male							**Total**	**48**

Table 4.S4: Microsatellite markers with their chromosomal location and primer sequences. The recommended annealing temperature and allele range were used as a guide. D = Dye labelled. Italic = CAG-tag added to three primers. Further detail: https://www-lgc.toulouse.inra.fr/pig/panel/panel2004.htm#D#D

Sl No	Name	Chrom.	Primer Sequence (5' - 3')	Dye	Ann. Tempt	Allele Range
1	*SW857*	14	D–TGAGAGGTCAGTTACAGAAGACC GATCCTCCTCCAAATCCCAT	HEX	55°C	138 – 156
2	*SW122*	6	D–CAAAAAAGGCAAAAGATTGACA TTGTCTTTTTATTTTGCTTTTGG	6-FAM	55°C	106 - 128
3	*IGF1*	5	D–GCTTGGATGGACCATGTTG CATATTTTTCTGCATAACTTGAACCT	6-FAM	55°C	193 - 209
4	*S0143*	12	***CAGTCGGGCGTCATCA*** ACTCACAGCTTGTCCTGGGTGT CAGTCAGCAGGCTGACAAAAAC	PET	55°C	150 - 167
5	*SW240*	2	D–AGAAATTAGTGCCTCAAATTGG AAACCATTAAGTCCCTAGCAAA	NED	55°C	92 - 124
6	*S0005*	5	D–TCCTTCCCTCCTGGTAACTA GCACTTCCTGATTCTGGGTA	NED	55°C	203 – 267
7	*SW951*	10	D– TTTCACAACTCTGGCACCAG GATCGTGCCCAAATGGAC	HEX	55°C	120 plus
8	*SW2406*	6	D–AATGTCACCTTTAAGACGTGGG AATGCGAAACTCCTGAATTAGC	NED	55°C	222 - 262
8	*SW936*	15	D– TCTGGAGCTAGCATAAGTGCC GTGCAAGTACACATGCAGGG	6-FAM	55°C	90 - 116
10	*S0226*	2	D–GCACTTTTAACTTTCATGATACTCC GGTTAAACTTTTNCCCCAATACA	6-FAM	55°C	180 - 210
11	*SW72*	3	***CAGTCGGGCGTCATCA***ATCAGAACAGTGCGCCGT TTTGAAAATGGGGTGTTTCC	NED	55°C	97 - 114
12	*SW632*	7	D–TGGGTTGAAAGATTTCCCAA GGAGTCAGTACTTTGGCTTGA	NED	55°C	148 – 178
13	*S0002*	3	D– GAAGCCAAAGAGACAACTGC GTTCTTTACCCACTGAGCCA	HEX	60°C	186 – 216
14	*S0155*	1	D–TGTTCTCTGTTTCTCCTCTGTTTG AAAGTGGAAAGAGTCAATGGCTAT	6-FAM	55°C	142 - 162
15	*S0090*	12	D–CCAAGACTGCCTTGTAGGTGAATA GCTATCAAGTATTGTACCATTAGG	6-FAM	55°C	227 - 249
16	*S0228*	6	***CAGTCGGGCGTCATCA***GGCATAGGCTGGCAGCAACA AGCCCACCTCATCTTATCTACACT	VIC	55°C	220 - 246
17	*S0068*	13	D–CCTTCAACCTTTGAGCAAGAAC AGTGGTCTCTCTCCCTCTTGCT	NED	55°C	211 - 262
18	*S0026*	16	D–AACCTTCCCTTCCCAATCAC CACAGACTGCTTTTTACTCC	HEX	55°C	87 - 105
19	*SW911*	9	D–CTCAGTTCTTTGGGACTGAACC CATCTGTGGAAAAAAAAAGCC	6-FAM	60°C	149 - 173
20	*S0355*	15	D–TCTGGCTCCTACACTCCTTCTTGATG TTGGGTGGGTGCTGAAAAATAGGA	FAM	50°C	244 - 271
21	*SW2008*	11	D– CAGGCCAGAGTAGCGTGC CAGTCCTCCCAAAAATAACATG	VIC	55°C	95 - 108

Table 4.S5: Overall heterozygosity in Bhutanese and Nepalese pigs.

	Bhutanese Pigs (n= 207)					**Nepalese Pigs (n=49)**				
Locus	**N**	**Na**	**Ne**	**Ho**	**He**	**N**	**Na**	**Ne**	**Ho**	**He**
IGF1	207	24.00	10.73	0.74	0.91	49	16	6.47	0.76	0.84
S0002	206	38.00	8.47	0.73	0.88	48	11	5.10	0.83	0.80
S0005	202	34.00	13.70	0.74	0.93	49	15	5.62	0.76	0.82
S0026	204	16.00	3.83	0.53	0.74	49	7.	3.56	0.51	0.72
S0068	201	35.00	12.24	0.75	0.92	49	17	8.38	0.71	0.88
S0090	205	18.00	6.10	0.64	0.84	48	15	5.76	0.65	0.83
S0143	206	16.00	6.13	0.67	0.84	49	11	4.17	0.80	0.76
S0155	206	25.00	7.00	0.43	0.86	49	11	5.03	0.55	0.80
S0226	205	20.00	5.52	0.61	0.82	49	13	6.56	0.74	0.85
S0228	207	18.00	7.94	0.70	0.87	48	14	5.72	0.83	0.83
S0355	207	22.00	7.06	0.66	0.86	49	12	5.94	0.84	0.83
SW72	207	22.00	5.91	0.66	0.83	49	13	6.00	0.80	0.83
SW122	207	24.00	13.47	0.76	0.93	48	14	6.64	0.44	0.85
SW240	207	24.00	8.20	0.76	0.88	48	12	7.31	0.85	0.86
SW632	207	14.00	4.72	0.63	0.79	49	8	3.79	0.69	0.74
SW857	206	16.00	6.77	0.54	0.85	49	9	5.53	0.86	0.82
SW911	206	25.00	6.45	0.68	0.84	49	12	3.74	0.53	0.73
SW936	207	28.00	7.52	0.29	0.87	48	16	8.60	0.52	0.88
SW951	207	14.00	3.67	0.39	0.73	49	5	3.31	0.53	0.70
SW2008	202	12.00	4.68	0.70	0.79	48	8	3.91	0.73	0.74
SW2406	204	29.00	5.41	0.65	0.82	49	15	4.95	0.65	0.80
Mean	205.52	22.57	7.41	0.63	0.85	48.67	12.10	5.53	0.69	0.81
SE	0.41	1.58	0.63	0.03	0.01	0.11	0.71	0.33	0.03	0.01

Key: Na = No. of Different Alleles; Ne = Effective No. of Alleles; Ho = Observed Heterozygosity; He = Expected Heterozygosity.

Table 4.S6: Summary of Chi-Square Tests for HWE in Bhutanese and Nepalese pigs.

	Bhutanese (n= 207)				Nepalese (n=49)			
Locus	**DF**	**ChiSq**	**Prob**	**Signif**	**DF**	**ChiSq**	**Prob**	**Signif**
IGF1	276	627.233	0.000	***	120	295.44	0.000	***
S0002	703	1012.080	0.000	***	55	142.42	0.000	***
S0005	561	1826.826	0.000	***	105	253.73	0.000	***
S0026	120	630.651	0.000	***	21	69.09	0.000	***
S0068	595	1371.504	0.000	***	136	245.42	0.000	***
S0090	153	1494.461	0.000	***	105	275.91	0.000	***
S0143	120	533.875	0.000	***	55	62.30	0.233	ns
S0155	300	1720.318	0.000	***	55	100.15	0.000	***
S0226	190	720.916	0.000	***	78	132.46	0.000	***
S0228	153	396.706	0.000	***	91	150.90	0.000	***
S0355	231	1243.847	0.000	***	66	137.41	0.000	***
SW72	231	803.351	0.000	***	78	209.39	0.000	***
SW122	276	699.554	0.000	***	91	241.86	0.000	***
SW240	276	450.707	0.000	***	66	105.21	0.002	**
SW632	91	386.730	0.000	***	28	35.46	0.157	ns
SW857	120	614.609	0.000	***	36	46.78	0.108	ns
SW911	300	1639.970	0.000	***	66	306.38	0.000	***
SW936	378	2122.778	0.000	***	120	270.59	0.000	***
SW951	91	842.371	0.000	***	10	42.99	0.000	***
SW2008	66	394.651	0.000	***	28	51.76	0.004	**
SW2406	406	1118.486	0.000	***	105	207.44	0.000	***

Key: HWE = Hardy-Weinberg Equilibrium; ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

Table 4.S7: HWE test and overall heterozygosity in both domestic and wild pigs of Sri Lanka.

Summary of Chi-Square Tests for HWE					**Overall Heterozygosity**				
Locus	**DF**	**ChiSq**	**Prob**	**Signif**	**N**	**Na**	**Ne**	**Ho**	**He**
IGF1	105	267.23	0.000	***	47	15	6.78	0.57	0.85
S0002	91	137.44	0.001	**	46	14	5.55	0.61	0.82
S0005	171	269.70	0.000	***	47	19	11.66	0.77	0.91
S0026	45	155.78	0.000	***	47	10	6.38	0.66	0.84
S0068	190	372.55	0.000	***	47	20	9.54	0.634	0.90
S0090	21	71.15	0.000	***	46	7.	2.79	0.30	0.64
S0143	28	86.91	0.000	***	46	8	5.90	0.59	0.83
S0155	78	186.32	0.000	***	47	13	5.78	0.62	0.83
S0226	55	183.35	0.000	***	47	11	4.78	0.34	0.79
S0228	105	104.80	0.487	ns	47	15	7.99	0.81	0.88
S0355	45	173.50	0.000	***	47	10	5.08	0.81	0.80
SW72	120	226.80	0.000	***	45	16	6.29	0.49	0.84
SW122	55	157.36	0.000	***	46	11	5.14	0.70	0.81
SW240	66	160.52	0.000	***	47	12	5.21	0.57	0.801
SW632	78	159.90	0.000	***	47	13	9.02	0.75	0.89
SW857	66	112.51	0.000	***	47	12	6.59	0.66	0.85
SW911	91	151.75	0.000	***	46	14	8.22	0.83	0.88
SW936	91	169.93	0.000	***	45	14	6.22	0.58	0.84
SW951	6	10.16	0.118	ns	47	4	3.32	0.53	0.70
SW2008	36	79.44	0.000	***	46	9	5.21	0.63	0.81
SW2406	171	273.00	0.000	***	47	19	7.14	0.77	0.86
					Mean	12.67 ± 0.88	6.41 ± 0.45	0.63 ± 0.03	0.83 ± 0.01

Table 4.S8: Mean heterozygosity over loci for each pig population in South Asia.

Country	Types	Pop	N	Na	Ne	Ho	He
Bhutan	Indigenous type	MMP1	60	12.57 ± 0.93	5.45 ± 0.43	0.63 ± 0.04	0.79 ± 0.02
		RGP2	24	8.10 ± 0.60	4.17 ± 0.38	0.62 ± 0.03	0.72 ± 0.03
		WWCP3	64	13. 48 ± 0.98	5.57± 0.62	0.65 ± 0.03	0.78 ± 0.02
		EECP4	48	14.14 ± 1.14	6.51 ± 0.53	0.60 ± 0.03	0.82 ± 0.02
		Mean		12.07 ± 0.91	5.43 ± 0.49	0.63 ± 0.03	0.78 ± 0.02
Bhutan	Wild Boar	BWB	10	8.76 ± 0.55	6.28 ± 0.53	0.67 ± 0.04	0.83 ± 0.01
Nepal	Indigenous	NKH	21	8.71 ± 0.56	4.62 ± 0.42	0.65 ± 0.04	0.74 ± 0.03
		NKD	24	8.14 ± 0.60	4.53 ± 0.33	0.76 ± 0.03	0.75 ± 0.02
		Mean		8.43 ± 0.58	4.58 ± 0.38	0.71 ± 0.04	0.75 ± 0.02
Sri Lanka	Indigenous	SLVP	26	9.29 ± 0.59	4.85 ± 0.33	0.64 ± 0.02	0.77 ± 0.02
	Wild Boar	SLWP	21	8.33 ± 0.72	4.28 ± 0.46	0.61 ± 0.05	0.69 ± 0.04
Outgroup	Australia Commercial	AC	15	6.76 ± 0.45	3.764 ± 0.33	0.64 ± 0.03	0.69 ± 0.03
		Total	313	9.86 ± 0.29	5.06 ± 0.16	0.65 ± 0.01	0.76 ± 0.01

Table 4.S9: F-statistics for each of the loci across South Asian pig populations

	Bhutanese			Nepalese			Sri Lankan		
Locus	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
IGF1	0.18	0.24	0.07	-0.03	0.07	0.10	0.25	0.33	0.11
S0002	0.12	0.18	0.07	-0.12	-0.04	0.07	0.04	0.26	0.23
S0005	0.14	0.20	0.07	-0.02	0.04	0.06	0.09	0.16	0.07
S0026	0.16	0.33	0.20	0.10	0.24	0.16	0.11	0.20	0.09
S0068	0.14	0.22	0.09	0.12	0.14	0.03	0.27	0.29	0.04
S0090	0.15	0.23	0.09	0.17	0.19	0.03	0.23	0.54	0.40
S0143	0.16	0.22	0.07	-0.15	-0.13	0.01	0.18	0.31	0.16
S0155	0.40	0.48	0.14	0.21	0.24	0.04	0.20	0.24	0.05
S0226	0.23	0.37	0.18	0.04	0.09	0.05	0.40	0.59	0.31
S0228	0.11	0.19	0.09	-0.06	-0.01	0.05	-0.04	0.06	0.10
S0355	0.17	0.26	0.10	-0.12	-0.05	0.06	-0.24	-0.02	0.18
SW72	0.20	0.24	0.05	-0.07	-0.04	0.03	0.32	0.42	0.14
SW122	0.16	0.21	0.06	0.48	0.50	0.03	0.10	0.14	0.04
SW240	0.10	0.16	0.06	-0.07	0.01	0.07	0.08	0.30	0.24
SW632	0.18	0.23	0.06	-0.04	0.05	0.09	0.07	0.16	0.10
SW857	0.23	0.30	0.10	-0.08	-0.04	0.04	0.11	0.23	0.13
SW911	0.11	0.22	0.13	0.10	0.20	0.11	-0.12	0.04	0.14
SW936	0.62	0.65	0.08	0.36	0.38	0.03	0.27	0.28	0.02
SW951	0.36	0.42	0.09	0.01	0.19	0.17	0.15	0.24	0.10
SW2008	0.07	0.16	0.10	-0.10	-0.03	0.07	-0.01	0.21	0.22
SW2406	0.10	0.20	0.10	0.06	0.17	0.12	-0.03	0.12	0.14
Mean	0.19	0.27	0.09	0.04	0.10	0.07	0.12	0.24	0.14
SE	0.03	0.03	0.01	0.04	0.03	0.01	0.03	0.03	0.02

Table 4.S10: Pairwise Population F_{ST} values (below diagonal) and genetic distance (below diagonal) (Nei 1972).

	MMP1	RGP2	WWCP3	EECP4	BWB	NKH	NKD	SLVP	SLWP	AC
MMP1	*****	0.60	0.33	0.45	1.10	0.62	0.25	0.81	1.69	1.03
RGP2	0.07	*****	0.43	0.41	1.16	0.64	0.67	0.88	1.89	1.08
WWCP3	0.04	0.06	*****	0.24	1.36	0.37	0.49	0.78	1.62	0.80
EECP4	0.04	0.05	0.03	*****	1.16	0.59	0.62	0.86	1.47	0.97
BWB	0.08	0.01	0.09	0.07	*****	1.17	1.01	0.88	1.54	0.96
NKH	0.07	0.08	0.05	0.06	0.09	*****	0.46	0.90	1.82	0.83
NKD	0.03	0.08	0.06	0.06	0.08	0.06	*****	0.95	1.99	1.17
SLVP	0.07	0.09	0.07	0.07	0.07	0.09	0.09	*****	1.32	0.34
SLWP	0.13	0.16	0.13	0.12	0.12	0.15	0.15	0.12	*****	1.26
AC	0.1	0.12	0.09	0.09	0.09	0.1	0.12	0.05	0.14	*****

Note: Bhutananese pig populations = MMP1, RGP2, WWCP3, EECP4, and BWB; Nepalese pig populations = NKH and NKD; Sri Lankan pig populations = SLVP and SLWP

Table 4.S11: Summary of expected heterozygosity and effective number of alleles used as estimators of genetic diversity. Values from wild boar are not included.

Country	No. of breeds/types used/detected	Total Pigs	Average He	Average Ne	Research Studies
Bhutan	4 (indigenous)	196	0.78 ± 0.02	5.43 ± 0.50	This study
Nepal	2 (indigenous)	45	0.75 ± 0.02	4.58 ± 0.38	
Sri Lanka	1 (village pigs)	26	0.77 ± 0.02	4.85 ± 0.33	
India (North)	2 (native)	50	0.80	5.17	Behl *et al.* (2002)
India (South)	1 (native)	26	0.83	5.34	Behl *et al.* (2006)
China	4 (indigenous)	61	0.72 ± 0.10	4.07 ± 1.46	Li *et al.* (2000)
China	7 (indigenous)	380	0.59	2.82	Fan *et al.* (2002)
China Tibet	1 (indigenous)	31	0.75	4.98	Fan *et al.* (2003)
China	3 (indigenous)	90	0.65	3.8	
China Tibet	1 (indigenous)	60	0.87	8.00	Yang *et al.* (2003)
China	17 (indigenous)	967	0.84	7.41	
China	10 (village pigs)	817	0.83	5.79	Fang *et al.* (2009)
China	10 (indigenous)	379	0.68	4.40	Li *et al.* (2004)
Vietnam	5 (indigenous)	152	0.75	4.54	Thuy *et al.* (2006)
European	10 (commercial + native)	471	0.53	2.19	Laval *et al.* (2000)
European	58 (commercial + native)	2737	0.56		SanCristobal *et al.* (2006)

Note: SE values for most studies were not available.

CHAPTER 5

5.0 Wild Boar Mitochondrial Phylogeography, Introgression, and Dispersal in South Asia

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5.1 ABSTRACT

The distribution and variation of porcine mitochondrial lineages in South Asia are not well understood. This study determined the distribution of porcine mtDNA lineages in South Asia, investigated their origins and relevance to domestication and dispersal of pigs within the region. DNA samples from 242 domestic pigs and wild boar from South Asia were amplified and 652bp of the control region of the mitochondrial DNA were sequenced. Seventy three haplotypes detected in South Asia were combined with Genbank sequences comprising 15 European, 289 East Asian haplotypes including previously reported sequences from South Asia, and 26 haplotypes from Thailand. These haplotypes represented sequences from almost 1800 wild boar and domestic pigs from all over the world. While our analyses revealed a very complex clustering pattern of porcine haplotypes, there were clear phylogeographic signals. The clear segregation of European and Asian pigs was consistent with independent domestication of pigs in Europe and Asia respectively. In addition, we observed three major mitochondrial porcine clades, which are unique to the Indian sub-continent. The shared haplotypes between domestic pigs of Bhutan, Northeast India, and Nepal with wild boar, possibly belonging to *Sus scrofa cristatus* from Northern India within Mixed Clade 1 (MC1) provides support for an independent centre of domestication in the foothills of Himalayas and Indian sub-continent. However, in the absence of corroborating archaeological or fossil evidence, this could also be explained by introgression of maternal genes from MC1 wild boar to domestic pigs. The two additional novel wild boar clades, the Northern South Asia (NSA) and Southern South Asia (SSA), could belong to previously recognised wild boar subspecies (*S. s. davidi* and *S. s. affinis*) from Northern South Asia and Sri Lanka respectively. Both NSA and SSA and two Bhutanese wild boar haplotypes, W17 and W12, have not been detected in any domestic pigs. The South Asian domestic pigs have also been influenced by haplotypes from widely dispersed East Asian and European pigs. The dispersal of pigs into and within the region may have been mediated by humans. These porcine haplotypes indicate that South Asia represents a unique and important source of genetic resources important for biodiversity, food security and rural development in the face of global climate change.

Key words: *Mitochondrial DNA, control region, haplotypes, genetic introgression, domestication, pig dispersal*

5.2 INTRODUCTION

The wild boar (*Sus scrofa*) is the most widely distributed species of the Old World Suidae. It is found from Western Europe and the Mediterranean basin to the Russian Taiga, through India and South-east Asia to the islands of Sri Lanka and Japan. It has also colonised Northern Europe including Britain (Erkinaro *et al.* 1982; Leaper *et al.* 1999; O' Connell 2008). More than 20 subspecies of wild boar have been reported based on their morphological characteristics (Ruvinsky & Rothschild 1998; Watson 2004), and geographical distribution in Asia, Europe and North Africa. Feral pigs, which are domestic pigs that have escaped or been released into the wild, have been introduced to the Americas (Barrett 1978; Grossi *et al.* 2006) and Pacific islands (Larson *et al.* 2005) from Europe and Asia.

The domestic pig (*S. s. domesticus*) was domesticated from wild boar (Giuffra *et al.* 2000; Larson *et al.* 2005). The mitochondrial DNA (mtDNA) sequences have also revealed several sites of domestication of pigs in Eurasia. Giuffra *et al.* (2000) reported clear evidence for independent domestication of both European and Asian subspecies of wild boar. Subsequently, Larson *et al.* (2005) suggested at least six independent domestications of pigs in Europe, Asia, India, and Southeast Asia in the past 9000 years. Using complete mtDNA sequences, Wu *et al.* (2007) suggested domestications in East Asia (EA), which have mainly occurred in the Mekong region and the middle and downstream regions of the Yangtze River. Tanaka *et al.* (2008) identified another unique haplotypes (MTSEA) in Souteast Asia suggesting another site of independent domestication in the mountainous areas of mainland Southeast Asia, particularly in Cambodia and Laos.

In 2010, Larson and his colleagues provided further in-depth discussion of independent domestications of indigenous wild boar populations in India, Peninsular Southeast Asia, and in Taiwan. In doing so, 13 clades of only wild boar (W1-W13) and four mixed clades (MC1-MC4) consisting of both wild and domestic pigs showed different clustering pattern with clear phylogeographic signals (Larson *et al.* 2010). Three clades (W1–W3) were found only in South Korea, one clade (W4) in Northeast Asia excluding South Korea, four (W5–W8) in Japan, Okinawa, Taiwan, and the southern Chinese island of Hainan, five clades (W9–W12) in Indo-Burma Biodiversity Hotspot or IBBH

(Mittermeier *et al.* 2005) and one clade (W13) was restricted to Central China although two samples are also found in northern Vietnam. Similarly, the mixed clade, MC1, which was previously designated as D3 (Larson *et al.* 2005), included both native wild boar and domestic pigs from India and Bhutan and provided evidence for a domestication event. The MC2 clade, which was referred to as the D6 or Pacific clade (Larson *et al.* 2005), consisted of domestic or feral pigs from Oceania clustered with wild boar from northern Vietnam, Yunnan province, and Laos thereby further supporting the fact that Oceania pigs were introduced domestic pigs from Southeast Asia. The third clade (MC3), referred to previously as MTSEA, belonged to both wild and domestic samples found almost exclusively in the IBBH (Table 5.1). MC4 was restricted to domestic pigs from South Chinese provinces and one wild boar from Vietnam.

However, besides MC1 and MC3 (Tanaka *et al.* 2008), not much sequence information has been generated from South Asian domestic and wild boar. An independent centre of domestication of pigs in the Indian-sub continent and foothills of the Himalayas has been speculated upon from the evidence of shared haplotypes between wild and domestic pigs within MC1 clade (Tanaka *et al.* 2008; Larson *et al.* 2010). However, in the absence of archaeological or fossil records, this scenario may be just a case of genetic introgression from local wild boar into the domestic pigs rather than an independent domestication event. Several general cluster haplotypes (Larson *et al.* 2010), which were previously designated as D2 haplotypes (Larson *et al.* 2005), were found at very high frequency within Bhutanese pigs (Tanaka *et al.* 2008). However, pigs from South Asia (Table 5.1) have not been adequately sampled and mitochondrially characterised.

Nidup (2006) emphasised a need for molecular characterization of pigs in the Hindu Kush Himalayan region, which includes parts of South Asia with focus on foothills of the Himalayas. In line with this, an assessment of biodiversity, population trends and importance of indigenous pigs of Bhutan and Nepal (Nidup *et al.* 2010; Nidup *et al.* 2011) including evaluation of genetic structure and diversity with microsatellites (Nidup *et al.* Submitted) has been recently completed. However, the distribution and variation of mtDNA lineages in South Asian pigs have not been well explored nor their origin or domestication well understood. This study will investigate distribution of porcine

mtDNA lineages in the South Asia, elucidate their possible origin and influence on domestication, assess genetic introgression and possible introduction of pigs in South Asia region. Various terms for pigs and geographical areas have been used in the text (Table 5.1).

The terms 'village' and 'domestic' pigs have often been used interchangeably. They refer to domestic indigenous pigs which are reared in villages of South Asian countries, namely Bhutan, Nepal, Sri Lanka and India. They do not exclude admixture or crossbreds that were phenotypically impossible to distinguish from pureline indigenous pigs during the sampling. 'Wild boar' refers to wild pigs belonging to *Sus scrofa* species that are free-roaming within the South Asian ecosystem. It does not include feral pigs, which are generally domestic pigs that have escaped human captivity or been introduced into the wild. The geographical definitions of regional areas used in this study are given in Table 5.1.

Table 5.1: Geographic areas referred to in the text. Some of the regions have been defined in line with Larson *et al.* (2010).

Region	Areas under each region
South Asia	Bhutan, Nepal, Sri Lanka, India, and Pakistan
Himalayas	Bhutan, Nepal, Indian States of Jammu and Kashmir, Arunachal Pradesh, Himachal Pradesh, Sikkim, and Darjeeling of West Bengal
Northern India	Indo-Gangetic Plain and Himalayas; include States of Jammu and Kashmir, Punjab, Himachal Pradesh, Haryana, Rajasthan, Union Territory of Chandigarh, and North Central Indian cultural zone, which includes Madhya Pradesh, Chhattisgarh, Haryana, Bihar, Jharkhand, Utter Pradesh, Uttarachand, and Delhi.
Northern South Asia	Northern India, Pakistan, and Nepal
Southern South Asia	Sri Lanka
Northeast India	Sikkim, parts of North Bengal (Darjeeling, Jalpaiguri, Cooch Bihar), Seven Sister States (Arunachal Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland, and Tripura).
West-central India	States of Gujarat, Goa, Maharashtra, and Madhya Pradesh
Central Province	Central province of British India. It consists of present-day Madhya Pradesh, Chhattisgarh and Maharashtra states.
Southern India	States of Andhra Pradesh, Karnataka, Kerala, Tamil Nadu
Eastern Bhutan	Districts: Trashigang, Mongar, S/Jhongkhar, Pemagatshel, Tashiyangtse, Lhuentse
East-central Bhutan	Districts: Zhemgang, Sarpang, Bumthang, Trongsa
Western Bhutan	Districts: Thimphu, Paro, Haa, Samtse, Chukha
West-central Bhutan	Districts: Wangdue, Punkha, Dagana, Tsirang, Gasa
IBBH	Vietnam, Laos, Cambodia, Myanmar, Thailand, and Chinese province of Yunnan Guangdong, and Guangxi Zhuang Nationality Autonomous Region
Northeast Asia	North Korea, South Korea, Japan, and northeast Chinese provinces of Liaoning, Jilin, Heilongjiang, and the northern portion of Inner Mongolia.
Central China	Central China (except the southern portion of Yunnan province), Guangxi, and Guangdong provinces, and the northeast provinces of Liaoning, Jilin, Heilongjiang,

Region	Areas under each region
	and the northern portion of Inner Mongolia
Oceania	Papua New Guinea and the islands of Sumatra, Java, Borneo, and all the islands to the east extending into the remote Pacific

5.3 MATERIALS AND METHODS

5.3.1 Sampling and DNA Extraction

The methods used for sampling and DNA purification from blood, hair and faecal samples are given in Nidup *et al.* (Submitted). A subset of 140 village pigs from Bhutan, 40 from Nepal, and 23 from Sri Lanka were carefully chosen based on their geographical distribution and phenotypic variation (Table 5.S3; Table 5.S4; Table 5.S5). All 12 Bhutanese and 15 Sri Lankan wild boar samples were used for this study. The only sample of Indian village pig, which was obtained from the livestock quarantine centre in Gelephu, Bhutan, was also used in this study.

5.3.2 PCR Amplification and Sequencing

The mtDNA hypervariable control region was amplified by PCR using the primer set employed in past studies (Kim *et al.* 2002; Gongora *et al.* 2003; Gongora *et al.* 2006): Kim L [(5'-CCA AGA CTC AAG GAA GGA GA-3' (Position 15,363-15,382 of the pig mtDNA, Accession # AJ002189)] and Kim H [5'-GGC GCG GAT ACT TGC ATG TG-3' (Position 115-134)]. A large fragment of mtDNA control region (>1.2 bp) was amplified and an internal reverse primer (> 600 bp), FAir 5'-GCA CCT TGT TTG GAT TGT CG-3' (Fang & Anderson 2006; Fang *et al.* 2006) was used as a precautionary measure to avoid possible contamination with numts (Bensasson *et al.* 2001) even though they have never been reported for porcine mitochondrial sequences.

PCR amplification was carried out using the following conditions. For each sample, 25 µl reaction was performed in a PTC-100^{TM} Peltier Thermal Cycler (MJ Research, Inc., Waltham). The reaction mixture contained 2 µl of forward and 2 µl reverse primers (both 20 *p*mol/µl), 3 µl DNA (50ng/µl), 0.2 unit of *Tli-Taq* polymerase, 1.5 µl dNTP (2mM), 1.5 µl $MgCl_2$ (25mM), 2.5 µl 10 buffer (100mM Tris.Cl, 15mM $MgCl_2$, 500mM KCl, pH 8.3), and 12.3 µl sterile Milli-Q water. The touchdown PCR (Don *et*

al. 1991) reaction profile consists of initial denaturation at 95°C for 4 minutes and 94°C for 1 minute followed by annealing at 65°C for 1 min and extension at 72°C for 1.5 min. This was followed by 7 cycles of 94°C for 1 min, 65°C for 1 min, and 72°C for 1.5 min. The second part of the profile had 30 cycles, each consisting of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1.5 min. The annealing temperature of the reaction was decreased by 0.5°C in every second cycle. The amplification was terminated after final a step of extension at 72°C for 10 minutes.

The PCR products were gel purified with a JETquick spin column (GeneWorks, Australia) and sequenced at Australian Genome Resource Facility (AGRF), Australia. About 200 DNA templates were sent directly to AGRF for PCR, purification and clean-up of amplicons, and for sequencing. After confirming the quality of sequences with reverse primer, only 652bp of the mtDNA control region from 231 pigs, corresponding 15433 to 16085 in the complete porcine mtDNA (Ursing & Arnason 1998) were used.

5.3.4 DNA Extraction, Amplification and Sequencing of Museum and Ancient Specimens

11 museum samples and 1 ancient specimen were extracted, amplified, and sequenced at the Henry Wellcome Ancient Biomolecules Centre (HWABC) in Oxford following the methods described by Shapiro *et al*. (2004). A 663bp fragment of the mitochondrial control region was generated for the 11 museum samples analysed at the HWABC using previously published primer combinations (Larson *et al.* 2007). In addition, two bones from ancient pigs from the site of Gotihawa in the Kapilbastu district of Nepal (Verardi 1998, 2007) were subjected to DNA extraction techniques, though only one of those bones yielded amplifiable DNA (Table 5.S6). All 11 museum specimens (Table 5.S6) yielded sufficient DNA to be combined with the modern samples in this study (Table 5.S3,Table 5.S4, & Table 5.S5).

5.3.5 Analysis of Sequence Data

CodonCode Aligner (©CodonCode Corporation, MA, NSA) was used for DNA sequence assembly, contig editing, and mutation detection. Ambiguous sequences were checked using sequence chromatograms and three reference sequences (AY463061,

AY463062, AF276926) from Genbank. To verify accuracy, DNA sequences were further aligned using MUSCLE (Edgar 2004b; Edgar 2004a) and ClustalW2 (Thompson *et al.* 1994; Larkin *et al.* 2007). The aligned sequences were collapsed to haplotypes using FaBox 1.35 (Villesen 2007) and verified using COLLAPSE 1.2 (Posada 2009). Final sequences from the South Asian pigs were submitted into Genbank (http://www.ncbi.nlm.nih.gov/genbank/submit.html) with accession numbers from HQ318287 to HQ318517 (Table 5.S3). The 11 novel museum sequences and one ancient sequence were submitted with accession numbers JF286589 to JF286600.

The phylogenetic analysis (Figure 5.1) was performed on 652bp sequences using Monte Carlo-Markov Chain (MCMC) with MrBayes 3.1.2 (Huelsenbeck *et al.* 2001; Ronquist & Huelsenbeck 2003) using model parameters (HKY+I+G) identified by ModelGenerator v0.85 (Keane *et al.* 2006) and ModelTest (Posada & Crandall 1998). This analysis consisted of 73 newly generated haplotypes from South Asia (Figure 5.1), 14 representative European haplotypes (Table 5.S8), 7 wild boar and 19 domestic pig haplotypes from Thailand (Table 5.S7), and 289 East Asian (EA) haplotypes (Table 8 A.1), which also include 12 previously reported South Asian sequences (Table 5.S7). These haplotypes represent almost 1800 wild and domestic pig sequences from all over the world.

To complement the Bayesian tree (Figure 5.1), the Median-Joining Network (MJN) analysis (Bandelt *et al.* 1999) was performed on shorter sequences (507bp) along with major representative haplotypes (Table 5.S8) that were used in a previous study (Tanaka *et al.* 2008). For this, sequences were further aligned, gaps and missing data within the sequences were removed using Fluxus's DNA alignment program (http://www.fluxus-engineering.com) to generate a multistate alignment rdf (Roehl data format), which was imported into Network 4.5.1.6. The frequency per haplotype, breed information and geographical distribution of the animals were specified. This dataset was star contracted (with threshold connection limit of 5) to further collapse very closely related sequences into major haplotypes. Star contraction simplifies networks and identifies star-like clusters of nodes and shrinks such nodes back towards the founder population (Forster *et al.* 2001). The MJN analysis was used to determine genealogies of haplotypes or further elucidate the differences among the varying haplotypes. It was performed using default parameters with all characters weighing equally. Post-processing analysis

(Polzin & Daneshmand 2003) was performed on MJN analyses output to identify unnecessary median vectors and links, which were switched off in the results.

To check the clustering of only ancient domestic pig sample (GL384) from Nepal, a Neighbour-Joining (NJ) genetic distance tree (Figure 5.S1) was generated using MEGA 4 (Tamura *et al.* 2007) by analysing 140bp sequence from this sample with major representative haplotypes.

5.4 RESULTS

5.4.1 Haplotype Distribution in South Asian Pigs

Analysis of mtDNA control region nucleotide sequences from 231 wild and village pigs in South Asia revealed 28 haplotypes in Bhutan, 16 in Nepal, and 18 in Sri Lanka (Table 5.S1). In addition to one village pig haplotype, we found 9 haplotypes from 10 Indian wild boar museum samples (Table 5.S2). There were 73 swine haplotypes in South Asia (Table 5.2). The number of individuals representing each haplotype for each country is given in Table 5.S3, Table 5.S4, and Table 5.S5.

Table 5.2: DNA samples used and haplotype generated in this study.

Sl No.	Category Of Pigs	Country	No. of Samples	Sequence Length (bp)	Genbank Accession Number	No. of Haplotypes	Total
1	Village	Bhutan	140	652	HQ318287-HQ318427	22	28
2	Wild boar		12	652	HQ318428-HQ318439	6	
3	Domestic	Nepal	40	652	HQ318440- HQ318479	15	16
4	Wild – Museum specimen		1			1	
5	Village	Sri Lanka	23	652	HQ318480- HQ318502	12	18
6	Wild boar		15	652	HQ318503- HQ318517	6	
7	Village	India	1	652	HQ318344	1	11
8	Wild – Museum specimen		10	663		10	
Total			**242**			**73**	**73**

5 of 22 haplotypes in village pigs and 3 of 12 haplotypes in wild boar in Bhutan were represented by a single sequence while others were found in at least two animals. In Sri Lanka, 9 of 22 village pig haplotypes and 2 of 6 wild boar haplotypes were represented by a single sequence while the rest were found in two or more animals. Similarly in

Nepal, 7 of 15 village pig haplotypes were represented by a single sequence while others were found in at least two animals.

The village pigs in South Asia share common haplotypes (Table 5.S1). Three haplotypes were shared between Bhutanese and Nepalese village pigs while two were shared amongst the former and Sri Lanka village pigs. Only one haplotype was shared between Nepalese and Sri Lankan village pigs. No identical haplotype of village pigs and wild boar was observed. Details of each individual are given in Table 5.S3, Table 5.S4 and Table 5.S5.

5.4.2 Phylogenetic Analysis of South Asian Pigs

5.4.2.1 Clustering of Haplotypes

The inclusion of numerous additional samples and haplotypes from South Asia has not changed the general shape of the consensus Bayesian trees (Figure 5.1), which is similar to ones derived in previous studies (Larson *et al.* 2005; Tanaka *et al.* 2008; Larson *et al.* 2010). Since the data were combined with EA haplotypes, nomenclature and labels used were consistent with Larson *et al.* (2010). All the haplotypes were broadly divided into six major groups, which were then divided into clades. The first major group contained the South Asian wild clades consisting of two distinct clades with high posterior probability support (97-100%). They were referred to as "Northern South Asia (NSA)" and "Southern South Asia (SSA)" clades. There is a clear phylogeographic signal for NSA and SSA, with the former being unique to Northern India, Pakistan, and Nepal (Northern South Asia) while the latter was found only in Sri Lankan wild boar and not in any village pigs.

The General Asian Cluster (GAC) consists of east Asian domestic pigs and wild boar, followed by the Domestic Cluster, which consists of 12 clades (D1-D8, D10-D12) belonging to various domestic pigs of Asia. The Wild Cluster comprised 17 Asian wild boar clades (W1-W17). There were 7 mixed clades (MC1-MC7) consisting of identical or closely related haplotype sequences from both domestic and wild samples. In addition to clades reported by Larson *et al.* (2010), 3 novel domestic (D10 - D12), four novel wild (W14 - W17), and 3 novel mixed clades (MC4 - MC7) were detected in this study. Surprisingly, D9 and W5, detected by Larson *et al.* (2010), were clustered under

GAC and MC6 respectively, confirming further complexity within Asian domestic and wild boar. Finally, the European clade consists of two clades belonging to European wild boar (W18) and domestic pigs (D13).

Except for the GAC, all the groups had good posterior probability support indicating the reliability of clades. Most clades had more than 50% posterior probability support. The Asian clades were clearly distinct from European clades confirming the independent domestication of pigs in Asia and Europe (Giuffra *et al.* 2000; Larson *et al.* 2005; Fang & Anderson 2006). The phylogenetic position of EA haplotypes with their phylogeographic provenance (Table 5.1) and detail of each clade has been thoroughly discussed by Larson *et al.* (2010). Here, the focus is on South Asian samples and novel clades detected in this study.

5.4.2.2 Clustering of haplotypes from South Asia

More than 37% of Bhutanese, 58% of Nepalese and 48% of Sri Lankan domestic pigs fall into the GAC, followed by MC1, previously designated D3 by Larson *et al.* (2005). About 50% of Bhutanese wild boar, 34% of Bhutanese domestic pigs, and 13% of Nepalese village pig sequences belong to MC1. There were shared haplotypes between MC1 wild boar and some domestic pigs from Bhutan and Nepal. This also includes sequence from one modern domestic pig of Northeast India and one ancient domestic pig sample from Nepal. This is consistent with geographical proximity of Northern Indian wild boar to Himalayan domestic pigs belonging to the MC1 clade. The D7 was exclusive to Bhutanese and Nepalese village pigs. Most Sri Lankan village pigs clustered in D4, MC4 and MC5. One Sri Lankan and one Bhutanese village pigs clustered as D12. Interestingly, some Bhutanese and Nepalese village pigs (Bhu98VP, Bhu164VP, Bhu47VP, NK44, and NK30) have clustered in MC7 along with wild boar from provinces of Hainan and Zhejiang of China. Likewise, some of the domestic pigs from the Western region of Bhutan and Chinese Hainan province clustered as D10. D11 was unique to Bhutanese pigs from East-central Bhutan. None of the village or wild pigs from South Asia clustered in the Pacific clade (MC2).

Interestingly, about 33% of the Bhutanese wild boar belongs to the W12 clade, which is equivalent to D5 haplotypes described previously (Larson *et al.* 2005). The wild boar

belonging to W12 spread across east-central and west-central regions of Bhutan. The museum sample (GL929_India) belonging to wild boar from the Indian state of Sikkim also clustered in W12. This is again phylogeographically consistent considering that Bhutan shares a border with Sikkim and other Northeast Indian states adjacent to Myanmar. Similarly, W17 was exclusive to wild boar from the East-central region of Bhutan. In contrast to Tanaka *et al.* (2008), there was no new evidence for MTSEA haplotypes (MC3) within Bhutanese village pigs or wild boar.

Table 5.3: Number of individuals representing each clade including general cluster across South Asia. The nomenclatures of the clades including the new ones detected in this study have been kept consistent with Larson *et al* (2010).

Major Clade	Clade	BVP	BWB	NVP	NWB	SLVP	SLWB	IVP	IWB
General Asian	GAC	52		23		11			
Domestic	D4					3			
	D7	21		4					
	D10	6							
	D11	1							
	D12	1				2			
Mixed	MC1	47	6	5				1	
	MC4					4			
	MC5					1			
	MC7	3		3					
Wild	W12		4						
	W17		2						
SA Wild	NSA				1				10
	SSA						15		
European	D13	9		5		2			
Total	**140**	**12**	**40**	**1**	**23**	**15**	**1**	**10**	

Note: SA = South Asia; Northern South Asia = NSA; Southern South Asia = SSA; BVP = Bhutanese village pigs; BWB = Bhutanese wild boar; NVP = Nepalese village pigs; NWB = Nepalese wild boar; SLVP = Sri Lankan village pigs; SWB = Sri Lankan wild boar; IVP = Indian village pig; IWB = Indian wild boar.

5.4.2.3 European haplotypes in South Asia

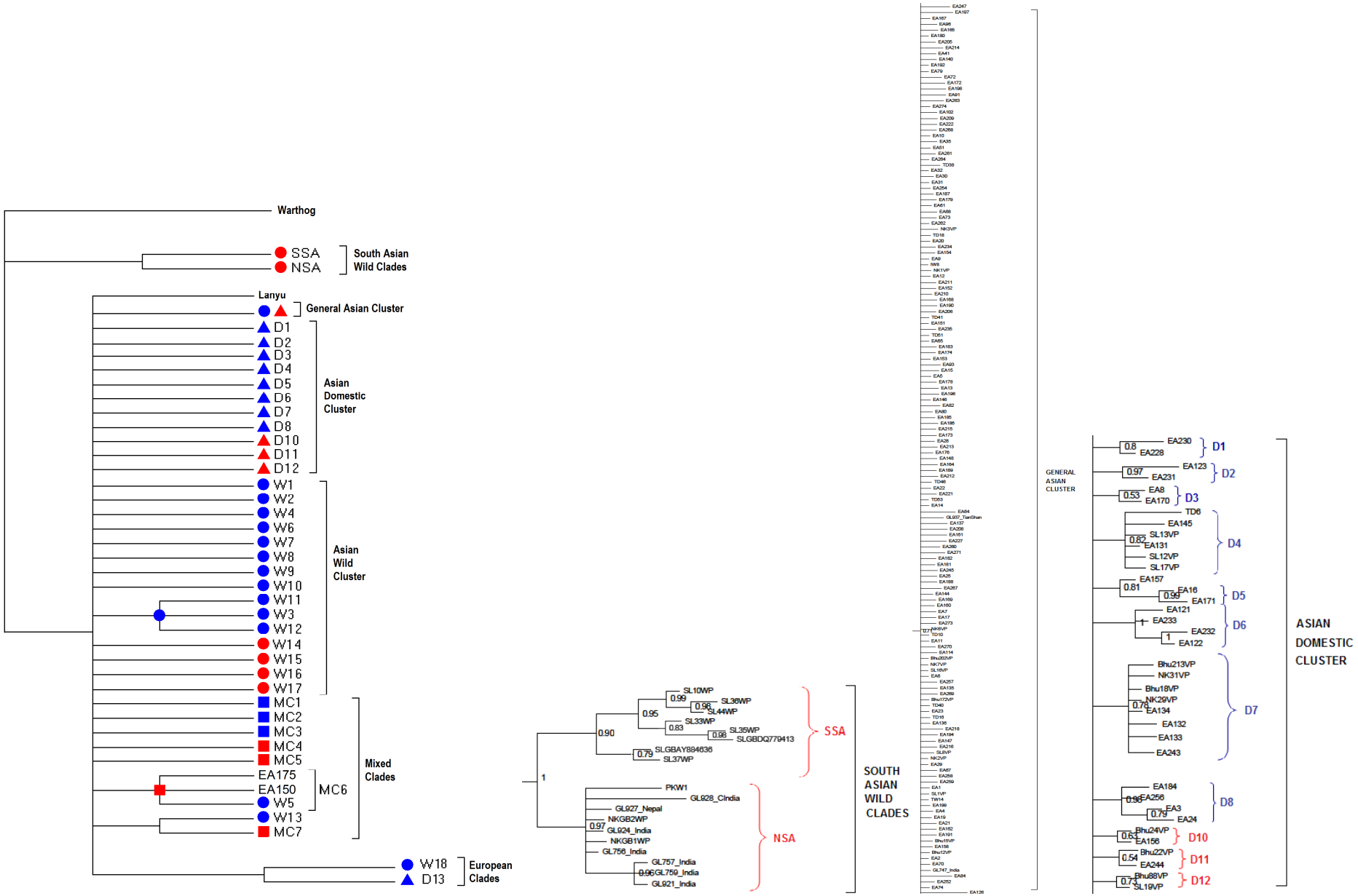
There was clear evidence for introgression of European genes into the domestic pigs of South Asia. About 6% of Bhutanese, 13% of Nepalese and 9% of Sri Lankan village pigs haboured European mitochondrial signatures (Table 5.3; Table 5.S5). Although the Nepalese NKD is a composite breed with genes potentially from European as well as Chinese breeds (Nidup *et al.* 2010), no European haplotypes were detected. On the other hand, the NKH breed contained European, GAC, and MC1 haplotypes.

5.4.3.4 Novel clades of East Asian haplotyes detected

In addition to the clades reported by Larson *et al.* (2010), we found three additional wild (W14-W16) and three mixed clades (MC5-MC7) from East Asian pigs. W14 and W15 contain wild boar from Vietnam, Laos, Burma, and Chinese provinces of Yunnan and Sichuan. W16 was exclusive to wild boar from Japan. Similarly, MC5 consisted of wild boar from Japan and domestic pigs from Chinese provinces of Zhejiang and Guandong. Some clades were well supported while others were not much (Figure 5.1).

5.4.3.5 Median Joining Network Analysis

The MJN analysis showed a similar pattern of clustering to the Bayesian tree, although only major representative haplotypes were used for this analysis. There was clear evidence for identical or closely related sequences in Bhutanese and Nepalese village pigs as well as wild boar from east-central Bhutan and Northern India. Additionally, the only domestic pig sequence from northern India (Datgari, Assam) also clustered in the same clade (MC1). Like the phylogenetic tree, MJN analysis showed clear segregation of NSA and SSA from the rest of the clades. Similarly, the mtDNA contamination of South Asian pigs with European genes was evident.


Warthog
SSA
NSA
South Asian Wild Clades
Lanyu
General Asian Cluster
D1
D2
D3
D4
D5
D6
D7
D8
D10
D11
D12
Asian Domestic Cluster
W1
W2
W4
W6
W7
W8
W9
W10
W11
W3
W12
W14
W15
W16
W17
Asian Wild Cluster
MC1
MC2
MC3
MC4
MC5
EA175
EA150
MC6
W5
W13
MC7
Mixed Clades
W18
D13
European Clades
SL10WP
SL36WP
SL44WP
SL33WP
SL35WP
SLGBDQJ779413
SLGBAY884636
SL37WP
SSA
PKW1
GL928_CIndia
GL927_Nepal
NKGB2WP
GL924_India
NKGB1WP
GL756_India
GL757_India
GL759_India
GL921_India
NSA
SOUTH ASIAN WILD CLADES
GENERAL ASIAN CLUSTER
EA230
EA228
D1
EA123
EA231
D2
EA8
EA170
D3
TD6
EA145
SL13VP
EA131
SL12VP
SL17VP
D4
EA157
EA16
EA171
D5
EA121
EA233
EA232
EA122
D6
Bhu213VP
NK31VP
Bhu18VP
NK29VP
EA134
EA132
EA133
EA243
D7
EA184
EA256
EA3
EA24
D8
Bhu24VP
EA156
D10
Bhu22VP
EA244
D11
Bhu88VP
SL19VP
D12
ASIAN DOMESTIC CLUSTER

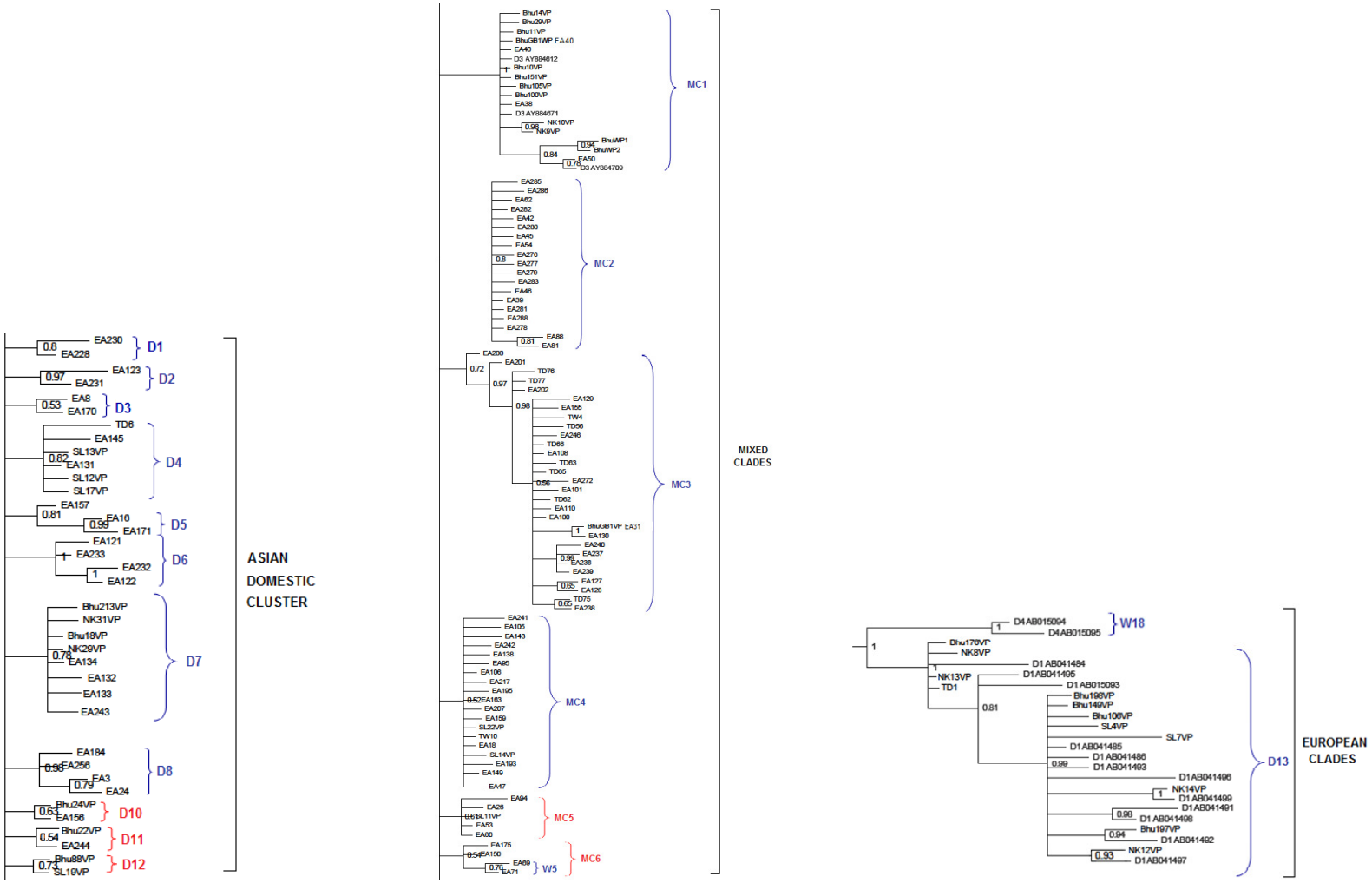


Figure 5.1: Bayesian (MCMC) consensus collapsed dendogram with corresponding trees of South Asian haplotypes (n = 73, 652bp) analysed with East Asian haplotypes. The digits at the nodes are posterior probability (>50%; 100 million generations) with *Phacochoerus aethiopicus* used as outgroup. **Note:** Bhu = Bhutan; NK = Nepal; VP = village pig; NKW = Nepalese wild boar; WP = wild pig/boar; IW= Indian wild boar; SL = Sri Lanka; TBD = Tibetan village pig; BhuGB = Bhutan Genbank; PKW = Pakistani wild boar. Clades in blue, which include some South Asian haplotypes, matched Larson et al (2010). Novel clades discovered in this study are shown in red.

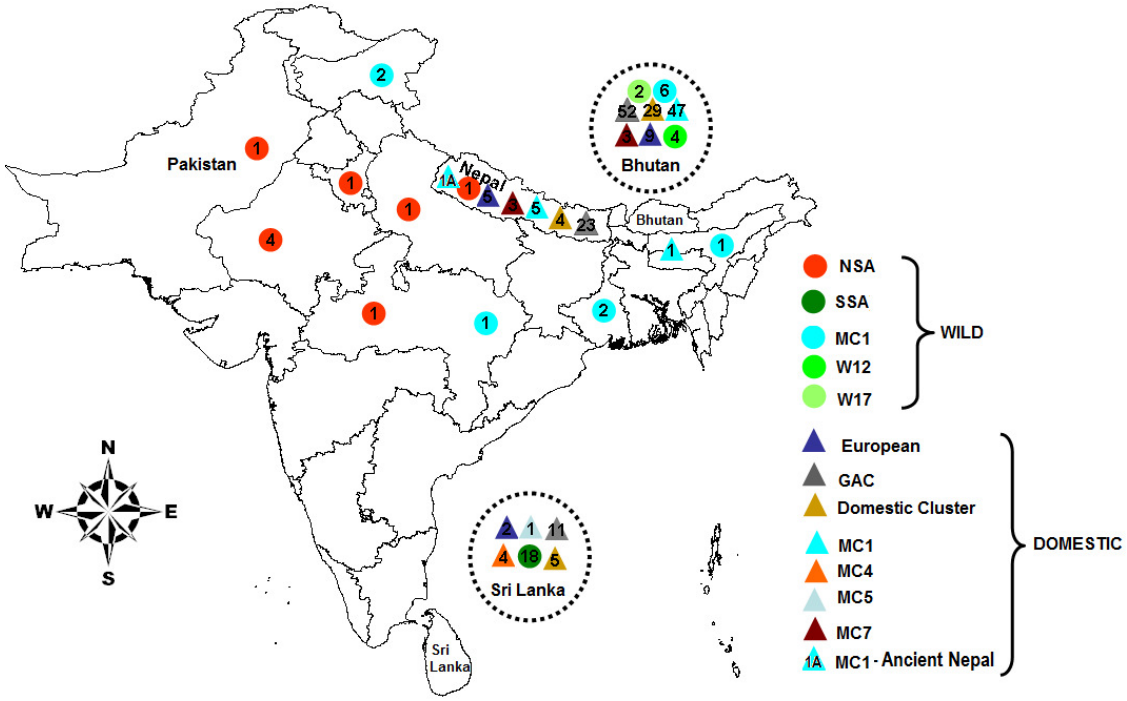


Figure 5.2: mtDNA control region haplotypes distribution amongst wild and domestic pigs in South Asia. GAC = General Asian cluster; NSA = Northern South Asia; SSA = Southern South Asia; MC = Mixed clade; W = Wild boar; 1A = Ancient sample from Gotihawa in Nepal. Coloured triangles represent haplotypes within domestic pigs while circles represent wild boar haplotypes. The numbers within the circles and triangles represent number of samples.

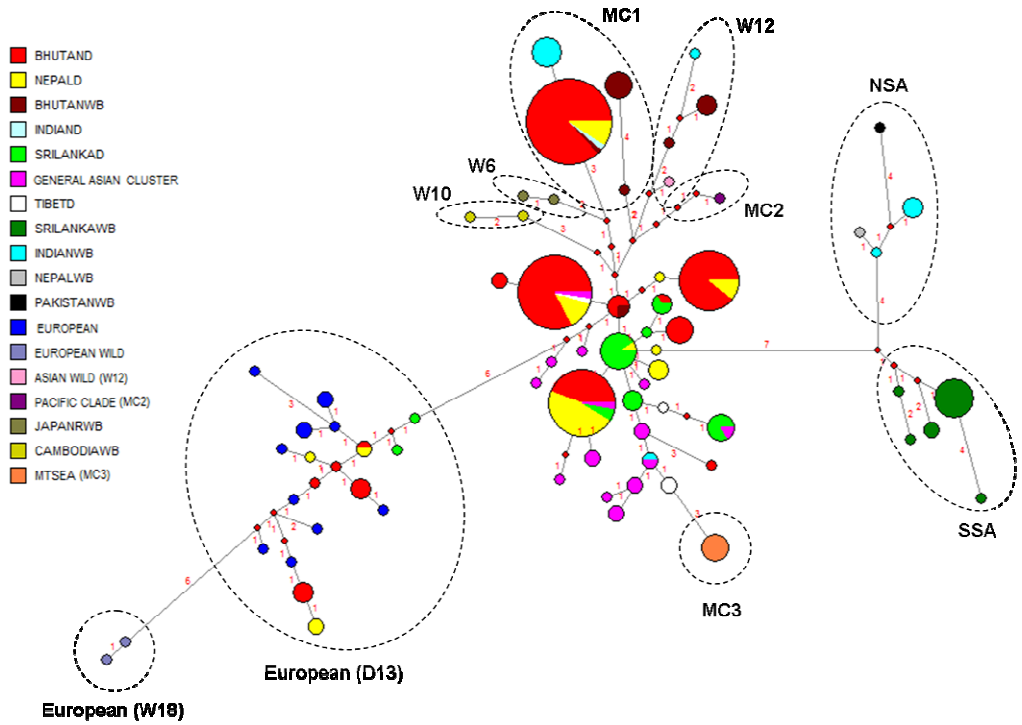


Figure 5.3: Relationship and clustering of South Asian haplotypes with representative mtDNA control region (507bp) haplotypes, which are all circled except for general cluster. Geographical locations of samples are given in colour and node size is proportional to the frequency of the corresponding haplotypes (Table 5.S3, Table 5.S4, & Table 5.S5). Small red dots are median vector (mv) representing hypothetical sequences that were not detected in this study. Numbers on the branch indicate number of mutations. Note: Letters after each country indicate either village pig (D) or wild boar (WB).

5.5 DISCUSSION

5.5.1 Mitochondrial DNA Lineages in South Asia

5.5.1.1 Porcine haplotypes in Northern South Asia

This study demonstrates the presence of more than one lineage of porcine mtDNA control region in South Asia. The MC1 haplotypes belonged to both wild boar and domestic pigs. The wild boar subspecies, *S. s. cristatus* is commonly found in the Himalayas (Figure 5.4) including Northern Indian states of Chhattishgarh and West Bengal (Larson *et al.* 2005; Tanaka *et al.* 2008). A large number of Himalayan (Bhutan & Nepal) domestic pigs shared identical or closely related haplotypes with wild boar suggesting close association and integration between these two subspecies.

(a) *(b)*

Figure 5.4: *(a)* Wild boar weighing more than 135 kg shot in Thinleygang, Bhutan. *(b)* Sow was trapped and then shot. Piglets were captured and raised by the farmers. (Courtesy: MoAF, 2004).

We confirm the presence of another distinct wild boar in Northern South Asia (Table 5.1), which we have referred to as NSA. The NSA haplotypes were found in Northern India (Rajasthan, Haryana, and Uttar Pradesh) and central provinces (Madhya Pradesh, Andhra Pradesh, Chhattisgarh, and Maharashtra). The states of Maharashtra, Madhya Pradesh and Rajasthan border Gujarat harbour *S. s. davidi,* one of the sixteen subspecies of wild boar, described by Groves (1981). It was also found in wild boar from Nepal, which shares a border with Uttar Pradesh. Pakistan shares a border with Rajasthan in Western India and its wild boar also clustered in the NSA clade. Together this provides a strong phylogeographic signal for one mitchondrially defined population of wild boar, which we suspect is from the subspecies *S. s. davidi,* a small light brown pig with a long

thick mane (Groves 1981; Groves 2008). As of now, there is no evidence of any maternal genetic contribution of NSA wild boar to modern domestic pigs. Conforming with size and shape variability of wild boar (Albarella *et al.* 2009) and previously reported MC1 haplotypes, it can be firmly confirmed that there are at least two mitochondrially defined populations of wild boar in Northern India and part of central province of India. We believe there could be some more lineages of wild boar in India, particularly South India, where future research must be focussed.

5.5.1.2 Unique Wild Boar in Southern South Asia

One of the most exciting findings in this study is the confirmation of a discrete mtDNA clade, SSA, within Sri Lankan wild boar, corresponding most likely to the subspecies *S. s. affinis* (Groves 1981; Groves 2008). The body weights of Sri Lankan wild boar have been reported as 74.0 (± 3.51 SE) and 75.0 (± 3.45 SE) kg for males and females respectively (Subalini *et al.* in press). On the other hand, the average weight of *S. s. cristatus* recorded in Bhutan was 110 kgs (70-140kgs) for males and 92kgs (50 to 130kgs) for sows (MoA 2004). This is in line with Groves (2008), who distinguished Sri Lanka and South Indian wild boar from *S. s. cristatus* solely by their smaller sizes. Considering their unique haplotypes, their isolated habitat in an island nation, the level of genetic diversity as determined by microsatellites (Nidup *et al.* Submitted) and their size variability (Groves 1981; Groves 2008), wild boar of Sri Lanka may be confirmed as another genuine subspecies, which has not contributed maternal genetic material to modern domestic pigs. Although many villagers practise scavenging types of pig farming which increases the chance of matings with male and sometimes female wild boar (Subalini *et al.* 2010), the mtDNA of Sri Lankan village pigs is very different from that of their wild counterparts. The lack of archaeological evidence and absence of any trace of maternal gene flow from wild to the village pigs suggest that the latter were not domesticated in Sri Lanka but rather are immigrants from other parts of the world. However, we cannot rule out a contribution of paternal genes from the local wild boar to the village pigs, although the microsatellite data make this very unlikely.

5.5.1.3 Novel Wild Boar haplotypes in Bhutan

In addition to wild boar with MC1 haplotypes, two distinct clades of Bhutanese wild boar were W12 and W17. The latter was exclusive to wild boar from the East-central region of Bhutan and did not have any influence on Bhutanese or any other village pigs in South Asia. On the other hand, the W12 clade, which was designated as D5 by Larson *et al.* (2005), was found in a substantial number of wild boar found throughout Bhutan and the neighbouring Indian state of Sikkim. Apart from the first report by Larson *et al.* (2005), no other sequences belonging to the W12 clade have been reported in previous studies (Tanaka *et al.* 2008; Larson *et al.* 2010). However, deduction of an independent centre of domestication in Myanmar, based on W12 haplotypes (Larson *et al.* 2005), may be contentious considering the fact that several animals with W12 haplotypes were found in Bhutan and one in Sikkim. Current data suggest that there is neither any evidence of cryptic domestication nor introgression of maternal genetic material from W12 wild boar into the domestic population. However, several Australian feral pigs (Larson *et al.* 2005) and one indigenous domestic pig in PNG (Nidup *et al.* unpublished-a) showed evidence for similar haplotypes suggesting possible maternal introduction of genuine wild boar to Australia or PNG from Southeast Asia or even from Northeast India.

5.5.2 Domestication and Genetic Introgression

5.5.2.1 Influence of Wild Boar

The sharing of haplotypes between Bhutanese, Northeast Indian, and Nepalese village pigs with wild boar from Northern India (Kashmir, West Bengal, and Chattishgarh) within MC1 clade supports an independent centre of "cryptic domestication" in the foothills of the Himalayas and Indian-sub continent. Similarly, the shared haplotypes between local wild boar and village pigs in Bhutan could possibly suggest a case for a local centre of cryptic domestication in East-central Bhutan. These are consistent with previous studies (Larson *et al.* 2005; Tanaka *et al.* 2008; Larson *et al.* 2010). However, in the absence of corroborating archaeological or fossil evidence, these could also be cases of introgression of maternal genes from wild boar to domestic pigs (Figure 5.2) rather than a proof of an independent centre of domestication. It is possible that both female piglets and adult females are occasionally caught, tamed, and raised to breed

with domestic pigs. During our field work in Bhutan, we have observed two such cases (Figure 5.5).

(a) *(b)*

Figure 5.5: *(a)* Female wild boar, which was captured as a piglet was raised to be crossbred with local indigenous boar in Kazi sub-district of Wangdue district, Bhutan; *(b)* Wild boar piglet captured in the nearby forest in Shingkhar Lauri sub-district of Samdrup Jhongkhar district, Bhutan. Like any other wild piglet, it has ochre, chocolate and cream coloured stripes lengthwise over its body (Courtesy: Karma Wangchuk).

Currently, lack of adequate ancient and modern boar samples from Nepal does not provide any evidence for a local centre of domestication or introgression of wild genes into domestic populations in Nepal. However, the only ancient domestic pig sample (GL384) from Nepal was clustered within the MC1 clade (Figure 5.S1). This demonstrates that at least 1,500 years ago at the site of Gotihawa (Verardi 1998, 2007), there were domestic pigs in Nepal that had a maternal signature that matched modern day MC1 wild and domestic pigs from the Himalayas and Northern India. While this is not an evidence for independent domestication of a novel population of wild boar, it clearly shows that any possible introgression of MC1 wild boar into domestic pigs is not a recent event. Considering the socio-cultural and economic importance of wild boar to the lives of several ethnic communities (Bista 2003; Nidup *et al.* 2010) and promotion of wild boar farming in Nepal (HMG 2003; Gajural 2004), we cannot rule out maternal genetic introgression and presence of MC1 wild boar in Nepal.

In contrast to recent reports emerging from Sri Lanka (Thangarajah 2009; Subalini *et al.* 2010; Subalini *et al.* in press), our study did not show any evidence of close affinity of wild boar with village pigs indicating that the latter did not originate in the present habitat from ancestors of the existing wild populations. In other words, local Sri Lanka

wild boar have not contributed to domestication in Sri Lanka. This is consistent with our microsatellite study which also indicated that Sri Lanka wild boar were very distinct from their domestic counterparts (Nidup *et al.* Submitted) in contrast to a previous study by Thangarajah (2009). However, we do not completely rule out the introgression of paternal genes from wild boar to the local village pigs because of the scavenging system of pig farming in the villages of South Asia (Nidup *et al.* 2010; Subalini *et al.* 2010; Nidup *et al.* 2011). With such farming practices, it is highly possible that domestic sow or gilt often mate with wild boar from nearby village forests and thus the introgression of nuclear genes would not be accompanied by mitochondrial DNA. However, our microsatellite data shows that any such introgression is minimal and probably does not happen.

5.5.2.2 Influence of European pigs

Only 6-10% of the village pigs in South Asia showed evidence of maternal introgression of European mitochondria. This level of introgression is relatively very low considering the fact that there has been rampant introduction of commercial pigs of European origins in South Asia (Rajamahendran *et al.* 1985; Nidup *et al.* 2010; Subalini *et al.* 2010; Nidup *et al.* 2011). However, this should not rule out the greater possibility of paternal introgression of nuclear genes into the village herds. The livestock development policy in the region has revolved around providing breeding boars to crossbreed with indigenous pigs. In such instances, paternal genetic contributions would be undoubtedly higher than via females. For instance, microsatellite evidence shows that Australian commercial pigs of European origin are closely related to Sri Lanka village pigs (Nidup *et al.* Submitted) suggesting possible paternal contamination of the latter with nuclear genes from European breeds.

5.5.3 Dispersal of Pigs in South Asia

Having found good evidence for W12 wild boar in Bhutan, we can ponder on whether W12 is actually indigenous to Myanmar. Geographically, it is likely, considering that Bhutan shares a border with the Northeast Indian states of Sikkim, parts of north Bengal, and other contiguous states around Myanmar. But the absence of Bhutanese domestic pigs within the W12 clade rules out dispersal mediated by human migration,

such as by Mon and Sharchop ethnic groups, from Myanmar and northern India into Bhutan (Worden 1991). On the contrary, it is also possible that W12 wild boar may have been dispersed from Northeast India into Myanmar during the migration of ethnic Naga people (Thohe Pou 2003). In addition, human mtDNA evidence supports dispersal from northeast India of the aboriginal Andamanese to Myanmar in the late Paleolithic (Wang *et al.* 2011). Sampling of indigenous domestic pigs from Naga communities in Myanmar and northeast States of India, particularly Nagaland, may provide better evidence for the presence of domestic pigs within W12, cryptic domestication, and possible movement of people in the region. At this stage, it is only sensible to deduce that the W12 clade is broadly distributed across Bhutan, Myanmar and possibly in northeast India.

During the recent excavations of the ruins of Drapham Dzong in the Chokor-toe valley (East-central Bhutan), several remains of bones from domestic animals including pigs were discovered (Lhamo 2011). This suggests that Bhutanese have raised pigs for more than 350 years. The shared haplotypes within and between village pigs of Bhutan, Nepal and Sri Lanka indicate some ancestral genetic signatures or movement of domestic pigs between countries, mediated either through historical human migration or a live-animal trading system. Many commercial breeds of European origins have been introduced into South Asia. It is intriguing to see large proportion of haplotypes (GAC) belonging to general Asian pigs of Chinese origin (Fang & Anderson 2006) present in South Asia. Here, we discuss some possible scenario on how pigs must have been introduced in South Asia, particularly in Bhutan.

5.5.3.1 Possible dispersal of pigs into Bhutan

Various ethnic groups of people have immigrated into Bhutan. The aboriginal Monpa arriving from northeast India and Burma; Ngalops or Buddhist Tibetans from Tibet; Sharchop populations from northeast India and Burma in the first millennium; and finally the Nepalese arriving in the late 19th centuries (Worden 1991) have shaped the modern geography of human language and culture in Bhutan. This is further supported by the preliminary genetic data from 15 autosomal Short Tandem Repeat (STR) loci of Bhutanese people (Kraaijenbrink *et al.* 2007; Thirsa *et al.* 2009). The evidence suggested that the people of Bhutan are likely to have originated from outside their current locations, in regions where their language families are spoken (Thirsa *et al.*

2009). Considering the ethnic diversity in Bhutan and assuming that the people must have moved animals with them, pigs in Bhutan could have been initially introduced from different places.

However, except for "*Sunggur"* in Nepali and "*Phap"* in Ngalop, none of the several languages or dialects in Bhutan have discrete terms for pigs. So, it is possible that pigs may have been introduced in Bhutan by Ngalop and Nepali speaking ethnic groups. There are several theories that support the possible introduction of pigs from Tibet (China) to Bhutan.

Firstly, palynological and extensive charcoal horizon data collected from high, mountain-locked Himalayan valleys in northwest Bhutan suggested that the most likely founder societies of Bhutan came from the Tibetan Plateau, where yak and barley based pastoralism and Neolithic settlements are known to have existed since the Mid Holocene (Meyer *et al.* 2009). It is possible that these people would have brought pigs along with their yaks and barley crop. Secondly, prior to the arrival of Buddhism, Tibetans firmly believed in the ancient practice of "Bonism", which involved sacrifice of animals (Wangmo 2008), including pigs (Dorji 2004), to worship local deities. Until recently, Bonism, which was introduced from Tibet (Wangmo 2008), was widely practised in several rural communities in Bhutan (Dorji 2004; Wangchuk 2005). Thirdly, Bhutan had trade relations with Tibet (Singh 1988; Sen 2003) prior to the occupation of the latter by China. Fourthly, Tanaka *et al.* (2008) suggested human-mediated dispersal of domestic pigs from north to the south during the historical expansion of Sino-Tibetan and Tai peoples. Finally, our study showed that a large number of domestic pigs from Bhutan had similar haplotypes to the majority of Chinese pigs belonging to the general Asian cluster (GAC). In addition to this, the MC7 clade has closely related haplotypes with some Himalayan domestic pigs and Chinese wild boar from Jiangxi and Hainan provinces. Considering all this evidence, it is highly possible that pigs of Chinese origin were introduced from Tibet (China) to Bhutan, which would have been feasible during the summer season when freezing temperatures across treacherous mountainous passes could be avoided.

On the basis of MC3 haplotypes, Tanaka *et al.* (2008) suggested introduction of pigs to Bhutan from Southeast Asia although our extensive and comprehensive analysis of

porcine haplotypes from South and East Asia do not support this claim. Another possible route of introduction of pigs into Bhutan could be from northeast India and Nepal, from where the Nepali speaking population migrated into Bhutan (Worden 1991). Our ancient sequence from Nepalese domestic pigs suggests that Nepalese were farming pigs at least 1,500 years ago and some castes within the ethnic Nepali in Nepal and Bhutan still sacrifice black pigs to implore local deities (*deota*) for bountiful crop yields, and good health and safety of the whole family (Nidup *et al.* 2010). Therefore, it is highly likely that some Nepalese speaking population have brought pigs with them when they have immigrated to Bhutan during the late 19th century (Worden 1991). The mitochondrial genome of the Bhutanese and Nepalese people could potentially provide better insight into the origins of Himalayan human populations and their livestock including pigs.

5.5.3.2 Dispersal of pigs into Sri Lanka

In Sri Lanka, except for the most recent introduction of exotic breeds of pigs (Chandrasi 2002), there was no historical record of introduction of any other types of pigs from any part of the world. However, the clustering within MC4 and MC5 suggested that the historical origin of some of Sri Lankan village pigs may be from IBBH region, possibly from Southeast Asia and Southern China. This fascinating evidence was complemented by the historical evidence of the ancient Silk Road that connected China, IBBH region, Sri Lanka, India, and central Asia (Christian 2000; Behera 2002; Kader 2010). Traders exchanged several items such as ceramics, glass, precious metals, ivory, gems, and medical herbs on the Silk Road (Christian 2000; Konkolewski 2011), which included Sri Lanka as one of the major ports. They also traded exotic animals and livestock (Christian 2000; Konkolewski 2011) that might have included pigs.

5.6 CONCLUSIONS

Two novel wild boar clades, NSA and SSA, have been confirmed in South Asia but there was no evidence to suggest that wild boar belonging to these clades have made any maternal genetic contribution to modern domestic pigs. From this study, we can conclude that there are three mitochondrially defined wild boar populations (MC1, NSA, and SSA) possibly belonging to three different wild boar subspecies (*S. s. cristatus*, *S. s. davidi*, and *S. s. affinis*), which are unique to the Indian sub-continent.

Currently, the W17 and W12 haplotypes have not been detected in South Asian domestic pigs. While W17 wild boar were exclusive to Bhutan, W12 wild boar may be geographically distributed from Bhutan through Northeast India to Myanmar. The South Asian domestic pigs have been mainly influenced by North Indian wild boar (MC1 clades). There were shared haplotypes between domestic pigs from Bhutan, Nepal and India with wild boar from North India. European mitochondria have also introgressed into South Asian domestic pigs. The dispersal of pigs in and within the region has been mainly human-mediated.

Wild boar have an enormously wide and ecologically diverse natural distribution. Wild boar from Pakistan, Southern India and other South Asian countries need to be researched. Analysis of their complete mitochondrial genome and nuclear genes including Y-chromosomal markers along with domestic pigs from the same regions may provide deeper insight into the evolution, introgression and propensity for independent domestication. They represent unique genetic resources and may contribute to future food security and rural development in the face of an ever changing global environment. Both *in* and *ex situ* conservation is not necessary but breeding and genetic improvement of wild boar for developing niche products and sustaining food production would be an interesting thought provoking process. Introgression of nuclear genes from genetically distinct wild boar may provide better physiological and environment fitness in domestic pigs in the face of global warming and climate change.

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5.8 REFERENCES

Albarella U., Dobney K. & Conwy R.P. (2009) Size and shape of the Eurasian wild boar *(Sus scrofa*), with a view to the reconstruction of its Holocene history. *Environmental Archaeology* **14**, 103-36.

Bandelt H.J., Forster P. & Rohl A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37-48.

Barrett R.H. (1978) The feral hog on Dye Creek Ranch, California. *Hilgardia*, 281-355.

Behera S. (2002) India's Encounter with the Silk Road. *Economic and Political Weekly* **37**, 5077-80.

Bensasson D., Zhang D., Hartl D.L. & Hewitt G.M. (2001) Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends in Ecology & Evolution* **16**, 314-21.

Bista P. (2003) Wild boar herding cattle in Lamitar. In: *The Kathmandu Post*. Nepalnews, Hadikhola, Manwanpure.

Chandrasi A.G.N. (2002) Country Report on the State of Animal Genetic Resources of Sri Lanka: Submitted to Food and Agriculture Organization of the United Nation. Veterinary Research Institute, Perideniya, Kandy, Sri Lanka.

Christian D. (2000) Silk Roads or Steppe Roads? The Silk Roads in World History. *Journal of World History* **11.1** 1-26.

Don R.H., Cox P.T., Wainwright B.J., Baker K. & Mattick J.S. (1991) Touchdown PCR to circumvent spurious primning during gene amplification. *Nucleic Acids Research* **19**, 4008-.

Dorji T. (2004) The Spider and the Piglet. In: *Proceedings of the First International Seminar on Bhutan Studies*. Centre for Bhutan Studies, Thimphu, Bhutan.

Edgar R.C. (2004a) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *Bmc Bioinformatics* ***5***, 113.

Edgar R.C. (2004b) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**, 1792 - 7.

Erkinaro E., Heikura K., Lindgren E., Pullianen E. & Sulkava S. (1982) Occurrence and spread of the wild boar (*Sus scrofa*) in eastern Fennoscandia. *Memoranda Flora and Fauna Fennoscandia* **58**, 39-47.

Fang M. & Anderson L. (2006) Mitochondrial diversity in European and Chinese pigs is consistent with population expansions that occurred prior to domestication. *Proceedings of the Royal Society* **273**, 1803-10.

Fang M., Berg F., Ducos A. & Andersson (2006) Mitochondrial haplotypes of European wild boars with 2n = 36 are closely related to those of European domestic pigs with 2n = 38. *Animal Genetics* **37**, 459-64.

Forster P., Torroni A., Renfrew C. & Röhl A. (2001) Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. *Molecular Biology Evolution* **18**, 1864-81.

Gajural D. (2004) Nepal authorizes wildlife farming for conservation. In: *ENS*, Kathamandu, Nepal, .

Giuffra E., Kijas J.M., Amarger V., Carlborg O., Jeon J.T. & Andersson L. (2000) The origin of the domestic pig: independent domestication and subsequent introgression. *Genetics* **154**, 1785-91.

Gongora J., Morales C., Bernal J.E. & Moran C. (2006) Phylogenetic divisions among Collared peccaries (*Pecari tajacu)* detected using mitochondrial and nuclear sequences. *Molecular Phylogenetics and Evolution* **41**, 1-11.

Gongora J., Peltoniemi O.A.T., Tammen I., Raadsma H. & Moran C. (2003) Analyses of possible domestic pig contribution in two populations of Finnish farmed wild boar. *Acta agriculturae Scandinavica* **53**, 161.

Grossi S.F., Lui J.F., Garcia J.E. & Meirelles F.V. (2006) Genetic diversity in wild (*Sus scrofa scrofa*) and domestic (*Sus scrofa domestica*) pigs and their hybrids based on polymorphism of a fragment of the D-loop region in the mitochondrial DNA *Genetics and Molecular Research* **5**, 564-8.

Groves C. (2008) Current views on taxonomy and zoogeography of the genus *Sus*. In: *Pigs and humans: 10,000 years of interaction* (eds. by Albarella U, Dobney K & Ervynck A), pp. 15-29. Oxford University Press, Oxford, UK.

Groves C.P. (1981) Ancestors for the Pigs: Taxonomy and Phylogeny of the Genus Sus. In: *Technical Bulletin 3*. Department of Prehistory, Research School of Pacific Studies, Australian National University, Canberra.

HMG (2003) Working policy on wildlife farming, breeding and research. Ministry of Forests and Soil Conservation, His Majesty's Government of Nepal, Singhdurbar, Kathmandu, Nepal.

Huelsenbeck J.P., Ronquist F., Nielsen R. & Bollback J.P. (2001) Evolution - Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294**, 2310-4.

Kader B.A. (2010) Sri Lanka aims to revive historic silk route. URL http://gulfnews.com/business/shipping/sri-lanka-aims-to-revive-historic-silk-route-1.671741.

Keane T.M., Creevey C.J., Pentony M.M., Naughton T.J. & McInerney J.O. (2006) Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified,. *BMC Evolutionary Biology* **6**, 29.

Kim K.I., J.H L., Li K., Zhang Y.P., Lee S.S., Gongora J. & Moran C. (2002) Phylogenetic relationships of Asian and European pig breeds determined by mitochondrial DNA D-loop sequence polymorphism. *Animal Genetics* **33**, 19-25.

Konkolewski N. (2011) Introduction to The Silk Road. URL http://www.cerritos.edu/jhaas/silk%20road%20project/silk_road.htm.

Kraaijenbrink T., van Driem G.L., Gaselo K.T.O. & de Knijff P. (2007) Allele frequency distribution for 21 autosomal STR loci in Bhutan. *Forensic Science International* **170**, 68-72.

Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J. & Higgins D.G. (2007) ClustalW and ClustalX version 2. *Bioinformatics 2007* **23**, 2947-8.

Larson G., Cucchi T., Fujita M., Matisoo-Smith E., Robins J., Anderson A., Rolett B., Spriggs M., Dolman G., Kim T.H., Thuy N.T.D., Randi E., Doherty M., Due R.A., Bollt R., Djubiantono T., Griffin B., Intoh M., Keane E., Kirch P., Li K.T., Morwood M., Pedrina L.M., Piper P.J., Rabett R.J., Shooter P., Van den Bergh G., West E., Wickler S., Yuan J., Cooper A. & Dobney K. (2007) Phylogeny and ancient DNA of Sus provides insights into neolithic expansion in island southeast Asia and Oceania. *Proceedings Of The National Academy Of Sciences Of The United States Of America* **104**, 4834-9.

Larson G., Dobney K., Albarella U., Fang M.Y., Matisoo-Smith E., Robins J., Lowden S., Finlayson H., Brand T., Willerslev E., Rowley-Conwy P., Andersson L. &

Cooper A. (2005) Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science* **307**, 1618-21.

Larson G., Liu R.R., Zhao X.B., Yuan J., Fuller D., Barton L., Dobney K., Fan Q.P., Gu Z.L., Liu X.H., Luo Y.B., Lv P., Andersson L. & Li N. (2010) Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 7686-91.

Leaper R., Massei G., Gorman M.L. & Aspinall R. (1999) The feasibility of reintroducing Wild Boar (*Sus scrofa*) to Scotland. *Mammal Rev.* **29**, 239-59.

Lhamo S. (2011) Of 16th century provenance. In: *Kuensel*. Kuensel Corporation, Thimphu. http://www.kuenselonline.com/2010/modules.php?name=News&file=article&sid=17936.

Meyer M.C., Hofmann C.-C., Gemmell A.M.D., Haslinger E., Ha¨usler H. & Wangda D. (2009) Holocene glacier fluctuations and migration of Neolithic yak pastoralists into the high valleys of northwest Bhutan. *Quaternary Science Reviews* **28**, 1217-37.

Mittermeier R.A., Gil P.R., Hoffman M., Pilgrim J., Brooks T., Mittermeier C.G., Lamoreux J. & Fonseca G.A.B. (2005) *Hotspots Revisited: Earth's biologically richest and most threatened terrestrial ecoregions*. University of Chicago Press, Chicago, USA.

MoAF (2004) Project completion report on wild pig conservation and management project. NCD, Ministry of Agriculture, Royal Government of Bhutan, Thimphu.

Nidup K. (2006) Swine resources in the Hindu Kush Himalayan region: a need for molecular genetic characterization. *SAARC Journal of Agriculture* **4**, 113-21.

Nidup K., Ayalew W., Danbaro G., Besari F., Gongora J. & Moran C. (unpublished) Genetic status of indigenous domestic pigs of Papua New Guinea. *Manuscript under preparation*.

Nidup K., Joshi D.D., Gongora J. & Moran C. (2010) Farming and Biodiversity of Indigenous Pigs in Nepal. *Biodiversity* **11**, 26-33.

Nidup K., Silva G.L.L.P., Joshi D.D., Pem R., Gongora J. & Moran C. (Submitted) Genetic structure and diversity of South Asian pigs *(Sus scrofa)* as determined by microsatellites. *Animal Genetics*.

Nidup K., Tshering D., Wangdi S., Gyeltshen C., Phuntsho T. & Moran C. (2011) Farming and Biodiversity of Pigs in Bhutan. *Animal Genetic Resources* **48**, 47-61.

O' Connell S. (2008) Back from dead: Could wolves and wild boar roam Britain again? In: *The Independent*. UK, UK.

Polzin T. & Daneshmand S.V. (2003) On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters* **31**, 12-20.

Posada D. (2009) Collapse 1.2: Describing haplotypes from sequence alignments. URL http://darwin.uvigo.es/.

Posada D. & Crandall K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817-8.

Rajamahendran R., Ravindran V., Goonewardene L.A., Sahaayaruban P. & Rajaguru A.S.B. (1985) The Native Pig of Sri Lanka. *Animal Genetics Resources Information* **4**, 9-13.

Ronquist F. & Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-4.

Ruvinsky A. & Rothschild M.F. (1998) *Systematics and evolution of the pig*. CAB International, Oxon (UK).

Sen S. (2003) The Duar War of 1865, and its Effect on the Indo-Bhutan Trade Relations. *The Himalayan Miscellany, Silver Jubilee Commemorative volume, December, 2003.*

Shapiro B., Drummond A.J., Rambaut A., Wilson M.C., Matheus P.E., Sher A.V., Pybus O.G., Gilbert M.T.P., Barnes I., Binladen J., Willerslev E., Hansen A.J., Baryshnikov G.F., Burns J.A., Davydov S., Driver J.C., Froese D.G., Harington C.R., Keddie G., Kosintsev P., Kunz M.L., Martin L.D., Stephenson R.O., Storer J., Tedford R., Zimov S. & Cooper A. (2004) Rise and fall of the Beringian steppe bison. *Science* **306**, 1561-5.

Singh A.K.J. (1988) *Himalayan Triangle: A historical survey of British India's relations with Tibet, Sikkim and Bhutan 1765-1950*. The British Library, UK.

Subalini E., Silva G.L.L.P. & Demetawewa C.M.B. (2010) Phenotypic Characterization and Production Performance of Village Pigs in Sri Lanka. *Tropical Agricultural Research* **21**, 198-208.

Subalini E., Silva G.L.L.P. & Demetawewa C.M.B. (in press) Phenotypic variations in native pig types: village and wild pigs in Sri Lanka. *Tropical Agricultural Research* **22**.

Tamura K., Dudley J., Nei M. & Kumar S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596 - 9.

Tanaka K., Iwaki Y., Takizawa T., Dorji T., Tshering G., Kurosawa Y., Maeda Y., Mannen H., Nomura K., Dang V.B., Chhum-Phith L., Bouahom B., Yamamoto Y., Daing T. & Namikawa T. (2008) Mitochondrial diversity of native pigs in the mainland South and South-east Asian countries and its relationships between local wild boars. *Animal Science Journal* **79**, 417-34.

Thangarajah S. (2009) Phenotypic and Genetic characterization of Wild boar and Village pigs in Sri Lanka. In: *Postgraduate Institute of Agriculture*. University of Peradeniya, Kandy, Sri Lanka.

Thirsa K., Parkin E.J., Carvalho-Silva D.R., van Driem G., Barbujani G., Tyler-Smith C., Jobling M.A. & de Knijff P. (2009) Genetic and linguistic borders in the Himalayan region. In: *Becoming Eloquent: Advances in the Emergence of Language, Human Cognition and Modern Cultures* (eds. by d'Errico F & Hombert J-M), pp. 81-201 John Benjamins, Amsterdam.

Thohe Pou R.B. (2003) The Myths of Naga Origin. URL http://nambon.com/history/nagas-myths/naga-origin.

Thompson J.D., G H. & Gibson T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673-80.

Ursing B.M. & Arnason U. (1998) The complete mitochondrial DNA sequence of the pig (Sus scrofa). *Journal of Molecular Evolution* **47**, 302-6.

Verardi G. (1998) Excavations at Gotihawa - a note on the results obtained during the first excavation campign in winter 1994-1995. *Ancient Nepal* **40**, 85-105.

Verardi G. (2007) *Excavations at Gotihawa and Pepri, Kapilbastu district, Nepal*. Istituto italiano per l'Africa e l'Oriente (IsIAO), Rome

Villesen P. (2007) FaBox: an online toolbox for fasta sequences. *Molecular Ecology Notes* **7**, 965-8.

Wang H.W., Mitra B., Chaudhuri T.K., Palanichamy M.G., Kong Q.P. & Zhang Y.P. (2011) Mitochondrial DNA evidence supports northeast Indian origin of the aboriginal Andamanese in the Late Paleolithic. *Journal of Genetics and Genomics* **38**, 117-22.

Wangchuk R. (2005) Bonism and Shamanism: an integral part of Bongop culture. In: *Kuensel*. Kuensel Corporation Ltd., Thimphu.

Wangmo S. (2008) Bon, Buddhism or both - What do we Bhutanese believe? In: *Kuensel*. Kuensel Co. Ltd., Thimphu.

Watson L. (2004) *The Whole Hog: Exploring the Extraordinary Potential of Pigs*. Smithsonian Books, New York.

Worden R.L. (1991) Bhutan. In: *Nepal and Bhutan: Country Studies* (ed. by Savada AM). Federal Research Division, United States Library of Congress, Washington D.C, USA.

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

5.9 SUPPLEMENTARY MATERIALS

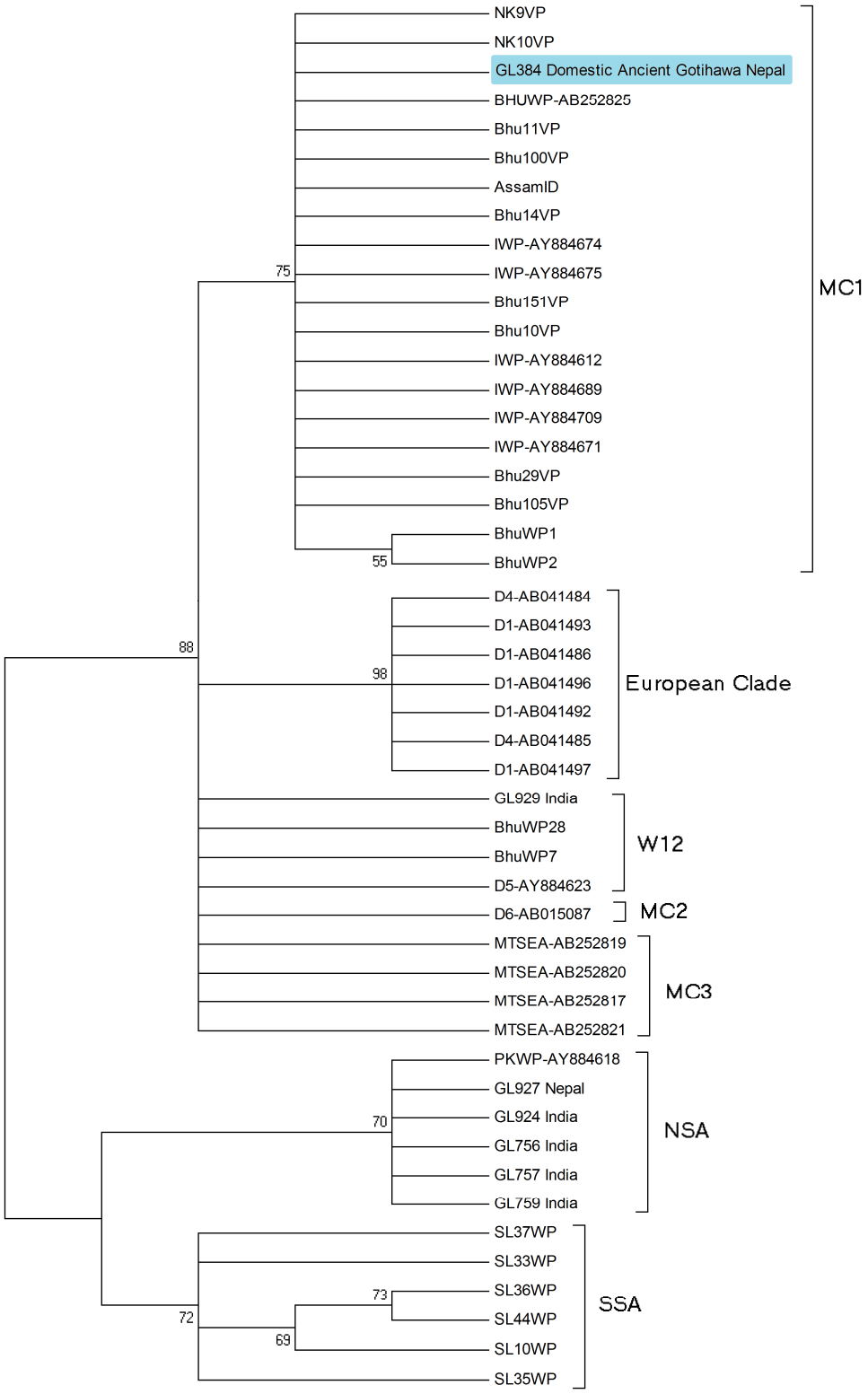

Figure 5.S1: NJ tree showing the clustering of ancient domestic pig sequence (140bp) from Nepal with MC1 wild and domestic samples from the Himalayas and Northern India.

Table 5.S1: Haplotypes in South Asian pigs. It also included shared haplotypes amongst Bhutan, Nepal and Sri Lanka pig populations including wild boar.

Haplotype number	Label	Number of sequences	Length of haplotype (bp)	Sequences belonging to haplotype
1	Bhu176VP	3	654	Bhu176VP Bhu174VP Bhu173VP
2	Bhu198VP	1	654	Bhu198VP
3	Bhu149VP	1	654	Bhu149VP
4	Bhu197VP	3	654	Bhu197VP Bhu195VP Bhu192VP
5	Bhu106VP	1	654	Bhu106VP
6	Bhu98VP	5	654	Bhu98VP Bhu164VP Bhu47VP NK44VP NK48VP
7	Bhu29VP	2	654	Bhu29VP Bhu87VP
8	Bhu11VP	2	654	Bhu11VP Bhu85VP
9	Bhu10VP	25	654	Bhu10VP Bhu193VP Bhu190VP Bhu183VP Bhu150VP Bhu147VP Bhu135VP Bhu134VP Bhu66VP Bhu65VP Bhu64VP Bhu63VP Bhu61VP Bhu53VP Bhu51VP Bhu50VP Bhu132VP Bhu57VP Bhu48VP Bhu116VP Bhu35VP Bhu148VP Bhu39VP Bhu153VP Bhu133VP
10	Bhu151VP	2	654	Bhu151VP Bhu139VP
11	Bhu14VP	1	654	Bhu14VP
12	Bhu100VP	14	654	Bhu100VP Bhu188VP Bhu185VP Bhu184VP Bhu181VP Bhu138VP Bhu102VP Bhu124VP Bhu101VP

Haplotype number	Label	Number of sequences	Length of haplotype (bp)	Sequences belonging to haplotype
				Bhu118VP Bhu104VP Bhu186VP Bhu103VP
13	Bhu105VP	2	654	Bhu105VP Bhu2VP
14	Bhu88VP	3	654	Bhu88VP SL19VP SL20VP
15	Bhu202VP	31	654	Bhu202VP Bhu129VP Bhu115VP Bhu110VP Bhu99VP Bhu93VP Bhu74VP Bhu161VP Bhu160VP Bhu159VP Bhu157VP Bhu156VP Bhu155VP Bhu167VP Bhu154VP NK7VP NK15VP NK35VP NK36VP NK37VP NK38VP NK39VP NK40VP NK41VP NK42VP NK43VP NK45VP NK46VP NK47VP NK50VP SL16VP
16	Bhu24VP	6	654	Bhu24VP Bhu32VP Bhu62VP Bhu40VP Bhu83VP Bhu38VP
17	Bhu22VP	1	654	Bhu22VP
18	Bhu15VP	4	654	Bhu15VP Bhu36VP Bhu7VP Bhu78VP
19	Bhu12VP	30	654	Bhu12VP Bhu30VP Bhu34VP Bhu37VP Bhu4VP Bhu41VP Bhu42VP

Haplotype number	Label	Number of sequences	Length of haplotype (bp)	Sequences belonging to haplotype
				Bhu8VP Bhu211VP Bhu206VP Bhu204VP Bhu203VP Bhu201VP Bhu191VP Bhu182VP Bhu137VP Bhu127VP Bhu123VP Bhu113VP Bhu54VP Bhu52VP Bhu49VP Bhu130VP Bhu68VP Bhu55VP Bhu33VP Bhu31VP Bhu17VP Bhu81VP Bhu9VP
20	Bhu172VP	3	654	Bhu172VP Bhu171VP Bhu152VP
21	Bhu18VP	22	654	Bhu18VP Bhu44VP Bhu69VP Bhu122VP Bhu109VP Bhu212VP Bhu208VP Bhu120VP Bhu112VP Bhu90VP Bhu86VP Bhu76VP Bhu89VP Bhu70VP Bhu209VP Bhu46VP Bhu19VP Bhu177VP Bhu94VP NK29VP NK33VP NK34VP
22	Bhu213VP	2	654	Bhu213VP Bhu210VP
23	BhuWP28	1	654	BhuWP28
24	BhuWP7	3	654	BhuWP7 BhuWP8 BhuWP6
25	BhuWP1	2	654	BhuWP1 BhuWP52
26	BhuWP2	4	654	BhuWP2 BhuWP53

Haplotype number	Label	Number of sequences	Length of haplotype (bp)	Sequences belonging to haplotype
				BhuWP3 BhuWP27
27	BhuWP4	1	654	BhuWP4
28	BhuWP5	1	654	BhuWP5
29	NK12VP	1	654	NK12VP
30	NK8VP	2	654	NK8VP NK11VP
31	NK14VP	1	654	NK14VP
32	NK30VP	1	654	NK30VP
33	NK10VP	3	654	NK10VP NK18VP NK24VP
34	NK9VP	2	654	NK9VP NK19VP
35	NK13VP	1	654	NK13VP
36	NK2VP	3	654	NK2VP NK4VP NK5VP
37	NK3VP	1	654	NK3VP
38	NK6VP	1	654	NK6VP
39	NK1VP	3	654	NK1VP NK16VP NK32VP
40	NK31VP	1	654	NK31VP
41	SL4VP	1	654	SL4VP
42	SL7VP	1	654	SL7VP
43	SL14VP	3	654	SL14VP SL27VP SL38VP
44	SL22VP	1	654	SL22VP
45	SL12VP	1	654	SL12VP
46	SL13VP	1	654	SL13VP
47	SL17VP	1	654	SL17VP
48	SL8VP	1	654	SL8VP
49	SL11VP	1	654	SL11VP
50	SL1VP	9	654	SL1VP SL2VP SL3VP SL15VP SL21VP SL23VP SL24VP SL25VP SL26VP
51	SL37WP	1	654	SL37WP
52	SL33WP	5	654	SL33WP SL40WP SL45WP SL46WP SL47WP
53	SL35WP	3	654	SL35WP SL41WP SL42WP
54	SL10WP	3	654	SL10WP SL29WP SL39WP
55	SL36WP	2	654	SL36WP

Haplotype number	Label	Number of sequences	Length of haplotype (bp)	Sequences belonging to haplotype
				SL43WP
56	SL44WP	1	654	SL44WP

Table 5.S2: Haplotypes in one village pig and 10 wild boar from India. One Nepalese wild boar is also included in this analysis. Only one shared haplotype was observed within Indian wild boar.

Haplotype number	Label	Number of sequences	Length of haplotype (bp)	Sequences belonging to haplotype
1	AssamID	1	652	AssamID
2	GL928_CIndia	1	663	GL928_CIndia
3	GL757_India	2	663	GL757_India GL921_India
4	GL759_India	1	663	GL759_India
5	GL756_India	1	663	GL756_India
6	GL924_India	1	663	GL924_India
7	GL929_India	1	663	GL929_India
8	GL747_India	1	663	GL747_India
1	GL927_Nepal	1	663	GL927_Nepal

Table 5.S3: Sampling areas and number of mtDNA control region sequences belonging to each halplogroup of the Bhutanese domestic pigs and wild boar.

Haplotype Number	Haplotype Label	No. of Sequences	Sample ID	Cluster/Clade	Genbank Access #	Sub-districts	Districts	Breed/ Population	Sex
1	Bhu176VP	3	Bhu176VP	D13	HQ318287	Sampheling	Chukha	Village pig	NR
			Bhu173VP	D13	HQ318289	Sampheling	Chukha	Village pig	NR
			Bhu174VP	D13	HQ318288	Sampheling	Chukha	Village pig	NR
2	Bhu198VP	1	Bhu198VP	D13	HQ318290	Khamdang	Trashiyangtse	Village pig	f
3	Bhu149VP	1	Bhu149VP	D13	HQ318291	Naga	Paro	Village pig	f
4	Bhu197VP	3	Bhu192VP	D13	HQ318294	Khamdang	Trashiyangtse	Village pig	f
			Bhu195VP	D13	HQ318293	Khamdang	Trashiyangtse	Village pig	m
			Bhu197VP	D13	HQ318292	Khamdang	Trashiyangtse	Village pig	m
5	Bhu106VP	1	Bhu106VP	D13	HQ318295	Bongo	Chukha	Village pig	f
6	Bhu98VP	3	Bhu98VP	MC7	HQ318296	Dekiling	Sarpang	Village pig	m
			Bhu164VP	MC7	HQ318297	Lhamoizingkha	Dagana	Village pig	m
			Bhu47VP	MC7	HQ318298	Eusu	Haa	Village pig	f
7	Bhu29VP	2	Bhu29VP	MC1	HQ318299	Katsho	Haa	Village pig	m
			Bhu87VP	MC1	HQ318300	Dotey	Paro	Village pig	m
8	Bhu11VP	2	Bhu11VP	MC1	HQ318301	Thoetsho	Wangdue	Village pig	f
			Bhu85VP	MC1	HQ318302	Dotey	Paro	Village pig	m
9	Bhu10VP	25	Bhu10VP	MC1	HQ318303	Thoetsho	Wangdue	Village pig	m
			Bhu193VP	MC1	HQ318304	Khamdang	Trashiyangtse	Village pig	f
			Bhu190VP	MC1	HQ318305	Khamdang	Trashiyangtse	Village pig	f
			Bhu183VP	MC1	HQ318306	Rangthangling	Tsirang	Village pig	f
			Bhu150VP	MC1	HQ318307	Naga	Paro	Village pig	m
			Bhu147VP	MC1	HQ318308	Naga	Paro	Village pig	f
			Bhu135VP	MC1	HQ318309	Tseza	Dagana	Village pig	m
			Bhu134VP	MC1	HQ318310	Drujegang	Dagana	Village pig	m
			Bhu66VP	MC1	HQ318311	Chubu	Punakha	Village pig	m
			Bhu65VP	MC1	HQ318312	Chubu	Punakha	Village pig	f
			Bhu64VP	MC1	HQ318313	Chubu	Punakha	Village pig	f
			Bhu63VP	MC1	HQ318314	Dzomi	Punakha	Village pig	f
			Bhu61VP	MC1	HQ318315	Phangyul-kazhi	Wangdue	Village pig	f

Haplotype Number	Haplotype Label	No. of Sequences	Sample ID	Cluster/Clade	Genbank Access #	Sub-districts	Districts	Breed/ Population	Sex
			Bhu53VP	MC1	HQ318316	Thoetsho	Wangdue	Village pig	f
			Bhu51VP	MC1	HQ318317	Thoetsho	Wangdue	Village pig	m
			Bhu50VP	MC1	HQ318318	Thoetsho	Wangdue	Village pig	f
			Bhu132VP	MC1	HQ318319	Tseza	Dagana	Village pig	f
			Bhu57VP	MC1	HQ318320	Thoetsho	Wangdue	Village pig	m
			Bhu48VP	MC1	HQ318321	Katsho	Haa	Village pig	f
			Bhu116VP	MC1	HQ318322	Darla	Chukha	Village pig	m
			Bhu35VP	MC1	HQ318323	Phangyul-kazhi	Wangdue	Village pig	f
			Bhu148VP	MC1	HQ318324	Naga	Paro	Village pig	f
			Bhu39VP	MC1	HQ318325	Eusu	Haa	Village pig	f
			Bhu153VP	MC1	HQ318326	Goshi	Dagana	Village pig	m
			Bhu133VP	MC1	HQ318327	Drujegang	Dagana	Village pig	m
10	Bhu151VP	2	Bhu151VP	MC1	HQ318328	Goshi	Dagana	Village pig	f
			Bhu139VP	MC1	HQ318329	Goshi	Dagana	Village pig	f
11	Bhu14VP	1	Bhu14VP	MC1	HQ318330	Katsho	Haa	Village pig	f
12	Bhu100VP	13	Bhu100VP	MC1	HQ318331	Gelephu	Sarpang	Village pig	m
			Bhu188VP	MC1	HQ318332	Tsholingkhor	Tsirang	Village pig	f
			Bhu185VP	MC1	HQ318333	Tsholingkhor	Tsirang	Village pig	f
			Bhu184VP	MC1	HQ318334	Tsholingkhor	Tsirang	Village pig	f
			Bhu181VP	MC1	HQ318335	Rangthangling	Tsirang	Village pig	m
			Bhu138VP	MC1	HQ318336	Drujegang	Dagana	Village pig	m
			Bhu102VP	MC1	HQ318337	Bongo	Chukha	Village pig	f
			Bhu124VP	MC1	HQ318338	Bongo	Chukha	Village pig	m
			Bhu101VP	MC1	HQ318339	Gelephu	Sarpang	Village pig	m
			Bhu118VP	MC1	HQ318340	Darla	Chukha	Village pig	m
			Bhu104VP	MC1	HQ318341	Bongo	Chukha	Village pig	f
			Bhu186VP	MC1	HQ318342	Tsholingkhor	Tsirang	Village pig	f
			Bhu103VP	MC1	HQ318343	Bongo	Chukha	Village pig	m
13	Bhu105VP	2	Bhu105VP	MC1	HQ318345	Bongo	Chukha	Village pig	f
			Bhu2VP	MC1	HQ318346	Drujegang	Dagana	Village pig	m
14	Bhu88VP	1	Bhu88VP	D12	HQ318347	Dotey	Paro	Village pig	f

Haplotype Number	Haplotype Label	No. of Sequences	Sample ID	Cluster/Clade	Genbank Access #	Sub-districts	Districts	Breed/ Population	Sex
15	Bhu202VP	15	Bhu202VP	GC	HQ318348	Ramjar	Trashiyangtse	Village pig	N
			Bhu129VP	GC	HQ318349	Bongo	Chukha	Village pig	m
			Bhu115VP	GC	HQ318350	Darla	Chukha	Village pig	m
			Bhu110VP	GC	HQ318351	Darla	Chukha	Village pig	m
			Bhu99VP	GC	HQ318352	Gelephu	Sarpang	Village pig	m
			Bhu93VP	GC	HQ318353	Darla	Chukha	Village pig	m
			Bhu74VP	GC	HQ318354	Trong	Zhemgang	Village pig	m
			Bhu161VP	GC	HQ318355	Umling	Sarpang	Village pig	m
			Bhu160VP	GC	HQ318356	Umling	Sarpang	Village pig	f
			Bhu159VP	GC	HQ318357	Umling	Sarpang	Village pig	m
			Bhu157VP	GC	HQ318358	Umling	Sarpang	Village pig	m
			Bhu156VP	GC	HQ318359	Umling	Sarpang	Village pig	f
			Bhu155VP	GC	HQ318360	Umling	Sarpang	Village pig	f
			Bhu167VP	GC	HQ318361	Lhamoizingkha	Dagana	Village pig	f
			Bhu154VP	GC	HQ318362	Umling	Sarpang	Village pig	f
16	Bhu24VP	6	Bhu24VP	D10	HQ318363	Samtse	Samtse	Village pig	m
			Bhu32VP	D10	HQ318364	Samtse	Samtse	Village pig	m
			Bhu62VP	D10	HQ318365	Dzomi	Punakha	Village pig	f
			Bhu40VP	D10	HQ318366	Samtse	Samtse	Village pig	f
			Bhu83VP	D10	HQ318367	Dogar	Paro	Village pig	m
			Bhu38VP	D10	HQ318368	Dogar	Paro	Village pig	m
17	Bhu22VP	1	Bhu22VP	D11	HQ318369	Chang (NPBC/R)	Thimphu	Village pig	f
18	Bhu15VP	4	Bhu15VP	GC	HQ318370	Thoetsho	Wangdue	Village pig	f
			Bhu36VP	GC	HQ318371	Thoetsho	Wangdue	Village pig	m
			Bhu7VP	GC	HQ318372	Thoetsho	Wangdue	Village pig	f
			Bhu78VP	GC	HQ318373	Bardo	Zhemgang	Village pig	m
19	Bhu12VP	30	Bhu12VP	GC	HQ318374	Bardo	Zhemgang	Village pig	m
			Bhu30VP	GC	HQ318375	Katsho	Haa	Village pig	m
			Bhu34VP	GC	HQ318376	Trong	Zhemgang	Village pig	m
			Bhu37VP	GC	HQ318377	Dzomi	Punakha	Village pig	m
			Bhu4VP	GC	HQ318378	Dogar	Paro	Village pig	f

Haplotype Number	Haplotype Label	No. of Sequences	Sample ID	Cluster/Clade	Genbank Access #	Sub-districts	Districts	Breed/ Population	Sex
			Bhu41VP	GC	HQ318379	Uzurong	Tashigang	Village pig	f
			Bhu42VP	GC	HQ318380	Uzurong	Tashigang	Village pig	m
			Bhu8VP	GC	HQ318381	Thoetsho	Wangdue	Village pig	f
			Bhu211VP	GC	HQ318382	Phobjikha	Wangdue	Village pig	f
			Bhu206VP	GC	HQ318383	Ramjar	Trashiyangtse	Village pig	NR
			Bhu204VP	GC	HQ318384	Ramjar	Trashiyangtse	Village pig	NR
			Bhu203VP	GC	HQ318385	Ramjar	Trashiyangtse	Village pig	NR
			Bhu201VP	GC	HQ318386	Ramjar	Trashiyangtse	Village pig	NR
			Bhu191VP	GC	HQ318387	Khamdang	Trashiyangtse	Village pig	f
			Bhu182VP	GC	HQ318388	Rangthangling	Tsirang	Village pig	f
			Bhu137VP	GC	HQ318389	Tsezg	Dagana	Village pig	m
			Bhu127VP	GC	HQ318390	Bongo	Chukha	Village pig	m
			Bhu123VP	GC	HQ318391	Darla	Chukha	Village pig	NR
			Bhu113VP	GC	HQ318392	Darla	Chukha	Village pig	f
			Bhu54VP	GC	HQ318393	Thoetsho	Wangdue	Village pig	f
			Bhu52VP	GC	HQ318394	Thoetsho	Wangdue	Village pig	f
			Bhu49VP	GC	HQ318395	Katsho	Haa	Village pig	f
			Bhu130VP	GC	HQ318396	Tseza	Dagana	Village pig	m
			Bhu68VP	GC	HQ318397	Chubu	Punakha	Village pig	f
			Bhu55VP	GC	HQ318398	Thoetsho	Wangdue	Village pig	m
			Bhu33VP	GC	HQ318399	Chang (NPBC/R)	Thimphu	Village pig	m
			Bhu31VP	GC	HQ318400	Bardo	Zhemgang	Village pig	f
			Bhu17VP	GC	HQ318401	Bardo	Zhemgang	Village pig	f
			Bhu81VP	GC	HQ318402	Dogar	Paro	Village pig	f
			Bhu9VP	GC	HQ318403	Phangyul-kazhi	Wangdue	Village pig	f
20	Bhu172VP	3	Bhu172VP	GC	HQ318405	Lhamoizingkha	Dagana	Village pig	m
			Bhu171VP	GC	HQ318404	Lhamoizingkha	Dagana	Village pig	m
			Bhu152VP	GC	HQ318406	Goshi	Dagana	Village pig	m
21	Bhu18VP	19	Bhu18VP	D7	HQ318407	Dotay	Paro	Village pig	f
			Bhu44VP	D7	HQ318408	Geyling	Chukha	Village pig	f
			Bhu69VP	D7	HQ318409	Dekiling	Sarpang	Village pig	m

Haplotype Number	Haplotype Label	No. of Sequences	Sample ID	Cluster/Clade	Genbank Access #	Sub-districts	Districts	Breed/ Population	Sex
			Bhu122VP	D7	HQ318410	Darla	Chukha	Village pig	f
			Bhu109VP	D7	HQ318411	Darla	Chukha	Village pig	m
			Bhu212VP	D7	HQ318412	Phobjikha	Wangdue	Village pig	m
			Bhu208VP	D7	HQ318413	Ramjar	Trashiyangtse	Village pig	NR
			Bhu120VP	D7	HQ318414	Darla	Chukha	Village pig	m
			Bhu112VP	D7	HQ318415	Darla	Chukha	Village pig	m
			Bhu90VP	D7	HQ318416	Darla	Chukha	Village pig	f
			Bhu86VP	D7	HQ318417	Dotey	Paro	Village pig	m
			Bhu76VP	D7	HQ318418	Trong	Zhemgang	Village pig	m
			Bhu89VP	D7	HQ318419	Darla	Chukha	Village pig	m
			Bhu70VP	D7	HQ318420	Dekiling	Sarpang	Village pig	m
			Bhu209VP	D7	HQ318421	Phobjikha	Wangdue	Village pig	m
			Bhu46VP	D7	HQ318422	Eusu	Haa	Village pig	f
			Bhu19VP	D7	HQ318423	Eusu	Haa	Village pig	f
			Bhu177VP	D7	HQ318424	Sampheling	Chukha	Village pig	NR
			Bhu94VP	D7	HQ318425	Geyling	Chukha	Village pig	m
22	Bhu213VP	2	Bhu213VP	D7	HQ318426	Phobjikha	Wangdue	Village pig	f
		140	Bhu210VP	D7	HQ318427	Phobjikha	Wangdue	Village pig	m
1	BhuWP28	1	BhuWP28	W12	HQ318428	Bjacho	Chukha	Wild boar	NR
2	BhuWP7	3	BhuWP7	W12	HQ318429	Bhur	Sarpang	Wild boar	NR
			BhuWP8	W12	HQ318430	Bhur	Sarpang	Wild boar	NR
			BhuWP6	W12	HQ318431	Shinkhar Lauri	S/Jhongkhar	Wild boar	NR
3	BhuWP1	2	BhuWP1	MC1	HQ318432	Kazi	Wangdue	Wild boar	NR
			BhuWP52	MC1	HQ318433	Thinlaygang	Thimphu	Wild boar	NR
4	BhuWP2	4	BhuWP2	MC1	HQ318434	Kazi	Wangdue	Wild boar	NR
			BhuWP53	MC1	HQ318435	Thinlaygang	Thimphu	Wild boar	NR
			BhuWP3	MC1	HQ318436	JSWNP	JSWN Park	Wild boar	NR
			BhuWP27	MC1	HQ318437	Kazi	Wangdue	Wild boar	NR
5	BhuWP4	1	BhuWP4	W17	HQ318438	Langthel	Trongsa	Wild boar	NR
6	BhuWP5	1	BhuWP5	W17	HQ318439	Shinkhar Lauri	S/Jhongkhar	Wild boar	NR

NR = Not recorded or determined; NPBC = National Pig Breeding Centre at Serbithang; R = Royal pig

Table 5.S4: Sampling areas and number of mtDNA control region sequences belonging to each halplogroup of the Nepalese pigs.

Haplotype Number	Haplotype Label	No. of Sequences	Sample ID	Cluster/Clade	Genbank Access #	Place of collection	Districts	Breed/ Population	Sex
1	NK12VP	1	NK12VP	D13	HQ318440	Talchikhel	Lalitpur	NKH - village pig	f
2	NK8VP	2	NK8VP	D13	HQ318441	Talchikhel	Lalitpur	NKH - village pig	m
			NK11VP	D13	HQ318442	Talchikhel	Lalitpur	NKH - village pig	m
3	NK14VP	1	NK14VP	D13	HQ318443	Talchikhel	Lalitpur	NKH - village pig	m
4	NK44VP	2	NK44VP	MC7	HQ318444	Lahan	Saptari	NKD - village pig	f
			NK48VP	MC7	HQ318445	Kurintar	Gorkha	Chwanche X	f
5	NK30VP	1	NK30VP	MC7	HQ318446	Naryananghat	Chitawan	NKD - village pig	m
6	NK10VP	3	NK18VP	MC1	HQ318447	Talchikhel	Lalitpur	NKH - village pig	f
			NK10VP	MC1	HQ318448	Talchikhel	Lalitpur	NKH - village pig	f
			NK24VP	MC1	HQ318449	Talchikhel	Lalitpur	NKH - village pig	m
7	NK9VP	2	NK9VP	MC1	HQ318450	Talchikhel	Lalitpur	NKH - village pig	f
			NK19VP	MC1	HQ318451	Talchikhel	Lalitpur	NKH - village pig	f
8	NK13VP	1	NK13VP	D13	HQ318452	Talchikhel	Lalitpur	NKH - village pig	m
9	NK2VP	3	NK2VP	GC	HQ318453	Talchikhel	Lalitpur	NKH - village pig	m
			NK4VP	GC	HQ318454	Talchikhel	Lalitpur	NKH - village pig	f
			NK5VP	GC	HQ318455	Talchikhel	Lalitpur	NKH - village pig	m
10	NK3VP	1	NK3VP	GC	HQ318456	Talchikhel	Lalitpur	NKH - village pig	m
11	NK7VP	15	NK7VP	GC	HQ318457	Talchikhel	Lalitpur	NKH - village pig	m
			NK15VP	GC	HQ318458	Talchikhel	Lalitpur	NKH - village pig	m
			NK35VP	GC	HQ318459	Kurintar	Gorkha	NKD - village pig	f
			NK36VP	GC	HQ318460	Kurintar	Gorkha	NKD - village pig	f
			NK37VP	GC	HQ318461	Kurintar	Gorkha	NKD - village pig	f
			NK38VP	GC	HQ318462	Kurintar	Gorkha	NKD - village pig	m
			NK39VP	GC	HQ318463	Kurintar	Gorkha	NKD - village pig	m
			NK40VP	GC	HQ318464	Kurintar	Gorkha	NKD - village pig	m
			NK41VP	GC	HQ318465	Kurintar	Gorkha	NKD - village pig	m
			NK42VP	GC	HQ318466	Kurintar	Gorkha	NKD - village pig	m
			NK43VP	GC	HQ318467	Lahan	Saptari	NKD - village pig	m

Haplotype Number	Haplotype Label	No. of Sequences	Sample ID	Cluster/Clade	Genbank Access #	Place of collection	Districts	Breed/ Population	Sex
			NK45VP	GC	HQ318468	Kurintar	Gorkha	Chwanche X	m
			NK46VP	GC	HQ318469	Kurintar	Gorkha	Chwanche X	m
			NK47VP	GC	HQ318470	Kurintar	Gorkha	Chwanche X	m
			NK50VP	GC	HQ318471	Lahan	Saptari	NKD - village pig	m
12	NK6VP	1	NK6VP	GC	HQ318472	Talchikhel	Lalitpur	NKH - village pig	m
13	NK1VP	3	NK1VP	GC	HQ318473	Talchikhel	Lalitpur	NKH - village pig	m
			NK16VP	GC	HQ318474	Talchikhel	Lalitpur	NKH - village pig	f
			NK32VP	GC	HQ318475	Naryananghat	Chitawan	NKD - village pig	m
14	NK29VP	3	NK29VP	D7	HQ318476	Naryananghat	Chitawan	NKD - village pig	m
			NK33VP	D7	HQ318477	Naryananghat	Chitawan	NKD - village pig	m
			NK34VP	D7	HQ318478	Kurintar	Gorkha	NKD - village pig	f
15	NK31VP	1	NK31VP	D7	HQ318479	Naryananghat	Chitawan	NKD - village pig	m

Note: NKD = Kalo Dharane Sunggur; NKH = Hurrah; Chwanche X = Chwanche cross and village pig; NSA = Upper South Asia.

Table 5.S5: Sampling areas and number of mtDNA control region sequences belonging to each halplogroup of the Sri Lanka.

Haplotype Number	Haplotype Label	No. of Sequences	Sample ID	Cluster/Clade	Genbank Access #	Village	Districts	Province	Breed	Sex
1	SL4VP	1	SL4VP	D13	HQ318480	Kudumbuwa	Kurunegala	North Western	Village pig	m
2	SL7VP	1	SL7VP	D13	HQ318481	Beruwila	Kalutara	Western	Village pig	f
3	SL14VP	3	SL27VP	MC4	HQ318482	Kudumbuwa	Kurunegala	North Western	Village pig	m
			SL14VP	MC4	HQ318483				Village pig	NRD
			SL38VP	MC4	HQ318484	Galaha	Kandy	Central	Village pigs	NRD
4	SL22VP	1	SL22VP	MC4	HQ318485	Marawila	Puttalam	North Western	Village pig	f
5	SL12VP	1	SL12VP	D4	HQ318486	Marawila	Puttalam	Western	Village pig	m
6	SL13VP	1	SL13VP	D4	HQ318487	Kudumbuwa	Kurunegala	North Western	Village pig	f
7	SL17VP	1	SL17VP	D4	HQ318488	Kalumulla	Kalutara	Western	Village pig	f
8	SL19VP	2	SL19VP	D12	HQ318489	Chilaw	Chilaw	North Western	Village pig	f
			SL20VP	D12	HQ318490	Beruwila	Kalutara	Western	Village pig	m
9	SL8VP	1	SL8VP	GC	HQ318491	Marawila	Puttalam	North Western	Village pig	f
10	SL11VP	1	SL11VP	MC5	HQ318492	Chilaw	Chilaw	North Western	Village pig	m
11	SL1VP	9	SL1VP	GC	HQ318493	Marawila	Puttalam	North Western	Village pig	f
			SL2VP	GC	HQ318494	Beruwila	Kalutara	Western	Village pig	m
			SL3VP	GC	HQ318495	Beruwila	Kalutara	Western	Village pig	m
			SL15VP	GC	HQ318496	Kalumulla	Kalutara	Western	Village pig	m
			SL21VP	GC	HQ318497	Kudumbuwa	Kurunegala	North Western	Village pig	m
			SL23VP	GC	HQ318498	Kalumulla	Kalutara	Western	Village pig	m
			SL24VP	GC	HQ318499	Kalumulla	Kalutara	Western	Village pig	f
			SL25VP	GC	HQ318500	Marawila	Puttalam	North Western	Village pig	m
			SL26VP	GC	HQ318501				Village pig	NR
12	SL16VP	1	SL16VP	GC	HQ318502	Kalumulla	Kalutara	Western	Village pig	f
1	SL37WP	1	SL37WP	SSA	HQ318503	Puliyankulama	Anuradhapura	Nothern central	Wild boar	f
2	SL33WP	5	SL33WP	SSA	HQ318504	Galaha	Kandy	Central	Wild boar	f
			SL40WP	SSA	HQ318505	Chiththandy	Batticaloa	Eastern	Wild boar	NR
				SSA						
			SL45WP		HQ318506	Manampittiya	Polonaruwa	Northern	Wild boar	f

Haplotype Number	Haplotype Label	No. of Sequences	Sample ID	Cluster/Clade	Genbank Access #	Village	Districts	Province	Breed	Sex
								Central		
			SL46WP	SSA	HQ318507	Kudumbuwa	Kurunegala	North western	Wild boar	f
			SL47WP	SSA	HQ318508	Puliyankulama	Anuradhapura	Northern Central	Wild boar	m
3	SL35WP	3	SL35WP	SSA	HQ318509	Kudumbuwa	Kurunegala	North western	Wild boar	m
			SL41WP	SSA	HQ318510	Walikandha	Batticaloa	Eastern	Wild boar	m
			SL42WP	SSA	HQ318511	Walikandha	Batticaloa	Eastern	Wild boar	m
4	SL10WP	3	SL10WP	SSA	HQ318512	Kudumbuwa	Kurunegala	North Western	Wild boar	NR
			SL29WP	SSA	HQ318513	Kudumbuwa	Kurunegala	North Western	Wild boar	f
			SL39WP	SSA	HQ318514	Kudumbuwa	Kurunegala	North Western	Wild boar	m
5	SL36WP	2	SL36WP	SSA	HQ318515	Manampittiya	Polonaruwa	Northern Central	Wild boar	f
			SL43WP	SSA	HQ318516	Puliyankulama	Anuradhapura	Northern Central	Wild boar	f
6	SL44WP	1	SL44WP	SSA	HQ318517	Manampittiya	Polonaruwa	Northern Central	Wild boar	f

Note: SSA = Southern South Asia

Table 5.S6: Modern domestic pig and museum wild boar sequences from India and ancient sequence from Nepal.

Haplotype Number	Haplotype Label	No. of Seq.	Sequences belonging to haplotype	Genbank Access #	Breed/Species	Sampling Areas
Modern domestic Indian pig Sample						
1	IDAssam	1	IDAssam	HQ318344	Village pig	Datgari, Assam, India
Indian wild boar museum specimens						
1	GL747-India	1	GL747-India		Wild boar	Agyas
2	GL929-India	1	GL929-India		Wild boar	Sampling area: Sikkim, India
3	GL756-India	1	GL756-India		Wild boar	Kheri, Oudh, Palia, Uttar Pradesh, India
4	GL757-India	2	GL757-India		Wild boar	Rajputana, Rajasthan, India
5			GL921-India		Wild boar	Jodhpur, Rajasthan, India
6	GL759-India	1	GL759-India		Wild boar	Rajputana, Rajasthan, India
7	GL924-India	1	GL924-India		Wild Boar	Shahabad, Haryana, India
8	GL928-India	1	GL928-India		Wild Boar	Central provinces, India
9	GL937-India	1	GL937-India		Thian Shan sus nigripes	India
10	GL925-India		GL925-India		Malaha, India	India
11	GL927_NKW	1	GL927_NKW		Wild boar	Kathmandu, Nepal
Nepal ancient sample						
1	GL384-Nepal	1	GL384-Nepal		Domestic	Gotihawa, Nepal

Note: NSA = Northern South Asia

Table 5.S7: Previously reported additional haplotype sequences of South Asian domestic and wild boar used in the analysis. Haplotypes from Thai wild and domestic pigs that were not analysed by Larson *et al.* (2010) were added to the overall analyses.

Country	Haplotype Label	Haplotype Group	Genbank Access #	Breed/Species	Sampling Areas	References
India	IWP-AY884709	MC1	AY884709	Wild boar	Ambikapur, Surguja district, Chhattishgarh state	Larson *et al.* 2005
	IWP-AY884612	MC1	AY884612	Wild boar	Valley of Kashmir, Jammu & Kashmir	
	IWP-AY884674	MC1	AY884674	Wild boar	Monghyr, Bengal	
	IWP-AY884689	MC1	AY884689	Wild boar	Woolar lake, Kashmir	
	IWP-AY884675	MC1	AY884675	Wild boar	Monghyr, Bengal	
	IWP-AY884671	MC1	AY884671	Wild boar; *S.s.cristatus*		
Pakistan	PKW1	NSA	AY884618		Sailkot Lahore	Larson *et al.* 2005
Sri Lanka	SLGB-AY884636	SSA	AY884636			Larson *et al.* 2005
	SLGB-DQ779423	SSA	DQ779423			Larson *et al.* 2007
	SLGB-DQ779413	SSA	DQ779413			
Nepal	NKW1-DQ779421	NSA	DQ779421	Wild boar	Dharan Bazar	Larson *et al.* 2007
	NKW2- DQ779421	NSA	DQ779421	Wild boar	Royal Chitawan National Park, Charara Grassland	
Thailand	Domestic	FM244674 (TD1); AM778828 (TD6); AM779906 (TD16); FM244468 (TD40); AM774644 (TD10); FM244496 (TD18); FM244679 (TD38); AM779914 (TD39); ; FM244495 (TD41); AM777926 (TD46); AM779910 (TD51); AM779915 (TD53); AM777922 (TD56); FM244681 (TD62); ; FM244471 (TD63); ; FM244682 (TD66); AM777919 (TD75); FM244680 (TD76); AM777918 (TD77)				
	Wild	DQ779402 (TW2); AY884630 (TW3); AM779933 (TW4); FM244686 (TW8); FM244688 (TW10); AM779935 (TW14); DQ779410 (TW15)				

Table 5.S8: Porcine mtDNA control region haplotypes from Genbank. To be consistent, exactly the same haplotype sequences used by Tanaka *et al.* (2008) were used in this study. Details of previously published sequences from South Asia are given in Table 5.S3, Table 5.S4, Table 5.S5, and Table 5.S6.

Region	**Cluster/Haplogroup**	**Genbank Accession No.**	**No. of Sequences**
European	D1	AB041484; AB041495; AB041485; AB041497; AB041493; AB041492; AB041491; AB041498; AB041499; AB041486; AB041496; AB015093	12
European	D4	AB015094; AB015095	2
Asian	D2	AB015086; D42171; AB041472; AB015084; AB041469**;** AB041467; AB041471; AB015085; D42173; D42174; D42178; D42181; D42182; AB041475; AB041476; AB041481	16
Myanmar	D5	AY884623	1
Pacific	D6	AY884678	1
Southeast Asia	MTSEA	AB252815; AB252820; AB252819; AB252816; AB252817; AB252821	6
Japanese wild boar	Ryukyu wild boar (*S. s. riukiuanus*)	AB015087; AB015088	2
Cambodia/Laos WB	Cambodia/Laos WB	AB252823; AB252824	2
		Total Haplotypes	**41**

Table 5.S9: Percentage of individuals in SA representing each clade. The nomenclature of the clades were kept consistent with Larson *et al* (2010).

Major Clade	Sub-clades	IN Larson *et al.* (2005)	Percentage (%)					
			BVP	BWB	NVP	SLVP	SLWB	IWB
General Asian	GAC	D2	37		58	48		
Domestic	D4					13		
	D7		15		10			
	D10		4					
	D11		1					
	D12		1			9		
Mixed	MC1	D3	34	50	13			
	MC4					17		
	MC5					4		
	MC7		2		8			
Wild	W12	D5		33				
	W17			17				
SA Wild	NSA							100
	SSA						100	
European	D13	D1	6		13	9		
Total		100	100	100	100	100	100	100

CHAPTER 6

Part I: Genetic Status of Indigenous Pigs of Papua New Guinea

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6.1 ABSTRACT

Pigs are the principal livestock species in Papua New Guinea (PNG) with significant socio-cultural and economic importance to hundreds of ethnic rural communities. The indigenous domestic pigs of PNG have never been genetically characterised. We have used 21 microsatellite markers and mitochondrial DNA (mtDNA) control region sequences (1044bp) to determine the genetic status of domestic pigs in three provinces of PNG. For the microsatellites, the mean number of observed and effective alleles were 14.19 (SE = 1.23) and 5.55 (SE = 0.50), respectively. The overall mean observed and expected heterozygosities were 0.63 (SE = 0.03) and 0.78 (SE = 0.02) respectively. With one exception, all loci deviated from Hardy-Weinberg equilibrium. All the five inferred populations, which had low to moderate genetic differentiation and correlated well with sampling localities, showed higher expected heterozygosities than the outgroup animals consisting of Australian commercial pigs of European composite breed. Our analyses of 24 mitochondrial haplotypes from 70 domestic pigs of PNG and 3 haplotypes from 5 Australian commercial pigs and 186 previously recognised major porcine haplotypes, revealed that mitochondria of domestic pigs of PNG belong mainly within Oceania (D6) haplotypes followed by General Asian (D2) and European (D1) haplotypes. The shared haplotypes between wild and domestic pigs within D6 suggested that the latter have been derived from wild or feral pigs in the Oceania region and within PNG. The D2 haplotypes, which are the most common haplotypes dispersed worldwide, may have been introduced along with D6 haplotypes during the expansion of Lapita and Polynesian culture. D2 may have also come via Europe following their introduction into European breeds during the 18th and 19th centuries and the subsequent introduction of "European" pigs into PNG. We also observed that Australian commercial pigs of European origin have been introgressed with D2 haplotypes. Interestingly, current evidence suggests that there is a genuine wild boar mtDNA signature (D5) within some Australian feral pigs and one domestic pig of PNG. Clearly, domestic pigs of PNG show reasonably high levels of biodiversity, which will be useful for future research and conservation programmes.

Keywords: *Microsatellites, genetic diversity, mitochondrial DNA, animal genetic resources, haplotype, domestication, conservation.*

6.2 INTRODUCTION

Papua New Guinea is one of the most ecologically and culturally diverse countries on Earth with several hundred languages and many traditional communities. More than 80% of the people live in rural areas and depend on agriculture for their livelihood. The first human settlements in Oceania (in areas from New Guinea to the Solomon Islands) are recorded between 40,000 and 49,000 years ago (Allen & O'Connell 2003; Summerhayes *et al.* 2010). Pigs, the principal livestock, have socio-cultural and economic importance in the lives of many people of PNG (Ayalew *et al.* 2011). Linguistic evidence proposes introduction of pigs by Austronesian speakers about 3500 years ago (Blust 1976), while accelerator mass spectroscopy of pigs' teeth (Blust 2002) and bones (Pasveer 2003) from various prehistoric sites dated pigs in PNG between 500-4000 year before present (BP). Golson & Hughes (1980) suggested that pigs were present by 6000 BP. Bones from two archaeological sites in the New Guinea Highlands suggest the presence of pigs in PNG between 6000 and 10000 years ago (Bulmer 1966, 1975). Another recent conflicting interpretation is that neither pig nor pottery arrived before 3,000 BP in mainland PNG (O'Connor *et al.* 2011).

DNA studies provide interesting insights into the introduction of pigs to the mainland of New Guinea (Larson *et al.* 2005; Lum *et al.* 2006; Larson *et al.* 2007; Larson *et al.* 2010). Lum *et al.* (2006) claim that Pacific island pigs were recently domesticated within Southeast Asia and dispersed during the human colonization of remote Oceania associated with the Lapita or Pacific Ocean archaeological culture. Larson *et al.* (2007) found evidence for early human mediated translocation of the Sulawesi warty pig (*Sus celebensis*) to Flores and Timor and two later separate human-mediated dispersals of domestic pig (*Sus scrofa*) through Island Southeast Asia into Oceania. This evidence was further supported by the discovery of additional wild boar samples from Laos and China possessing Pacific Clade haplotypes thereby adding support to the hypothesis that the Pacific Clade is indigenous to peninsular Southeast Asia (Larson *et al.* 2010).

Hide (2003) reviewed the pig husbandry systems, historical and cultural views on pigs, possible origins and breeds, population trends, diseases, impact on environment, and various research trials of pigs in PNG, but with very little mention of the need for conservation and sustainable utilization of genetic resources of indigenous pigs. Quartermain (2002) proposed genetic characterization and conservation of indigenous pigs, but no mechanism was suggested for sustainable development and the promotion of genetic resources of indigenous stock. The colonial government policy of introducing exotic breeds of pigs continues to the present (Ayalew *et al.* 2011). With such practices, the indigenous stock may become marginalised, as seen in many developing countries (Rajamahendran *et al.* 1985; Nidup *et al.* 2010; Nidup *et al.* 2011).

Loss of livestock genetic biodiversity is a pervasive problem throughout the world (FAO 2007b). To combat erosion of genetic diversity and promote sustainable management of animal genetic resources, the *Global Plan of Action (GPA) for Animal Genetic Resources* has been adopted following the *Interlaken Declaration on Animal Genetic Resources* (FAO 2007a). The international community confirmed common and individual responsibilities for the conservation, sustainable use and development of animal genetic resources for world food security, improving human nutrition, and for rural development. PNG is a signatory to this declaration. In an effort to implement the GPA, Ayalew *et al.* (2011) performed phenotypic characterization of indigenous domestic pigs and discussed their cultural significance to the people of PNG.

PNG pigs have gone through various morphological and phenotypic changes over several decades (Jenness & Ballantyne 1920; AMVSU 1946; Anderson 1972). They are medium sized, have either black or grey coarse bristly coats, sharp backs, mostly short and cylindrical straight snouts, sometimes distinct manes, and pricked ears (Ayalew *et al.* 2011). They are non-differentiable phenotypically across a wide and isolated range of geography. There are reports of the mtDNA (Larson *et al.* 2005; Larson *et al.* 2007; Larson *et al.* 2010) and microsatellite (Spencer *et al.* 2006) studies on feral pigs, which are defined as pigs either released or escaped from human captivity. Some of these get hunted; some are captured alive and raised; and some get re-feralised and become wild.

In this study, we will use the term "indigenous domestic" for pigs under human captivity; "wild or feral" for pigs that are free roaming in the wild. Reports of mtDNA (Larson *et al.* 2005; Larson *et al.* 2007; Larson *et al.* 2010) and microsatellite studies for feral pigs exist. However, the indigenous domestic pigs, which are very close to the hearts of hundreds of ethnic rural communities, have not been genetically characterised except for couple of a reports on chromosome banding and karyotyping (Popescu *et al.* 1982; Popescu *et al.* 1989). Therefore, the main objective of this study was to determine the genetic structure, diversity, and origin of indigenous domestic pigs of PNG using microsatellite markers and the control region of mtDNA sequences. The ultimate goal is to generate prerequisite genetic information for further research and development leading to conservation programs of biodiversity and sustainable utilization of swine genetic resources in PNG.

6.3 MATERIALS AND METHODS

6.3.1 Sampling and DNA Extraction

Blood and hair samples of 70 indigenous pigs, excluding first degree relatives, were collected from three provinces (Morobe, Western Highlands, and Enga) comprising eight locations (Tambul, Nawaeb, Finschhafen, Garaina, Huon Gulf, Kandep, Bulolo, and Boana) in six districts (Figure 6.1). These locations were chosen because they were considered to be less affected by the introduction of exotic breeds and their crossbreeding with indigenous stock. Additionally, fifteen pigs of mixed ancestry (Large White and Landrace composite) sampled from a large commercial piggery at Corowa, New South Wales (Australia) were used as an outgroup. DNA from blood and hair samples was purified using QIAamp® DNA Blood Mini Kit (QIAGEN).

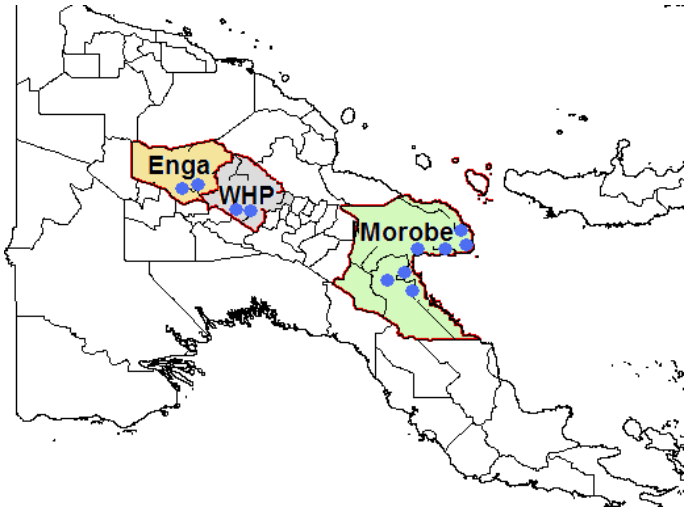

Figure 6.1: Shows three provinces where sampling has been conducted in PNG. Specific sampling locations are not shown on the map.

6.3.2 Microsatellite Genotyping

6.3.2.1 PCR and Genotyping

Twenty one polymorphic loci were selected for this study (Table 6.S2). Except for marker *SW951*, which has been used in feral pig studies (Hampton *et al.* 2004; Cowled *et al.* 2008), all others were recommended by FAO and the International Society for Animal Genetics (FAO 2004; Hoffmann *et al.* 2009). No null alleles have been reported for these markers and linkage disequilibrium was not expected because of the minimum spacing of at least 30 centiMorgans (Groenen *et al.* 2003).

PCR was performed in 15µl volume containing 40ng of porcine genomic DNA, 80nM of each dye-labelled (FAM, VIC, NED, and PET) primers, 10 x *Taq* DNA polymerase buffer, 1.5mM $MgCl_2$ and 100µM dNTPs. After denaturation at 95˚C for 10 minutes and then holding at 80˚C, 1U of *Taq* polymerase enzyme was added. A touchdown program was used (44 cycles 95ºC 40 seconds, 62-55ºC 60 seconds, 72ºC 60 seconds, 1 cycle 72 ºC for 20 minutes) for all markers, using a PTC-100^{TM} Programmable Thermal Controller (MJ Research, Inc, Waltham, MA). The amplified products, which were pooled based on their size and dye types, were analysed by ABI PRISM 3730 DNA Analyser (Applied

Biosystems, Warrington, UK) at the Australian Genomic Resource Facility (AGRF), Victoria, Australia. GeneScan500 Liz (PN4322682, Applied Biosystems) was used as a size standard and the alleles were scored using GeneMapper v3.7.

6.3.2.2 Genotype Data Analysis

All loci were tested for Hardy-Weinberg Equilibrium (HWE) using Genepop (Raymond & Rousset 1995). Bayesian clustering, implemented in STRUCTURE (Pritchard *et al.* 2000), was used to investigate heterozygosity and genetic structure. Simulation of 1-10 inferred populations (K = 1-10) with a burn-in period of 100,000 iterations and 10^6 iterations of Markov Chain Monte Carlo (MCMC) simulation were used in an admixture model. For each estimate of the number of inferred populations (K), five iterations were specified to check for consistency between runs. K was plotted against mean log likelihood (Figure 6.3) and the number of inferred populations determined from the point where the mean log likelihood plateaued.

Genetic diversity indices including number of alleles, heterozygosity, genetic distance (Nei 1972, 1978), and F-statistics (Weir & Cockerham 1984) for inferred populations were determined using GenAlEX (Peakall & Smouse 2006). Matrices of genetic distances between pairs of inferred populations were summarised diagrammatically in a Neighbour-joining (NJ) tree (Saitou & Nei 1987). Analysis of molecular variance (AMOVA) and Principal Coordinates Analysis (PCA) were calculated using GenAlEx. All these analyses were verified using PopGene (Yeh *et al.* 1999) and Genepop (Raymond & Rousset 1995) to ensure computational accuracy and consistency of interpretation.

6.3.3 Mitochondrial DNA Sequences

6.3.3.1 PCR and Sequencing

Previously employed porcine primers (Kim *et al.* 2002), L-strand [(5'-CCAAGACTC AAGGAAGGAGA-3' (Position 15,363-15,382 of the pig mtDNA, AJ002189)] and H-strand [5'-GGCGCGGATACT TGCATGTG-3' (Position 115-134)] were used to amplify 1044bp mtDNA control region. PCR reactions were performed following the published protocols (Gongora *et al.* 2004; Gongora *et al.* 2006) for up to ten samples, whose products were gel purified with a JETquick spin column (GeneWorks, Australia) and sent to the Australian Genome Research Facility Ltd (AGRF: http://www.agrf.org.au) in Brisbane, Australia, for direct sequencing. Sixty DNA samples were sent directly to AGRF for PCR, purification or clean-up of amplicons, and for sequencing.

CodonCode Aligner (©CodonCode Corporation, MA, USA) was used to assemble and edit sequences along with three reference sequences (AY463061, AY463062, AF276926) from Genbank. DNA sequences were aligned (Thompson *et al.* 1994; Edgar 2004b; Larkin *et al.* 2007) and collapsed to haplotypes (Villesen 2007). Final sequences were deposited in Genbank database (http://www.ncbi.nlm.nih.gov/genbank/submit.html) with accession numbers from HQ318518 to HQ318592 (Table 6.S8).

6.3.3.2 Sequence Data Analysis

To accommodate every possible major porcine haplotype, a total of 186 representative porcine haplotypes (Table 6.S9; Table 6.S10; Table 6.S11) from each clade reported in Larson *et al.* (2010) and Tanaka *et al.* (2008) along with some additional haplotypes (Table 6.S11) from Southeast Asia and Oceania were retrieved from Genbank. They were combined with haplotypes obtained from 70 PNG indigenous pigs and 5 Australian commercial pigs. The latter were used to verify the authenticity of their maternal origin as they were perceived to be European pigs.

Bayesian Monte Carlo-Markov Chain (MCMC) analysis (Drummond & Rambaut 2007) was performed to generate a phylogenetic tree using model parameters (HKY+I+G) identified by ModelGenerator v0.85 (Keane *et al.* 2006) and ModelTest (Posada & Crandall 1998). In addition to the phylogenetic tree, a Median-Joining Network (MJN) analysis (Bandelt *et al.* 1999) was performed using truncated (507bp) sequences to enable incorporation of all major haplotypes reported in Genbank. Sequences were further aligned, gaps and missing data were removed, and a multistate alignment rdf (Roehl data format) file was generated using Fluxus's DNA alignment program (http://www.fluxus-engineering.com) for analysis with Network 4.5.1.6 genetic program. Population information, geographical distribution and frequency of sequence per haplotype were specified. Closely related sequences were collapsed into major haplogroups using star contraction, which identifies star-like clusters of nodes and shrinks such nodes back towards the founder population (Forster *et al.* 2001).

With default parameters and weighting all characters equally, MJN analysis was performed to determine genealogies of haplotypes. This was followed by post-processing analysis (Polzin & Daneshmand 2003) on MJN analyses to identify unnecessary median vectors and links that were switched off in the results. Based on the phylogenetic tree, the haplotypes of PNG pigs were assorted into major clades (Figure 6.5) with nomenclatures assigned similarly to past studies (Giuffra *et al.* 2000; Larson *et al.* 2005; Fang & Anderson 2006; Tanaka *et al.* 2008; Larson *et al.* 2010). To be consistent, the geographic regions (Table 6.1) used in this study are the same as those referred by Larson *et al.* (2010).

Table 6.1: Geographic regions and areas referred to in the text.

Region	Areas within each region
Northeast Asia	North Korea, South Korea, and northeast Chinese provinces of Liaoning, Jilin, Heilongjiang, and the northern portion of Inner Mongolia.
Oceania	Papua New Guinea and the islands of Sumatra, Java, Borneo, and all the islands to the east extending into the remote Pacific
Indo-Burma Biodiversity Hotspot (IBBH)	Vietnam, Laos, Cambodia, Myanmar, Thailand, and Chinese province of Yunnan Guangdong, and Guangxi Zhuang Nationality Autonomous Region
Central China	Central China (except the southern portion of Yunnan province), Guangxi, and Guangdong provinces, and the northeast provinces of Liaoning, Jilin, Heilongjiang, and the northern portion of Inner Mongolia
South Asia	Bhutan, Nepal, Sri Lanka, India, Bangladesh, Maldives, and Pakistan

6.4 RESULTS

6.4.1 Population Genetics of PNG Pigs

6.4.1.1 Population Structure

Observed allele numbers ranged from 6 (*S0005*, *S0090*) to 24 (*SW2406*) with a mean of 14.19 ± 1.23 (Table 6.S4). Similarly, the effective number of alleles varied from 2.18 (*S0026*) to 11 (*S0068*) with a mean of 5.5 ± 0.50. All loci, except *IGF1*, deviated significantly from Hardy-Weinberg Equilibrium (Table 6.S3). The overall observed and expected heterozygosities (Table 6.S1) varied from 0.34 to 0.86 (0.63 ± 0.03) and 0.54 to 0.91 (0.78 ± 0.02) respectively. The locus *S0068* had the highest heterozygosity while *S0026* had the lowest heterozygosity.

STRUCTURE analyses revealed five inferred populations (Figure 6.2), which correspond well with the sampling localities (Figure 6.3). Pigs from two adjacent districts of Huon Gulf and Bulolo of Morobe province have been inferred in the same group and were referred to as "MHGB" while individuals from Boana village of Nawaeb district and Finschhafen village of Finschhafen district in Morobe province were inferred as "MBF". Surprisingly, pigs from two widely separated districts of Bulolo (Garaina) and Kandep from Morobe and Enga provinces respectively clustered as "MGK". The fourth inferred population consisting only of pigs from the Nawaeb district of Morobe province was referred to as "MN". The final population, which includes pigs from the Tambul district of Western Highland Province (WHP) was referred to as "WHPT". Clearly, Morobe province contains very diverse populations of pigs. Some hybrids were observed in all the inferred populations except in the AC outgroup. There was no evidence of introgression of European genes, as exemplified by the AC outgroup, into any of these inferred indigenous populations.

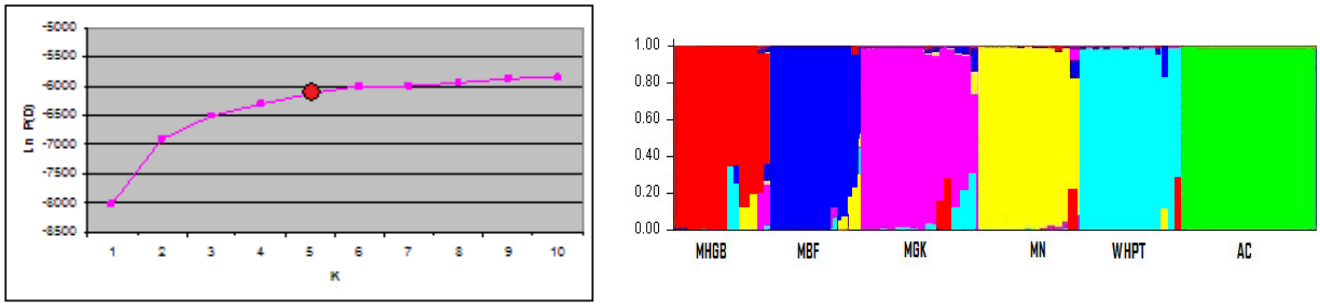

Figure 6.2: Structure output showing the number of inferred populations (*K)* and its corresponding graph for indigenous domestic pigs in PNG.

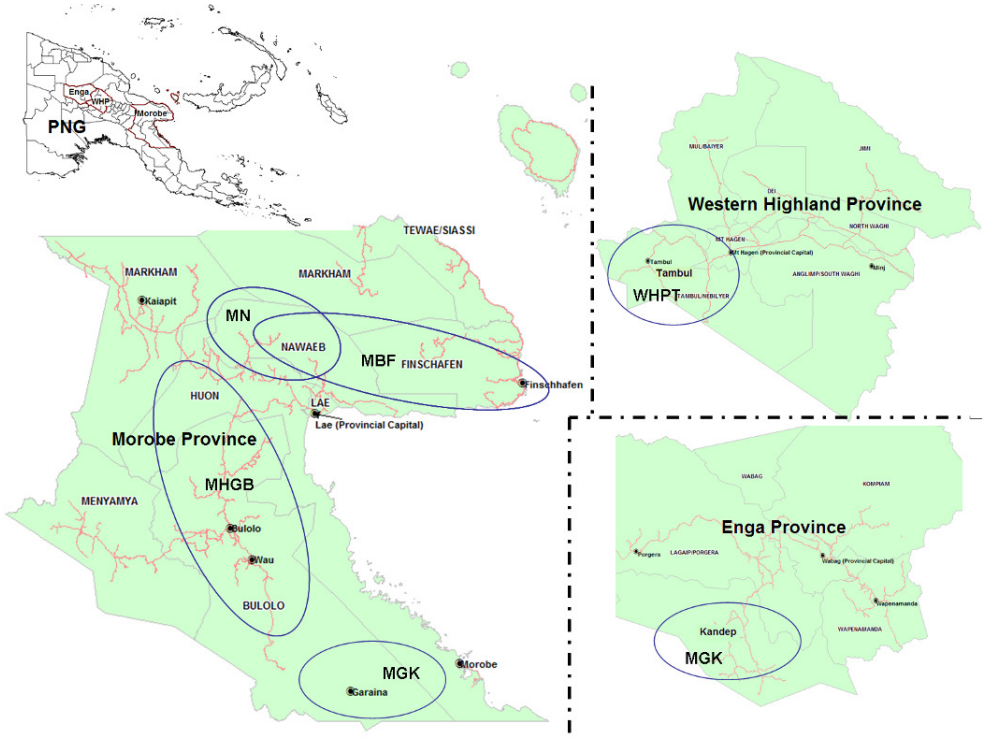

Figure 6.3: Distribution of inferred pig populations in PNG provinces of Enga, Western Highlands and Morobe. Pigs from Nawaeb were inferred in both MN and MBF clusters.

6.4.1.2 Genetic Diversity

The inferred populations; MHGB, MBF, MGK, MN, and WHPT showed a moderate level of genetic diversity with mean expected heterozygosities of 0.74 ± 0.02, 0.71 ± 0.03, 0.70 ± 0.03, 0.73 ± 0.02 and 0.75 ± 0.02 respectively. The heterozygosities of all the inferred populations were greater than for AC (0.67 ± 0.03), despite these being a composite of Large White and Landrace.

When the "rule of thumb" for interpretation of F_{ST} values and genetic differentiation (Wright 1978) was applied, the inferred population of pigs from the Tambul district of WHP was considered to be moderately differentiated (F_{ST} = > 0.05 - 0.15) from the rest of the inferred populations, while there was low genetic differentiation (F_{ST} = < 0.05) between MN and the other three populations (MHGB, MBF, MGK). There was a moderate genetic differentiation between MGK and MHGB (F_{ST} = 0.05). There was also low differentiation between the former MGK and MBF. Overall, there was low to moderate genetic differentiation between the inferred populations in PNG.

The Nei's D_A genetic distances and NJ tree (Figure 6.4) showed the pattern of genetic relatedness among inferred populations and clear segregation from AC. Although the bootstrap values were not particularly strong, there was correlation between the inferred populations and their geographical distribution. Similarly, the PCA (Figure 6.S1) showed the relative position of the inferred populations including the outgroup population, which was found to be the most distant outlier. The inferred populations of PNG were closely clustered with most genetic variation within population (79%) and 21% among inferred populations.

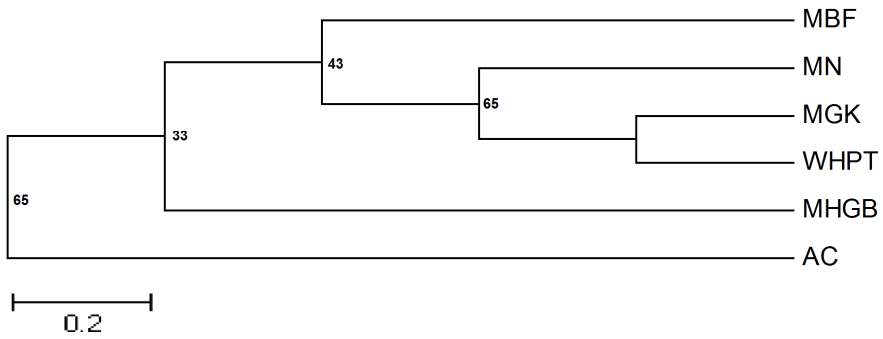

Figure 6.4: Neighbour-Joining dendogram of five inferred pig populations of PNG and the Australian commercial pigs based on Nei's (1972) genetic distance. The bootstrap values (1000 replicates) are given as a percentage at the nodes.

6.4.2 Mitochondrial DNA Haplotypes of PNG pigs

The mtDNA control region sequences from 70 indigenous pigs of PNG comprise twenty four haplotypes (H1-H24). Sixteen haplotypes were represented by a single sequence while the others were found in at least two animals (Table 6.S1). Both phylogenetic (Figure 6.5) and MJN (Figure 6.6) analyses indicated clearly segregated European, Asian, and Pacific clades. The European clade consists of D1 and D4, clearly branching from the rest of the groups with 100% posterior probability support. The Asian group comprises the South Asian clade (D3), the Indo-Burma Biodiversity Hotspot (IBBH-I), Northeast Asia and IBBH-2 (D5), Japanese *S. s. riukiuanus*, and the General Asian clade (GAC). The Pacific clade (D6), which is elsewhere referred to as MC2 (Larson *et al.* 2010), contains sequences from wild and domestic pigs from Oceania (Larson *et al.* 2005; Larson *et al.* 2007), together with wild boar from Vietnam, Yunnan Province, and Laos (Larson *et al.* 2010).

Except for GAC, which had only 65% branch support, all the clades were reliably assigned with good posterior probability support ranging from 91 to 100%. However, focus will be on clades bearing haplotypes from wild and indigenous domestic pigs of PNG. The mtDNA sequences from indigenous pigs from PNG segregated 50% into D6, 37% into GAC, 11% into D1, and a sequence from a single animal belonging to D5 (Table 6.2), thus showing a predominant mitochondrial contribution from pigs from the Pacific region. No sequences belonged to IBBH-1, D3, D4, or *S. s. riukiuanus*. Surprisingly, sequences from five

Australian commercial pigs, which were genotyped as an out-group, clustered within the heterogenous GAC clade.

Table 6.2: Number of PNG indigenous pigs representing each major clade. Five commercial pigs from Australia were also included.

Clade	No. of PNG pigs	%	Australian commercial pigs	%
D1	8	11	0	0
GAC	26	37	5	100
D5	1	1	0	0
D6	35	50	0	0
	70	**100**	**5**	**100**

The D6 clade can be divided into two substructures. D6-1 was present in two individuals from the Tambul district of WH province, one from Nawaeb and five from the Garaina district of Morobe province, and represents five haplotypes (PNG-H8 to PNG-H12). D6-1 clusters with Indonesian wild pigs from Halmahera, Morotai (North Halmahera), Ternate (West of Halmahera), and Flores. Wild boar from Laos and Yunnan province of China are also clustered in this substructure, indicating the potential origins for D6-1. D6-2 consists of 6 haplotypes (PNG-H13 to PNG-H18) from 27 individuals from all three provinces of Morobe, Enga and WHP. Haplotype PNG-H15 was present in 22 different animals from these provinces. D6-2 clusters with previously reported wild pigs of PNG and several Islands of Indonesia (Sumatra, Ceram, Soemba, Timor, Flores, Ternate, and West Bali), French Polynesia, Vanuatu, Cook Islands, and Hawai (USA). The wild boar haplotype reported from Northern Vietnam also clustered in D6-2. Clearly, the D6 mitochondria of PNG pigs have been derived from wild or feral ancestral animals, which have their initial origins from wild boar of Laos, Northern Vietnam, and Yunnan province of China (Larson *et al.* 2010).

D5-1 consists of wild boar from South Korea categorised as W3 by Larson *et al.* (2010). The PNG-H6 haplotype from the Nawaeb district of Morobe province of PNG clusters closely with Australian feral pigs in D5-2. The subclade D5-3 consists of sequences from wild boar from IBBH referred to as W11 and W12 by (Larson *et al.* 2010). The general pattern indicates that mitochondria of some PNG and Australian feral pigs originated from wild boar from IBBH or South Korea. The D3 cluster is clearly a distinct group belonging to Indian wild boar but as expected does not have any influence on PNG pigs.

However, GAC, initially designated as D2 (Larson *et al.* 2005) and later as GC (Larson *et al.* 2010) was the largest clade observed. The GAC clade is polyphyletic, highly heterogeneous, and complex. It was impossible to ascertain a clear substructure and this is consistent with Fang and Andersson (2006). Even after adding all the representative clades from Larson *et al*. (2010), the GAC was still not well supported although it provided some form of clustering patterns within which some haplotypes, particularly those representative haplotypes, taken from Larson *et al.* (2010), were well supported. Irrespective of the branch support, we have divided GAC into four substructures with a broad branching pattern (Figure 6.5).

Three GAC-1 haplotypes from PNG pigs clustered with mitochondrial sequences from indigenous pig breeds from China (Erhualian breed), Bhutan, Thailand, and Vietnam (Figure 6.5; Table 6.S12). One of the Australian commercial pig sequences also clustered in this substructure together with sequences from New Zealand feral pigs (Figure 6.5;). The wild boar sequences, W1 and W2 from South Korea (Larson *et al.* 2010), are also clustered in this substructure.

Three GAC-2 haplotypes of PNG indigenous pigs cluster with indigenous domestic pig sequences from the Jeju native black pig of Korea, native pigs of Myanmar, Thailand, Vietnam and several Chinese breeds (example: Jinhua, Lepinghua, Shanggao, Wei, Jian-Qu-Hai, Qingping, small Mei-Shan, Dong-Lan etc; Figure 6.5; Table 6.S12). Also included in this substructure were one haplotype from an Australian commercial pig, two haplotypes of Australian feral pigs and one from a New Zealand feral pig.

The GAC-3 grouping consists only of sequences from wild boar from China (Hai-Nan), Taiwan, Japan (*S. s. leucomystax*), and Vietnam. The wild boar sequences comprising W1, W5, and W7-W9 (Larson *et al.* 2010) are all included under this substructure. None of the PNG pigs or any of the indigenous domestic pig sequences clustered in this group.

The final GAC substructure, GAC-4, previously referred to as MTSEA (Tanaka *et al.* 2008) or MC3 (Larson *et al.* 2010), contains one haplotype from PNG pigs clustered with both indigenous domestic and wild boar sequences from IBBH, South Korea and China (Jiangxi, Fujian, Guizhou, Yunnan). Interestingly, all the indigenous pigs with GAC

haplotypes were found in Morobe province, close to the coast, but not in highland provinces of Enga and WHP. Similarly, most D1 individuals were found in the coastal Morobe province with a few individuals in WHP suggesting introgression of European mitochondria into indigenous pigs across PNG. Morobe province, which is mainly dominated by haplotypes from the GAC cluster, also harbours one D5 and a large proportion of D6 haplotypes.

Similarly, the MJN analysis also provided an interesting clustering pattern, consistent with past studies (Larson *et al.* 2005; Tanaka *et al.* 2008) despite using only half the length (507bp) of most available sequences (Figure 6.1). It clearly showed shared haplotypes between PNG indigenous domestic and wild pigs in the Pacific region including the local wild or feral population as indicated in the D6 cluster.

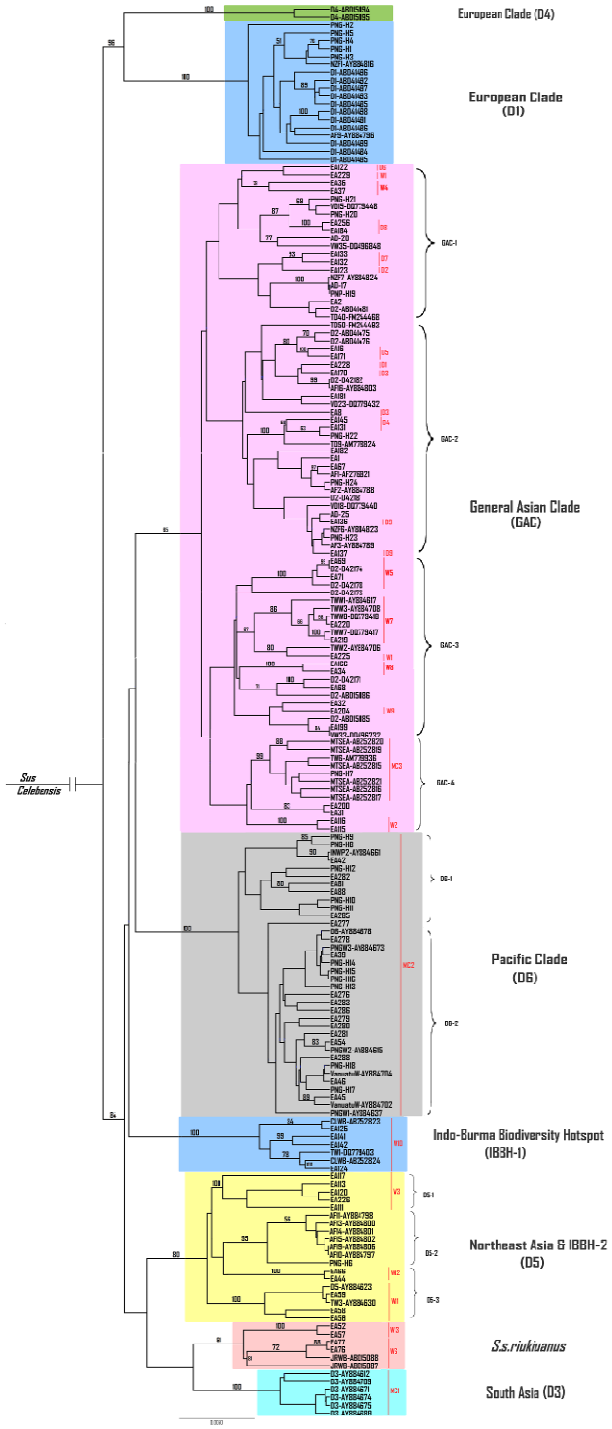

Figure 6.5: Bayesian (MCMC) consensus tree of PNG pig haplotypes (n = 24, 1044bp) analysed with representative haplotypes from past studies (Tanaka *et al.* 2008; Larson *et al.* 2010) and additional sequences (Table 6.S1) retrieved from Genbank. Posterior probability of more than 50% is shown. MCMC simulation used was 100 million generations. *Sus celebensis* was used as an outgroup. Fonts in red, "D" refer to domestic clades and "W" refers to wild clades in Larson *et al.* (2010). **Note:** PNG-H = PNG pig mtDNA haplotype; EA = East Asian haplotypes used in Larson *et al.* (2010); INWP = Indonesian wild pig; TWW = Taiwan wild boar; AF = Australian feral pigs; AD = Australian commercial pig; VSPW = Vanuatu wild pig.

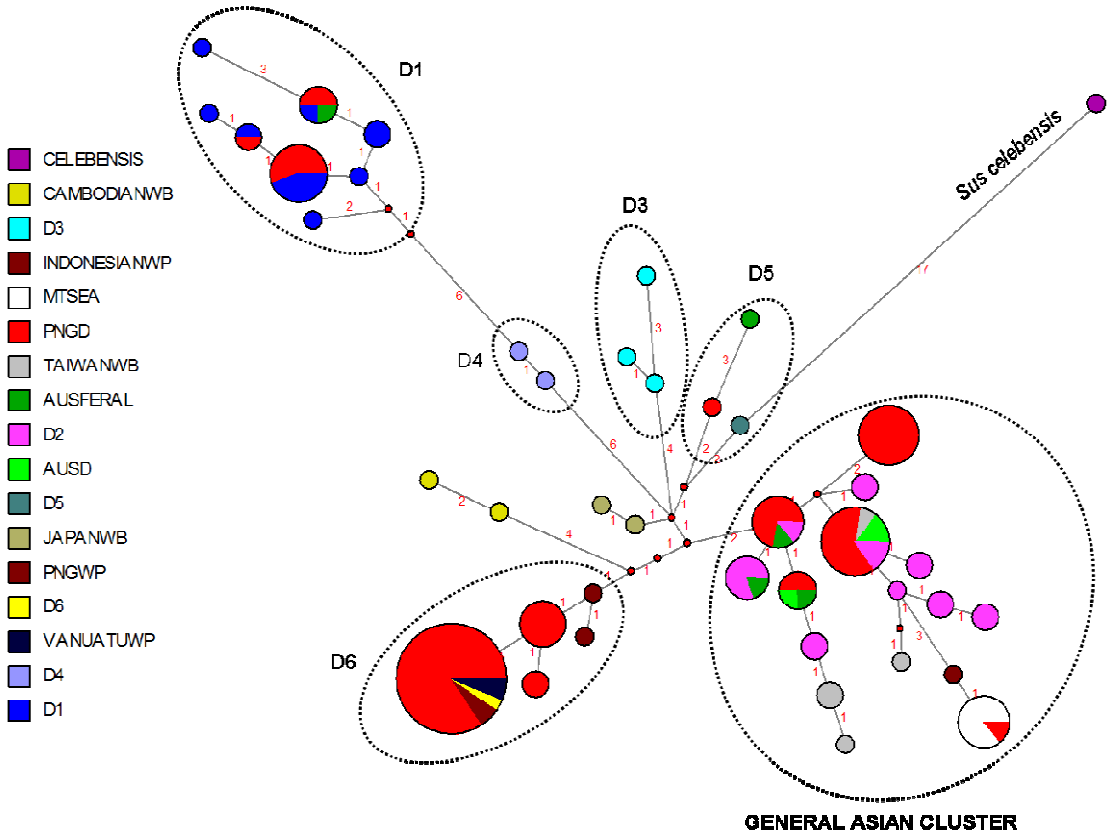

Figure 6.6: Relationship and clustering mtDNA control region (507bp) haplotypes of PNG pigs with major haplogroups, which are all circled except for Japanese (*S. s. riukiuanus)* and Cambodian wild boar. Geographical locations of samples are given in colour and node size is proportional to the frequency of the corresponding haplotypes. Small red dots are median vector (mv) representing hypothetical sequences that were not detected in this study. Numbers on the branch indicate number of mutations. **Notes:** CambodianWB = Haplotypes of Camobdian and Laos wild boar; D3 = South Asian haplotypes belonging to North Indian wild boar; IndonesinWB = Wild pig of Indonesia; MTSEA = Southeast Asian haplotypes; PNGD = Indigenous pigs of PNG; TaiwanWB = Haplotypes of Taiwanese wild boar; Ausferal = haplotypes of Australian feral pigs; General Asian Clade = General haplotypes observed in most part of the world; D5 = Myanmar; JapanWB = Japanese wild boar; PNGWP = wild pig of PNG; D6 = Haplotypes from Oceania; VanuatuWP = Wild pig of Vanuatu Island; D1 & D4 = European haplotypes

6.5 DISCUSSION

6.5.1 Genetic Structure and Diversity of PNG pigs

The overall heterozygote deficiencies indicate genetic structure within PNG pigs. The inferred populations from STRUCTURE analyses are generally consistent with their geographical distribution (Figure 6.3). Pigs from adjacent districts were clustered together as discrete populations as observed in MHGB and MBF. However, it is intriguing to see that pigs from two distant sites of Garaina in the Bulolo district, and Kandep in Enga Province belong to the same inferred population. There are two provinces separating these sites and there is no obvious link in terms of trade or socio-cultural exchanges. The Lutheran Development Services (LDS), a local Non-Government Organization (NGO), maintained breeding herds of crossbred (exotic X native or *vice versa*) and exotic breeds at its research facilities in both highland (Western Highlands) and lowland (Morobe) regions between 1970 and 1980. They provided breeding boars and sows to the remote communities of Garaina and Tambul of Morobe and Western Highland provinces respectively in order to improve village pig production. It is possible that genetically related boars or sows may have been distributed to these communities. Tambul is located adjacent to Kandep district of Enga province and the people of these two districts have close cultural and traditional links including trading of livestock, particularly pigs, which are prized assets in the rural communities. There are settlers or farmers migrating with their pigs from one province to another. For instance, one of the samples in Garaina was taken from a pig of a farmer, who had migrated from Kandep in Enga province. There are clear indications of translocation and human-mediated dispersal of pigs from one province to another from the earliest days of European settlement (Hide 2003).

Microsatellite markers indicate a low to moderate level of genetic diversity amongst inferred groups of PNG pigs, particularly WHPT (Table 6.S5). This is possibly due to the free range system of rearing indigenous village pigs whereby there is no selection pressure but instead allows unselected mating both within indigenous as well as with wild or feral

pigs in nearby woods. Of the 91 pigs surveyed (Ayalew *et al.* 2011) about 11% had been obtained from neighbouring provinces indicating the possibility of considerable genetic exchange within the country and this is a useful step towards minimizing inbreeding. MGK consists of pigs from two distant areas and this inferred population has the lowest within-population genetic diversity when compared with other inferred populations. The relatively small numbers of pigs maintained in the Garaina area may have contributed to higher rates of drift and loss of diversity compared with other populations.

Several exotic breeds of pigs such as Large White, Landrace, Berkshire, Tamworth, Saddleback, and Large Black, have been introduced in PNG from the earliest days of European settlement (Malynicz 1973a, b; Hide 2003) and therefore hybrids of exotic breeds, particularly the Landrace breed, might be expected. Surprisingly, there was no evidence of hybridity of PNG pigs with AC, which would be expected if there were recent crosses involving European breed animals. If there is any influence of European commercial pigs on indigenous pigs, it is most likely to be in the MHGB inferred population which is from the Huon Gulf and Bulolo districts of Morobe province. This is indicated by the relatively low genetic distance between AC and MHGB when the former was compared with rest of the inferred populations. Current data suggest that the pigs from Tambul district in the Western Highlands province appear to be the least affected by introgression from European commercial pigs. Generally, the genetic relationship amongst the inferred pig populations in PNG is geographically consistent (Figure 6.3).

The overall heterozygosity of PNG pigs was compared to other indigenous domestic pigs from various countries (Table 6.S7). The genetic diversity of indigenous pigs of PNG is similar to that of indigenous pigs of Vietnam (Thuy *et al.* 2006) and South Asia (Behl *et al.* 2002; Behl *et al.* 2006; Nidup *et al.* Submitted) but lower than that of Chinese pig breeds (Yang *et al.* 2003) including village pigs (Fang *et al.* 2009). However, their genetic diversity is greater than European pig breeds (Laval *et al.* 2000; SanCristobal *et al.* 2006) and naturalised Brazilian pigs (Sollero *et al.* 2009).

The indigenous domestic pigs of PNG have often been described as similar to the wild pigs, with males often castrated, and females free to mate with wild pigs (Anderson 1972; Hide 2003). Many piglets are still born with ochre, chocolate and cream coloured stripes lengthwise over their bodies suggesting gene flow from wild pigs. Unfortunately, we could not genotype samples from wild or feral pigs but Spencer *et al.* (2006) revealed a considerable degree of genetic diversity (0.77 ± 0.03) within a small sample of 26 feral pigs in Western Province when analysed with 14 microsatellite markers. Interestingly, this is very similar to the values (0.78 ± 0.02; Table 6.S4) observed within the indigenous indigenous domestic pigs in this study. This implies that both indigenous and wild pigs may form a single genetic pool as previously speculated (Groves 1981). Compared to other inferred populations, the genetic diversity of WHPT (0.75 ± 0.02) was closer to that of feral pigs (Spencer *et al.* 2006), suggesting that indigenous domestic pigs in Western Highland province may be more influenced by wild pigs. As for the PNG feral pigs (Spencer *et al.* 2006), our study also suggests that PNG indigenous pigs retain a reasonably high level of genetic diversity. These animals could provide resources for future development of productive but well adapted pigs for PNG agricultural conditions.

6.5.2 Mitochondrial DNA Haplotypes of Indigenous Pigs of PNG

6.5.2.1 Clustering of PNG pigs

The majority of indigenous domestic pig mitochondrial sequences from PNG belong to D6, which is a dominant group in Oceania. The wild boar haplotypes from northern Vietnam, Yunnan province of China and Laos all clustered within the Pacific Clade. All the haplotype sequences from Oceania also clustered in the same Clade (Figure 6.5 & Figure 6.6). This study further supports the fact that the Pacific Clade, which was indigenous to peninsular Southeast Asia, has been dispersed into Island South East Asia by Neolithic farmers of Austroasiatic language speakers along the major Southeast Asian rivers from Yunnan (Higham 2003; Larson *et al.* 2007; Larson *et al.* 2010). This was later followed by later dispersal into Wallacea and remote Oceania during the migration of Lapita and Polynesian people (Larson *et al.* 2007).

Despite their being indigenous to Southeast Asia, we did not observe D6 haplotypes in indigenous domestic pigs in any other part of the world except in PNG. This is thought to be due to replacement of native pigs by introduced pigs from China (Larson *et al.* 2010) during the demographic expansion of several ethnic groups (Van Driem 1998; Pawley 2003; Blench 2005) into the Indo-Burma Biodiversity Hotspot or IBBH (Mittermeier *et al.* 2005). The replacement process did not go beyond IBBH thereby leaving populations of Oceania intact. It has been suggested that people carrying D6 domestic pigs may have left the IBBH before Chinese domestic pigs were introduced (Larson *et al.* 2010). However, our current study also shows that PNG also harbours a large number of Asian sequences belonging to the General Asian Cluster, previously described as D2 haplotypes (Larson *et al.* 2005). This implies that not just pigs with D6 haplotypes but also pigs with D2 haplotypes may have been dispersed by migrating Lapita and Polynesian people. However, it may also be possible that D2 in PNG indigenous domestic pigs is due to recent introduction of European commercial pigs that happened to carry the Asian D2 haplotypes as a result of historical introgression into Europe during the 17th and 18th centuries (Fang & Anderson 2006).

6.5.2.2 Dispersal and Introgression

The phylogenetic and MJN analyses also indicated that the indigenous domestic pigs of PNG belonging to D6 haplotypes were closely related to local wild pigs within PNG and wild pigs from neighbouring Islands of Indonesia and other Pacific Islands including Vanuatu, French Polynesia, and Hawai. This genetic relatedness may have been facilitated by the movement of Lapita populations (ca. 3300–2200 BP) and their pigs within the region (Shaw *et al.* 2009; Shaw *et al.* 2010).

The shared haplotypes between wild and indigenous domestic pigs within PNG (Figure 6.6) possibly indicate that these two populations are mitochondrially a single population forming a common gene pool, an observation consistent with past studies (Groves 1981). This retention of local wild haplotypes in the indigenous domestic pigs may be evidence for introgression rather than an independent centre of indigenous domestication as suggested in previous studies (Larson *et al.* 2005; Larson *et al.* 2010). Firstly, given the current lack of

archaeological evidence for any earlier pig material, it may be that there were no pigs, wild boar, feral, or domestic, in PNG until the arrival of Lapita and Polynesian people into Wallacea and remote Oceania. Secondly, the introduced pigs were initially indigenous domestic pigs while transported to PNG. However, it is also possible that people may have carried captured wild boar with them considering their habits of fruit gathering and hunting. Thirdly, some introduced pigs may have escaped human captivity or been deliberately released into the wild to become feral in the same way as the later European explorers released pigs on uninhabited islands for hunting and food supplies. Taking account of all these cases, the shared haplotypes between wild and indigenous domestic pigs are more likely to be from introgression of genes from wild or feral pigs rather than from a domestication event.

6.5.2.3 General Asian haplotypes

The general or east Asian haplotype (D2) is the most widely dispersed worldwide. It is found particularly among Chinese pig breeds and even East Asian wild boar (Okumura *et al.* 2001; Larson *et al.* 2005; Fang & Anderson 2006; Wu *et al.* 2007). More than one third (37%) of the PNG pigs had sequences belonging to the GAC, although this frequency within PNG pigs is similar to that in other breeds (Fang & Anderson 2006; Tanaka *et al.* 2008). Phylogenetic analysis suggests that D2 haplotypes from PNG pigs are more closely related to those from domestic pigs in Southeast Asia and East Asia (Figure 6.5). However, the route of introduction of D2 haplotypes into PNG pigs cannot be determined. It is possible that pigs with D2 haplotypes may have been introduced along with pigs with D6 haplotypes during the expansion of Lapita and Polynesian culture. It is also possible that D2 haplotypes came via Europe following their introduction into European breeds during the 18th and 19th centuries and the subsequent introduction of these "European" pigs into PNG. Our analyses have shown that mitochondrial sequences from Australian commercial pigs of European origin were ultimately derived from Southeast China and related pigs, since they unambiguously clustered with D2 haplotypes. Fang & Anderson (2006) have made similar observations on Asian mitochondrial sequences segregating in European populations. There is good historical evidence on the use of Asian pigs to improve European pig breeds during the 18th and early 19th centuries (Darwin 1868; Jones 1998).

6.5.2.4 European Haplotypes in PNG pigs

The main purpose of introducing exotic breeds of pigs into developing countries and distributing them to farmers is to crossbreed with indigenous pigs and produce F1 or subsequent generations combining preferred traits (such as growth rate, reproductive performance, and hardiness) from both exotic and indigenous backgrounds. Exotic breeds of pigs have been recorded in New Guinea since at least 1912 (Hide 2003), and most likely were introduced even earlier. By the early 1930's, exotic breeds of pigs were crossed regularly with local pigs (Strathern 1980; Hide 2003). However, despite extensive introduction of exotic breeds of pigs since the earliest days of European influence (Malynicz 1973a, b; Hide 2003), the evidence of introgression of maternal genes from pigs of European origin into indigenous pigs of PNG is relatively slight (Figure 6.5; Figure 6.6). It is estimated that about 11% of the PNG pigs contained European haplotypes (D1). One possible reason for the low incidence of maternal gene introgression could be that the European breed introductions were mainly via the use of exotic boars rather than sows. As expected, there was no evidence of the influence of D4 European haplogroup on PNG pigs.

6.5.2.5 Wild Boar mtDNA Signature in Australian Feral pigs

We observed that the mitochondrial DNAs of feral pigs of Australia have contribution of both Asian and European domestic origin (Figure 6.5; Figure 6.6; Figure 6.S2). Furthermore, previous studies (Gongora *et al.* 2004; Larson *et al.* 2005) have reported a genetic contribution of Asian wild boar to feral pigs in various areas in and around Northern Queensland (Kowanyama, Rutland, West coast of Cape York, Cuddle Springs of Darling river basin). To complement the Bayesian analyses, a Neighbour-Joining (NJ) analysis (Figure 6.S2), of Australian feral pig sequences from previous studies (Gongora *et al.* 2004; Larson *et al.* 2005), and the major porcine haplotypes (Larson *et al.* 2005; Tanaka *et al.* 2008) revealed that Asian wild boar with the D5 haplotype have contributed mitochondria via an unknown pathway to some Australian feral pigs. To date, D5 haplotypes have not been detected in any Asian or European domestic pigs despite the screening of almost 1800 porcine sequences (Larson *et al.* 2010; Nidup *et al.* unpublished-

b). It is possible that a domestic population derived from D5 wild boar has not yet been identified possibly because it is extinct or adequate sampling has not yet been carried out. However, in contrast to previous speculation (Larson *et al.* 2005), the current evidence suggests that the D5 wild boar have not been domesticated, similar to several other wild boar clades whose DNA haplotypes have never been found in modern population of domestic pigs (Larson *et al.* 2010; Nidup *et al.* unpublished-b). One could speculate that wild boar carrying the D5 haplotype have been introduced directly into Australia from Southeast Asia. Clearly more comprehensive sampling in Southeast Asia and neighbouring regions is required to better understand the origin and distribution of the D5 haplotype. However, an explanation for human-mediated dispersal of pigs between peninsular Southeast Asia and Northeast Australia would still appear to be necessary.

The presence of the D5 haplotype within one PNG domestic pig only contributes to the confusion. Did it acquire the D5 haplotype in a similar fasion to Australian feral pigs? Did it come directly from Southeast Asia or indirectly from Australian feral animals? This situation warrants further in-depth investigation.

6.5.2.6 Relationship of PNG pigs with Sus celebensis

It has been suggested that PNG pigs are a result of hybridisation between *Sus scrofa vittatus* and *Sus celebensis* (Groves 1981; Flannery 1995; Quartermain 2002). However, our analyses show that the mitochondrial sequences from PNG pigs clearly belong to *Sus scrofa,* and are very different from *Sus celebensis* sequences (Figure 6.5; Figure 6.6). Our results are consistent and further support Larson *et al.* (2005) who initially reported the lack of affinity of the New Guinea pigs with *Sus celebensis.* It also supports the archaeological evidence (Bulmer 1998) which has contradicted previous claims (Groves 1981; Flannery 1995; Quartermain 2002).

6.6 CONCLUSIONS

Indigenous pigs clearly play very important roles in the livelihood of many ethnic rural communities in PNG. Both microsatellite and mtDNA suggest a reasonably high level of genetic structure and diversity within PNG pigs. These PNG domestic pigs have been influenced mainly by wild pigs from Oceania, followed by Asian and European pigs. The shared haplotypes between wild or feral with domestic pigs suggest introgression of genes from local wild or feral pigs to domestic pigs - and possibly from the latter to the former considering that both of these populations are ancient ferals introduced during the expansion of Lapita and Polynesian culture. The PNG indigenous domestic pigs have also been influenced by D2 haplotypes that may have been introgressed directly by east Asian pigs or via either from introducing general Asian pigs or via European pigs carrying the D2 haplotype. We have also observed the D2 haplotype within Australian commercial pigs of European origin. Additionally, some PNG pigs have D1 haplotypes, which reflects several decades of official promotion of exotic and crossbred pigs into traditional pig populations with the view to improving production from these populations. The observation of D5 haplotypes within some Australian feral pigs and one PNG domestic pig indicate the presence of maternally genuine wild boar, which have not yet contributed to domestication. Our findings, which show reasonably high levels of biodiversity, provide strong foundations on which future research or decision on conservation programme for pig genetic resources in PNG must be based.

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6.8 REFERENCES

Allen J. & O'Connell J.F. (2003) The Long and Short of it: Archaeological Approaches to Determining when Humans First Colonised Australia and New Guinea. *Australian Archaeology* **57**, 5-19.

AMVSU (1946) Report on the animal diseases survey of Papua and the mandated territory of New Guinea. p. 151. Australian Mobile Veterinary Survey Unit (AMVSU).

Anderson J.L. (1972) Livestock. In: *Encyclopaedia of Papua and New Guinea* (ed. by Ryan P), pp. 645-6. Melbourne University Press in association with the University of Papua and New Guinea, Melbourne.

Ayalew W., Danbaro G., Dom M., Amben S., Besari F., Moran C. & Nidup K. (2011) Genetic and cultural significance of indigenous pigs in Papua New Guinea and their phenotypic characteristics. *Animal Genetic Resources* **48**, 37-46.

Bandelt H.J., Forster P. & Rohl A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37-48.

Behl R., Kaul R., Sheoran N., Behl J., Tantia M.S. & Vijh R.K. (2002) Genetic identity of two Indian pig types using microsatellite markers. *Animal Genetics* **33**, 158-9.

Behl R., Sheoran N., Behl J. & Vijh R.K. (2006) Genetic analysis of Ankamali pigs of India using microsatellite markers and their comparison with other domesticated Indian pig types. *Journal of Animal Breeding and Genetics* **123**, 131-5.

Blench R.M. (2005) *Perspectives on the Phylogeny of East Asian Languages*. Curzon, London, UK.

Blust R. (1976) Austronesian culture history: some linguistic inferences and their relations to the archaeological record. *World Archaeology* **8**, 19-43.

Blust R. (2002) The history of faunal terms in Austronesian languages. *Oceanic Linguistics* **41(1)**, 89-139.

Bulmer S. (1966) Pig bone from two archaeological sites in the New Guinea Highlands. *Journal of the Polynesian Society* **75 (4)**, 504-5.

Bulmer S. (1975) Settlement and economy in prehistoric Papua New Guinea: a review of the archaeological evidence. *J. Soc. Ocean* **31**, 7-75.

Bulmer S. (1998) The natural and cultural history of the pig in New Guinea - the archaeological evidence. In: *International Congress on Archaeozoology*, Victoria, British Columbia.

Cowled B.D., Aldenhoven J., Odeh I.O.A., Garrett T., Moran C. & Lapidge S.J. (2008) Feral pig population structuring in the rangelands of eastern Australia: applications for designing adaptive management units. *Conservation Genetics* **9**, 211-24.

Darwin C. (1868) *The variation of animals and plants under domestication*. John Murray, London.

Drummond A.J. & Rambaut A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.

Edgar R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**, 1792 - 7.

Fang M. & Anderson L. (2006) Mitochondrial diversity in European and Chinese pigs is consistent with population expansions that occurred prior to domestication. *Proceedings of the Royal Society* **273**, 1803-10.

Fang M., Hu X., Jin W., Li N. & Wu C. (2009) Genetic uniqueness of Chinese village pig populations inferred from microsatellite markers. *Journal of Animal Science* **87**, 3445-50.

FAO (2007a) Global Plan of Action for Animal Genetic Resources and the Interlaken Declaration. FAO, Rome.

FAO (2007b) *The State of the World's Animal Genetic Resources for Food and Agriculture*. FAO, Rome.

Flannery T.F. (1995) *Mammals of New Guinea*. Cornell University Press, New York, USA.

Forster P., Torroni A., Renfrew C. & Röhl A. (2001) Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. *Molecular Biology Evolution* **18**, 1864-81.

Giuffra E., Kijas J.M., Amarger V., Carlborg O., Jeon J.T. & Andersson L. (2000) The origin of the domestic pig: independent domestication and subsequent introgression. *Genetics* **154**, 1785-91.

Golson J. & Hughes P.J. (1980) The appearance of plant and animal domestication in New Guinea. *J. Soc. Ocean* **36**, 294-303.

Gongora J., Fleming P., Spencer P.B.S., Mason R., Garkavenko O., Meyer J.N., Droegemueller C., Lee J.H. & Moran C. (2004) Phylogenetic relationships of Australian and New Zealand feral pigs assessed by mitochondrial control region sequence and nuclear GPIP genotype. *Molecular Phylogenetics and Evolution* **33**, 339-48.

Gongora J., Morales C., Bernal J.E. & Moran C. (2006) Phylogenetic divisions among Collared peccaries (*Pecari tajacu)* detected using mitochondrial and nuclear sequences. *Molecular Phylogenetics and Evolution* **41**, 1-11.

Groves C.P. (1981) Ancestors for the Pigs: Taxonomy and Phylogeny of the Genus Sus. In: *Technical Bulletin 3*. Department of Prehistory, Research School of Pacific Studies, Australian National University, Canberra.

Hampton J.O., Spencer P.B.S., Alpers D.L., Twigg L.E., Woolnough A.P., Doust J., Higgs T. & Pluske J. (2004) Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. *Journal of Applied Ecology* **41**, 735-43.

Hide R. (2003) *Pig husbandry in New Guinea. A literature review and bibliography*. ACIAR Monograph 108, Australian Centre for International Agricultural Research., Canberra.

Higham C. (2003) *Examining the farming/language dispersal hypothesis*. McDonald Institute for Archaeological Research, Cambridge.

Jenness D. & Ballantyne A. (1920) *The Northern D'Entrecasteaux*. Clarendon Press, Oxford, UK.

Jones G.F. (1998) Genetic aspects of domestication, common breeds and their origin. In: *The Genetics of the Pig* (eds. by Rothschild MF & Ruvinsky A), pp. 17-50. CAB International, Wallingford, Oxon, UK.

Keane T.M., Creevey C.J., Pentony M.M., Naughton T.J. & McInerney J.O. (2006) Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified,. *BMC Evolutionary Biology* **6**, 29.

Kim K.I., J.H L., Li K., Zhang Y.P., Lee S.S., Gongora J. & Moran C. (2002) Phylogenetic relationships of Asian and European pig breeds determined by mitochondrial DNA D-loop sequence polymorphism. *Animal Genetics* **33**, 19-25.

Kimura M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111-20.

Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J. & Higgins D.G. (2007) ClustalW and ClustalX version 2. *Bioinformatics 2007* **23**, 2947-8.

Larson G., Cucchi T., Fujita M., Matisoo-Smith E., Robins J., Anderson A., Rolett B., Spriggs M., Dolman G., Kim T.H., Thuy N.T.D., Randi E., Doherty M., Due R.A., Bollt R., Djubiantono T., Griffin B., Intoh M., Keane E., Kirch P., Li K.T., Morwood M., Pedrina L.M., Piper P.J., Rabett R.J., Shooter P., Van den Bergh G., West E., Wickler S., Yuan J., Cooper A. & Dobney K. (2007) Phylogeny and ancient DNA of Sus provides insights into neolithic expansion in island southeast Asia and Oceania. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 4834-9.

Larson G., Dobney K., Albarella U., Fang M.Y., Matisoo-Smith E., Robins J., Lowden S., Finlayson H., Brand T., Willerslev E., Rowley-Conwy P., Andersson L. & Cooper A. (2005) Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science* **307**, 1618-21.

Larson G., Liu R.R., Zhao X.B., Yuan J., Fuller D., Barton L., Dobney K., Fan Q.P., Gu Z.L., Liu X.H., Luo Y.B., Lv P., Andersson L. & Li N. (2010) Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 7686-91.

Laval G., Iannuccelli N., Legault C., Milan D., Groenen M.A.M., Giuffra E., Andersson L., Nissen P.H., Jargensen C.B., Beeckmann P., Geldermann H., Foulley J.-L., Chevalet C. & Ollivier L. (2000) Genetic diversity of eleven European pig breeds. *Genetic Selection and Evolution* **32**, 187-203.

Lum J.K., McIntyre J.K., Greger D.L., Huffman K.W. & Vilar M.G. (2006) Recent Southeast Asian domestication and Lapita dispersal of sacred male pseudohermaphroditic "tuskers" and hairless pigs of Vanuatu. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 17190-5.

Malynicz G.L. (1973a) The productivity of exotic and indigenous pigs under village conditions: Part 1. *Papua New Guinea Agricultural Journal* **24**, 16-9.

Malynicz G.L. (1973b) The productivity of exotic and indigenous pigs under village conditions: Part 2. *Papua New Guinea Agricultural Journal* **24**, 20-2.

Mittermeier R.A., Gil P.R., Hoffman M., Pilgrim J., Brooks T., Mittermeier C.G., Lamoreux J. & Fonseca G.A.B. (2005) *Hotspots Revisited: Earth's biologically richest and most threatened terrestrial ecoregions*. University of Chicago Press, Chicago, USA.

Nidup K., Joshi D.D., Gongora J. & Moran C. (2010) Farming and Biodiversity of Indigenous Pigs in Nepal. *Biodiversity* **11**, 26-33.

Nidup K., Larson G., Gongora J., Joshi D.D., Silva G.L.L.P. & Moran C. (unpublished) Wild boar mitochondrial phylogeography, introgression, and dispersal in South Asia. *Manuscript under preparation*.

Nidup K., Silva G.L.L.P., Joshi D.D., Pem R., Gongora J. & Moran C. (Submitted) Genetic structure and diversity of South Asian pigs *(Sus scrofa)* as determined by microsatellites. *Animal Genetics*.

Nidup K., Tshering D., Wangdi S., Gyeltshen C., Phuntsho T. & Moran C. (2011) Farming and Biodiversity of Pigs in Bhutan. *Animal Genetic Resources* **48**, 47-61.

O'Connor S., Barham A., Aplin K., Dobney K., Fairbairn A. & Richards M. (2011) The Power of Paradigms: Examining the Evidential Basis for Early to Mid-Holocene Pigs and Pottery in Melanesia. *Journal of Pacific Archaeology* **2**, 1-25.

Okumura N., Kurosawa Y., Kobayashi E., Watanobe T., Ishiguro N., Yasue H. & Mitsuhashi T. (2001) Genetic relationship amongst the major non-coding regions of mitochondrial DNAs in wild boars and several breeds of domesticated pigs. *Animal Genetics* **32**, 139-47.

Pasveer J. (2003) The Djief Hunters: 26,000 years of lowland rainforest exploitation on the Bird's Head of Papua, Indonesia. p. 476. Rijksuniversiteit Groningen, Groningen, Netherlands.

Pawley A. (2003) *Examining the Farming/Language Dispersal Hypothesis*. McDonald Institute for Archaeological Research, Cambridge, UK.

Polzin T. & Daneshmand S.V. (2003) On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters* **31**, 12-20.

Popescu C.P., Boscher J. & Malynicz G.L. (1989) Chromosome R-banding patterns and NOR homologies in the European wild pig and four breeds of domestic pig. *Annales de Génétique,* **32**, 136-40.

Popescu C.P., Lauvergne J.J. & Malynicz G.L. (1982) Le caryotype du porc villageois de Papouasie Nouvelle-Guinée. *Annales de Génétique et de Sélection Animales* **14**, 237-40.

Posada D. & Crandall K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817-8.

Quartermain A.R. (2002) Conservation of domestic animal genetic resources in Papua New Guinea. NARI Technical Bulletin No 4, National Agricultural Research Institute, Lae, Papua New Guinea.

Rajamahendran R., Ravindran V., Goonewardene L.A., Sahaayaruban P. & Rajaguru A.S.B. (1985) The Native Pig of Sri Lanka. *Animal Genetics Resources Information* **4**, 9-13.

Raymond M. & Rousset F. (1995) GENEPOP (Version 1.2): Population genetic software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-9.

SanCristobal M., Chevalet C., Haley C.S., Joosten R., Rattink A.P., Harlizius B., Groenen M.A.M., Amigues Y., Boscher M.Y., Russell G., Law A., Davoli R., Russo V., Desautes C., Alderson L., Fimland E., Bagga M., Delgado J.V., Vega-Pla J.L., Martinez A.M., Ramos M., Glodek P., Meyer J.N., Gandini G.C., Matassino D., Plastow G.S., Siggens K.W., Laval G., Archibald A.L., Milan D., Hammond K. & Cardellino R. (2006) Genetic diversity within and between European pig breeds using microsatellite markers. *Animal Genetics* **37**, 189-98.

Shaw B., Buckley H., Summerhayes G., Anson D., Garling S., Valentin F., Mandui H., Stirling C. & Reid M. (2010) Migration and mobility at the Late Lapita site of Reber-Rakival (SAC), Watom Island using isotope and trace element analysis: a new insight into Lapita interaction in the Bismarck Archipelago. *Journal of Archaeological Science* **37**, 605-13.

Shaw B.J., Summerhayes G.R., Buckley H.R. & Baker J.A. (2009) The use of strontium isotopes as an indicator of migration in human and pig Lapita populations in the Bismarck Archipelago, Papua New Guinea. *Journal of Archaeological Science* **36**, 1079-91.

Sollero B.P., Paiva S.R., Faria D.A., Guimaraes S.E.F., Castro S.T.R., Egito A.A., Albuquerque M.S.M., Piovezan U., Bertani G.R. & Mariante A.D. (2009) Genetic diversity of Brazilian pig breeds evidenced by microsatellite markers. *Livestock Science* **123**, 8-15.

Spencer P.B.S., Hampton J., Lapidge S.J., Mitchell J., Lee J. & J.R. P. (2006) An assessment of the genetic diversity and structure within and among populations of wild pigs (*Sus scrofa*) from Australia and Papua New Guinea. *Journal of Genetics* **85**, 63-6.

Strathern A. (1980) The central and the contingent: bridewealth among the Melpa and the Wiru. In: *The Meaning of Marriage Payments* (ed. by Comaroff J), pp. 49-67. Academic press, London, UK.

Summerhayes G.R., Leavesley M., Fairbairn A., Mandui H., Field J., Ford A. & Fullagar R. (2010) Human Adaptation and Plant Use in Highland New Guinea 49,000 to 44,000 Years Ago. *Science* **330**.

Tamura K., Dudley J., Nei M. & Kumar S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596 - 9.

Tanaka K., Iwaki Y., Takizawa T., Dorji T., Tshering G., Kurosawa Y., Maeda Y., Mannen H., Nomura K., Dang V.B., Chhum-Phith L., Bouahom B., Yamamoto Y., Daing T. & Namikawa T. (2008) Mitochondrial diversity of native pigs in the mainland South and South-east Asian countries and its relationships between local wild boars. *Animal Science Journal* **79**, 417-34.

Thompson J.D., G H. & Gibson T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673-80.

Thuy N.T.D., Melchinger-Wild E., Kuss A.W., Cuong N.V., Bartenschlager H. & Geldermann H. (2006) Comparison of Vietnamese and European pig breeds using microsatellites. *Journal of Animal Science* **84**, 2601-8.

Van Driem G. (1998) *Archaeology and Language II: Archaeological Data and Linguistic Hypotheses*. Routledge, London, UK.

Villesen P. (2007) FaBox: an online toolbox for fasta sequences. *Molecular Ecology Notes* **7**, 965-8.

Weir B.S. & Cockerham C.C. (1984) Estimating F-Statistics for the analysis of population structure. *Evolution* **38**, 1358-70.

Wright S. (1978) *Evolution and the genetics of populations. Variability within and among natural populations.* The University of Chicago Pess, Chicago.

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

Yang S.-L., Wang Z.-G., Liu B., Zhang G.-X., Zhao S.-H., Yu M., Fan B., Li M.-H., Xiong T.-A. & Li K. (2003) Genetic variation and relationships of eighteen Chinese indigenous pig breeds. *Genet Sel Evol* **35**, 657-71.

Yeh F., Yang R. & Boyle T. (1999) PopGene 2 (Version1.31): Microsoft windows-based freeware for population genetics analysis. University of Alberta and Centre for International Forestry Research.

6.9 SUPPLEMENTARY MATERIALS

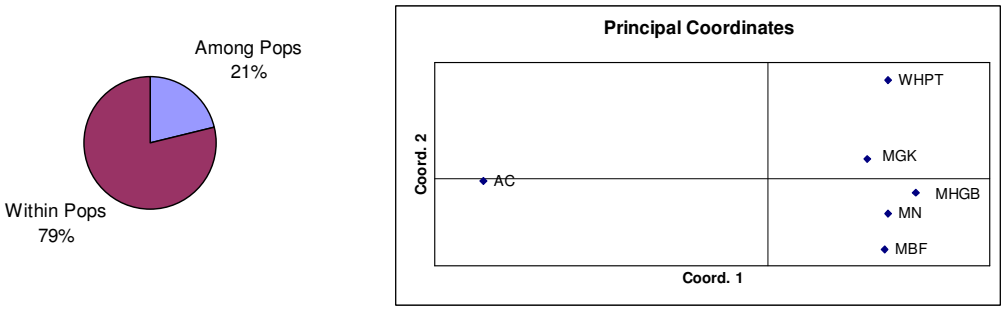

Figure 6.S1: Molecular variance (AMOVA) and Principal Coordinate Analysis (PCA) of the PNG inferred pig populations.

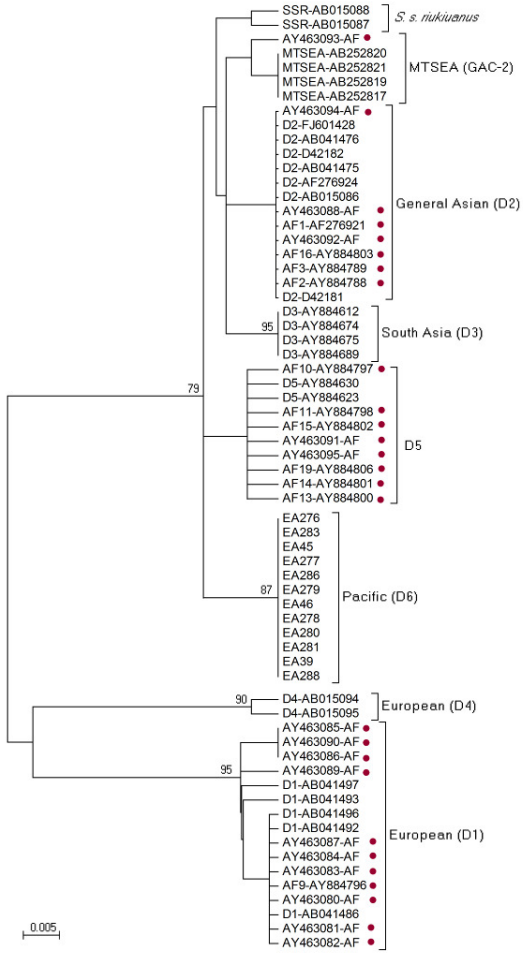

Figure 6.S2: NJ dendogram drawn from control region sequences from Australian feral pigs (Gongora *et al.* 2004; Larson *et al.* 2010) and major representative porcine haplotypes (Table 6.S12; check accession no.) retrieved from Genbank. Bootstrap values (%; 10,000 replicates) greater than 50% are indicated on branches. Genetic distances were computed using the Kimura-2 parameter (Kimura 1980) substitution model and the phylogenetic analysis was conducted in MEGA4 (Tamura *et al.* 2007).

Table 6.S1: Details of samples of indigenous pigs used for microsatellite analysis.

Sl No	Sample Codes	Type of samples	Province	District	Village	Approx. DNA Conc (ng/ul)
1	PNG_1	Blood	WHP	Tambul	Tambul	150
2	PNG_2	Hair	WHP	Tambul	Tambul	15
3	PNG_5	Hair	WHP	Tambul	Tambul	10
4	PNG_12	Blood	WHP	Tambul	Tambul	200
5	PNG_13	Blood	WHP	Tambul	Tambul	180
6	PNG_14	Blood	WHP	Tambul	Tambul	150
7	PNG_15	Blood	WHP	Tambul	Tambul	200
8	PNG_16	Blood	WHP	Tambul	Tambul	120
9	PNG_17	Hair	WHP	Tambul	Tambul	20
10	PNG_18	Blood	WHP	Tambul	Tambul	130
11	PNG_20	Hair	WHP	Tambul	Tambul	100
12	PNG_21	Hair	WHP	Tambul	Tambul	15
13	PNG_22	Hair	WHP	Tambul	Tambul	15
14	PNG_23	Hair	WHP	Tambul	Tambul	20
15	PNG_25	Hair	WHP	Tambul	Tambul	100
16	PNG_26	Hair	WHP	Tambul	Tambul	10
17	PNG_B1	Blood	Morobe	Nawaeb	Talec	150
18	PNG_B2		Morobe	Nawaeb	Talec	30
19	PNG_B3	Blood	Morobe	Nawaeb	Tikaleng	10
20	PNG_B4	Blood	Morobe	Nawaeb	Tikaleng	30
21	PNG_B5	Blood	Morobe	Nawaeb	Tikaleng	150
22	PNG_B6	Blood	Morobe	Nawaeb	Bukam	150
23	PNG_B7	Blood	Morobe	Nawaeb	Belem	15
24	PNG_F1	Blood	Morobe	Finschaffen	Finschaffen	40
25	PNG_F2	Blood	Morobe	Finschaffen	Finschaffen	140
26	PNG_F3	Blood	Morobe	Finschaffen	Finschaffen	130
27	PNG_F4	Blood	Morobe	Finschaffen	Finschaffen	40
28	PNG_F5	Blood	Morobe	Finschaffen	Finschaffen	20
29	PNG_G1	Blood	Morobe	Bulolo	Garaina	20
30	PNG_G10	Blood	Morobe	Bulolo	Garaina	10
31	PNG_G12	Blood	Morobe	Bulolo	Garaina	10
32	PNG_G17	Blood	Morobe	Huon Gulf	Pille	100
33	PNG_G18	Hair	Morobe	Huon Gulf	Pille	10
34	PNG_G2	Blood	Morobe	Bulolo	Garaina	15
35	PNG_G3	Blood	Morobe	Bulolo	Garaina	40
36	PNG_G4	Blood	Morobe	Bulolo	Garaina	40
37	PNG_G5	Blood	Morobe	Bulolo	Garaina	30
38	PNG_G6	Blood	Morobe	Bulolo	Garaina	10
39	PNG_G7	Blood	Morobe	Bulolo	Garaina	40
40	PNG_G8	Blood	Morobe	Bulolo	Garaina	40
41	PNG_G9	Blood	Morobe	Bulolo	Garaina	15
42	PNG_I	Blood	Morobe	Nawaeb	Boana	10
43	PNG_III	Blood	Morobe	Nawaeb	Boana	80
44	PNG_IV	Blood	Morobe	Nawaeb	Boana	15
45	PNG_K1	Blood	Enga	Kandep	Kandep	200

Sl No	Sample Codes	Type of samples	Province	District	Village	Approx. DNA Conc (ng/ul)
46	PNG_K2	Blood	Enga	Kandep	Kandep	150
47	PNG_K3	Hair	Enga	Kandep	Kandep	140
48	PNG_K4	Hair	Enga	Kandep	Kandep	20
49	PNG_MU3	Blood	Morobe	Bulolo	Katne	15
50	PNG_MU5	Blood	Marobe	Bulolo	Zenag	15
51	PNG_MU6	Blood	Marobe	Bulolo	Zenag	20
52	PNG_MU7	Blood	Marobe	Bulolo	Zenag	20
53	PNG_MU8	Blood	Marobe	Bulolo	Latep	15
54	PNG_MU9	Blood	Marobe	Bulolo	Zimban	10
55	PNG_N11	Blood	Morobe	Nawaeb	Gobari	10
56	PNG_N12	Blood	Morobe	Nawaeb	Pom	30
57	PNG_N13	Blood	Morobe	Nawaeb	Pom	90
58	PNG_N14	Blood	Morobe	Nawaeb	Pom	100
59	PNG_N15	Blood	Morobe	Nawaeb	Hobu	120
60	PNG_N16	Hair	Morobe	Nawaeb	Hobu	20
61	PNG_N9	Hair	Morobe	Nawaeb	Gobari	15
62	PNG_VI	Hair	Morobe	Nawaeb	Boana	15
63	PNG_VII	Blood	Morobe	Nawaeb	Boana	130
64	PNG_VIII	Blood	Morobe	Nawaeb	Boana	50
65	PNG_XIV	Blood	Morobe	Nawaeb	Boana	100
66	PNG_Y24	Blood	Morobe	Huon Gulf	Yalu	130
67	PNG_Y25	Hair	Morobe	Huon Gulf	Yalu	20
68	PNG_Y28	Blood	Morobe	Huon Gulf	Munum	30
69	PNG_Y29	Blood	Morobe	Huon Gulf	Munum	15
70	PNG_Y30	Blood	Morobe	Nawaeb	Munkip	15
71	PNG_Y31	Hair	Morobe	Nawaeb	Munkip	20
72	PNG_Y32	Blood	Morobe	Nawaeb	Munkip	30
73	PNG_Y33	Blood	Morobe	Nawaeb	Munkip	25

Table 6.S2: Panel of FAO/ISAG recommended microsatellite markers used in this study.

Sl No	Name	Chromosome	Primer Sequence (5' - 3')	Dye	Annealing Tempt.	Allele Range
1	*SW857*	14	D-TGAGAGGTCAGTTACAGAAGACC GATCCTCCTCCAAATCCCAT	FAM	55°C	138 – 156
2	*SW122*	6	D-CAAAAAAGGCAAAAGATTGACA TTGTCTTTTTATTTTGCTTTTGG	VIC	55°C	106 - 128
3	*IGF1*	5	D- GCTTGGATGGACCATGTTG CATATTTTTCTGCATAACTTGAACCT	FAM	55°C	193 - 209
4	*S0143*	12	D-ACTCACAGCTTGTCCTGGGTGT CAGTCAGCAGGCTGACAAAAAC	NED	55°C	150 - 167
5	*SW240*	2	D- AGAAATTAGTGCCTCAAATTGG AAACCATTAAGTCCCTAGCAAA	NED	55°C	92 - 124
6	*S0005*	5	D- TCCTTCCCTCCTGGTAACTA GCACTTCCTGATTCTGGGTA	FAM	55°C	203 – 267
7	*SW951*	10	D- TTTCACAACTCTGGCACCAG GATCGTGCCCAAATGGAC	PET	55°C	120 plus
8	*SW2406*	6	D- AATGTCACCTTTAAGACGTGGG AATGCGAAACTCCTGAATTAGC	PET	55°C	222 - 262
8	*SW936*	15	D- TCTGGAGCTAGCATAAGTGCC GTGCAAGTACACATGCAGGG	FAM	55°C	90 - 116
10	*S0226*	2	D- GCACTTTTAACTTTCATGATACTCC GGTTAAACTTTTNCCCCAATACA	VIC	55°C	180 - 210
11	*SW72*	3	D-ATCAGAACAGTGCGCCGT TTTGAAAATGGGGTGTTTCC	VIC	55°C	97 - 114
12	*SW632*	7	D- TGGGTTGAAAGATTTCCCAA GGAGTCAGTACTTTGGCTTGA	VIC	55°C	148 – 178
13	*S0002*	3	D- GAAGCCAAAGAGACAACTGC GTTCTTTACCCACTGAGCCA	PET	60°C	186 – 216
14	*S0155*	1	D- TGTTCTCTGTTTCTCCTCTGTTTG AAAGTGGAAAGAGTCAATGGCTAT	FAM	55°C	142 - 162
15	*S0090*	12	D- CCAAGACTGCCTTGTAGGTGAATA GCTATCAAGTATTGTACCATTAGG	VIC	55°C	227 - 249
16	*S0228*	6	D-GGCATAGGCTGGCAGCAACA AGCCCACCTCATCTTATCTACACT	NED	55°C	220 - 246
17	*S0068*	13	D- CCTTCAACCTTTGAGCAAGAAC AGTGGTCTCTCTCCCTCTTGCT	NED	55°C	211 - 262
18	*S0026*	16	D- AACCTTCCCTTCCCAATCAC CACAGACTGCTTTTTACTCC	FAM	55°C	87 - 105
19	*SW911*	9	D- CTCAGTTCTTTGGGACTGAACC CATCTGTGGAAAAAAAAAGCC	NED	60°C	149 - 173
20	*S0355*	15	D- TCTGGCTCCTACACTCCTTCTTGATG TTGGGTGGGTGCTGAAAAATAGGA	VIC	50°C	244 - 271
21	*SW2008*	11	D- CAGGCCAGAGTAGCGTGC CAGTCCTCCCAAAAATAACATG	NED	55°C	95 - 108

Table 6.S3: Summary of Chi-Square Tests for Hardy-Weinberg Equilibrium in PNG pigs (n = 67).

Locus	DF	ChiSq	Probability	Significance
IGF1	55	66.050	0.146	ns
S0002	120	298.877	0.000	***
S0005	253	321.437	0.002	**
S0026	15	117.239	0.000	***
S0068	190	377.105	0.000	***
S0090	15	27.279	0.027	*
S0143	55	117.781	0.000	***
S0155	45	115.381	0.000	***
S0226	171	345.183	0.000	***
S0228	120	184.026	0.000	***
S0355	190	549.646	0.000	***
SW72	36	222.657	0.000	***
SW122	91	444.065	0.000	***
SW240	171	306.087	0.000	***
SW632	91	259.611	0.000	***
SW857	21	85.098	0.000	***
SW911	28	146.312	0.000	***
SW936	105	263.886	0.000	***
SW951	45	235.343	0.000	***
SW2008	190	497.330	0.000	***
SW2406	276	787.415	0.000	***

Key: ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

Table 6.S4: Overall heterozygosity in PNG pigs (n = 67).

Locus	N	Na	Ne	Ho	He
IGF1	67	11.00	6.31	0.78	0.84
S0002	66	16.00	5.33	0.53	0.81
S0005	67	23.00	6.05	0.81	0.83
S0026	67	6.00	2.18	0.34	0.54
S0068	66	20.00	11.00	0.86	0.91
S0090	65	6.00	4.48	0.66	0.78
S0143	66	11.00	4.04	0.62	0.75
S0155	67	10.00	4.89	0.70	0.80
S0226	67	19.00	7.64	0.75	0.87
S0228	67	16.00	6.44	0.79	0.84
S0355	66	20.00	6.71	0.62	0.85
SW72	67	9.00	3.49	0.49	0.71
SW122	67	14.00	3.75	0.51	0.73
SW240	67	19.00	9.86	0.78	0.90
SW632	67	14.00	5.38	0.58	0.81
SW857	67	7.00	2.26	0.49	0.56
SW911	67	8.00	3.10	0.60	0.68
SW936	67	15.00	5.86	0.73	0.83

Locus	N	Na	Ne	Ho	He
SW951	67	10.00	3.47	0.51	0.71
SW2008	67	20.00	7.46	0.55	0.87
SW2406	67	24.00	6.77	0.45	0.85
Mean	66.71	14.19	5.55	0.63	0.78
SE	0.12	1.23	0.50	0.03	0.02

Key: Na = No. of Different Alleles; Ne = Effective No. of Alleles; Ho = Observed Heterozygosity; He = Expected Heterozygosity; Mean He = Average He across the populations; Mean Ho = Average Ho across the populations

Table 6.S5: Mean heterozygosity over loci for each inferred pig population in PNG and Outgroup animals (AC).

Pop	N	Na	Ne	Ho	He
MHGB	13	6.52 ± 0.41	4.28 ± 0.28	0.53 ± 0.04	0.74 ± 0.02
MBF	12	6.24 ± 0.48	4.16 ± 0.35	0.56 ± 0.05	0.71 ± 0.03
MGK	15	6.52 ± 0.49	3.91 ± 0.33	0.67 ± 0.04	0.70 ± 0.03
MN	14	6.76 ± 0.45	4.18 ± 0.29	0.61 ± 0.05	0.73 ± 0.02
WHPT	13	7.81 ± 0.62	4.77 ± 0.47	0.76 ± 0.04	0.75 ± 0.02
Mean	13	6.77 ± 0.49	4.26 ± 0.34	0.63 ± 0.04	0.73 ± 0.02
AC	15.00 ± 0.00	5.90 ± 0.51	3.54 ± 0.32	0.65 ± 0.03	0.67 ± 0.03

Table 6.S6: Matrix of Nei's (1972) genetic distance (above diagonal) and pairwise population differentiation using estimates of FST (Weir and Cockerham 1984; below diagonal) between the pig populations sampled in this study.

Pop	MHGB	MBF	MGK	MN	WHPT	AC
MHGB	****	0.38	0.32	0.31	0.36	2.46
MBF	0.06	****	0.26	0.23	0.48	2.59
MGK	0.05	0.04	****	0.19	0.30	2.86
MN	0.04	0.04	0.03	****	0.39	2.77
WHPT	0.05	0.06	0.05	0.05	****	3.19
AC	0.16	0.18	0.18	0.17	0.17	****

Table 6.S7: Summary of expected heterozygosity and effective number of alleles used as estimators of genetic diversity in various studies. Values from wild boar were omitted.

Country	No. of breeds/types/ population detected	Total Pigs	Average He	Average Ne	Research Studies
PNG pigs	5 (indigenous)		0.73 ± 0.02	4.26 ± 0.34	This Study
Bhutan	4 (indigenous)	196	0.78 ± 0.02	5.43 ± 0.50	Nidup *et al.* (2009; submitted)
Nepal	2 (indigenous)	45	0.75 ± 0.02	4.58 ± 0.38	
SL	1 (village pigs)	26	0.77 ± 0.02	4.85 ± 0.33	
India (North)	2 (native)	50	0.80	5.17	Behl *et al.* (2002)
India (South)	1 (native)	26	0.83	5.34	Behl *et al.* (2006)
China	4 (indigenous)	61	0.72 ± 0.10	4.07 ± 1.46	Li *et al.* (2000)
China	7 (indigenous)	380	0.59	2.82	Fan *et al.* (2002)
China Tibet	1 (indigenous)	31	0.75	4.98	Fan *et al.* (2003)
China	3 (indigenous)	90	0.65	3.8	
China Tibet	1 (indigenous)	60	0.87	8.00	Yang *et al.* (2003)
China	17 (indigenous)	967	0.84	7.41	
China	10 (village pigs)	817	0.83	5.79	Fang *et al.* (2009)
China	10 (indigenous)	379	0.68	4.40	Li *et al.* (2004)
Vietnam	5 (indigenous)	152	0.75	4.54	Thuy *et al.* (2006)
European	10 (commercial + native)	471	0.53	2.19	Laval *et al.* (2000)
European	58 (commercial + native)	2737	0.56		SanCristobal *et al.* (2006)

Note: SE values for most studies were not available

Table 6.S8: Sampling areas and number of mtDNA control region sequences belonging to each halplogroup and haplogroup of the PNG indigenous pigs.

Haplotype Labelled As	No. of Sequences	Sample ID	Haplogroup	Genbank Access #	Village	District	Province	Breed
PNG-H1	1	PNG-18	D1	HQ318518	Tambul	Tambul	WHP	Indigenous
PNG-H2	1	PNG-Y25	D1	HQ318519	Yalu	Huon Gulf	Morobe	Indigenous
PNG-H3	1	PNG-G5	D1	HQ318520	Garaina	Bulolo	Morobe	Indigenous
PNG-H4	1	PNG-01	D1	HQ318521	Tambul	Tambul	WHP	Indigenous
PNG-H5	4	PNG-MU8	D1	HQ318522	Latep	Bulolo	Marobe	Indigenous
		PNG-MU7	D1	HQ318523	Zenag	Bulolo	Marobe	Indigenous
		PNG-MU6	D1	HQ318524	Zenag	Bulolo	Marobe	Indigenous
		PNG-IV	D1	HQ318525	Boana	Boana	Morobe	Indigenous
PNG-H6	1	PNG-B1	D5	HQ318526	Talec	Nawaeb	Morobe	Indigenous
PNG-H7	1	PNG-B5	GAC	HQ318527	Tikaleng	Nawaeb	Morobe	Indigenous
PNG-H8	1	PNG-2	D6	HQ318528	Tambul	Tambul	WHP	Indigenous
PNG-H9	1	PNG-21	D6	HQ318529	Tambul	Tambul	WHP	Indigenous
PNG-H10	1	PNG-G9	D6	HQ318530	Garaina	Bulolo	Morobe	Indigenous
PNG-H11	1	PNG-G4	D6	HQ318531	Garaina	Bulolo	Morobe	Indigenous
PNG-H12	4	PNG-N11	D6	HQ318532	Gobari	Nawaeb	Morobe	Indigenous
		PNG-G2	D6	HQ318533	Garaina	Bulolo	Morobe	Indigenous
		PNG-G10	D6	HQ318534	Garaina	Bulolo	Morobe	Indigenous
		PNG-G1	D6	HQ318535	Garaina	Bulolo	Morobe	Indigenous
PNG-H13	1	PNG-G17	D6	HQ318536	Pille	Huon Gulf	Morobe	Indigenous
PNG-H14	1	PNG-16	D6	HQ318537	Tambul	Tambul	WHP	Indigenous
PNG-H15	22	PNG-Y29	D6	HQ318538	Munum	Huon Gulf	Morobe	Indigenous
		PNG-Y24	D6	HQ318539	Yalu	Huon Gulf	Morobe	Indigenous
		PNG-XIV	D6	HQ318540	Boana	Nawaeb	Morobe	Indigenous
		PNG-VIII	D6	HQ318541	Boana	Nawaeb	Morobe	Indigenous
		PNG-VII	D6	HQ318542	Boana	Nawaeb	Morobe	Indigenous
		PNG-N15	D6	HQ318543	Hobu	Nawaeb	Morobe	Indigenous
		PNG-MU9	D6	HQ318544	Zimban	Bulolo	Marobe	Indigenous
		PNG-MU5	D6	HQ318545	Zenag	Bulolo	Marobe	Indigenous
		PNG-MU3	D6	HQ318546	Katne	Bulolo	Marobe	Indigenous
		PNG-K4	D6	HQ318547	Kandep	Kandep	Enga	Indigenous

Haplotype Labelled As	No. of Sequences	Sample ID	Haplogroup	Genbank Access #	Village	District	Province	Breed
		PNG-K3	D6	HQ318548	Kandep	Kandep	Enga	Indigenous
		PNG-K2	D6	HQ318549	Kandep	Kandep	Enga	Indigenous
		PNG-K1	D6	HQ318550	Kandep	Kandep	Enga	Indigenous
		PNG-B4	D6	HQ318551	Tikaleng	Nawaeb	Morobe	Indigenous
		PNG-5	D6	HQ318552	Tambul	Tambul	WHP	Indigenous
		PNG-4	D6	HQ318553	Boana	Nawaeb	Morobe	Indigenous
		PNG-25	D6	HQ318555	Tambul	Tambul	WHP	Indigenous
		PNG-23	D6	HQ318556	Tambul	Tambul	WHP	Indigenous
		PNG-22	D6	HQ318557	Tambul	Tambul	WHP	Indigenous
		PNG-20	D6	HQ318558	Tambul	Tambul	WHP	Indigenous
		PNG-17	D6	HQ318559	Tambul	Tambul	WHP	Indigenous
		PNG-13	D6	HQ318560	Tambul	Tambul	WHP	Indigenous
PNG-H16	1	PNG-26	D6	HQ318554	Tambul	Tambul	WHP	Indigenous
PNG-H17	1	PNG-Y28	D6	HQ318561	Munum	Huon Gulf	Morobe	Indigenous
PNG-H18	1	PNG-15	D6	HQ318562	Tambul	Tambul	WHP	Indigenous
PNG-H19	2	PNP-G7	GAC	HQ318563	Garaina	Bulolo	Morobe	Indigenous
		PNG-N14	GAC	HQ318564	Pom	Nawaeb	Morobe	Indigenous
PNG-H20	1	PNG-N13	GAC	HQ318565	Pom	Nawaeb	Morobe	Indigenous
PNG-H21	5	PNG-N12	GAC	HQ318566	Pom	Nawaeb	Morobe	Indigenous
		PNG-F3	GAC	HQ318567	Finschaffen	Finschaffen	Morobe	Indigenous
		PNG-F2	GAC	HQ318568	Finschaffen	Finschaffen	Morobe	Indigenous
		PNG-F1	GAC	HQ318569	Finschaffen	Finschaffen	Morobe	Indigenous
		PNG-B6	GAC	HQ318570	Bukam	Nawaeb	Morobe	Indigenous
PNG-H22	10	PNG-Y31	GAC	HQ318571	Munkip	Nawaeb	Morobe	Indigenous
		PNG-VI	GAC	HQ318572	Boana	Boana	Morobe	Indigenous
		PNG-N9	GAC	HQ318573	Gobari	Nawaeb	Morobe	Indigenous
		PNG-N16	GAC	HQ318574	Hobu	Nawaeb	Morobe	Indigenous
		PNG-I	GAC	HQ318575	Boana	Nawaeb	Morobe	Indigenous
		PNG-G8	GAC	HQ318576	Garaina	Bulolo	Morobe	Indigenous
		PNG-G6	GAC	HQ318577	Garaina	Bulolo	Morobe	Indigenous
		PNG-G3	GAC	HQ318578	Garaina	Bulolo	Morobe	Indigenous
		PNG-B3	GAC	HQ318579	Tikaleng	Nawaeb	Morobe	Indigenous

Haplotype Labelled As	No. of Sequences	Sample ID	Haplogroup	Genbank Access #	Village	District	Province	Breed
		PNG-B2	GAC	HQ318580	Talec	Nawaeb	Morobe	Indigenous
PNG-H23	2	PNG-F4	GAC	HQ318581	Finschaffen	Finschaffen	Morobe	Indigenous
		PNG-F5	GAC	HQ318582	Finschaffen	Finschaffen	Morobe	Indigenous
PNG-H24	5	PNG-Y33	GAC	HQ318583	Munkip	Nawaeb	Morobe	Indigenous
		PNG-Y32	GAC	HQ318584	Munkip	Nawaeb	Morobe	Indigenous
		PNG-Y30	GAC	HQ318585	Munkip	Nawaeb	Morobe	Indigenous
		PNG-G18	GAC	HQ318586	Pille	Huon Gulf	Morobe	Indigenous
		PNG-B7	GAC	HQ318587	Bulem	Nawaeb	Morobe	Indigenous
AD-20	2	Aus-OG20	GAC	HQ318588	Australia	NSW, Corowa	QAF Meat Industries	Commercial
		Aus-OG1	GAC	HQ318589	Australia	NSW, Corowa	QAF Meat Industries	Commercial
AD25	2	Aus-OG25	GAC	HQ318590	Australia	NSW, Corowa	QAF Meat Industries	Commercial
		Aus-OG15	GAC	HQ318591	Australia	NSW, Corowa	QAF Meat Industries	Commercial
AD-17	1	Aus-OG17	GAC	HQ318592	Australia	NSW, Corowa	QAF Meat Industries	Commercial

Table 6.S9: Representative porcine mtDNA control region haplotypes used by Tanaka *et al.* (2008) retrieved from Genbank.

Clade	Genbank Accession No.	No. of Sequences
D1	AB041484; AB041495; AB041485; AB041497; AB041493; AB041492; AB041491; AB041498; AB041499; AB041486; AB041496	11
D4	AB015094; AB015095	2
General Asian Cluster (GC)	AB015086; D42171; AB041472; AB015084; AB041469**;** AB041467; AB041471; AB015085; D42173; D42174; D42178; D42181; D42182; AB041475; AB041476; AB041481; AB252815; AB252816; AB252820; AB252821; AB252819; AB252818	22
D3	AY884709; AY884612; AY884674; AY884689; AY884675; AY884671	6
D5	AY884623	1
D6	AY884678	1
Ryukyu wild boar (*S.s. riukiuanus*)	AB015087; AB015088	2
Cambodia-Laos Wild Boar	AB252823; AB252824	2
	Total	**47**

Table 6.S10: Representative haplotypes of each clade (D1-D9; W1-W12; MC1-MC3) illustrated in Larson *et al.* (2010) retrieved from Genbank and used in this study. This Table must be used in conjunction with Table 6.S12, which provides complete detail for all the haplotypes including Genbank accession numbers.

Clade	Haplotype ID	No. of Sequences
General Clade	EA1; EA2; EA31; EA32; EA199; EA200; EA257; EA258; EA181; EA182	10
Domestic		
D1	EA228	2
D2	EA123	1
D3	EA8; EA170	2
D4	EA131; EA145	2
D5	EA16; EA171	2
D6	EA122	1
D7	EA132; EA133	2
D8	EA184; EA124	2
D9	EA136; EA137	2
Wild		
W1	EA229; EA225	2
W2	EA115; EA116	2
W3	EA226; EA120; EA111; EA113; EA117	5
W4	EA36; EA37	2
W5	EA69; EA71	2
W6	EA76; EA77	2
W7	EA219; EA220	2
W8	EA34; EA166	2
W9	EA204; EA256	2
W10	EA141; EA142; EA124; EA125	4
W11	EA56; EA59	2
W12	EA44; EA66	2
W13	EA52; EA57	2

Clade	Haplotype ID	No. of Sequences
Mixed Clade		
MC1	EA40; EA38; EA40	3
MC2	EA39; EA42; EA45; EA46; EA54; EA81; EA88; EA278; EA279; EA276; EA277; EA280; EA281; EA282; EA283; EA284; EA285; EA286; EA288	19
MC3	EA100;EA127; EA128; EA129; EA202; EA238	6
	Total	**85**

Table 6.S11: Additional haplotypes from Southeast Asia and Pacific region retrieved and used in the analyses. References for each accession number may be checked in Genbank.

Country	Breed/species	Genbank Accession No.	No. of Sequences
Papua New Guinea	Wild pig	AY884637; AY884615; AY884673	3
Indonesia	Wild pig	AY884661; AY884688	2
Vanuatu	Wild pig	AY884702;AY884704	2
Cook Islands	Domestic	AF182446	1
Australia	Feral	AF276921; AY884788; AY884789; AY884796; AY884797; AY884798; AY884800; AY884801; AY884802; AY884803;AY884806; AY463080, AY463081, AY463082; AY463083, AY463084, AY463085; AY463086, AY463087, AY463088; AY463089, AY463090, AY463091; AY463092, AY463093, AY463094; AY463095	27
New Zealand	Feral	AY884816; AY884823; AY884824	3
Thailand	Wild boar	Q779403; AY884630; AM779936	3
	Domestic	AM778824; FM244468; FM244493	3
Vietnam	Wild	DQ496732; DQ496848	2
	Domestic	DQ779448; DQ779440; DQ779432	3
Taiwan	Wild boar	AY884617; AY884706; AY884708; DQ779417; DQ779418	5
		Total	**54**

Table 6.S12: Details of representative haplotypes and their sequences from Larson *et al.* (2010) selected for this study.

Sample No.	Accession NO.	Country	Location	Status	Breeds	Haplotype	Clade	Reference
1	AB252786	Myanmar	Kachin state	Domestic		EA228	D1	Tanaka *et al.* (2008)
1	AY879787	South Korea		Domestic	Jeju Native Pig	EA228	D1	Cho et al 2003, 2004 & 2005 (unpublished)
1	AY243480	South Korea		Domestic	Jeju native black	EA230	D1	Cho et al 2003, 2004 & 2005 (unpublished)
1	DQ779287	China	Modern restaurant, Daoqing	Domestic		EA231	D2	Larson *et al.*2007
1	AY230827	China		Domestic	Meishan	EA231	D2	Yue 2003 (unpulished)
1	AY230821	China		Domestic	Erhualian	EA123	D2	Yue 2003 (unpulished)
1	DQ496986	China	Shandong	Domestic	Yimenghei	EA170	D3	Wu *et al.*2007
1	DQ496917	China	Anhui	Domestic	Wei	EA8	D3	Wu *et al.* (2007)
3	AB252810	Myanmar	Kachin State	Domestic		EA131	D4	Tanaka *et al.* (2008)
1	DQ496451	China	-	Domestic	Huzhu	EA171	D5	Wu *et al.* (2007)
1	DQ496624	China	Hubei	Domestic	Qingping	EA16	D5	Wu *et al.* (2007)
1	DQ496626	China	Hubei	Domestic	Qingping	EA16	D5	Wu *et al.* (2007)
1	DQ496629	China	Hubei	Domestic	Qingping	EA16	D5	Wu *et al.* (2007)
1	DQ496635	China	Hubei	Domestic	Qingping	EA16	D5	Wu *et al.* (2007)
1	DQ496638	China	Hubei	Domestic	Qingping	EA16	D5	Wu *et al.* (2007)
1	AF486865	China		Domestic	QingPing	EA16	D5	Yang et al 2003
1	DQ152888	China	Qinghai	Domestic	Huzhu	EA171	D5	Fang & Andersson (2006)
1	AY230826	China		Domestic	Erhualian	EA232	D6	Yue 2003 (unpulished)
1	AY230824	China		Domestic	Erhualian	EA122	D6	Yue 2003 (unpulished)
1	AB252798	Bhutan	Haa	Domestic		EA132	D7	Tanaka *et al.* (2008)
1	AB252797	Bhutan	Haa	Domestic		EA133	D7	Tanaka *et al.* (2008)
1	AF486861	China		Domestic	Erhualian	EA256	D8	Yang et al 2003
1	DQ152889	China	Jiangsu	Domestic	Jiangquhai	EA184	D8	Fang & Andersson (2006)
1	AB252785	Myanmar	Bago division	Domestic		EA136	D9	Tanaka *et al.* (2008)

Sample No.	Accession NO.	Country	Location	Status	Breeds	Haplotype	Clade	Reference
1	AB252784	Myanmar	Kayin state	Domestic		EA137	D9	Tanaka *et al.* (2008)
1	DQ496306	China	Yunnan	Domestic	-	EA2	GC	Wu *et al.* (2007)
1	DQ496659	China	Yunnan	Domestic	Saba	EA2	GC	Wu *et al.* (2007)
1	DQ496395	China	Yunnan, Diqing	Domestic	Zang	EA1	GC	Wu *et al.* (2007)
1	DQ496396	China	Yunnan, Diqing	Domestic	Zang	EA1	GC	Wu *et al.* (2007)
1	DQ496397	China	Yunnan, Diqing	Domestic	Zang	EA1	GC	Wu *et al.* (2007)
1	DQ496326	China	Yunnan	Domestic	Baoshan	EA200	GC	Wu *et al.* (2007)
1	DQ496315	China	Yunnan, Baoshan	Domestic	Baoshan	EA32	GC	Wu *et al.* (2007)
1	DQ496568	China	Northeast	Domestic	Min	EA1	GC	Wu *et al.* (2007)
1	DQ496965	China	Guizhou	Domestic	Xiang	EA1	GC	Wu *et al.* (2007)
1	DQ496442	China	-	Domestic	Hanhei	EA1	GC	Wu *et al.* (2007)
1	DQ496463	China	-	Domestic	Huzhu	EA1	GC	Wu *et al.* (2007)
1	DQ496443	China	Northwest	Domestic	Hanhei	EA2	GC	Wu *et al.* (2007)
1	DQ496263	China	Sichuan, Aba	Domestic		EA2	GC	Wu *et al.* (2007)
1	DQ496619	China	Guizhou	Domestic	Domestic	EA2	GC	Wu *et al.* (2007)
1	DQ496620	China	Guizhou	Domestic	Domestic	EA2	GC	Wu *et al.* (2007)
1	DQ496591	China	Sichuan	Domestic	Neijiang	EA2	GC	Wu *et al.* (2007)
1	DQ496583	China	Sichuan	Domestic	Neijiang	EA2	GC	Wu *et al.* (2007)
1	DQ496644	China	Sichuan	Domestic	Rongchang	EA1	GC	Wu *et al.* (2007)
1	DQ496645	China	Sichuan	Domestic	Rongchang	EA1	GC	Wu *et al.* (2007)
1	DQ496649	China	Sichuan	Domestic	Rongchang	EA1	GC	Wu *et al.* (2007)
1	DQ496735	China	Anhui	Domestic	Wannanhua	EA1	GC	Wu *et al.* (2007)
1	DQ496736	China	Anhui	Domestic	Wannanhua	EA1	GC	Wu *et al.* (2007)
1	DQ496738	China	Anhui	Domestic	Wannanhua	EA1	GC	Wu *et al.* (2007)
1	DQ496739	China	Anhui	Domestic	Wannanhua	EA1	GC	Wu *et al.* (2007)
1	DQ496734	China	Anhui	Domestic	Wannanhua	EA1	GC	Wu *et al.* (2007)
1	DQ496740	China	Anhui	Domestic	Wannanhua	EA1	GC	Wu *et al.* (2007)
1	DQ496923	China	Anhui	Domestic	Wei	EA2	GC	Wu *et al.* (2007)
1	DQ496921	China	Anhui	Domestic	Wei	EA2	GC	Wu *et al.* (2007)
1	DQ496922	China	Anhui	Domestic	Wei	EA2	GC	Wu *et al.* (2007)
1	DQ496928	China	Anhui	Domestic	Wei	EA2	GC	Wu *et al.* (2007)

Sample No.	Accession NO.	Country	Location	Status	Breeds	Haplotype	Clade	Reference
1	DQ496913	China	Anhui	Domestic	Wei	EA1	GC	Wu *et al.* (2007)
1	DQ496914	China	Anhui	Domestic	Wei	EA1	GC	Wu *et al.* (2007)
1	DQ496478	China	Hubei	Domestic	Jianli	EA1	GC	Wu *et al.* (2007)
1	DQ496627	China	Hubei	Domestic	Qingping	EA2	GC	Wu *et al.* (2007)
1	DQ496637	China	Hubei	Domestic	Qingping	EA2	GC	Wu *et al.* (2007)
1	DQ496632	China	Hubei	Domestic	Qingping	EA1	GC	Wu *et al.* (2007)
1	DQ496495	China	Jiangsu	Domestic	Jiangquhai	EA1	GC	Wu *et al.* (2007)
1	DQ496497	China	Jiangsu	Domestic	Jiangquhai	EA1	GC	Wu *et al.* (2007)
1	DQ496540	China	Jiangxi	Domestic	Lepinghua	EA2	GC	Wu *et al.* (2007)
1	DQ496538	China	Jiangxi	Domestic	Lepinghua	EA2	GC	Wu *et al.* (2007)
1	DQ496816	China	Jiangxi	Domestic	Wild boar	EA2	GC	Wu *et al.* (2007)
1	DQ496992	China	Jiangxi	Domestic	Yujiang	EA2	GC	Wu *et al.* (2007)
1	DQ496991	China	Jiangxi	Domestic	Yujiang	EA2	GC	Wu *et al.* (2007)
1	DQ496993	China	Jiangxi	Domestic	Yujiang	EA2	GC	Wu *et al.* (2007)
1	DQ496987	China	Jiangxi	Domestic	Yushan	EA2	GC	Wu *et al.* (2007)
1	DQ496988	China	Jiangxi	Domestic	Yushan	EA2	GC	Wu *et al.* (2007)
1	DQ496277	China	Zhejiang	Domestic	Bihu	EA2	GC	Wu *et al.* (2007)
1	DQ496275	China	Zhejiang	Domestic	Bihu	EA2	GC	Wu *et al.* (2007)
1	DQ496278	China	Zhejiang	Domestic	Bihu	EA2	GC	Wu *et al.* (2007)
1	DQ496281	China	Zhejiang	Domestic	Bihu	EA1	GC	Wu *et al.* (2007)
1	DQ496330	China	Zhejiang	Domestic	Chalu	EA1	GC	Wu *et al.* (2007)
1	DQ496332	China	Zhejiang	Domestic	Chalu	EA1	GC	Wu *et al.* (2007)
1	DQ496333	China	Zhejiang	Domestic	Chalu	EA1	GC	Wu *et al.* (2007)
1	DQ496413	China	Zhejiang	Domestic	Erhualian	EA2	GC	Wu *et al.* (2007)
1	DQ496414	China	Zhejiang	Domestic	Erhualian	EA2	GC	Wu *et al.* (2007)
1	DQ496421	China	Zhejiang	Domestic	Erhualian	EA2	GC	Wu *et al.* (2007)
1	DQ496422	China	Zhejiang	Domestic	Erhualian	EA2	GC	Wu *et al.* (2007)
1	DQ496487	China	Zhejiang	Domestic	Jiaxinghei	EA2	GC	Wu *et al.* (2007)
1	DQ496488	China	Zhejiang	Domestic	Jinhua	EA2	GC	Wu *et al.* (2007)
1	DQ496705	China	Zhejiang	Domestic	Shengxianhua	EA2	GC	Wu *et al.* (2007)
1	DQ496707	China	Zhejiang	Domestic	Shengxianhua	EA2	GC	Wu *et al.* (2007)

Sample No.	Accession NO.	Country	Location	Status	Breeds	Haplotype	Clade	Reference
1	DQ496709	China	Zhejiang	Domestic	Shengxianhua	EA1	GC	Wu *et al*. (2007)
1	AF486856	China		Domestic	Zang	EA2	GC	Yang et al 2003
1	AF276930	China		Domestic	Jinhua	EA258	GC	Kim *et al.* (2002)
1	AF276924	China		Domestic	Wanan	EA1	GC	Kim *et al.* (2002
1	AF276926	China		Domestic	Wannanhua	EA1	GC	Kim *et al.* (2002
1	AF276932	China		Domestic	Wanhua	EA1	GC	Okumura et al. 1996, Watanobe, *et al.* 1999, Watanobe *et al*. 2001
1	AY486116	China		Domestic	Tibetan	EA2	GC	Gongora J. 2004
1	DQ152870	China	Jiangxi	Domestic	Lepinghua	EA181	GC	Fang & Andersson (2006)
1	DQ152873,	China	Jiangxi	Domestic	Yushan	EA2	GC	Fang & Andersson (2006)
1	DQ379133	China	Jiangsu	Domestic	Jiangquhai	EA2	GC	Fang & Andersson (2006)
1	DQ379134	China	Jiangxi	Domestic	Lepinghua	EA2	GC	Fang & Andersson (2006)
1	DQ379135	China	Guizhou	Domestic	Quanbei	EA2	GC	Fang & Andersson (2006)
1	DQ379136	China	Zhejiang	Domestic	Shengxianhua	EA2	GC	Fang & Andersson (2006)
1	DQ379137	China	Zhejiang	Domestic	Shengxianhua	EA2	GC	Fang & Andersson (2006)
1	DQ379138	China	Hubei	Domestic	Tongcheng	EA2	GC	Fang & Andersson (2006)
1	DQ379139	China	Guizhou	Domestic	Xiang	EA2	GC	Fang & Andersson (2006)
1	DQ379140	China	Hunan	Domestic	Xiangxi	EA2	GC	Fang & Andersson (2006)
1	DQ379141	China	Jiangxi	Domestic	Yushan	EA2	GC	Fang & Andersson (2006)
1	DQ379142	China	Sichuan	Domestic	Neijiang	EA2	GC	Fang & Andersson (2006)
1	DQ379143	China	Sichuan	Domestic	Neijiang	EA2	GC	Fang & Andersson (2006)
1	DQ152884	China	Jiangxi	Domestic	Shanggao	EA182	GC	Fang & Andersson (2006)
1	FJ601393	China	Hubei, jianli county	Domestic	Jian-Li	EA1	GC	Larson *et al.* (2010)
1	FJ601407	China	Sichuan, Hongya county	Domestic	Ya-Nan	EA2	GC	Larson *et al.* (2010)
1	FJ601457	China	Anhui, Xuancheng county	Domestic	Wei	EA1	GC	Larson *et al.* (2010)
1	FJ601458	China	Anhui, Xuancheng county	Domestic	Wei	EA1	GC	Larson *et al.* (2010)
1	FJ601491	China	Shan-Dong	Domestic	Ju-Nan	EA1	GC	Larson *et al.* (2010)
1	FJ601399	China	Jiangsu, Hai-an county	Domestic	Jiang-Qu-Hai	EA1	GC	Larson *et al.* (2010)
1	FJ601401	China	Jiangsu, Haimen county	Domestic	Sha-Wu-Tou	EA1	GC	Larson *et al.* (2010)
1	FJ601419	China	Jiangsu, Taicang county	Domestic	Small Mei-Shan	EA2	GC	Larson *et al.* (2010)

Sample No.	Accession NO.	Country	Location	Status	Breeds	Haplotype	Clade	Reference
1	FJ601420	China	Jiangsu, Taicang county	Domestic	Small Mei-Shan	EA2	GC	Larson *et al.* (2010)
1	FJ601429	China	Hunan, Xiangxi county	Domestic	Shan-Di	EA1	GC	Larson *et al.* (2010)
1	FJ601430	China	Hunan, Xiangxi county	Domestic	Shan-Di	EA1	GC	Larson *et al.* (2010)
1	FJ601431	China	Sichuan, Rongchang county	Domestic	Rong-Chang	EA2	GC	Larson *et al.* (2010)
1	FJ601448	China	Hainan, Lingao county	Domestic	Lin-Gao	EA1	GC	Larson *et al.* (2010)
1	FJ601449	China	Guizhou, Jiangkou county	Domestic	Jiang-Ko-Luo-Bo	EA1	GC	Larson *et al.* (2010)
1	FJ601450	China	Guizhou, Jiangkou county	Domestic	Jiang-Ko-Luo-Bo	EA1	GC	Larson *et al.* (2010)
6	FJ601463	China	Shanxi, Datong city	Domestic	Ma-Shen	EA2	GC	Larson *et al.* (2010)
1	FJ601464	China	Shanxi, Datong city	Domestic	Ma-Shen	EA2	GC	Larson *et al.* (2010)
1	FJ601467	China	Hubei, Yangxin county	Domestic	Yang-Xin	EA1	GC	Larson *et al.* (2010)
1	FJ601482	China	Fujian, Wuyishan city	Domestic	Wu-Yi black	EA1	GC	Larson *et al.* (2010)
1	FJ601483	China	Fujian, Wuyishan city	Domestic	Wu-Yi black	EA2	GC	Larson *et al.* (2010)
1	FJ601486	China	Jiangsu, Changshu city	Domestic	Er-Hua-Lian	EA2	GC	Larson *et al.* (2010)
1	FJ601493	China	Hunan, Taoyuan county	Domestic	Tao-Yuan	EA2	GC	Larson *et al.* (2010)
1	FJ601502	China	Guangxi, Donglan county	Domestic	Dong-Lan	EA1	GC	Larson *et al.* (2010)
1	FJ601503	China	Guangxi, Donglan county	Domestic	Dong-Lan	EA2	GC	Larson *et al.* (2010)
1	FJ601514	China	Hunan, Ningxiang county	Domestic	Ning-Xiang	EA1	GC	Larson *et al.* (2010)
1	FJ601517	China	Hubei, Gongan county	Domestic	E-Xi black	EA2	GC	Larson *et al.* (2010)
1	DQ496860	China	Yunnan	Wild		EA88	GC	Wu *et al*. (2007)
1	DQ496862	China	Yunnan	Wild		EA2	GC	Wu *et al*. (2007)
1	DQ496881	China	Yunnan	Wild		EA2	GC	Wu *et al*. (2007)
1	DQ496883	China	Yunnan	Wild		EA2	GC	Wu *et al*. (2007)
1	DQ496884	China	Yunnan	Wild		EA2	GC	Wu *et al*. (2007)
1	DQ496871	China	Yunnan	Wild		EA2	GC	Wu *et al*. (2007)
1	DQ496826	China	Guizhou	Wild		EA2	GC	Wu *et al*. (2007)
1	DQ496828	China	Guizhou	Wild		EA31	GC	Wu *et al*. (2007)
1	DQ496777	China	Fujian	Wild		EA2	GC	Wu *et al*. (2007)
1	DQ496815	China	Jiangxi	Wild		EA31	GC	Wu *et al*. (2007)
1	DQ496819	China	Jiangxi	Wild		EA31	GC	Wu *et al*. (2007)

Sample No.	Accession NO.	Country	Location	Status	Breeds	Haplotype	Clade	Reference
1	DQ496907	China	Zhejiang	Wild		EA32	GC	Wu *et al*. (2007)
1	DQ496912	China	Zhejiang	Wild		EA32	GC	Wu *et al*. (2007)
4	AB015092	Japan		Domestic	Okinawan	EA1	GC	Okumura et al. 1996, Watanobe, *et al.* 1999, Watanobe *et al*. 2001
1	DQ496725	Laos		Domestic		EA2	GC	Wu *et al*. (2007)
1	DQ496719	Laos		Domestic		EA2	GC	Wu *et al*. (2007)
1	DQ496820	Laos		Wild		EA2	GC	Wu *et al*. (2007)
8	AB252790	Laos,Cambodia,Myanmar	Laos:Borikamxai Province,Cambodia:Mondulkiri (5) Myanmar:Kachin(2)	Domestic		EA1	GC	Tanaka *et al.* (2008)
1	AB252800	Myanmar	Kachin State	Domestic		EA32	GC	Tanaka *et al.* (2008)
1	AF276935	South Korea	Cheju Island	Domestic	Cheju	EA257	GC	Kim et al 2002
1	DQ779443	Vietnam	North Central Coast	Domestic	Co	EA1	GC	Larson *et al*. (2007)
1	DQ779444	Vietnam	Tay Nguyen province, highland area (south central)	Domestic	Soc + Wild	EA1	GC	Larson *et al*. (2007)
1	DQ779445	Vietnam	Tay Nguyen province, highland area (south central)	Domestic	Soc + Wild	EA1	GC	Larson *et al*. (2007)
1	DQ496732	Vietnam		Wild		EA199	GC	Wu *et al*. (2007)
1	DQ496733	Vietnam		Wild		EA199	GC	Wu *et al*. (2007)
1	AM040641		Taiwan	Domestic	Taoyuan	EA1	GC	Yen 2005 (unpublished)
1	AM040642		Taiwan	Domestic	Taoyuan	EA1	GC	Yen 2005 (unpublished)
1	AM040643		Taiwan	Domestic	Taoyuan	EA1	GC	Yen 2005 (unpublished)
1	AM040644		Taiwan	Domestic	Taoyuan	EA1	GC	Yen 2005 (unpublished)
1	AM040645		Taiwan	Domestic	Taoyuan	EA1	GC	Yen 2005 (unpublished)
1	AM040646		Taiwan	Domestic	Taoyuan	EA1	GC	Yen 2005 (unpublished)
1	AM040653		Taiwan	Domestic	Taoyuan	EA1	GC	Yen 2005 (unpublished)
1	DQ152876	China	Hunan	Domestic	Xiangxi	EA1	GC	Fang & Andersson (2006)
1	DQ379146	China	Hubei	Domestic	Tongcheng	EA1	GC	Fang & Andersson (2006)
1	DQ379147	China	Hubei	Domestic	Tongcheng	EA1	GC	Fang & Andersson (2006)
1	DQ379148	China	Zhejiang	Domestic	Jinhua	EA1	GC	Fang & Andersson (2006)
1	DQ379149	China	Zhejiang	Domestic	Jinhua	EA1	GC	Fang & Andersson (2006)

Sample No.	Accession NO.	Country	Location	Status	Breeds	Haplotype	Clade	Reference
1	DQ379150	China	Jiangsu	Domestic	Jiangquhai	EA1	GC	Fang & Andersson (2006)
1	AY884612	India	Kashmir, Valley of Kashmir	Wild		EA40	MC1	Larson *et al*. (2005)
1	AY884671	India		Wild		EA38	MC1	Larson *et al*. (2005)
1	AY884674	India	Monghyr, Bengal	Wild		EA40	MC1	Larson *et al*. (2005)
1	AY884689	India	Kashmir, Woolar Lake	Wild		EA40	MC1	Larson *et al*. (2005)
1	DQ496743	China	Yunnan, Banna	Wild		EA81	MC2	Wu *et al*. (2007)
1	DQ496875	China	Yunnan, Jinghong	Wild		EA81	MC2	Wu *et al*. (2007)
1	DQ779528	Cook Islands	Tangatatau Rock Shelter, Mangaia			EA288	MC2	Larson *et al*. (2007)
1	DQ444710	French Polynesia	Tetiaroa, Society Islands	Feral		EA39	MC2	Larson *et al*.2005
1	DQ779373	French Polynesia	Marquesas, Hiva Oa	Feral		EA39	MC2	Larson *et al*. (2007)
1	DQ779375	French Polynesia	Marquesas, Hiva Oa	Feral		EA39	MC2	Larson *et al*. (2007)
1	DQ779376	French Polynesia	Marquesas, Hiva Oa	Feral		EA39	MC2	Larson *et al*. (2007)
1	DQ779429	French Polynesia	Marquesas, Tahuata (Hanatuuna)	Feral		EA39	MC2	Larson *et al*. (2007)
1	DQ779430	French Polynesia	Marquesas, Hiva Oa (Hanaui)	Feral		EA39	MC2	Larson *et al*. (2007)
1	DQ779431	French Polynesia	Marquesas, Hiva Oa (Hanaui)	Feral		EA279	MC2	Larson *et al*. (2007)
1	DQ841948	Indonesia	Waingapoe, Soemba island	Domestic		EA276	MC2	Larson *et al*. (2007)
1	DQ779425	Indonesia	Flores, Arkenas	Feral		EA39	MC2	Larson *et al*. (2007)
1	DQ779312	Indonesia	Timor	Feral		EA276	MC2	Larson *et al*. (2007)
1	DQ779346	Indonesia	West New Guinea, Salawatti, Sagobos	Feral		EA279	MC2	Larson *et al*. (2007)
1	DQ779341	Indonesia	Morotai island, North of Halmahera	Feral		EA282	MC2	Larson *et al*. (2007)
1	DQ779352	Indonesia	Ternate - small island west of Halmahera	Feral		EA282	MC2	Larson *et al*. (2007)
1	DQ779343	Indonesia	Koerik (S.W. coast) bij Merauke, Ned. New Guinea	Feral		EA283	MC2	Larson *et al*. (2007)
1	DQ779349	Indonesia	Tobati, New Guinea	Feral		EA284	MC2	Larson *et al*. (2007)
1	DQ779408	Indonesia	Flores, Arkenas	Feral		EA285	MC2	Larson *et al*. (2007)
1	DQ779347	Indonesia	Omtrak van Bivak-Eiland, Central New Guinea	Feral		EA286	MC2	Larson *et al*.2007
1	DQ779350	Indonesia	Manikion-Gabied, on bird's head West N.G.	Feral		EA54	MC2	Larson *et al*. (2007)
1	DQ779337	Indonesia	Flores? Java?	Wild		EA276	MC2	Larson *et al*. (2007)
1	DQ779320	Indonesia	Tandjong Morawa Deli (Sumatra)	Wild		EA277	MC2	Larson *et al*. (2007)
1	DQ779328	Indonesia	Ceram (Selidewai)	Wild		EA278	MC2	Larson *et al*. (2007)

Sample No.	Accession NO.	Country	Location	Status	Breeds	Haplotype	Clade	Reference
1	DQ779326	Indonesia	Ceram (asaoede)	Wild		EA278	MC2	Larson *et al.* (2007)
1	DQ779329	Indonesia	Bali (Sendang, W. Bali)	Wild		EA279	MC2	Larson *et al.* (2007)
1	DQ779330	Indonesia	Sendang, W. Bali	Wild		EA280	MC2	Larson *et al.* (2007)
1	DQ779338	Indonesia	Flores? Java?	Wild		EA281	MC2	Larson *et al.* (2007)
1	DQ779327	Indonesia	Ceram (Leciela)	Wild		EA284	MC2	Larson *et al.* (2007)
1	AY884688	Indonesia	Halmahera island, Wasile district, Kampung, Loleba	Wild		EA42	MC2	Larson *et al.* (2005)
1	DQ496824	Laos		Wild		EA88	MC2	Wu *et al.* (2007)
1	DQ779409	Papua New Guinea	Admiralty islands, 'wild island'	Feral		EA39	MC2	Larson *et al.* (2007)
1	AY884615	Papua New Guinea	San Sapor	Feral		EA54	MC2	Larson *et al.* (2005)
1	AY884673	Papua New Guinea		Feral		EA39	MC2	Larson *et al.* (2005)
1	AY884821	Papua New Guinea	Doido	Feral		EA39	MC2	Larson *et al.* (2005)
1	AY884822	Papua New Guinea	Kairouk,Jimi Valley	Feral		EA39	MC2	Larson *et al.* (2005)
1	DQ779530	Solomon Islands	Lomlom Island (Reef Islands)			EA39	MC2	Larson *et al.* (2007)
1	AY884678	USA	Oahu, Hawaii	Feral		EA39	MC2	Larson *et al.* (2005)
1	AY884702	Vanuatu	Gaua, New Hebrides	Feral		EA45	MC2	Larson *et al.* (2005)
1	AY884704	Vanuatu		Feral		EA46	MC2	Larson *et al.* (2005)
2	AB053610	Vietnam	Purchased nr Hanoi / Hunted in North Vietnam	Wild		EA46	MC2	Hongo *et al.* 2002
14	AB252820	Cambodia,Laos	Cambodia:Mondul Kiri& Ratanakiri(12),Laos:Champasak	Domestic		EA127	MC3	Tanaka *et al.* (2008)
1	AB252819	Laos	Xieng Khuang Province	Domestic		EA128	MC3	Tanaka *et al.* (2008)
6	AB252816	Laos,Cambodia	Laos:Xieng KhuangProvince,Cambodia: Ratanakiri(5)	Domestic		EA100	MC3	Tanaka *et al.* (2008)
4	AB252817	Laos,Myanmar	Laos:Vientiane Province,Myanmar:Shan& Bago	Domestic		EA202	MC3	Tanaka *et al.* (2008)
1	AB252821	Myanmar	Shan State	Domestic		EA238	MC3	Tanaka *et al.* (2008)
1	AB252815	Myanmar	Bago division	Domestic		EA129	MC3	Tanaka *et al.* (2008)
1	AY879782	South Korea		Wild		EA229	W1	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AB053627		Taiwan	Wild		EA225	W1	Hongo *et al.* 2002
2	AB252824	Cambodia	Kampong Cham	wild		EA124	W10	Tanaka *et al.* (2008)

Sample No.	Accession NO.	Country	Location	Status	Breeds	Haplotype	Clade	Reference
2	AB252823	Cambodia	Mondul Kiri	wild		EA125	W10	Tanaka *et al.* (2008)
1	AB326939	Vietnam		wild		EA141	W10	Ishiguro *et al.*2008
1	AB326938	Vietnam		wild		EA142	W10	Ishiguro *et al.*2008
1	AY884623	Burma	Tenasserim, Bok Pyin	Wild		EA56	W11	Larson *et al.* (2005)
1	AY884629	Burma	Tenasserim, Kisseraing Island	Wild		EA58	W11	Larson *et al.* (2005)
1	AY884712	Burma	Tenasserim, Champang	Wild		EA56	W11	Larson *et al.* (2005)
1	AY884630	Thailand	Trang Trong	Wild		EA59	W11	Larson *et al.* (2005)
1	AY884647	Burma	Tenasserim, Tanjong Budak	Wild		EA66	W12	Larson *et al.* (2005)
1	AY884695	Burma	Tenasserim, Boyse's Point	Wild		EA44	W12	Larson *et al.* (2005)
1	DQ496788	China	Gansu	Wild		EA57	W13	Wu *et al.* (2007)
1	DQ496790	China	Gansu	Wild		EA57	W13	Wu *et al.* (2007)
1	DQ496837	China	Shaanxi	Wild		EA57	W13	Wu *et al.* (2007)
1	DQ496838	China	Shaanxi	Wild		EA57	W13	Wu *et al.* (2007)
1	AY884610	China	Sichuan, Wen Chuan	Wild		EA52	W13	Larson *et al.* (2005)
1	AY884627	China	Shanxi Fen Chow	Wild		EA57	W13	Larson *et al.* (2005)
1	AY884639	China	Shensi, Yen-An-Fu	Wild		EA57	W13	Larson *et al.* (2005)
1	AY884684	China	Shanxi Tai-Yuan-Fu	Wild		EA57	W13	Larson *et al.* (2005)
1	AY879781	South Korea		Wild		EA115	W2	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AY879780	South Korea		Wild		EA116	W2	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AB053624	South Korea		Wild		EA111	W3	Hongo *et al.* 2002
1	AY879779	South Korea		Wild		EA111	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AY879784	South Korea		Wild		EA111	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AY879783	South Korea		Wild		EA111	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AY879778	South Korea		Wild		EA111	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AY879777	South Korea		Wild		EA111	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)

Sample No.	Accession NO.	Country	Location	Status	Breeds	Haplotype	Clade	Reference
1	AY879771	South Korea		Wild		EA113	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AY534288	South Korea		Wild		EA113	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AY534284	South Korea		Wild		EA113	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AY879772	South Korea		Wild		EA226	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AY534287	South Korea		Wild		EA226	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AY879776	South Korea		Wild		EA117	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AY534286	South Korea		Wild		EA120	W3	Cho *et al.* (2003, 2004 & 2005, Direct Submission)
1	AB041466	China		Wild		EA37	W4	Larson *et al.*2007
1	AY751460	China	Northeast	wild		EA36	W4	Zhang D. 2004 (unpublished)
1	DQ496753	China	Northeast	Wild		EA36	W4	Wu *et al.* (2007)
1	DQ496769	China	Northeast	Wild		EA36	W4	Wu *et al.* (2007)
1	DQ496771	China	Northeast	Wild		EA36	W4	Wu *et al.* (2007)
1	DQ496772	China	Northeast	Wild		EA36	W4	Wu *et al.* (2007)
1	DQ496744	China	Northeast	Wild		EA37	W4	Wu *et al.* (2007)
33	D42174	Japan		Wild		EA69	W5	Okumura et al. 1996, Watanobe, *et al.* 1999, Watanobe *et al.* 2001
1	D42178	Japan		Wild		EA71	W5	Okumura et al. 1996, Watanobe, *et al.* 1999, Watanobe *et al.* 2001
7	AB015089	Japan		Wild		EA76	W6	Okumura et al. 1996, Watanobe, *et al.* 1999, Watanobe *et al.* 2001

Sample No.	Accession NO.	Country	Location	Status	Breeds	Haplotype	Clade	Reference
1	AB015090	Japan		Wild		EA77	W6	Okumura et al. 1996, Watanobe, *et al.* 1999, Watanobe *et al*. 2001
1	DQ779417		Taiwan	Wild		EA219	W7	Larson *et al*. (2007)
1	DQ779418		Taiwan	Wild		EA220	W7	Larson *et al*. (2007)
1	FJ601524	China	Hainan, Sanya city	wild	Hai-Nan wildboar	EA166	W8	Larson *et al*. (2010)
1	DQ496793	China	Hainan	Wild		EA166	W8	Wu *et al*. (2007)
1	DQ496795	China	Hainan	Wild		EA166	W8	Wu *et al*. (2007)
1	DQ496796	China	Hainan	Wild		EA166	W8	Wu *et al*. (2007)
1	DQ496892	China	Zhejiang	Wild		EA34	W8	Wu *et al*. (2007)
1	DQ496893	China	Zhejiang	Wild		EA34	W8	Wu *et al*. (2007)
1	DQ496856	China	Yunnan	Wild		EA204	W9	Wu *et al*. (2007)
1	AB326943	Vietnam		wild		EA250	W9	Ishiguro *et al*.2008

Part II: Genetic and Cultural Significance of Indigenous Pigs in Papua New Guinea and their Phenotypic Characteristics

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Genetic and cultural significance of indigenous pigs in Papua New Guinea and their phenotypic characteristics

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Summary
Pigs are the most important livestock species in Papua New Guinea (PNG) from economic as well as cultural perspectives. Most of the estimated population of 1.8 million pigs are maintained by smallholder farmers. The genetic attributes, differentiation and production capacities of indigenous pigs are largely unknown. But the rich socio-cultural diversity of rural communities living in geographically isolated pristine environments, with long and strong attachments with indigenous pigs implies that indigenous pigs may harbour unique genetic diversity. This study reports preliminary survey of indigenous pigs sampled from major pig farming areas of the country as part of a South Asia-wide regional indigenous pig genetic diversity study. It assesses farmers' perceptions about the origin, population trend and utility value of indigenous pigs, as well as their trait preferences. Average herd sizes and external physical forms and appearances of pigs are described. About 19 percent of the sampled indigenous pigs were identified through pedigree checks to have an admixtured genotype with some distant indigenous or exotic parentage. The importance of indigenous pig genetic resources in PNG requires a policy and legislative framework to support sustainable utilization. As a first step in informing such development, a comprehensive molecular genetic study is required to elucidate the genetic attributes of this unique genetic resource.

Keywords: *Papua New Guinea, indigenous pigs, genetic diversity, phenotypic characterization*

Résumé
En Papouasie-Nouvelle-Guinée, les porcs représentent l'espèce d'animaux d'élevage la plus importante tant du point de vue économique que culturel. La plupart de la population estimée de 1,8 million de porcs est élevée par des petits exploitants. Les caractères génétiques et les capacités de différenciation et de production des porcs indigènes sont largement inconnus. Mais la riche diversité socio-culturelle des communautés rurales qui vivent dans des environnements vierges isolés et ont des liens forts et de longue durée avec les porcs indigènes laisse supposer que ces derniers pourraient conserver une diversité génétique unique. Cette étude présente le rapport d'une enquête préliminaire sur les porcs indigènes des principales zones d'élevage du pays, dans le cadre d'une vaste étude régionale sur la diversité génétique des porcs, mise en place en Asie du Sud. Dans cette étude, on évalue les perceptions des agriculteurs sur l'origine, l'évolution de la population et la valeur d'utilité des porcs indigènes, ainsi que leurs préférences de caractères. On y décrit les tailles moyennes des troupeaux et les formes et aspects physiques extérieurs des porcs. Par le biais des contrôles généalogiques, on a déterminé qu'environ 19 pour cent des porcs indigènes pris en considération présentent un génotype provenant d'un mélange avec une ascendance lointaine indigène ou exotique. L'importance des ressources génétiques des porcs indigènes de la Papouasie-Nouvelle-Guinée demande un cadre politique et législatif soutenant leur utilization durable. La première étape pour accompagner ce développement consiste à entreprendre une étude génétique moléculaire détaillée pouvant préciser les caractères génétiques de cette ressources génétique unique.

Mots-clés: *Papouasie-Nouvelle-Guinée, porcs indigènes, diversité génétique, caractérization phénotypique*

Resumen
El cerdo representa la especie de ganado más importante en Papúa Nueva Guinea (PNG) tanto desde un punto de vista económico como cultural. La mayor parte de la población estimada, de 1,8 millones de cerdos, es mantenida por granjeros minifundistas. Las características genéticas y las capacidades de diferenciación y producción de los cerdos indígenas se desconocen ampliamente. Pero la riqueza de la diversidad socio-cultural de las comunidades rurales que viven aisladas geográficamente en ambientes en perfecto estado, con antiguas y fuertes relaciones con cerdos indígenas, implica que los cerdos indígenas posean una diversidad genética única. Este trabajo está basado en el estudio preliminar de cerdos indígenas muestreados en las más importantes áreas de cría de cerdos del país, como parte de un estudio de la diversidad genética de una amplia región del sur de Asia. Se valora la percepción de los ganaderos acerca del origen, tendencia de la población, valor de utilidad de los cerdos indígenas, así como sus rasgos preferidos. Se describe

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el tamaño medio de las piaras, sus formas físicas externas y la apariencia de los cerdos. Alrededor del 19 percent de los cerdos indígenas muestreados fueron identificados por medio del pedigrí como poseedores de una mezcla genética con algún origen lejano indígena o exótico. La importancia de los recursos genéticos porcinos en PNG requiere de un marco de trabajo político y legislativo para apoyar su utilización sostenible. Como primer paso para informar de tal desarrollo, se ha requerido un exhaustivo estudio genético molecular para aclarar las características genéticas de este recurso genético único.

Palabras clave: *Papúa Nueva Guinea, cerdos indígenas, diversidad genética, caracterización fenotípica*

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Introduction

Country background

Papua New Guinea (PNG) is located in the South Pacific region from longitude 141°E to 156°E and latitude 1°S to 11°S, sharing a land border only with Indonesia to the East (Figure 1). It has a total land area of about 470 000 km^2 constituting the mainland and more than 700 Islands including atolls. It is mostly mountainous with coastal lowlands and rolling foothills. The Owen Stanley Ranges, which has a peak elevation of 4 793 m at Mt. Wilhelm runs through the middle of the mainland of PNG from west to east. Up to 60 percent of the land mass is covered by pristine natural forest providing natural habitat to a wide range of flora and fauna (Kambuou, 1996; MoA&L, 2004). It also has numerous rivers and over 5 000 lakes with an extensive system of marshes located on the north and south parts of the country.

About 87 percent of the total population depend on subsistence agriculture for their livelihood. The main livestock species are pigs, poultry, cattle, goats, sheep and rabbits. Although PNG harbours large numbers of introduced exotic pigs of European origin, indigenous pigs are by far the most important to the farmers considering their social and cultural roles. They are raised by several hundred ethnic groups (Reilly, 2008) residing in various agro-ecological zones. Historically and culturally, pigs have been associated with PNG people much earlier than any other livestock species. However, the genetic structure and diversity of this invaluable resource remains unknown.

Introduction of pigs to PNG

Archaeological and linguistic evidences have suggested the first introduction of pigs to PNG between 10 000 and 2 500 years ago (Hide, 2003). However, the origin, taxonomy and diversity of the indigenous pigs of PNG are not well understood. Recent DNA evidence suggests that the indigenous pigs of PNG belong to the species *Sus scrofa* and were introduced by humans to the main island of PNG (Allen *et al.*, 2001; Larson *et al.*, 2005; Lum *et al.*, 2006; Larson *et al.*, 2007). Larson *et al.* (2007) suggest that Austronesian-speaking people (Kirch, 1997), whose putative homeland was present-day Taiwan introduced pigs into the region some 3 500 years ago, although pigs involved were not of Taiwanese origin. They argued further that the so-called wild pigs of PNG are probably feral pigs derived from the initial introductions from Southeast Asia. This argument is supported by observations in some parts of the country where hunters capture young piglets alive and take them home to be raised (Figure 2c).

Socio-cultural importance of pigs

In the largely traditional and subsistence rural communities of PNG, in particular in the fertile and densely populated highlands, pigs have strong socio-cultural significance. Pigs are culturally the most important animals used extensively in many forms of exchange, alms-giving, feasting, compensation and as symbols of social status and rank. Pigs are used for bride prize payments, as gifts to establish or maintain social relations and as payments to resolve social disputes or strengthen relationships between individuals, families, clans and tribes. Pigs are also slaughtered during initiation rites, funerals for the dead and during elaborate ceremonies or gatherings where feasting is involved. Lemonnier (2002) surmises that the use of pigs in these ways symbolizes the transformation of strength and maleness embodied in the pig into a substitute or token of life suitable for compensation and exchange. In many parts of PNG, the number of pigs owned and their body size and conditioning is used to judge the social rank of a person. Furthermore, the pig husbandry system followed reflects gender relationships whereby women in some communities look after the pig but do not own them, although pigs cannot be disposed off without their consent (Dwyer, 2006).

Traditional methods of pig breeding and feeding in PNG often portray cultural differences among various ethnic communities. In the central highlands and some lowland areas, boars are often castrated and sows mated with wild boars; young piglets are captured from the wild and either eaten or cared for by women who sometimes carry them in string bags or walk them on tether to food gardens till they reach maturity. In some coastal and eastern highland areas, however, wild pigs are less common. Availability of feed resources varies among various communities. For instance, surplus sweet potato tubers and vines are commonly used for feeding pigs in the Eastern

Figure 1. Political map of Papua New Guinea and location of three sample sites (*source:* http://www.infoplease.com/atlas/country/papuanewguinea.html#axzz0zSNfxZKM 25 March 2010)

Highlands province. Pigs are allowed to root in fallow food gardens and this is seen as a way of cultivating the land in some places. In coastal and island regions, however, coconuts, taro and sago are often used to feed pigs. All these differences in feeds and feeding system suggest that the indigenous pigs are adapted to dealing with various types of feeds.

Pig population

The pig population of PNG is not known with certainty. The frequently quoted figure is 1.8 million, estimated in 2003 (FAOSTAT, 2010). Quartermain (2002) estimated that at least one million of this population could be genetically isolated indigenous populations maintained by rural communities, with the rest being increasingly influenced by introduced and cross-bred pigs that are mainly raised by market-oriented pig farmers. Gibson and Rozelle (cited by Hide, 2003) estimated the village (indigenous) pig numbers at 1.7 million in 1998 based on a sample of 830 rural households from different parts of PNG. The number of indigenous pigs per household in the four regions of Papua, Highlands, Momase and New Guinea Islands was 1.9, 3.3, 1.7 and 1.3, respectively.

The current pig population of PNG consists of indigenous, exotic and cross-bred pigs. Only one indigenous type is reported in FAO's Domestic Animal Diversity Information System (FAO, 2010). This is because indigenous pigs are regarded as non-descriptive type without any geographical pattern of morphological variation (Figures 1 and 2).

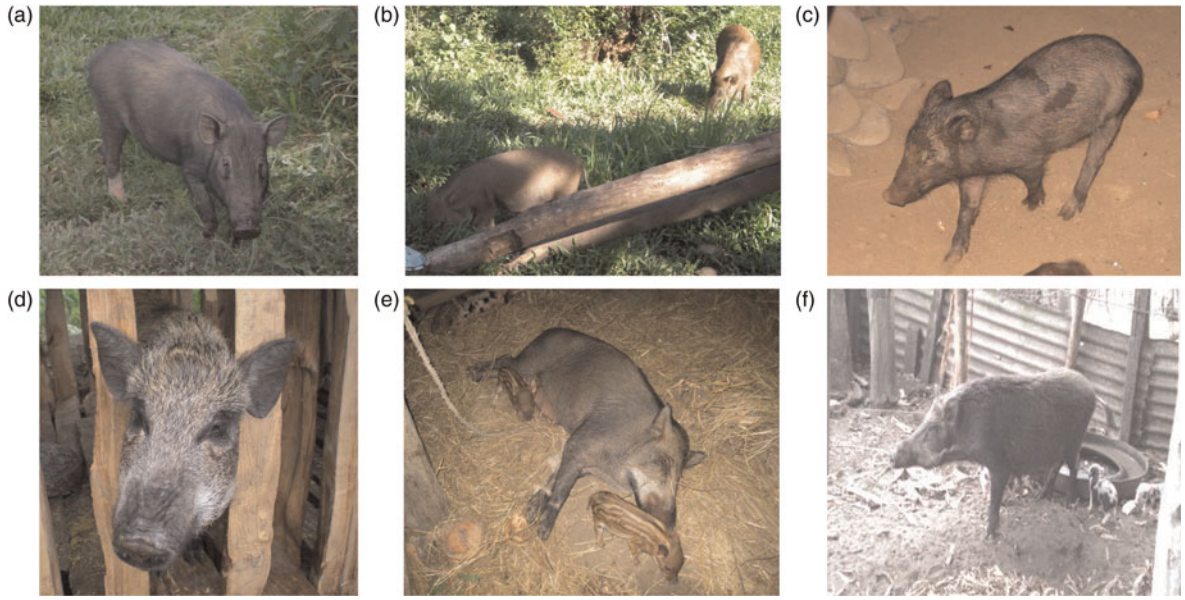

Figure 2. Indigenous pigs of Papua New Guinea. (a, b) Typical indigenous pigs seen in Nawaeb district; (c) captured wild pig being raised in confinement; (d) indigenous sow in a sty at Kabwum village in Finschhafen district; (e) a nursing indigenous sow in Finschhafen village, Finschhafen district. Piglets have ochre, chocolate and cream coloured stripes lengthwise over their bodies suggesting gene flow from wild pigs; (f) nursing indigenous sow in Tambul village, Tambul district.

However, it does not imply lack of variation among indigenous pigs. For instance, Near East sheep that are all of "generic type" phenotypically and not easy to tell apart, display much more genetic variation than readily distinguishable European breeds (Peter *et al.*, 2007). In addition to this, the preliminary analyses of microsatellite data also suggest the presence of at least four distinct populations of indigenous pigs in PNG (Nidup *et al.*, 2010). Therefore, indigenous pigs of PNG may be shown to have a high level of genetic variation in productive ability regardless of the fact that there is no obvious partitioning into breeds.

Exotic pigs (Figure 3a) are mostly European breeds, such as Large White, Landrace, Berkshire, Tamworth, Saddleback and Large Black. These breeds are mostly used for intensive commercial production and cross-breeding with the indigenous pigs (Figure 3b, c).

Cross-breeding between exotic and indigenous pigs was a policy of the Australian colonial government soon after Second World War in an effort to restock and improve pig populations which were devastated by the war in many parts of PNG (Malynicz *et al.*, 1973a, b; Hide 2003). Unabated loose disseminations of these European breeds and their cross-breds now constitute a threat to the continued survival of indigenous pigs.

Importance of genetic diversity

Present and potential future animal production is underpinned by the genetic diversity in the existing breeding populations. Even though it is not clear whether or not the indigenous pigs of PNG belong to the same breed, it is conceivable that, through natural and artificial selection, the different populations found in various parts of the country, including those in geographically isolated valleys, plateaus and islands, may have accumulated various specific genetic adaptations to different environmental challenges leading to population differentiation. Indigenous pigs are vital for supporting household economies, livelihoods and cultures of a significant proportion of the human population. However, they have received very little attention from the policy-makers because they

Figure 3. (a) Introduced exotic pigs of Large Black and Duroc origins; (b) Cross-bred sow with her highly heterogeneous looking piglets; (c) progenies of wild pig and improved or exotic sows. Large ear and belted-white phenotype suggest exotic pig origin while stripes indicate wild pig origin.

are regarded as "low producers" without considering important traits such as adaptability to poor feed quality, resistance to several diseases and adaptation to wide range of environments. It should be noted that indigenous pig genetic resources will continue to be useful for food security and rural development of current and future generations. To meet the needs of the present as well as future generations, it is necessary to document and characterize the indigenous pigs for conservation, promotion and sustainable utilization.

Objective of the study

This study was conducted in the larger framework of an analysis of genetic diversity in South Asian pigs (Nidup *et al.*, unpublished data). It has the specific objectives of providing a preliminary phenotypic description of indigenous pigs and prevailing husbandry practices in major pig-growing regions of PNG as a prelude to the genetic analyses.

Materials and methods

Three divergent sampling sites (Table 1; Figure 1) from major pig-raising areas of the country were selected for the study. The first site (Tambul) covered a wide area in the highlands region of the country from Kandep district in Enga province to Tambul district in the Western Highlands. The second site (Garaina) consisted of agriculturally fertile valleys in Bulolo and Huon Gulf districts in the west of Morobe province. The isolated villages in Finschhafen and Nawaeb districts on the east side of Morobe province were the third sampling areas (Finschhafen). Purposeful sampling was employed to identify major pig raising villages far from urban centres, which are considered less likely to have been influenced or contaminated by the expanding distribution of cross-bred pigs. Hair and blood samples of 91 pigs (Table 1) from three sampling sites were collected for microsatellite and mitochondrial DNA studies. At least 82 households from six districts of three provinces were interviewed on a semi-structured questionnaire (Table 1). Each of the respondents was asked to identify their indigenous pigs by breed type. The questionnaires covered origins and sources of pigs, reasons for keeping them, current herd sizes, husbandry practices as well as their perceptions of pig population trends. Linear body measurements, which were taken with standard measuring tape coated with plastic film graduated to the next half-centimetre, were recorded from adult pigs. The external body forms and appearances of pigs were also documented. Although every possible attempt was made to ensure that only households with indigenous pigs were included in the study, some of the households later revealed that one or more of their pigs were of mixed (cross-bred × indigenous) or of unknown genetic background. Data were collected between November 2009 and March 2010. Simple descriptive statistics were used to analyse these data.

Results and discussion

Local names and purpose of raising pigs

Three quarters of the respondents ($n = 82$, Table 1) mentioned local names of pigs, which appear to be local linguistic terms given to pigs in general (e.g. Buc, Ambi, Pi, Kong, and Kareh) and have some similarities between sampling sites. The names relate more to linguistic differences between sites rather than genetic distinctiveness of the pigs. Although indigenous and cross-bred pigs were clearly distinguishable, the term "breed" was neither clearly understood nor used. The pedigree observation on 91 "indigenous pigs" revealed that 19 percent have a known parent that is considered a cross-bred with either known exotic cross or an indigenous type from a distant source within the country. At site level, this figure is 14 percent for both Tambul and Finschhafen but as high as 30 percent for Garaina. This merely indicates the extent of spread of various levels of exotic cross-bred pigs but not necessarily commercialization tendencies.

The main reasons of keeping pigs (Figure 4) are meat production (25 percent) followed by breeding (23 percent), wealth creation (22 percent) and savings (22 percent), with slight differences between those for males and

Table 1. Number of households interviewed and samples collected from six districts of PNG.

Sampling site	District	Province	Number of samples (blood and hair)	Total number of samples from each site	Total number of households interviewed from each site[a]	Households with herd size data
Tambul	Kandep	Enga	4	21	19	4
	Tambul	Western highlands	17			13
Garaina	Bulolo	Morobe (west)	17	27	22	14
	Huon Gulf	Morobe (west)	10			5
Finschhafen	Finschhafen	Morobe (east)	10	43	41	10
	Nawaeb	Morobe (east)	33			28
Total			91	91	82	74

[a]Some households without herd size data were also interviewed.

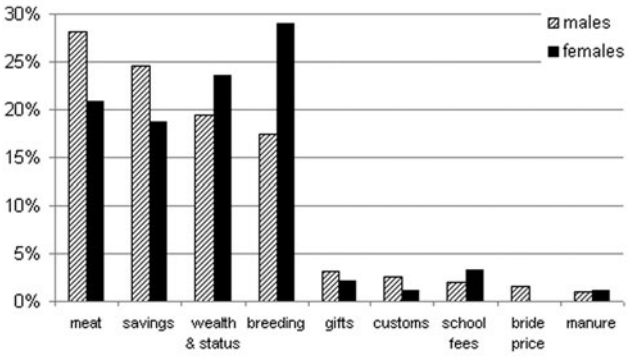

Figure 4. Frequency of reported reasons for raising indigenous pigs ($n = 91$).

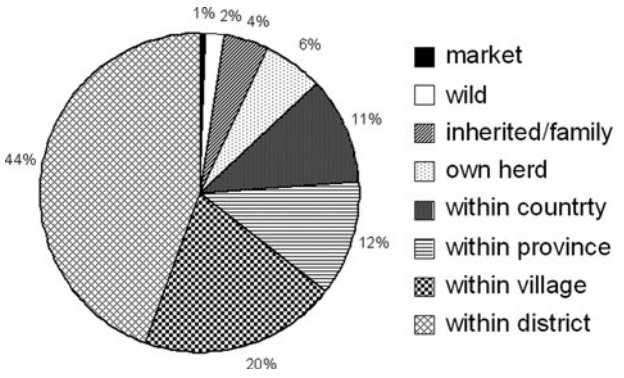

Figure 5. Frequency of reported sources of sample indigenous pigs at study sites ($n = 91$).

females and between sites. Male pigs are preferred for meat, savings and bride prices, as are females for breeding and status symbol. The other reasons include needs to honour social obligations (gifts, deity), pay school fees and secure manure for farmyards.

Sources of pigs

Over three quarters of the pigs originated from within the study district, 12 percent from outside the district in the same province and another 11 percent from a neighbouring province within the country, indicating a large-scale gene flow from one part of the country to another (Figure 5). Farmers also mentioned multiple sources of boars used in their herds. Only 12 percent reported using boars from their own herd, 39 percent used boars in their village, while 20 percent depended on communal boars. Half of the respondents did not have any control of mating as they rely on communal mating, or any available boar for mating. A few wild pigs (2 percent) also contributed as breeding boars.

Herd size and composition

The herd size differed between sampling areas ranging from 3.24 head in Tambul to 9.21 in Garaina (Table 2) with an average of 6.23 heads per household. Individual herd sizes ranged from 1 to 138. Breeding sows and boars constitute 14 and 8 percent, respectively, with the number of boars varying from zero to five.

Eighty percent were identified by their owners as having known indigenous dam and boar parentage, whereas 20 percent had a mixed parentage of known and unknown genotype. One of the farmers maintained a breeding boar of feral origin. Two other boars with known feral parentage were also observed giving a total frequency of 4 percent for pigs regarded by owners as half-feral or wild.

Population trend

Farmers had mixed views on the population trend of the indigenous pigs. From the 64 responses, just over a third indicated that the population of indigenous pigs in their area is increasing while the rest indicated decreasing (31 percent), stable (30 percent) or unknown (5 percent) trends. Some of the reasons stated for a decreasing trend of indigenous pigs are decreased interest of farmers who have switched to other alternative livelihoods (41 percent), unavailability of breeding stock (29 percent), competition from exotic and cross-bred pigs (8 percent) and competition from pigs of neighbouring areas (8 percent).

Important traits

When pig owners were asked to indicate the importance of a selected set of common desirable production and adaptation traits as unimportant, marginally important, moderately important and important, at least half of the respondents identified the following traits as important in descending order of ranking; scavenging ability, meat taste, general appearance, compact body, feeding habits, coat hair cover and tolerance to heat or heat load, most of which relate to adaptation attributes (Figure 6). Traits that were least frequently identified as important were mothering ability, reproductive performance, general appearance and longevity/durability.

External features

Overall breeding males measured 78.2 cm on heart girth and 56.6 cm on body length; the respective averages for sows were 78.3 and 54.4 cm (Table 3). But some of these values are those of growing boars and sows and hence cannot be taken as typical values of the adult populations. Females have from 7 to 16 teats when compared with males with 8 to 12 teats (Table 3).

Only preliminary descriptions of phenotype of the whole sample herd of 54 growing sows and 29 young boars sampled for genetic diversity study (Nidup *et al.*, 2010) are presented here:

The snout is often short (37 percent), cylindrical (34 percent) or elongated, thin and long (26 percent), but rarely (2 percent) concave. The head profile is mostly (79 percent) straight and convex profiles appear only in 6 percent

Table 2. Herd size and percentage of different categories of pigs in three sampling sites.

Sampling sites	Number of pigs sampled at each site	Piglets (%)		Growers (%)		Adults (%)		Average herd size
		Female	Male	Female	Male	Female	Male	
(1) Finschhafen	38	22	25	18	12	12	10	6.03
(2) Garaina	19	18	18	13	38	11	2	9.21
(3) Tambul	17	14	15	24	2	25	20	3.24
Total	74	18	20	17	23	14	8	6.23

of the cases. Slightly concave foreheads are also observed in low (15 percent) frequencies. Ear sizes can be small (27 percent), medium (57 percent) or large (16 percent), with mostly (88 percent) prick orientation. Few droopy (2 percent) and semi-lopping (10 percent) forms are also observed on both indigenous and admixture genotypes. Ear orientations are dominated by those that point upwards (65 percent) and backwards (24 percent), but some (11 percent) have forward pointing ears. Bristles are mostly (69 percent) found on the dorsal backline, and also form parallel lines on the backline (15 percent) or found scattered throughout the body (16 percent). The hair coat is normally straight (53 percent) and dense (18 percent); about 16 percent of pigs of both sexes have sparse hair throughout the body. Both smooth and wrinkled forms of skin are evenly observed on both sexes.

Tails on the indigenous pigs are normally straight (93 percent) but can also be curly or twisted (7 percent). None of the pigs identified as admixtures were observed with curly or twisted tails. Most pigs had normal udder, while 5 percent of pigs had asymmetrical udder. Most (83 percent) of the pigs were identified by their owners as having a placid and friendly temperament, with the rest 17 percent showing various levels of aggressiveness both in indigenous and admixture pigs.

Pig housing

No patterns were apparent in the type of housing provided, with semi-intensive sties and pens observed only in 41 percent of the sample households (Figure 2c–f). One in ten farmers keep their pigs tethered all the time around the homestead and gardens, and another 21 percent combine tethering and day-time scavenging. About one in ten pig farmers do not provide any shelter at all, and another 17 percent provide only night sheds, indicating that the rearing practices are predominantly of very low input. Generally, farmers reside in village settlements with at least several households residing close together, with the food gardens not far away. It is therefore expected that pigs are often tethered or kept in pen enclosures around the homestead.

Feeding and watering

Only 10 percent of the pig farmers mentioned the use of commercial feeds, which were fed to their pigs at least

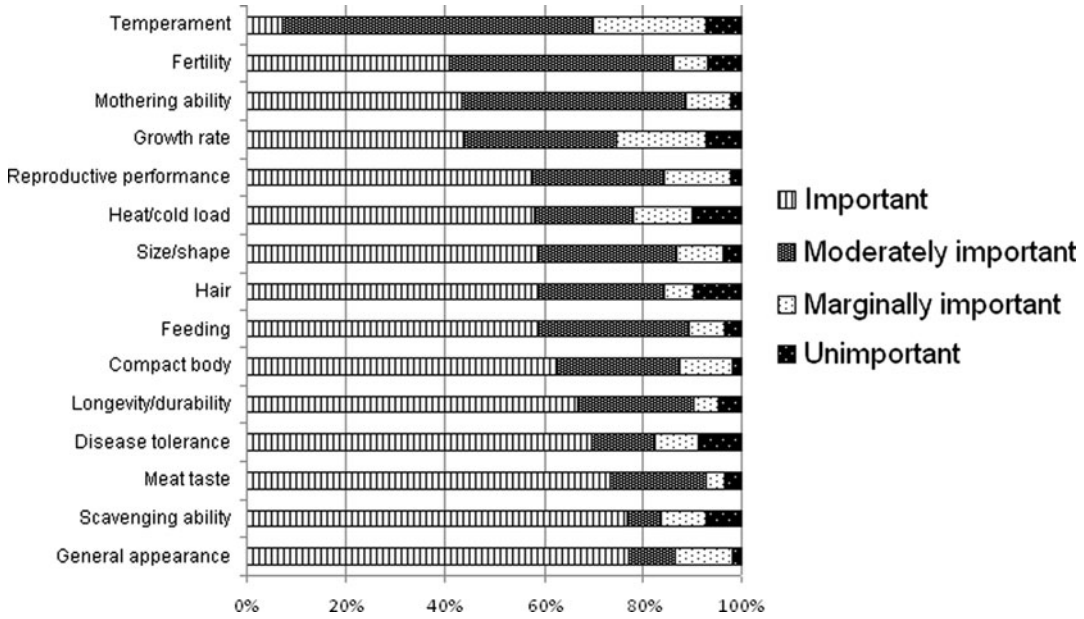

Figure 6. Rankings of importance of selected traits of indigenous pigs by their owners ($n = 82$) in three sampling sites.

Table 3. Body measurements (cm) of breeding males and females.

Statistical measures	Boar			Sow		
	Heart girth	Body length	Number of teats	Heart girth	Body length	Number of teats
Number of pigs measured (*n*)	22	22	29	46	46	54
Mean	78.2	56.6	10.1	78.3	54.4	10.7
Median	80	51	10	74.5	52	10
Minimum	48	39	8	45	30	7
Maximum	104	90	12	125	105	16
Standard deviation	17	15.9	1.06	20.3	16	1.54
Standard error of mean	3.62	3.31	0.2	3	2.34	0.21
CV (%)	21.7	28	10.4	26	29.4	14.4

once a week. The majority of farmers use a variety of local feeds (52 percent), let the animals freely scavenge (9 percent) or a combination (29 percent) of these two. Supplementary feeds are provided twice a day in some cases. Water supply to pigs was never mentioned as a constraint to pig production, but when water is made available, it is mostly (71 percent) supplied once everyday.

Relevant policy and legislation

PNG needs a national strategy for the identification, sustainable use and conservation of its pig genetic resources. Such a strategy is needed to provide direction and guidance to maintain and develop the vital livelihood support and socio-cultural services of PNG indigenous pigs in much of the country. It can provide a common platform for interaction and collaboration between various stakeholders such as livestock owners and farmers, policy-makers, scientists, private sector, civil society organizations and donors. In planning the effective use of this genetic resource, it is necessary to take account of limited human and financial resources available within the country. It is therefore important to engage all stakeholders in drawing up a national strategy to ensure comprehensive understanding of the potential of these pig genetic resources towards their optimal utilization for the benefit of present generations without unduly compromising options for future generations.

A critical constraint towards the development of a national strategy is the incomplete (or lack of) baseline information on the identity, diversity, distribution, utilization and current status of the indigenous pig genetic resources of the country. It is a general concern that in the face of uncontrolled continued spread of exotic and cross-bred pig genotypes through commercialization in hitherto unaffected natural habitats of PNG indigenous pig populations, no indigenous pig genetic evaluation or improvement activities are underway or firmly planned. This is compounded by the inadequate technical and logistic capacity in the design and operationalization of appropriate programmes for the management of pig genetic resources.

PNG has legislation governing quarantine and sanitary aspects of management of animal genetic resources, implemented by the National Agricultural Quarantine and Inspection Authority. This legislation prevents indiscriminate import of animal genetic materials and imposes strict quarantine protocols in the movement of both indigenous and exotic genetic resources into and out of the country. A standard (generic) germplasm transfer form is also available to regulate the import and export of live animals or biological samples from them. However, no action is being taken to at least sensitize the general public on the negative long-term effects of uncontrolled distribution of unknown grades of cross-bred pigs emanating from commercial piggeries for mating in the villages. The unintended and undesirable consequences of indiscriminate mating of these crosses with indigenous pig populations need to be discussed at various research and development forums in the country and acted upon.

Existing sanitary and quarantine legislation concerning food production standards does not affect the actual use, development and conservation of animal genetic resources. There is not a single operational conservation farm or herd of indigenous pigs in the country, although the National Agricultural Research Institute (NARI) plans to establish such herds at Labu in the lowlands and/or Tambul in the highlands.

Needs for research and development

A primary consideration for research and development of PNG indigenous pigs is a need to have a national inventory of the indigenous pig genetic resources and their production environment through a baseline survey. This study should also cover estimation of pig population size, their structure and dynamics. Along with comprehensive phenotypic characterization, the genetic differentiation of PNG indigenous pig populations needs to be explored through molecular genetic studies. The apparently age-long practice of castrating boars in village herds to allow sows to mate with wild pigs may have genetic significance. Sampling of wild (feral) pigs in PNG may be needed to provide molecular genetic data that could be integrated

with available morphological and biogeographical information to help elucidate the origin, taxonomic affinities and level of differentiation among the different populations of indigenous pigs of PNG. This could help to identify evolutionarily significant units (Moritz, 1994) and the planning of management plans for indigenous pigs in PNG.

As established through nation-wide stakeholder consultation by NARI, the public livestock research system should focus on addressing: (1) inadequate feeds and nutrition for both subsistent and commercial production operations; (2) high mortality of piglets and (3) low reproductive rates (NARI 2006a, b). Action is also needed to address the limited preparedness to manage likely major outbreaks of contagious diseases such as swine flu and swine cholera. The public veterinary services do not have the capacity to handle this. Although a limited number of large commercial piggeries continue to struggle with rising cost of feed made from imported ingredients, the formal pork market supplies imported cuts at competitive prices. On the other hand, emerging smallholder market-oriented pig growers, required to comply with safety standards to enter the market chain, are constrained by limited capacity as well as high cost of public inspection protocols. These policy related issues need to be closely investigated with the view of encouraging participation of smallholder farmers in the fast growing domestic pig meat market and providing immediate incentives for sustainable use of indigenous pigs in the country.

Conclusion and recommendations

The largely indigenous but loosely interbreeding population of pigs of PNG has considerable economic, genetic and cultural significance to the people of PNG. This population has remained under continuous genetic introgression from introduced local and cross-bred pigs from commercial piggeries as well as from public extension services that unsuccessfully promoted cross-breeding with exotic pigs to increase subsistent pig production at village level. To date even in isolated rural villages up to a third of pigs maintained by subsistent pig farmers are considered to be admixtures of indigenous and introduced genotypes. A third of surveyed pig farmers believe that the population of indigenous pigs is decreasing, while another third thinks it is on the increase.

Local names of PNG indigenous pigs do not suggest breed identities, and animals show variable body form and appearance across major pig growing areas of the country. Village pig herds range in size from 1 to 138, with an overall average of 6.23. Breeding boar numbers vary from zero to five, but only 12 percent reported to be using their own boar, whereas the rest mentioned use of boars in their village (39 percent), rely on known communal boars (20 percent), or opt to leave the sows to freely mate. Very little, if any, external inputs are used in raising pigs, and much of feed comes from scavenging, garden fodder and kitchen waste.

The genetic differentiation of this population is yet to be explored. It is therefore recommended that a nation-wide baseline survey of indigenous pig populations be conducted to identify and document genetically distinct indigenous pig populations in the country to provide the basis for a national management plan. It is also recommended that comprehensive molecular genetic studies be undertaken to elucidate the genetic attributes and differentiation of this probably unique genetic resource.

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References

Allen, M.S., Matisoo-Smith, E. & Horsburgh, A. 2001. Pacific 'Babes: issues in the origins and dispersal of pacific pigs and the potential of mitochondrial DNA analysis. *Int. J. Osteoarchaeol.*, 11: 4–13.

Dwyer, P.D. 2006. People, pigs and parasites in New Guinea: relational contexts and epidemiological possibilities. *Parasitol. Int*., 55: 167–173.

FAO. 2010. Domestic Animal Diversity Information System (DAD-IS). Food and Agriculture Organization (available at www.fao.org/dad-is). Accessed on 14 September 2010.

FAOSTAT. 2010. FAO Statistics Division 2010 (available at http://faostat.fao.org/site/573/DesktopDefault.aspx?PageID=573#ancor). Accessed on 14 September 2010.

Hide, R. 2003. Pig Husbandry in New Guinea. A Literature Review and Bibliography. ACIAR Monograph No. 108, xvi + 291 p., Canberra, Australian Centre for International Agricultural Research.

Kambuou, R. 1996. The current status and prospects of plant genetic resources in Papua New Guinea. *Harvest*, 18(1–2): 26–70.

Kirch, P.V. 1997. *The Lapita peoples: ancestors of the oceanic world*. Oxford, Blackwell.

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

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. & Cooper, A. 2005.

Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science*, 307: 1618–1621.

Lemonnier, P. 2002. Pigs as ordinary wealth: technical logic, exchange and leadership in New Guinea. *Technological choices: transformations in material cultures since the Neolithic*. pp. 126–155, Cornwall (UK), TJ International Ltd.

Lum, J.K., Mcintryre, J.K., Greger, D.L., Huffman, K.W. & Vilar, M.G. 2006. Recent Southeast Asian domestication and Lapita dispersal of sacred male pseudohermaphroditic "tuskers" and hairless pigs of Vanuatu. *Proc. Natl. Acad. Sci. USA*, 103: 17190–17195.

Malynicz, G.L. 1973a. The productivity of exotic and indigenous pigs under village conditions: Part 1. *Papua New Guinea Agric. J*., 24(1): 16–19.

Malynicz, G.L. 1973b. The productivity of exotic and indigenous pigs under village conditions: Part 2. *Papua New Guinea Agric. J*., 24 (1): 20–22.

MoA&L (Ministry of Agriculture and Livestock of Papua New Guinea). 2004. Papua New Guinea Country Report to the State of the World's Animal genetic Resources. November 2004. MoA&L. Port Moresby, Papua New Guinea. 54 p.

Moritz, C.C. 1994. Defining 'evolutionarily significant units' for conservation. *Trends Ecol. Evol*. 9: 373–375.

NARI (National Agricultural Research Institute of Papua New Guinea). 2006a. NARI Strategic Programme Implementation Plan for the period 2006 to 2010: Programmes and projects. NARI Corporate Document No. 4, Volume 2, 97 pp.

NARI (National Agricultural Research Institute of Papua New Guinea). 2006b. Strategic Plan: 2006–2015, Towards Innovative Agricultural Development in Papua New Guinea. NARI Corporate Document No. 3. NARI, Lae, PNG. 47 pp.

Nidup, K., Ayalew, W., Danbaro, G., Gongora, J. & Moran, C. 2010. *Genetic structure and diversity of indigenous pigs of Papua New Guinea. Annual Postgraduate Conference*. P53, 1–2 November 2010. Camperdown Campus, Faculty of Veterinary Science, University of Sydney, Australia.

Peter, C., Bruford, M., Perez, T., Dalamitra, S., Hewitt, G., Erhardt, G. & the ECONOGENE Consortium. 2007. Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. *Anim. Genet*., 38 (1): 37–44.

Quartermain, A.R. 2002. Conservation of animal genetic resources in PNG. Lae Papua New Guinea., National Agricultural Research Institute. NARI Technical Bulletin, No. 4.

Reilly, B. 2008. Ethnic conflict in Papua New Guinea. *Asia Pacific Viewpoint*, 49(1): 12–22.

CHAPTER 7

7.0 General Discussion

7.1 INTRODUCTION

This thesis has investigated and documented the physical characteristics of indidgenous domestic pigs, population trends, farming practices, and their socio-cultural and economic importance to the livelihood of many rural communities in South Asia and PNG. Findings demonstrated the versatility of microsatellite markers and mitochondrial DNA sequences in analyses of population structure, genetic diversity, origin, and domestication of pigs. This research has shown genetic structure and high levels of biodiversity within and among South Asian and PNG pig populations. These are useful baseline scientific information on which any policy or holistic conservation decision related to pigs in the regions should be based. This chapter will focus on research gaps in current studies and how this and related porcine research in South Asia and PNG might be addressed in future.

7.2 GAPS IN CURRENT RESEARCH

7.2.1 Biodiversity and Sampling Strategy

Genetic biodiversity is necessary for a species to adapt and survive. A species that has a large degree of genetic diversity among its population will have more variation from which natural selection can choose. Species that have very little genetic variation are at a greater risk. The indigenous pig populations of both South Asia and PNG are morphologically uniform but exhibit reasonably high genetic diversity and variability when assessed with standard neutral molecular markers. It is not possible to anticipate which component of hidden genetic variation will be useful for indigenous pigs to survive or outperform exotic breeds or to contribute to the gene pool of domestic animals in developed countries and presence of any such novel genetic traits remains to

be explored. The fact is that the indigenous pigs have thrived well under harsh rural conditions and this may be attributed to their adaptedness coupled with their socio-cultural importance to the rural poor communities. However, farmers have not been able to make extensive use of indigenous pigs in the transition from village scavenging to semi-intensive production systems, due to slow growth rate and poorer reproductive performance when compared to exotic breeds, which were promoted by the governments in South Asia and PNG for several decades. This does not mean that the native pigs could not contribute useful genes for disease resistance and climatic adaptation in a suitable backcrossing program to produce more suitable pigs for semi-intensive production (or alternatively introducing genes for high growth and fecundity into a village pig genetic background). Thus, no opportunity has been provided to discover and exploit such genetic variation and it risks being lost due to lack of promotion and conservation of indigenous pigs in the region. Consequently, the declining population of indigenous pigs is a major concern and may compromise options for enhancing future food security in the face of global climate change and subsequent environmental problems. Several studies have shown that population size, genetic variability and different fitness related traits (e.g. fertility and viability) were positively correlated with each other (Lundqvist *et al.* 2008). Considering this and other reasons outlined in previous chapters, it was necessary to evaluate the current level of genetic diversity and variability of indigenous pig populations in the regions.

However, further study leading to identification of novel genetic variation underlying complex traits is the essential and difficult next step in biodiversity management and conservation. Although measurement or assessment of biologically complex traits such as disease resistance or unique physiological or climatic adaptation would be preferable to recording morphological traits such as size and colour and would provide greater justification for biodiversity conservation, such traits could not possibly be measured in this first stage study, where even obtaining the DNA samples alone was a major logistic challenge.

Biodiversity sampling is a very challenging task and there is no perfect sampling strategy. Nevertheless, it is best to sample the entire population or use simple random sampling method but the fact is researchers seldom have the luxury of time, money, resources, and logistic arrangements to access the whole population. Therefore,

sampling in both PNG (Chapter 6, Part II) and South Asia (Sections 2.1, 2.2 & 2.3) employed a stratified sampling method (random within target groups) (Denzin & Lincoln, 2000; Ferrarini, 2012) for collecting most samples of domestic pigs. In addition, some domestic samples were collected using opportunistic or non-strategic sampling. However, only opportunistic sampling was used for collecting wild boar samples.

The non-strategic method, which was also used for sampling a small number of domestic pig samples (<10) is unlikely to have had any significant impact on the overall findings. However, it was not possible to test the effect of non-strategic sampling on the level of biodiversity of wild boar in the region. Irrespective of the methods of sampling, samples of wild boar come from various parts of the country (See Table 2.2 & Figure 4.1c). This indicates meaningful 'representativeness' of the diversity of genetic composition of wild boar across the country. Having said this, it would be useful, to acquire through appropriate strategic sampling, as many phenotypic details and DNA samples as possible to further improve our understanding through deeper insight into the distribution and optimum biodiversity of both domestic and wild pigs in South Asia and PNG**.**

7.2.2 South Asia

There are more than two mitochondrially defined wild boar populations in India (Chapter 5). In addition to D3 (Larson *et al.* 2005) or MC1 haplotypes (Larson *et al.* 2010; Nidup *et al.* unpublished), the Northern South Asia (NSA) haplotypes belonging to wild boar from central and northern India have been confirmed in Chapter 5. However, samples from South Indian wild boar, which is believed to have similar body size to that of the Sri Lankan wild boar (Groves 2008) but to be smaller than wild boar within MC1 clade*,* have not been analysed. It would be interesting from the phylogeographic point of view to check the possibility of gene flow from other (if any) mitochondrially defined Indian wild boar to local domestic pigs, which comprises 78% of the total 13 million pigs in India (LCI 2007). Apart from difficulties arising from the limited number of wild boar sequences and lack of any archaeological data, there are no mtDNA sequences from Indian domestic pigs in Genbank. This has limited the scope

for investigating further independent and the local centres of domestication of pigs in India.

The current evidence for the W12 haplotype in Bhutan and the northeast Indian state of Sikkim (Chapter 5) provides a strong motivation for analysing both wild and domestic pig samples from other northeast Indian States, including Assam, West Bengal, Arunachal Pradesh, Meghalaya, Manipur, and Nagaland. It would not only provide insights into domestication of wild boar in the region but also possible movement of people from northeast India to Myanmar, where W12 was been initially thought to have originated and an independent centre of domestication has been assumed (Larson *et al.* 2005). Interestingly, domestication, which is often defined in terms of identical mtDNA sequences or shared haplotypes between wild boar and domestic pig, is still arguable as no domestic pig having W12 has been detected until now except in Australian feral pigs and one indigenous domestic pig of PNG. While no further mtDNA sequences from Bhutanese domestic pigs are required, the Bhutanese wild boar are very much under-represented and further sampling would be valuable.

In Nepal, two indigenous domestic pig breeds, Chwanche and Bampudke, have yet to be analysed. While the population of Chwanche is stable, the Bampudke breed is almost extinct (Nidup *et al.* 2010). Currently, only two wild boar sequences (DQ779421 & DQ779422), including one generated as part of the studies included in this thesis from a museum sample, are available in Genbank. With limited sequences, it was not possible to gain a clear a picture of variation of mtDNA of wild boar in Nepal. Therefore, analyses of DNA samples from Chwanche, Bampudke, and wild boar in any future studies would provide a better picture of genetic biodiversity of pigs in Nepal.

Domestic pig farming in Islamic states (Pakistan, Bangladesh, Afghanistan) across South Asia is virtually non-existent (Nidup 2006) due to religious edicts. However, wild boar commonly invade cultivated fields and have been described by the locals as a "perennial menace" (Bedi 2002; Maher 2009). There is currently only one wild boar mtDNA sequence from Pakistan available in Genbank, despite the large population of this invasive pest. Even worse, not one sequence of wild boar from neighbouring Afghanistan has been reported, although wild boar is quite intrusive in the region.

Therefore, it is important to acquire and analyse additional samples to better map the biodiversity and phylogeography of wild boar in the region.

7.2.3 Papua New Guinea

PNG has more than twenty provinces but indigenous domestic pigs have been sampled from only three provinces, namely, Morobe, Enga and Western Highlands. Having found a considerable level of microsatellite and mitochondrial diversity in just three provinces, it would be interesting to investigate more thoroughly the pig genetic resources that PNG may be harbouring in the remaining provinces that were not sampled in this study. Considering the long and close association of pigs with humans, who live in remote and ethnically diverse communities, there could be more distinct populations of both indigenous domestic and wild pigs in PNG. Unfortunately, for the studies reported in this thesis, it was not possible to obtain samples of wild or feral pigs from PNG. In contrast to the earlier reports that feral pigs do not exist in the highlands of PNG (Hide 2003), pig farmers in the Tambul-Nebilyer, Mul-Baiyer and Jimi districts of Western Highlands province confirmed the existence of feral pigs in their areas and frequent cases of mating with their domestic sows (Ayalew, *Personal communication*). It will be important that future studies of PNG pigs include wild pigs so as to determine the level of gene flow from wild to domestic or *vice versa.* Larson *et al.* (2007) proposed that the wild pigs of PNG are ancient ferals of Southeast Asian domestic origin introduced into PNG thousands of years ago. However, analysis of ancient DNA as well as extensive sampling of modern wild pig will be pivotal to better understand the origins and biodiversity of pigs in PNG.

7.2.4 Pooling of Genotype Data for Meta-Analysis

In the analyses reported in this thesis, several attempts were made to access genotype data, which were generated during European pig biodiversity projects (Archibald *et al.* 1995; Ollivier *et al.* 2003; Ollivier 2009). Unfortunately, the pig genetic diversity database maintained at the Roslin Institute (Russell *et al.* 2003) has not been maintained and has become non-functional. Genotype data from other studies were not available either except for allele frequency summaries, which cannot be used for comparative studies due to the need to calibrate allele sizes between studies. However, Dr Louis

Ollivier in France has kindly provided raw genotype data from the European pig genetic diversity projects (Ollivier *et al.* 2005). To enable utilisation of these data, two European PigMap Reference samples (F9110010 and F9119912) obtained from Dr Denis Milan, INRA Toulouse, France were genotyped. Using allele size calls from the reference samples, an attempt was made to calibrate the fragment sizes for the genotype data obtained from South Asian and European pigs. While the heterozygosity showed similar patterns, there were some unresolvable discrepancies in allele sizes, probably resulting from differences in electrophoresis systems and fluorescent detection methods (e.g. ABI 730 vs ABI 377), different size standard, and the methods employed for calling alleles. It became apparent that pooling of genotype data generated in different laboratories and calibrating them based solely on a small number of standard reference genotypes would induce large errors leading to misinterpretation of population data. Therefore, the result from such analysis was excluded from this thesis. However, another attempt will be made in future to analyse limited subsets of the data where pooling may be possible and also to report on the major hurdles associated with such pooled analyses.

Another problem with microsatellite technology is the difficulty to integrate such data with Single Nucleotide Polymorphism (SNP) data. Microsatellites generally have multiple alleles per locus, often very large numbers of alleles, and are relatively highly mutable. For SNPs, two alleles per site is almost inevitably the case and the mutation rate at a SNP site is orders of magnitude lower than for a microsatellite locus. This means that there are fundamental differences in the way in which microsatellites and SNPs behave in an evolutionary sense and thus in which they are analysed and interpreted in population data. However, by amalgamating SNPs into haplotypes, the effect is similar to having multiple alleles. If Next Generation Sequencing (NGS) is used for genotyping, then both SNP and microsatellite data may both be acquired simultaneously, with reliability of genotyping depending on the depth of sequencing.

7.3 CHOOSING THE APPROPRIATE TECHNOLOGY

7.3.1 Future of Microsatellites

Today, microsatellite technology is fast becoming outdated and is being replaced by SNP genotyping and NGS (Mardis 2008), especially for well studied and characterised species. During the 32nd ISAG conference in Edinburgh in 2010, UK, there was debate about completely "doing-away" with microsatellite based phylogenetic and populations analyses of well characterised domestic animal species, due to difficulties in pooling genotype data across different laboratories and from past and present studies for meta-analysis (Section 7.2.4), the high cost, and the labour intensive and error-prone scoring of alleles (e.g. sample swapping, cross contamination, artefacts). At the same conference, in a workshop session on "Publishing and Participating in *Animal Genetics* – Advice for Early Career Scientists" chaired by the editor–in-chief of *Animal Genetics*, Professor Chris Moran of the University of Sydney, there was discussion about the future publishability of microsatellite-based population and phylogenetic studies in domestic species. During the open discussion, there were indications from both chief and associate editors of the journal that in the near future, manuscripts arising from microsatellite studies on well characterised domestic species may not be favourably considered for review and publication in *Animal Genetics*, unless they were well integrated with previous studies. The FAO has also indicated that it will ultimately switch to application of SNPs or NGS technologies for whole genome analyses to perform molecular characterization of farm animal genetic diversity (Boettcher *et al.* 2010). The future for microsatellite-based studies is thus bleak. It is a technology which has contributed enormously in understanding of population structures and relationships in well characterised species and will continue to do so for less-well-characterised species, such as new species in aquaculture, but future research or grant proposals based on microsatellites in well-characterised species of domestic animals such as pigs, poultry or major ruminant species would be very unlikely to be favourably considered by granting agencies. Therefore, any future research efforts on pigs or any other major livestock in South Asia and PNG must be directed towards SNP genotyping or possibly even NGS technologies.

7.3.2 High-Throughput Sequencing for Future Research

7.3.2.1 Sequencing Technologies

High-Throughput Sequencing (HTS), also called NGS or Deep-Sequencing, is based on massively parallel sequencing of millions of different DNA fragments, therefore resulting in ultra-high-throughput of data. Massively Parallel Signature Sequencing or MPSS was the first of the "next-generation" sequencing technologies developed in the 1990s (Brenner *et al.* 2000) followed by several others (Shendure *et al.* 2005; Totty 2005). Today, technologies such as 454 pyrosequencing by Roche (Margulies *et al.* 2005), Analyzer II or GAII (Solexa) by Illumina (Bennett 2004; Mardis 2008), SOLiD by Applied Biosystems (Rubin *et al.* 2010), Helicos (Milos 2008) and Pacific Biosciences real time sequencing (Eid *et al.* 2009) allow the generation of large volumes of sequencing data in a fast, accurate and inexpensive way (Rothberg *et al.* 2011). The advent of these technologies is already revolutionizing the field of animal genetics including porcine genetics and genomics. One of the areas where these technologies have been widely applied is SNP discovery in the pig genome (Wiedmann *et al.* 2008; Amaral *et al.* 2009; Ramos *et al.* 2009).

7.3.2.2 Porcine SNPs

Hundreds of thousands of SNPs in the porcine genome have been identified (Ramos *et al.* 2009; Groenen *et al.* 2010) using the Illumina GAII sequencing platform. Together with a few hundreds of SNPs generated using conventional Sanger sequencing, these SNPs generated with NGS or HTS were used to develop the Illumina PorcineSNP60 Beadchip, which is the most comprehensive genome-wide genotyping array for the porcine genome, providing superior power to interrogate genetic variation across several pig breeds including wild boar populations. It features more than 62,000 evenly spaced SNPs covering the entire porcine genome (Ramos *et al.* 2009) thereby enabling a diverse range of genetic research applications. Using this chip, around 2400 pigs from 60 breeds including local and commercial ones, and 30 different wild boar populations distributed throughout Eurasia have been genotyped (Groenen *et al.* 2010). Besides enabling whole-genome characterization of linkage disequilibrium and haplotype structure in pigs (Amaral *et al.* 2008; Amaral 2010), the chip also allows investigation of signatures of selection associated with domestication and breed formation as well as

elucidating demographic events such as population bottlenecks, and providing insight into the complex origin of domesticated populations by examining the variability of haplotypes (Groenen *et al.* 2010). The breed characterization studies, which previously have been relatively expensive, are now becoming more affordable although there are some limitations with the use of SNPs in population biodiversity studies (Nidup & Moran 2010; Chapter 1, Part II). Despite their requirement for extensive computational analysis, the tools for which are improving (Kerstens 2010) the popularity of HTS and SNPs is rising rapidly Any future porcine conservation and biodiversity research in South Asia and PNG should consider using HTS technologies, either to identify new SNPs or possibly as an ultimate genotyping tool.

7.4 CONTROL REGION OR COMPLETE MITOCHONDRIAL GENOME

Many mtDNA studies, particularly on porcine mtDNA, have been based on D-loop or control region sequences. This was mainly due to high costs associated with conventional capillary based Sanger sequencing. With HTS technologies, complete mitochondrial genome sequences are produced as a by-product of nuclear genome sequencing at no extra cost. Therefore, future mitochondrial DNA studies should be based on complete mitochondrial genome sequences. Recent evidence suggests that partial control region inference can be misleading and may contradict whole mitochondrial genome analysis with populations of highest level of endangerment not being accurately resolved (Knight *et al.* 2009). A significant excess of amino acid changes, indicative of molecular adaptation, has been observed within the whole mitochondrial genome, while this obviously will not be visible to the control region analyses. Additionally, the impact of recurrent mutation appears mostly in closely related haplotypes, due to the low level of evolutionary signal (unique mutations that mark lineages) relative to evolutionary noise (recurrent, shared mutation in unrelated haplotypes). Knaus *et al.* (2011) proved that populations of greatest conservation concern may be at the highest risk of being misidentified by control region sequencing alone. In addition to these considerations, the complete mitochondrial genome provides a more refined estimate of divergence time as demonstrated recently by Fernandez *et al.* (2010).

7.5 Y-CHROMOSOME ANALYSES AND PATERNAL ORIGIN

Due to the low levels of polymorphism detected on the porcine Y-chromosome (Ramirez *et al.* 2009) and the high cost associated with conventional sequencing, no attempt was made to assess male-mediated introgression and polymorphism in South Asian and PNG pigs. Now that the HTS technologies have defined Y-chromosome polymorphisms in domestic pig breeds (Cliffe *et al.* 2010), it would be worth using Y-chromosome polymorphisms in investigating the genetic diversity and paternal origins for South Asian and PNG pigs. Ideally, it is essential to use nuclear gene, mitochondria, and Y-chormosome in phylogenetic and population biodiversity studies.

7.6 PORCINE GENOME

The porcine genome sequence, which has been obtained primarily using conventional technology (Schook *et al.* 2005; Humphray *et al.* 2007; Ramos *et al.* 2009; Archibald *et al.* 2010), is already contributing to many areas of swine research including those discussed in this thesis. It is allowing researchers to pinpoint genes that are useful to pork production or are involved in immunity or other important physiological processes in the pig. It will also enhance breeding practices, offer insight into diseases that afflict pigs (and, sometimes, also humans) and will assist in efforts to preserve the global heritage of rare, endangered and wild pigs. It will also be important for the study of human health because pigs are very similar to humans in their physiology, behaviour and nutritional needs and are used extensively in medical research.

7.7 CONCLUSION

South Asian countries, particularly Bhutan, Nepal, and Sri Lanka, and Papua New Guinea are signatories to the Convention on Biological Diversity (CBD) (UN 1992) and the *Interlaken Declaration on Animal Genetic Resources* (FAO 2007), whereby respective governments have confirmed their commitment to the conservation, sustainable use and development of animal genetic resources for improving human nutrition and for rural development. To implement the *Global Plan of Action* (FAO 2007) on animal genetic resources, indigenous pigs must be genetically and phenotypically characterised, utilised in a sustainable manner, promoted and protected

from extinction. To achieve this, the information presented in this thesis contributes baseline information for rationalising the conservation of South Asian and PNG pig genetic resources, and provides directions for future research.

Decisions on management of farm animal genetic resources should be based on a holistic understanding of the extent and pattern of genetic diversity in these resources, the patterns of variation in priority economic traits and specific adaptive attributes of existing populations as well as the population size and status (level of endangerment) of known breeds. Decisions should also be based on the availability of essential infrastructure for research and development and financial resources. To fully exploit priority economic and adaptative traits, whole genome sequencing using SNP and/or large scale HTS genotyping, are the way of the future for building on the results from conventional microsatellite genotyping and capillary-based sequencing. It is imperative that scientists must anticipate the rapid developments of HTS and to adapt them for use in phylogenetic, biodiversity or any other related studies.

7.8 REFERENCES

Amaral A.J. (2010) Nucleotide variation and footprints of selection in the porcine and chicken genomes. PhD Thesis. In: *Wageningen Institute of Animal Sciences (WIAS) Graduate School*, p. 160 pp. Wageningen University, Wageningen, Netherlands.

Amaral A.J., Megens H.J., Crooijmans R., Heuven H.C.M. & Groenen M.A.M. (2008) Linkage disequilibrium decay and haplotype block structure in the pig. *Genetics* **179**, 569-79.

Amaral A.J., Megens H.J., Kerstens H.H.D., Heuven H.C.M., Dibbits B., Crooijmans R., Den Dunnen J.T. & Groenen M.A.M. (2009) Application of massive parallel sequencing to whole genome SNP discovery in the porcine genome. *Bmc Genomics* **10**.

Archibald A.L., Bolund L., Churcher C., Fredholm M., Groenen M.A.M., Harlizius B., Lee K.T., Milan D., Rogers J., Rothschild M.F., Uenishi H., Wang J., Schook L.B. & Swine Genome Sequencing C. (2010) Pig genome sequence - analysis and publication strategy. *Bmc Genomics* **11**.

Archibald A.L., Haley C.S., Brown J.F., Couperwhite S., McQueen H.A., Nicholson D., Coppieters W., Vandeweghe A., Stratil A., Wintero A.K., Fredholm M., Larsen N.J., Nielsen V.H., Milan D., Woloszyn N., Robic A., Dalens M., Riquet J., Gellin J., Caritez J.C., Burgaud G., Ollivier L., Bidanel J.P., Vaiman M., Renard C., Geldermann H., Davoli R., Ruyter D., Verstege E.J.M., Groenen M.A.M., Davies W., Hoyheim B., Keiserud A., Andersson L., Ellegren H., Johansson M., Marklund L., Miller J.R., Dear D.V.A., Signer E., Jeffreys A.J., Moran C., Letissier P., Muladno, Rothschild M.F., Tuggle C.K., Vaske D., Helm J., Liu H.C., Rahman A., Yu T.P., Larson R.G. & Schmitz C.B. (1995) The PigMaP consortium linkage map of the pig (Sus scrofa). *Mammalian Genome* **6**, 157-75.

Ayalew W. (*Personal communication*) Existence of wild pigs in Highlands region of Papua New Guinea - information provided to W. Ayalew by agriculture extension staff (J. Pakatul and S. Amben). Papua New Guinea, Livestock Research Programme, National Agricultural Research Institute, Labu, Morobe Province.

Bedi R. (2002) Wild boar infiltrate Line of Control. URL http://www.atimes.com/ind-pak/DF13Df02.html.

Bennett S. (2004) Solexa Ltd. *Pharmacogenomics* **5**, 433-8.

Boettcher P., J, Lenstra J.A. & Ajmone-Marsan P. (2010) FAO guidelines on molecular genetic characterization of animal genetic resources (P3043). In: *32nd Conference of the International Society for Animal Genetics*, p. 76, Edinburgh, Scotland (UK).

Brenner S., Johnson M., Bridgham J., Golda G., Lloyd D.H., Johnson D., Luo S.J., McCurdy S., Foy M., Ewan M., Roth R., George D., Eletr S., Albrecht G., Vermaas E., Williams S.R., Moon K., Burcham T., Pallas M., DuBridge R.B., Kirchner J., Fearon K., Mao J. & Corcoran K. (2000) Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. *Nature Biotechnology* **18**, 630-4.

Cliffe K.M., Day A.E., Bagga M., Siggens K., Quilter C.R., Lowden S., Finlayson H.A., Palgrave C.J., Li N., Huang L., Blott S.C. & Sargent C.A. (2010) Analysis of the non-recombining Y chromosome defines polymorphisms in domestic pig

breeds: ancestral bases identified by comparative sequencing. *Animal Genetics* **41**, 619-29.

Denzin N.K. & Lincoln Y.S. (2000) *Handbook of qualitative research*. Sage Publications, London.

Eid J., Fehr A., Gray J., Luong K., Lyle J., Otto G., Peluso P., Rank D., Baybayan P., Bettman B., Bibillo A., Bjornson K., Chaudhuri B., Christians F., Cicero R., Clark S., Dalal R., Dewinter A., Dixon J., Foquet M., Gaertner A., Hardenbol P., Heiner C., Hester K., Holden D., Kearns G., Kong X.X., Kuse R., Lacroix Y., Lin S., Lundquist P., Ma C.C., Marks P., Maxham M., Murphy D., Park I., Pham T., Phillips M., Roy J., Sebra R., Shen G., Sorenson J., Tomaney A., Travers K., Trulson M., Vieceli J., Wegener J., Wu D., Yang A., Zaccarin D., Zhao P., Zhong F., Korlach J. & Turner S. (2009) Real-Time DNA Sequencing from Single Polymerase Molecules. *Science* **323**, 133-8.

FAO (2007) Global Plan of Action for Animal Genetic Resources and the Interlaken Declaration. FAO, Rome.

Fernandez A.I., Alves E., Ovilo C., Rodriguez M.C. & Silio L. (2010) Divergence time estimates of East Asian and European pigs based on multiple near complete mitochondrial DNA sequences. *Animal Genetics* **42**, 86-8.

Ferrarini A. (2012) Biodiversity optimal sampling: an algorithmic solution. *Proceedings of the International Academy of Ecology and Environmental Sciences* **2**, 50-2.

Groenen M.A.M., Megens H.J., Croojimans R.P.M.A., Larson G., Muir W.M., Enciso M.P., Archibald A., Ma J. & Schook L.B. (2010) Genome-wide assessment of diversity in the pig and other suids. In: *32nd International Society for Animal Genetics*, pp. 37, W0005, July 26-30, Edinburgh, UK.

Groves C. (2008) Current views on taxonomy and zoogeography of the genus *Sus*. In: *Pigs and humans: 10,000 years of interaction* (eds. by Albarella U, Dobney K & Ervynck A), pp. 15-29. Oxford University Press, Oxford, UK.

Hide R. (2003) *Pig husbandry in New Guinea. A literature review and bibliography*. ACIAR Monograph 108, Australian Centre for International Agricultural Research., Canberra.

Humphray S.J., Scott C.E., Clark R., Marron B., Bender C., Camm N., Davis J., Jenks A., Noon A., Patel M., Sehra H., Yang F.T., Rogatcheva M.B., Milan D., Chardon P., Rohrer G., Nonneman D., de Jong P., Meyers S.N., Archibald A., Beever J.E., Schook L.B. & Rogers J. (2007) A high utility integrated map of the pig genome. *Genome Biology* **8**.

Kerstens H.H. (2010) Bioinformatics' approaches to detect genetic variation in whole genome sequencing data. PhD thesis. In: *Wageningen Institute of Animal Sciences (WIAS) Graduate School*, p. 182. Wageningen University, Wageningen.

Knight J.T., Nock C.J., Elphinstone M.S. & Baverstock P.R. (2009) Conservation implications of distinct genetic structuring in the endangered freshwater fish Nannoperca oxleyana (Percichthyidae). *Marine and Freshwater Research* **60**, 34-44.

Larson G., Cucchi T., Fujita M., Matisoo-Smith E., Robins J., Anderson A., Rolett B., Spriggs M., Dolman G., Kim T.H., Thuy N.T.D., Randi E., Doherty M., Due R.A., Bollt R., Djubiantono T., Griffin B., Intoh M., Keane E., Kirch P., Li K.T., Morwood M., Pedrina L.M., Piper P.J., Rabett R.J., Shooter P., Van den Bergh G., West E., Wickler S., Yuan J., Cooper A. & Dobney K. (2007) Phylogeny and ancient DNA of Sus provides insights into neolithic expansion in island

southeast Asia and Oceania. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 4834-9.

Larson G., Dobney K., Albarella U., Fang M.Y., Matisoo-Smith E., Robins J., Lowden S., Finlayson H., Brand T., Willerslev E., Rowley-Conwy P., Andersson L. & Cooper A. (2005) Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science* **307**, 1618-21.

Larson G., Liu R.R., Zhao X.B., Yuan J., Fuller D., Barton L., Dobney K., Fan Q.P., Gu Z.L., Liu X.H., Luo Y.B., Lv P., Andersson L. & Li N. (2010) Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 7686-91.

LCI (2007) Livestock census of India. URL http://www.indiaagristat.com/agriculture/2/animalhusbandrylivestock/48/stats.aspx.

Lundquist A.C., Andersson S. & Lonn M. (2008) Genetic variation in wild plants and animals in Sweden - a review of case studies from perspective of conservation genetics. Swedish Environmental Agency, Stockholm, Sweden.

Maher A. (2009) Pakistan's new menace: Wild boars URL http://news.bbc.co.uk/2/hi/7697485.stm.

Mardis E.R. (2008) Next-generation DNA sequencing methods. *Annual Review of Genomics and Human Genetics* **9**, 387-402.

Margulies M., Egholm M., Altman W.E., Attiya S., Bader J.S., Bemben L.A., Berka J., Braverman M.S., Chen Y.J., Chen Z.T., Dewell S.B., Du L., Fierro J.M., Gomes X.V., Godwin B.C., He W., Helgesen S., Ho C.H., Irzyk G.P., Jando S.C., Alenquer M.L.I., Jarvie T.P., Jirage K.B., Kim J.B., Knight J.R., Lanza J.R., Leamon J.H., Lefkowitz S.M., Lei M., Li J., Lohman K.L., Lu H., Makhijani V.B., McDade K.E., McKenna M.P., Myers E.W., Nickerson E., Nobile J.R., Plant R., Puc B.P., Ronan M.T., Roth G.T., Sarkis G.J., Simons J.F., Simpson J.W., Srinivasan M., Tartaro K.R., Tomasz A., Vogt K.A., Volkmer G.A., Wang S.H., Wang Y., Weiner M.P., Yu P.G., Begley R.F. & Rothberg J.M. (2005) Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**, 376-80.

Milos P. (2008) Helicos BioSciences. *Pharmacogenomics* **9**, 477-80.

Nidup K. (2006) Swine resources in the Hindu Kush Himalayan region: a need for molecular genetic characterization. *SAARC Journal of Agriculture* **4**, 113-21.

Nidup K., Joshi D.D., Gongora J. & Moran C. (2010) Farming and Biodiversity of Indigenous Pigs in Nepal. *Biodiversity* **11**, 26-33.

Nidup K., Larson G., Gongora J., Joshi D.D., Silva G.L.L.P. & Moran C. (unpublished) Wild boar mitochondrial phylogeography, introgression, and dispersal in South Asia. *Manuscript under preparation*.

Ollivier L. (2009) European pig genetic diversity: a minireview. *Animal* **3**, 915-24.

Ollivier L., Alderson L., Gandini G.C., Foulley J.L., Haley C.S., Joosten R., Rattink A.P., Harlizius B., Groenen M.A.M., Amigues Y., Boscher M.Y., Russell G., Law A., Davoli R., Russo V., Matassino D., Desautes C., Fimland E., Bagga M., Delgado J.V., Vega-Pla J.L., Martinez A.M., Ramos A.M., Glodek P., Meyer J.N., Plastow G.S., Siggens K.W., Archibald A.L., Milan D., San Cristobal M., Laval G., Hammond K., Cardellino R. & Chevalet C. (2005) An assessment of European pig diversity using molecular markers: Partitioning of diversity among breeds. *Conservation Genetics* **6**, 729-41.

Ollivier L., Amigues Y. & Boscher M.Y. (2003) An EC-funded project on characterization of genetic variation in the European pig. Objectives, organization, breed sampling, DNA preparation, and circulation. *Arch. Zootec* **52**, 137-44.

Ramirez O., Ojeda A., Tomas A., Gallardo D., Huang L.S., Folch J.M., Clop A., Sanchez A., Badaoui B., Hanotte O., Galman-Omitogun O., Makuza S.M., Soto H., Cadillo J., Kelly L., Cho I.C., Yeghoyan S., Perez-Enciso M. & Amills M. (2009) Integrating Y-Chromosome, Mitochondrial, and Autosomal Data to Analyze the Origin of Pig Breeds. *Molecular Biology and Evolution* **26**, 2061-72.

Ramos A.M., Crooijmans R.P.M.A., Affara N.A., Amaral A.J., Archibald A.L., Beever J.E., Bendixen C., Churcher C., Clark R., Dehais P., Hansen M.S., Hedegaard J., Hu Z.L., Kerstens H.H., Law A.S., Megens H.J., Milan D., Nonneman D.J., Rohrer G.A., Rothschild M.F., Smith T.P.L., Schnabel R.D., Tassell C.P.v., Taylor J.F., Wiedmann R.T., Schook L.B. & Groenen M.A.M. (2009) Design of a high density SNP genotyping assay in the pig using SNPs identified and characterized by next generation sequencing technology. *Plos One*, e6524.

Rothberg J.M., Hinz W., Rearick T.M., Schultz J., Mileski W., Davey M., Leamon J.H., Johnson K., Milgrew M.J., Edwards M., Hoon J., Simons J.F., Marran D., Myers J.W., Davidson J.F., Branting A., Nobile J.R., Puc B.P., Light D., Clark T.A., Huber M., Branciforte J.T., Stoner I.B., Cawley S.E., Lyons M., Fu Y.T., Homer N., Sedova M., Miao X., Reed B., Sabina J., Feierstein E., Schorn M., Alanjary M., Dimalanta E., Dressman D., Kasinskas R., Sokolsky T., Fidanza J.A., Namsaraev E., McKernan K.J., Williams A., Roth G.T. & Bustillo J. (2011) An integrated semiconductor device enabling non-optical genome sequencing. *Nature* **475**, 348-52.

Rubin C.J., Zody M.C., Eriksson J., Meadows J.R.S., Sherwood E., Webster M.T., Jiang L., Ingman M., Sharpe T., Ka S., Hallbook F., Besnier F., Carlborg O., Bed'hom B., Tixier-Boichard M., Jensen P., Siegel P., Lindblad-Toh K. & Andersson L. (2010) Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature* **464**, 587-U145.

Russell G.A., Archibald A.L., Haley C.S. & Law A.S. (2003) The pig genetic diversity database and the WWW. *Archivos de Zootecnia* **52**, 165-72.

Schook L.B., Beever J.E., Rogers J., Humphray S., Archibald A., Chardon P., Mllan D., Rohrer G. & Eversole K. (2005) Swine Genome Sequencing Consortium (SGSC): a strategic roadmap for sequencing the pig genome. *Comparative and Functional Genomics* **6**, 251-5.

Shendure J., Porreca G.J., Reppas N.B., Lin X.X., McCutcheon J.P., Rosenbaum A.M., Wang M.D., Zhang K., Mitra R.D. & Church G.M. (2005) Accurate multiplex polony sequencing of an evolved bacterial genome. *Science* **309**, 1728-32.

Totty M. (2005) "A Better Idea". URL http://online.wsj.com/public/article/SB112975757605373586-Z9c37xOR0aRxcPzd_hkq9fcF8Ls_20061024.html.

UN (1992) The Convention on Biological Diversity. URL http://www.cbd.int/convention/convention.shtml.

Wiedmann R.T., Smith T.P.L. & Nonneman D.J. (2008) SNP discovery in swine by reduced representation and high throughput pyrosequencing. *Bmc Genetics* **9**, 7.

CHAPTER 8

Appendix

Appendix 1: Porcine haplotypes from Asia and Oceania analysed with South Asian porcine haplotypes.

Table 8 A.1: East Asian haplotypes, which were identified by Larson *et al.* (2010) were combined and analysed with South Asian haplotypes in this study. These haplotypes represent more than 1700 pigs comprising both domestic and wild pigs from Asian and Oceania.

Accession No.	Country	Location	Status	Haplotype
Wu *et al*. 2007				
DQ496498	China	Shandong	Domestic	EA3
DQ496500	China	Shandong	Domestic	EA6
DQ496503	China	Shandong	Domestic	EA6
DQ496504	China	Shandong	Domestic	EA6
DQ496506	China	Shandong	Domestic	EA6
DQ496507	China	Shandong	Domestic	EA6
DQ496508	China	Shandong	Domestic	EA6
DQ496509	China	Shandong	Domestic	EA6
DQ496510	China	Shandong	Domestic	EA6
DQ496511	China	Shandong	Domestic	EA6
DQ496502	China	Shandong	Domestic	EA7
DQ496505	China	Shandong	Domestic	EA7
DQ496501	China	Shandong	Domestic	EA144
DQ496499	China	Shandong	Domestic	EA144
DQ496541	China	Shandong	Domestic	EA7
DQ496542	China	Shandong	Domestic	EA7
DQ496543	China	Shandong	Domestic	EA7
DQ496544	China	Shandong	Domestic	EA7
DQ496545	China	Shandong	Domestic	EA7
DQ496546	China	Shandong	Domestic	EA7
DQ496547	China	Shandong	Domestic	EA7
DQ496979	China	Shandong	Domestic	EA3
DQ496980	China	Shandong	Domestic	EA3
DQ496981	China	Shandong	Domestic	EA3
DQ496983	China	Shandong	Domestic	EA3
DQ496978	China	Shandong	Domestic	EA168
DQ496982	China	Shandong	Domestic	EA168
DQ496984	China	Shandong	Domestic	EA168
AF486868	China	Shandong	Domestic	EA168
DQ496976	China	Shandong	Domestic	EA144
DQ496977	China	Shandong	Domestic	EA169
DQ496986	China	Shandong	Domestic	EA170

Accession No.	Country	Location	Status	Haplotype
DQ496985	China	Shandong	Domestic	EA6
DQ496435	China	Sichuan, Ganzi	Domestic	EA151
DQ496306	China	Yunnan	Domestic	EA2
DQ496307	China	Yunnan	Domestic	EA65
DQ496304	China	Yunnan	Domestic	EA10
DQ496303	China	Yunnan	Domestic	EA10
DQ496314	China	Yunnan	Domestic	EA188
DQ496358	China	Yunnan	Domestic	EA14
DQ496359	China	Yunnan	Domestic	EA14
DQ496360	China	Yunnan	Domestic	EA14
DQ496363	China	Yunnan	Domestic	EA10
DQ496361	China	Yunnan	Domestic	EA11
DQ496380	China	Yunnan	Domestic	EA14
DQ496382	China	Yunnan	Domestic	EA14
DQ496387	China	Yunnan	Domestic	EA14
DQ496366	China	Yunnan	Domestic	EA14
DQ496369	China	Yunnan	Domestic	EA14
DQ496370	China	Yunnan	Domestic	EA14
DQ496378	China	Yunnan	Domestic	EA14
DQ496379	China	Yunnan	Domestic	EA14
DQ496367	China	Yunnan	Domestic	EA65
DQ496374	China	Yunnan	Domestic	EA179
DQ496386	China	Yunnan	Domestic	EA179
DQ496377	China	Yunnan	Domestic	EA11
DQ496423	China	Yunnan	Domestic	EA149
DQ496553	China	Yunnan	Domestic	EA189
DQ496580	China	Yunnan	Domestic	EA189
DQ496572	China	Yunnan	Domestic	EA14
DQ496573	China	Yunnan	Domestic	EA14
DQ496549	China	Yunnan	Domestic	EA146
DQ496550	China	Yunnan	Domestic	EA146
DQ496548	China	Yunnan	Domestic	EA146
DQ496576	China	Yunnan	Domestic	EA188
DQ496577	China	Yunnan	Domestic	EA188
DQ496578	China	Yunnan	Domestic	EA188
DQ496655	China	Yunnan	Domestic	EA190
DQ496656	China	Yunnan	Domestic	EA190
DQ496660	China	Yunnan	Domestic	EA190
DQ496676	China	Yunnan	Domestic	EA190
DQ496677	China	Yunnan	Domestic	EA190
DQ496679	China	Yunnan	Domestic	EA190
DQ496680	China	Yunnan	Domestic	EA190
DQ496678	China	Yunnan	Domestic	EA14
DQ496663	China	Yunnan	Domestic	EA14
DQ496683	China	Yunnan	Domestic	EA14
DQ496688	China	Yunnan	Domestic	EA14
DQ496664	China	Yunnan	Domestic	EA14
DQ496667	China	Yunnan	Domestic	EA14
DQ496681	China	Yunnan	Domestic	EA14
DQ496691	China	Yunnan	Domestic	EA14
DQ496659	China	Yunnan	Domestic	EA2

Accession No.	Country	Location	Status	Haplotype
DQ496668	China	Yunnan	Domestic	EA188
DQ496859	China	Yunnan	Wild	EA14
DQ496858	China	Yunnan	Wild	EA79
DQ496860	China	Yunnan	Wild	EA88
DQ496863	China	Yunnan	Wild	EA14
DQ496874	China	Yunnan	Wild	EA14
DQ496862	China	Yunnan	Wild	EA2
DQ496881	China	Yunnan	Wild	EA2
DQ496883	China	Yunnan	Wild	EA2
DQ496884	China	Yunnan	Wild	EA2
DQ496871	China	Yunnan	Wild	EA2
DQ496783	China	Yunnan	Wild	EA10
DQ496880	China	Yunnan	Wild	EA18
DQ496867	China	Yunnan	Wild	EA18
DQ496879	China	Yunnan	Wild	EA65
DQ496872	China	Yunnan	Wild	EA10
DQ496851	China	Yunnan	Wild	EA191
DQ496857	China	Yunnan	Wild	EA192
DQ496854	China	Yunnan	Wild	EA192
DQ496878	China	Yunnan	Wild	EA14
DQ496392	China	Yunnan	Domestic	EA193
DQ496402	China	Yunnan	Domestic	EA146
DQ496403	China	Yunnan	Domestic	EA146
DQ496400	China	Yunnan	Domestic	EA188
DQ496406	China	Yunnan	Domestic	EA188
DQ496407	China	Yunnan	Domestic	EA188
DQ496394	China	Yunnan	Domestic	EA194
DQ496381	China	Yunnan	Domestic	EA65
DQ496743	China	Yunnan, Banna	Wild	EA81
DQ496310	China	Yunnan, Baoshan	Domestic	EA18
DQ496320	China	Yunnan, Baoshan	Domestic	EA18
DQ496311	China	Yunnan, Baoshan	Domestic	EA149
DQ496312	China	Yunnan, Baoshan	Domestic	EA149
DQ496317	China	Yunnan, Baoshan	Domestic	EA195
DQ496321	China	Yunnan, Baoshan	Domestic	EA196
DQ496327	China	Yunnan, Baoshan	Domestic	EA196
DQ496316	China	Yunnan, Baoshan	Domestic	EA65
DQ496318	China	Yunnan, Baoshan	Domestic	EA65
DQ496319	China	Yunnan, Baoshan	Domestic	EA65
DQ496324	China	Yunnan, Baoshan	Domestic	EA65
DQ496322	China	Yunnan, Baoshan	Domestic	EA197
DQ496409	China	Yunnan, Diqing	Domestic	EA163
DQ496405	China	Yunnan, Diqing	Domestic	EA195
DQ496395	China	Yunnan, Diqing	Domestic	EA1
DQ496396	China	Yunnan, Diqing	Domestic	EA1
DQ496397	China	Yunnan, Diqing	Domestic	EA1
DQ496792	China	Yunnan, Gaoligongshan	Wild	EA86
DQ496875	China	Yunnan, Jinghong	Wild	EA81
DQ496877	China	Yunnan, Jinghong	Wild	EA83
DQ496864	China	Yunnan, Jinghong	Wild	EA5
DQ496868	China	Yunnan, Jinghong	Wild	EA5

Accession No.	Country	Location	Status	Haplotype
DQ496662	China	Yunnan	Domestic	EA14
DQ496684	China	Yunnan	Domestic	EA14
DQ496690	China	Yunnan	Domestic	EA198
DQ496674	China	Yunnan	Domestic	EA149
DQ496675	China	Yunnan	Domestic	EA149
DQ496685	China	Yunnan	Domestic	EA217
DQ496686	China	Yunnan	Domestic	EA217
DQ496687	China	Yunnan	Domestic	EA217
DQ496692	China	Yunnan	Domestic	EA65
DQ496673	China	Yunnan	Domestic	EA65
DQ496661	China	Yunnan	Domestic	EA151
DQ496665	China	Yunnan	Domestic	EA151
DQ496666	China	Yunnan	Domestic	EA151
DQ496657	China	Yunnan	Domestic	EA218
DQ496658	China	Yunnan	Domestic	EA218
DQ496669	China	Yunnan	Domestic	EA218
DQ496309	China	Yunnan	Domestic	EA108
DQ496326	China	Yunnan	Domestic	EA200
DQ496389	China	Yunnan	Domestic	EA29
DQ496390	China	Yunnan	Domestic	EA29
DQ496383	China	Yunnan	Domestic	EA147
DQ496371	China	Yunnan	Domestic	EA108
DQ496373	China	Yunnan	Domestic	EA201
DQ496391	China	Yunnan	Domestic	EA108
DQ496388	China	Yunnan	Domestic	EA108
DQ496365	China	Yunnan	Domestic	EA202
DQ496368	China	Yunnan	Domestic	EA202
DQ496372	China	Yunnan	Domestic	EA202
DQ496375	China	Yunnan	Domestic	EA202
DQ496376	China	Yunnan	Domestic	EA202
DQ496384	China	Yunnan	Domestic	EA202
DQ496385	China	Yunnan	Domestic	EA202
DQ496305	China	Yunnan	Domestic	EA202
DQ496308	China	Yunnan	Domestic	EA202
DQ496551	China	Yunnan	Domestic	EA29
DQ496579	China	Yunnan	Domestic	EA29
DQ496574	China	Yunnan	Domestic	EA29
DQ496575	China	Yunnan	Domestic	EA29
DQ496852	China	Yunnan	Wild	EA85
DQ496876	China	Yunnan	Wild	EA78
DQ496850	China	Yunnan	Wild	EA203
DQ496856	China	Yunnan	Wild	EA204
DQ496855	China	Yunnan	Wild	EA205
DQ496853	China	Yunnan	Wild	EA29
DQ496741	China	Yunnan	Wild	EA108
DQ496882	China	Yunnan	Wild	EA202
DQ496869	China	Yunnan	Wild	EA108
DQ496870	China	Yunnan	Wild	EA202
DQ496398	China	Yunnan	Domestic	EA216
DQ496408	China	Yunnan	Domestic	EA216
DQ496315	China	Yunnan, Baoshan	Domestic	EA32

Accession No.	Country	Location	Status	Haplotype
DQ496323	China	Yunnan, Baoshan	Domestic	EA216
DQ496313	China	Yunnan, Baoshan	Domestic	EA210
DQ496325	China	Yunnan, Baoshan	Domestic	EA210
DQ496399	China	Yunnan, Diqing	Domestic	EA216
DQ496401	China	Yunnan, Diqing	Domestic	EA216
DQ496404	China	Yunnan, Diqing	Domestic	EA216
DQ496861	China	Yunnan, Jinghong	Wild	EA29
DQ496873	China	Yunnan, Jinghong	Wild	EA29
DQ496885	China	Yunnan, ruili	Wild	EA84
DQ496682	China	Yunnan	Domestic	EA180
DQ496689	China	Yunnan	Domestic	EA180
DQ496670	China	Yunnan	Domestic	EA210
DQ496671	China	Yunnan	Domestic	EA210
DQ496672	China	Yunnan	Domestic	EA210
DQ496764	China	Northeast	Wild	EA35
DQ496768	China	Northeast	Wild	EA35
DQ496770	China	Northeast	Wild	EA35
DQ496569	China	Northeast	Domestic	EA14
DQ496564	China	Northeast	Domestic	EA14
DQ496555	China	Northeast	Domestic	EA14
DQ496561	China	Northeast	Domestic	EA14
DQ496556	China	Northeast	Domestic	EA14
DQ496566	China	Northeast	Domestic	EA14
DQ496554	China	Northeast	Domestic	EA7
DQ496557	China	Northeast	Domestic	EA7
DQ496558	China	Northeast	Domestic	EA7
DQ496559	China	Northeast	Domestic	EA7
DQ496560	China	Northeast	Domestic	EA7
DQ496562	China	Northeast	Domestic	EA7
DQ496563	China	Northeast	Domestic	EA7
DQ496565	China	Northeast	Domestic	EA7
DQ496570	China	Northeast	Domestic	EA7
DQ496571	China	Northeast	Domestic	EA7
DQ496568	China	Northeast	Domestic	EA1
DQ496753	China	Northeast	Wild	EA36
DQ496769	China	Northeast	Wild	EA36
DQ496771	China	Northeast	Wild	EA36
DQ496772	China	Northeast	Wild	EA36
DQ496744	China	Northeast	Wild	EA37
DQ496339	China	Guangdong	Domestic	EA9
DQ496340	China	Guangdong	Domestic	EA9
DQ496341	China	Guangdong	Domestic	EA9
DQ496342	China	Guangdong	Domestic	EA9
DQ496343	China	Guangdong	Domestic	EA60
DQ496344	China	Guangdong	Domestic	EA53
DQ496345	China	Guangdong	Domestic	EA53
DQ496346	China	Guangdong	Domestic	EA53
DQ496347	China	Guangdong	Domestic	EA53
DQ496348	China	Guangdong	Domestic	EA53
DQ496349	China	Guangdong	Domestic	EA53
DQ496350	China	Guangdong	Domestic	EA53

Accession No.	Country	Location	Status	Haplotype
DQ496351	China	Guangdong	Domestic	EA53
DQ496352	China	Guangdong	Domestic	EA53
DQ496353	China	Guangdong	Domestic	EA60
DQ496354	China	Guangdong	Domestic	EA60
DQ496355	China	Guangdong	Domestic	EA53
DQ496356	China	Guangdong	Domestic	EA53
DQ496357	China	Guangdong	Domestic	EA9
DQ496364	China	Guangdong	Domestic	EA53
DQ496528	China	Guangdong	Domestic	EA10
DQ496514	China	Guangdong	Domestic	EA10
DQ496515	China	Guangdong	Domestic	EA10
DQ496521	China	Guangdong	Domestic	EA10
DQ496522	China	Guangdong	Domestic	EA10
DQ496527	China	Guangdong	Domestic	EA10
DQ496529	China	Guangdong	Domestic	EA206
DQ496512	China	Guangdong	Domestic	EA9
DQ496513	China	Guangdong	Domestic	EA9
DQ496516	China	Guangdong	Domestic	EA9
DQ496517	China	Guangdong	Domestic	EA9
DQ496518	China	Guangdong	Domestic	EA9
DQ496519	China	Guangdong	Domestic	EA9
DQ496520	China	Guangdong	Domestic	EA9
DQ496523	China	Guangdong	Domestic	EA9
DQ496524	China	Guangdong	Domestic	EA9
DQ496525	China	Guangdong	Domestic	EA9
DQ496526	China	Guangdong	Domestic	EA9
DQ496530	China	Guangdong	Domestic	EA9
DQ496622	China	Guizhou	Domestic	EA6
DQ496617	China	Guizhou	Domestic	EA6
DQ496609	China	Guizhou	Domestic	EA6
DQ496965	China	Guizhou	Domestic	EA1
DQ496802	China	Hainan	Wild	EA27
DQ496806	China	Hainan	Wild	EA27
DQ496808	China	Hainan	Wild	EA27
DQ496803	China	Hainan	Wild	EA87
DQ496794	China	Hainan	Wild	EA29
DQ496798	China	Hainan	Wild	EA29
DQ496810	China	Hainan	Wild	EA29
DQ496814	China	Hainan	Wild	EA29
DQ496799	China	Hainan	Wild	EA91
DQ496800	China	Hainan	Wild	EA214
DQ496797	China	Hainan	Wild	EA167
DQ496807	China	Hainan	Wild	EA167
DQ496809	China	Hainan	Wild	EA167
DQ496811	China	Hainan	Wild	EA167
DQ496812	China	Hainan	Wild	EA167
DQ496813	China	Hainan	Wild	EA167
DQ496804	China	Hainan	Wild	EA33
DQ496793	China	Hainan	Wild	EA166
DQ496795	China	Hainan	Wild	EA166
DQ496796	China	Hainan	Wild	EA166

Accession No.	Country	Location	Status	Haplotype
DQ496801	China	Hainan	Wild	EA215
DQ496442	China	-	Domestic	EA1
DQ496462	China	-	Domestic	EA11
DQ496463	China	-	Domestic	EA1
DQ496452	China	-	Domestic	EA67
DQ496457	China	-	Domestic	EA67
DQ496458	China	-	Domestic	EA67
DQ496451	China	-	Domestic	EA171
DQ496257	China	Sichuan, Aba	Domestic	EA6
DQ496258	China	Sichuan, Aba	Domestic	EA6
DQ496259	China	Sichuan, Aba	Domestic	EA6
DQ496261	China	Sichuan, Aba	Domestic	EA6
DQ496268	China	Sichuan, Aba	Domestic	EA6
DQ496282	China	Northwest	Domestic	EA173
DQ496283	China	Northwest	Domestic	EA173
DQ496284	China	Northwest	Domestic	EA173
DQ496285	China	Northwest	Domestic	EA173
DQ496286	China	Northwest	Domestic	EA173
DQ496288	China	Northwest	Domestic	EA173
DQ496289	China	Northwest	Domestic	EA173
DQ496291	China	Northwest	Domestic	EA173
DQ496287	China	Northwest	Domestic	EA14
DQ496292	China	Northwest	Domestic	EA14
DQ496290	China	Northwest	Domestic	EA14
DQ496439	China	Northwest	Domestic	EA14
DQ496444	China	Northwest	Domestic	EA14
DQ496441	China	Northwest	Domestic	EA14
DQ496446	China	Northwest	Domestic	EA174
DQ496445	China	Northwest	Domestic	EA174
DQ496443	China	Northwest	Domestic	EA2
DQ496440	China	Northwest	Domestic	EA175
DQ496453	China	Northwest	Domestic	EA65
DQ496466	China	Northwest	Domestic	EA12
DQ496460	China	Northwest	Domestic	EA12
DQ496461	China	Northwest	Domestic	EA12
DQ496459	China	Northwest	Domestic	EA12
DQ496454	China	Northwest	Domestic	EA176
DQ496464	China	Northwest	Domestic	EA153
DQ496455	China	Northwest	Domestic	EA153
DQ496465	China	Northwest	Domestic	EA153
DQ496456	China	Northwest	Domestic	EA174
DQ496260	China	Sichuan	Domestic	EA12
DQ496265	China	Sichuan	Domestic	EA14
DQ496651	China	Sichuan	Domestic	EA178
DQ496251	China	Sichuan, Aba	Domestic	EA168
DQ496254	China	Sichuan, Aba	Domestic	EA65
DQ496270	China	Sichuan, Aba	Domestic	EA65
DQ496271	China	Sichuan, Aba	Domestic	EA65
DQ496272	China	Sichuan, Aba	Domestic	EA65
DQ496255	China	Sichuan, Aba	Domestic	EA179
DQ496256	China	Sichuan, Aba	Domestic	EA65

Accession No.	Country	Location	Status	Haplotype
DQ496262	China	Sichuan, Aba	Domestic	EA153
DQ496266	China	Sichuan, Aba	Domestic	EA153
DQ496267	China	Sichuan, Aba	Domestic	EA174
DQ496263	China	Sichuan, Aba	Domestic	EA2
DQ496252	China	Sichuan	Domestic	EA179
DQ496269	China	Sichuan	Domestic	EA28
DQ496293	China	Guangxi: Bama	Domestic	EA207
DQ496294	China	Guangxi: Bama	Domestic	EA207
DQ496295	China	Guangxi: Bama	Domestic	EA207
DQ496296	China	Guangxi: Bama	Domestic	EA207
DQ496297	China	Guangxi: Bama	Domestic	EA207
DQ496298	China	Guangxi: Bama	Domestic	EA207
DQ496299	China	Guangxi: Bama	Domestic	EA207
DQ496300	China	Guangxi: Bama	Domestic	EA207
DQ496301	China	Guangxi: Bama	Domestic	EA207
DQ496302	China	Guangxi: Bama	Domestic	EA207
DQ496619	China	Guizhou	Domestic	EA2
DQ496620	China	Guizhou	Domestic	EA2
DQ496621	China	Guizhou	Domestic	EA80
DQ496438	China	Guizhou	Domestic	EA18
DQ496436	China	Guizhou	Domestic	EA18
DQ496437	China	Guizhou	Domestic	EA106
DQ496606	China	Guizhou	Domestic	EA146
DQ496607	China	Guizhou	Domestic	EA146
DQ496608	China	Guizhou	Domestic	EA146
DQ496610	China	Guizhou	Domestic	EA146
DQ496611	China	Guizhou	Domestic	EA146
DQ496612	China	Guizhou	Domestic	EA146
DQ496613	China	Guizhou	Domestic	EA146
DQ496614	China	Guizhou	Domestic	EA146
DQ496615	China	Guizhou	Domestic	EA146
DQ496616	China	Guizhou	Domestic	EA146
DQ496618	China	Guizhou	Domestic	EA146
DQ496829	China	Guizhou	Wild	EA208
DQ496827	China	Guizhou	Wild	EA41
DQ496831	China	Guizhou	Wild	EA41
DQ496830	China	Guizhou	Wild	EA14
DQ496826	China	Guizhou	Wild	EA2
DQ496640	China	Guizhou	Domestic	EA18
DQ496642	China	Guizhou	Domestic	EA18
DQ496643	China	Guizhou	Domestic	EA9
DQ496639	China	Guizhou	Domestic	EA185
DQ496641	China	Guizhou	Domestic	EA185
DQ496966	China	Guizhou	Domestic	EA25
DQ496967	China	Guizhou	Domestic	EA25
DQ496964	China	Guizhou	Domestic	EA25
DQ496969	China	Guizhou	Domestic	EA25
DQ496968	China	Guizhou	Domestic	EA25
DQ496828	China	Guizhou	Wild	EA31
DQ496338	China	Sichuan	Domestic	EA14
DQ496336	China	Sichuan	Domestic	EA14

Accession No.	Country	Location	Status	Haplotype
DQ496337	China	Sichuan	Domestic	EA14
DQ496335	China	Sichuan	Domestic	EA14
DQ496591	China	Sichuan	Domestic	EA2
DQ496583	China	Sichuan	Domestic	EA2
DQ496582	China	Sichuan	Domestic	EA10
DQ496584	China	Sichuan	Domestic	EA10
DQ496585	China	Sichuan	Domestic	EA10
DQ496586	China	Sichuan	Domestic	EA10
DQ496587	China	Sichuan	Domestic	EA10
DQ496588	China	Sichuan	Domestic	EA10
DQ496589	China	Sichuan	Domestic	EA10
DQ496592	China	Sichuan	Domestic	EA10
DQ496593	China	Sichuan	Domestic	EA10
DQ496594	China	Sichuan	Domestic	EA10
DQ496595	China	Sichuan	Domestic	EA10
DQ496596	China	Sichuan	Domestic	EA10
DQ496590	China	Sichuan	Domestic	EA10
DQ496647	China	Sichuan	Domestic	EA10
DQ496653	China	Sichuan	Domestic	EA10
DQ496652	China	Sichuan	Domestic	EA80
DQ496646	China	Sichuan	Domestic	EA6
DQ496648	China	Sichuan	Domestic	EA6
DQ496650	China	Sichuan	Domestic	EA6
DQ496644	China	Sichuan	Domestic	EA1
DQ496645	China	Sichuan	Domestic	EA1
DQ496649	China	Sichuan	Domestic	EA1
DQ496843	China	Sichuan	Wild	EA85
DQ496844	China	Sichuan	Wild	EA63
DQ496845	China	Sichuan	Wild	EA63
DQ496846	China	Sichuan	Wild	EA210
DQ496832	China	Sichuan	Wild	EA210
DQ496970	China	Sichuan	Domestic	EA185
DQ496975	China	Sichuan	Domestic	EA185
DQ496973	China	Sichuan	Domestic	EA151
DQ496974	China	Sichuan	Domestic	EA158
DQ496971	China	Sichuan	Domestic	EA158
DQ496972	China	Sichuan	Domestic	EA211
DQ496434	China	Sichuan	Domestic	EA14
DQ496424	China	Sichuan	Domestic	EA14
DQ496425	China	Sichuan	Domestic	EA14
DQ496426	China	Sichuan	Domestic	EA14
DQ496427	China	Sichuan	Domestic	EA14
DQ496429	China	Sichuan	Domestic	EA14
DQ496430	China	Sichuan	Domestic	EA14
DQ496431	China	Sichuan	Domestic	EA14
DQ496432	China	Sichuan	Domestic	EA14
DQ496264	China	Sichuan, Aba	Domestic	EA212
DQ496428	China	Sichuan, Ganzi	Domestic	EA213
DQ496433	China	Sichuan, Ganzi	Domestic	EA213
DQ496735	China	Anhui	Domestic	EA1
DQ496736	China	Anhui	Domestic	EA1

Accession No.	Country	Location	Status	Haplotype
DQ496738	China	Anhui	Domestic	EA1
DQ496739	China	Anhui	Domestic	EA1
DQ496734	China	Anhui	Domestic	EA1
DQ496740	China	Anhui	Domestic	EA1
DQ496923	China	Anhui	Domestic	EA2
DQ496916	China	Anhui	Domestic	EA3
DQ496920	China	Anhui	Domestic	EA3
DQ496926	China	Anhui	Domestic	EA3
DQ496915	China	Anhui	Domestic	EA4
DQ496921	China	Anhui	Domestic	EA2
DQ496922	China	Anhui	Domestic	EA2
DQ496928	China	Anhui	Domestic	EA2
DQ496927	China	Anhui	Domestic	EA5
DQ496918	China	Anhui	Domestic	EA5
DQ496919	China	Anhui	Domestic	EA5
DQ496925	China	Anhui	Domestic	EA5
DQ496924	China	Anhui	Domestic	EA6
DQ496930	China	Anhui	Domestic	EA6
DQ496931	China	Anhui	Domestic	EA6
DQ496929	China	Anhui	Domestic	EA7
DQ496913	China	Anhui	Domestic	EA1
DQ496914	China	Anhui	Domestic	EA1
DQ496917	China	Anhui	Domestic	EA8
DQ496447	China	Fujian	Domestic	EA9
DQ496448	China	Fujian	Domestic	EA9
DQ496450	China	Fujian	Domestic	EA9
DQ496601	China	Fujian	Domestic	EA10
DQ496602	China	Fujian	Domestic	EA10
DQ496599	China	Fujian	Domestic	EA9
DQ496600	China	Fujian	Domestic	EA6
DQ496603	China	Fujian	Domestic	EA6
DQ496605	China	Fujian	Domestic	EA6
DQ496604	China	Fujian	Domestic	EA11
DQ496787	China	Fujian	Wild	EA12
DQ496777	China	Fujian	Wild	EA2
DQ496783	China	Fujian	Wild	EA10
DQ496776	China	Fujian	Wild	EA9
DQ496598	China	Fujian	Domestic	EA13
DQ496479	China	Hubei	Domestic	EA11
DQ496478	China	Hubei	Domestic	EA1
DQ496477	China	Hubei	Domestic	EA11
DQ496627	China	Hubei	Domestic	EA2
DQ496637	China	Hubei	Domestic	EA2
DQ496633	China	Hubei	Domestic	EA14
DQ496631	China	Hubei	Domestic	EA5
DQ496630	China	Hubei	Domestic	EA15
DQ496636	China	Hubei	Domestic	EA14
DQ496628	China	Hubei	Domestic	EA14
DQ496625	China	Hubei	Domestic	EA6
DQ496632	China	Hubei	Domestic	EA1
DQ496624	China	Hubei	Domestic	EA16

Accession No.	Country	Location	Status	Haplotype
DQ496626	China	Hubei	Domestic	EA16
DQ496629	China	Hubei	Domestic	EA16
DQ496635	China	Hubei	Domestic	EA16
DQ496638	China	Hubei	Domestic	EA16
DQ496493	China	Jiangsu	Domestic	EA11
DQ496496	China	Jiangsu	Domestic	EA11
DQ496492	China	Jiangsu	Domestic	EA17
DQ496494	China	Jiangsu	Domestic	EA17
DQ496495	China	Jiangsu	Domestic	EA1
DQ496497	China	Jiangsu	Domestic	EA1
DQ496537	China	Jiangxi	Domestic	EA14
DQ496540	China	Jiangxi	Domestic	EA2
DQ496538	China	Jiangxi	Domestic	EA2
DQ496539	China	Jiangxi	Domestic	EA6
DQ496533	China	Jiangxi	Domestic	EA6
DQ496534	China	Jiangxi	Domestic	EA6
DQ496535	China	Jiangxi	Domestic	EA6
DQ496536	China	Jiangxi	Domestic	EA6
DQ496695	China	Jiangxi	Domestic	EA18
DQ496701	China	Jiangxi	Domestic	EA18
DQ496697	China	Jiangxi	Domestic	EA19
DQ496694	China	Jiangxi	Domestic	EA19
DQ496696	China	Jiangxi	Domestic	EA6
DQ496699	China	Jiangxi	Domestic	EA6
DQ496700	China	Jiangxi	Domestic	EA6
DQ496702	China	Jiangxi	Domestic	EA6
DQ496703	China	Jiangxi	Domestic	EA6
DQ496704	China	Jiangxi	Domestic	EA6
DQ496816	China	Jiangxi	Domestic	EA2
DQ496818	China	Jiangxi	Domestic	EA14
DQ496817	China	Jiangxi	Domestic	EA20
DQ496992	China	Jiangxi	Domestic	EA2
DQ496991	China	Jiangxi	Domestic	EA2
DQ496990	China	Jiangxi	Domestic	EA9
DQ496993	China	Jiangxi	Domestic	EA2
DQ496987	China	Jiangxi	Domestic	EA2
DQ496988	China	Jiangxi	Domestic	EA2
DQ496998	China	Jiangxi	Domestic	EA21
DQ496989	China	Jiangxi	Domestic	EA3
DQ496994	China	Jiangxi	Domestic	EA3
DQ497000	China	Jiangxi	Domestic	EA22
DQ496995	China	Jiangxi	Domestic	EA6
DQ496996	China	Jiangxi	Domestic	EA6
DQ496997	China	Jiangxi	Domestic	EA6
DQ496999	China	Jiangxi	Domestic	EA6
DQ496910	China	Zhejiang,Taizhou	Wild	EA22
DQ496277	China	Zhejiang	Domestic	EA2
DQ496275	China	Zhejiang	Domestic	EA2
DQ496278	China	Zhejiang	Domestic	EA2
DQ496279	China	Zhejiang	Domestic	EA23
DQ496280	China	Zhejiang	Domestic	EA23

Accession No.	Country	Location	Status	Haplotype
DQ496274	China	Zhejiang	Domestic	EA6
DQ496276	China	Zhejiang	Domestic	EA6
DQ496281	China	Zhejiang	Domestic	EA1
DQ496328	China	Zhejiang	Domestic	EA6
DQ496329	China	Zhejiang	Domestic	EA6
DQ496334	China	Zhejiang	Domestic	EA6
DQ496330	China	Zhejiang	Domestic	EA1
DQ496332	China	Zhejiang	Domestic	EA1
DQ496333	China	Zhejiang	Domestic	EA1
DQ496331	China	Zhejiang	Domestic	EA14
DQ496419	China	Zhejiang	Domestic	EA24
DQ496416	China	Zhejiang	Domestic	EA24
DQ496417	China	Zhejiang	Domestic	EA24
DQ496418	China	Zhejiang	Domestic	EA24
DQ496413	China	Zhejiang	Domestic	EA2
DQ496414	China	Zhejiang	Domestic	EA2
DQ496421	China	Zhejiang	Domestic	EA2
DQ496422	China	Zhejiang	Domestic	EA2
DQ496415	China	Zhejiang	Domestic	EA3
DQ496487	China	Zhejiang	Domestic	EA2
DQ496483	China	Zhejiang	Domestic	EA18
DQ496486	China	Zhejiang	Domestic	EA6
DQ496480	China	Zhejiang	Domestic	EA6
DQ496481	China	Zhejiang	Domestic	EA6
DQ496482	China	Zhejiang	Domestic	EA6
DQ496484	China	Zhejiang	Domestic	EA6
DQ496485	China	Zhejiang	Domestic	EA25
DQ496488	China	Zhejiang	Domestic	EA2
DQ496490	China	Zhejiang	Domestic	EA14
DQ496581	China	Zhejiang	Domestic	EA26
DQ496705	China	Zhejiang	Domestic	EA2
DQ496707	China	Zhejiang	Domestic	EA2
DQ496706	China	Zhejiang	Domestic	EA6
DQ496708	China	Zhejiang	Domestic	EA6
DQ496709	China	Zhejiang	Domestic	EA1
DQ496890	China	Zhejiang	Wild	EA27
DQ496891	China	Zhejiang	Wild	EA27
DQ496897	China	Zhejiang	Wild	EA14
DQ496898	China	Zhejiang	Wild	EA28
DQ496899	China	Zhejiang	Wild	EA14
DQ496886	China	Zhejiang	Wild	EA6
DQ496895	China	Zhejiang	Wild	EA6
DQ496900	China	Zhejiang	Wild	EA6
DQ496901	China	Zhejiang	Wild	EA6
DQ496887	China	Zhejiang	Wild	EA6
DQ496896	China	Zhejiang	Wild	EA6
DQ496902	China	Zhejiang	Wild	EA6
DQ496903	China	Zhejiang	Wild	EA6
DQ496904	China	Zhejiang	Wild	EA6
DQ496905	China	Zhejiang	Wild	EA6
DQ496908	China	Zhejiang	Wild	EA6

Accession No.	Country	Location	Status	Haplotype
DQ496909	China	Zhejiang	Wild	EA6
DQ496911	China	Zhejiang	Wild	EA6
DQ496449	China	Fujian	Domestic	EA29
DQ496775	China	Fujian	Wild	EA30
DQ496779	China	Fujian	Wild	EA30
DQ496786	China	Fujian	Wild	EA30
DQ496782	China	Fujian	Wild	EA30
DQ496784	China	Fujian	Wild	EA30
DQ496785	China	Fujian	Wild	EA30
DQ496773	China	Fujian	Wild	EA30
DQ496774	China	Fujian	Wild	EA30
DQ496781	China	Fujian	Wild	EA30
DQ496780	China	Fujian	Wild	EA30
DQ496815	China	Jiangxi	Wild	EA31
DQ496819	China	Jiangxi	Wild	EA31
DQ496906	China	Zhejiang	Wild	EA30
DQ496907	China	Zhejiang	Wild	EA32
DQ496912	China	Zhejiang	Wild	EA32
DQ496888	China	Zhejiang	Wild	EA29
DQ496889	China	Zhejiang	Wild	EA33
DQ496892	China	Zhejiang	Wild	EA34
DQ496893	China	Zhejiang	Wild	EA34
DQ496789	China	Gansu	Wild	EA89
DQ496788	China	Gansu	Wild	EA57
DQ496790	China	Gansu	Wild	EA57
DQ496791	China	Gansu	Wild	EA172
DQ496833	China	Shaanxi	Wild	EA177
DQ496839	China	Shaanxi	Wild	EA177
DQ496841	China	Shaanxi	Wild	EA177
DQ496842	China	Shaanxi	Wild	EA177
DQ496837	China	Shaanxi	Wild	EA57
DQ496838	China	Shaanxi	Wild	EA57
DQ496835	China	Shaanxi	Wild	EA90
DQ496834	China	Shaanxi	Wild	EA63
DQ496840	China	Shaanxi	Wild	EA63
DQ496836	China	Shaanxi	Wild	EA172
DQ496956	Laos		Domestic	EA22
DQ496725	Laos		Domestic	EA2
DQ496710	Laos		Domestic	EA18
DQ496711	Laos		Domestic	EA18
DQ496716	Laos		Domestic	EA18
DQ496720	Laos		Domestic	EA18
DQ496721	Laos		Domestic	EA18
DQ496723	Laos		Domestic	EA18
DQ496933	Laos		Domestic	EA18
DQ496934	Laos		Domestic	EA18
DQ496935	Laos		Domestic	EA18
DQ496936	Laos		Domestic	EA18
DQ496937	Laos		Domestic	EA18
DQ496940	Laos		Domestic	EA18
DQ496943	Laos		Domestic	EA18

Accession No.	Country	Location	Status	Haplotype
DQ496944	Laos		Domestic	EA18
DQ496947	Laos		Domestic	EA18
DQ496948	Laos		Domestic	EA18
DQ496951	Laos		Domestic	EA18
DQ496952	Laos		Domestic	EA18
DQ496953	Laos		Domestic	EA18
DQ496954	Laos		Domestic	EA18
DQ496959	Laos		Domestic	EA18
DQ496963	Laos		Domestic	EA18
DQ496932	Laos		Domestic	EA18
DQ496722	Laos		Domestic	EA106
DQ496726	Laos		Domestic	EA106
DQ496939	Laos		Domestic	EA106
DQ496942	Laos		Domestic	EA106
DQ496955	Laos		Domestic	EA106
DQ496957	Laos		Domestic	EA106
DQ496960	Laos		Domestic	EA106
DQ496962	Laos		Domestic	EA106
DQ496717	Laos		Domestic	EA65
DQ496728	Laos		Domestic	EA234
DQ496719	Laos		Domestic	EA2
DQ496714	Laos		Domestic	EA6
DQ496945	Laos		Domestic	EA6
DQ496946	Laos		Domestic	EA6
DQ496950	Laos		Domestic	EA6
DQ496958	Laos		Domestic	EA6
DQ496961	Laos		Domestic	EA6
DQ496821	Laos		Wild	EA83
DQ496822	Laos		Wild	EA83
DQ496824	Laos		Wild	EA88
DQ496820	Laos		Wild	EA2
AB041479	Vietnam		Domestic	EA235
DQ496597	Vietnam		Domestic	EA235
DQ496938	Laos		Domestic	EA29
DQ496941	Laos		Domestic	EA29
DQ496949	Laos		Domestic	EA187
DQ496713	Laos		Domestic	EA236
DQ496718	Laos		Domestic	EA236
DQ496724	Laos		Domestic	EA236
DQ496712	Laos		Domestic	EA237
DQ496715	Laos		Domestic	EA108
DQ496727	Laos		Domestic	EA108
DQ496823	Laos		Wild	EA78
DQ496825	Laos		Wild	EA78
DQ496865	Laos		Wild	EA187
DQ496848	Vietnam		Wild	EA12
DQ496732	Vietnam		Wild	EA199
DQ496733	Vietnam		Wild	EA199
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DQ152868	China	Southwest	Domestic	EA180
DQ379101	China	Southwest	Domestic	EA180

Accession No.	Country	Location	Status	Haplotype
DQ379102	China	Southwest	Domestic	EA180
DQ152869	China	Hunan	Domestic	EA14
DQ379103	China	Qinghai	Domestic	EA14
DQ379104	China	Qinghai	Domestic	EA14
DQ152870	China	Jiangxi	Domestic	EA181
DQ152871	China	Jiangxi	Domestic	EA6
DQ379105	China	Shandong	Domestic	EA6
DQ379106	China	Shandong	Domestic	EA6
DQ379107	China	Shandong	Domestic	EA6
DQ379108	China	Shandong	Domestic	EA6
DQ379109	China	Shandong	Domestic	EA6
DQ379110	China	Jiangxi	Domestic	EA6
DQ379111	China	Jiangxi	Domestic	EA6
DQ379112	China	Guizhou	Domestic	EA6
DQ379113	China	Jiangxi	Domestic	EA6
DQ379114	China	Zhejiang	Domestic	EA6
DQ379115	China	Hubei	Domestic	EA6
DQ379116	China	Guizhou	Domestic	EA6
DQ379130	China	jiangxi	Domestic	EA6
DQ152872	China	Sichuan	Domestic	EA10
DQ379131	China	Sichuan	Domestic	EA10
DQ379132	China	Sichuan	Domestic	EA10
DQ152873,	China	Jiangxi	Domestic	EA2
DQ379133	China	Jiangsu	Domestic	EA2
DQ379134	China	Jiangxi	Domestic	EA2
DQ379135	China	Guizhou	Domestic	EA2
DQ379136	China	Zhejiang	Domestic	EA2
DQ379137	China	Zhejiang	Domestic	EA2
DQ379138	China	Hubei	Domestic	EA2
DQ379139	China	Guizhou	Domestic	EA2
DQ379140	China	Hunan	Domestic	EA2
DQ379141	China	Jiangxi	Domestic	EA2
DQ379142	China	Sichuan	Domestic	EA2
DQ379143	China	Sichuan	Domestic	EA2
DQ152874	China	Southwest	Domestic	EA165
DQ152875	China	Hubei	Domestic	EA9
DQ152876	China	Hunan	Domestic	EA1
DQ379146	China	Hubei	Domestic	EA1
DQ379147	China	Hubei	Domestic	EA1
DQ379148	China	Zhejiang	Domestic	EA1
DQ379149	China	Zhejiang	Domestic	EA1
DQ379150	China	Jiangsu	Domestic	EA1
DQ152877	China	Zhejiang	Domestic	EA162
DQ379152	China	Zhejiang	Domestic	EA162
DQ152878	China	Jiangxi	Domestic	EA3
DQ379153	China	Shandong	Domestic	EA3
DQ379154	China	Shandong	Domestic	EA3
DQ379155	China	Zhejiang	Domestic	EA3
DQ379156	China	Jiangsu	Domestic	EA3
DQ379157	China	-	Domestic	EA3
DQ379158	China	-	Domestic	EA3

Accession No.	Country	Location	Status	Haplotype
DQ379159	China	-	Domestic	EA3
DQ379160	China	-	Domestic	EA3
DQ379161	China	-	Domestic	EA3
DQ379162	China	-	Domestic	EA3
DQ152879	China	Qinghai	Domestic	EA153
DQ379163	China	Qinghai	Domestic	EA153
DQ379164	China	Qinghai	Domestic	EA153
DQ152880	China	Shandong	Domestic	EA7
DQ379165	China	Shandong	Domestic	EA7
DQ379166	China	Shandong	Domestic	EA7
DQ379167	China	Shandong	Domestic	EA7
DQ379168	China	Shandong	Domestic	EA7
DQ379169	China	Northeast	Domestic	EA7
DQ379170	China	Northeast	Domestic	EA7
DQ379171	China	Northeast	Domestic	EA7
DQ379172	China	Northeast	Domestic	EA7
DQ379173	China	Northeast	Domestic	EA7
DQ152881	China	Guizhou	Domestic	EA152
DQ379174	China	Guizhou	Domestic	EA152
DQ152882	China	Guizhou	Domestic	EA146
DQ152883	China	Shandong	Domestic	EA144
DQ379175	China	Shandong	Domestic	EA144
DQ379176	China	Zhejiang	Domestic	EA144
DQ379177	China	Zhejiang	Domestic	EA144
DQ152884	China	Jiangxi	Domestic	EA182
DQ152885	China	Jiangxi	Domestic	EA19
DQ379178	China	Jiangxi	Domestic	EA19
DQ152886	China	Qinghai	Domestic	EA183
DQ152887	China	Qinghai	Domestic	EA67
DQ379179	China	Qinghai	Domestic	EA67
DQ379180	China	Qinghai	Domestic	EA67
DQ152888	China	Qinghai	Domestic	EA171
DQ152889	China	Jiangsu	Domestic	EA184
DQ379181	China	Guangxi	Domestic	EA18
DQ379182	China	Guangxi	Domestic	EA18
DQ379183	China	Guangxi	Domestic	EA18
DQ379184	China	Guangxi	Domestic	EA18
DQ379185	China	Guangxi	Domestic	EA18
DQ379186	China	Jiangxi	Domestic	EA18
DQ152891	China	Guizhou	Domestic	EA11
DQ379200	China	Jiangsu	Domestic	EA11
DQ379201	China	Guizhou	Domestic	EA11
DQ152892	China	Southwest	Domestic	EA185
DQ152893	China	Guizhou	Domestic	EA186
DQ152894	China	Hunan	Domestic	EA187
DQ152895	China	Hunan	Domestic	EA151
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AY884610	China	Sichuan, Wen Chuan	Wild	EA52
AY884612	India	Kashmir, Valley of Kashmir	Wild	EA40
AY884617		Taiwan ,Nan Tou, Jen-Ai, Hua-Chi Village	Wild	EA55
AY884623	Burma	Tenasserim, Bok Pyin	Wild	EA56

Accession No.	Country	Location	Status	Haplotype
AY884627	China	Shanxi Fen Chow	Wild	EA57
AY884629	Burma	Tenasserim, Kisseraing Island	Wild	EA58
AY884630	Thailand	Trang Trong	Wild	EA59
AY884634	Japan	Honshu, Tokio market	Wild	EA61
AY884639	China	Shensi, Yen-An-Fu	Wild	EA57
AY884640	China	Sichuan, Mt Omei	Wild	EA63
AY884641	China	Hunan Kiangsu	Wild	EA64
AY884642	China	Gansu Nanking	Wild	EA6
AY884647	Burma	Tenasserim, Tanjong Budak	Wild	EA66
AY884671	India		Wild	EA38
AY884674	India	Monghyr, Bengal	Wild	EA40
AY884675	India	Monghyr, Bengal	Domestic	EA40
AY884683	China	Hunan Yochow /yueyang	Wild	EA14
AY884684	China	Shanxi Tai-Yuan-Fu	Wild	EA57
AY884685	China	Sichuan, Suifu /yibin	Wild	EA41
AY884689	India	Kashmir, Woolar Lake	Wild	EA40
AY884691	Russia	Vladivostock	Wild	EA43
AY884692	Russia	Vladivostock	Wild	EA43
AY884695	Burma	Tenasserim, Boyse's Point	Wild	EA44
AY884702	Vanuatu	Gaua, New Hebrides	Feral	EA45
AY884704	Vanuatu		Feral	EA46
AY884705	India	Andaman Is	Feral	EA47
AY884706		Taiwan	Wild	EA48
AY884709	India	Surguja, Ambikapur	Wild	EA50
AY884712	Burma	Tenasserim, Champang	Wild	EA56
AY884615	Papua New Guinea	San Sapor	Feral	EA54
AY884637	PNG	Admiralty Islands, Ponam Islet, North of Manus Is	Feral	EA62
AY884673	PNG		Feral	EA39
AY884821	PNG	Doido	Feral	EA39
AY884822	PNG	Kairouk,Jimi Valley	Feral	EA39
AY884688	Indonesia	Halmahera island, Wasile district, Kampung, Loleba	Wild	EA42
AY884708		Formosa (Taiwan)	Wild	EA49
AY884678	USA	Oahu, Hawaii	Feral	EA39
DQ444710	French Polynesia	Tetiaroa, Society Islands	Feral	EA39
AF136567	China		Domestic	EA3
AF136564	Japan		Wild	EA51
AF136565	Japan		Wild	EA70
AF486855	China		Domestic	EA26
AF486856	China		Domestic	EA2
AF486857	China		Domestic	EA9
AF486859	China		Domestic	EA14
AF486860	China		Domestic	EA14
AF486861	China		Domestic	EA256
AF486862	China		Domestic	EA6
AF486863	China		Domestic	EA144
AF486864	China		Domestic	EA7
AF486865	China		Domestic	EA16
AF486867	China		Domestic	EA10
AF486868	China		Domestic	EA168
AF486869	China		Domestic	EA108

Accession No.	Country	Location	Status	Haplotype
AF486870	China		Domestic	EA9
AF486871	China		Domestic	EA6
Kim *et al.* 2002				
AF486872	China		Domestic	EA6
AF486873	China		Domestic	EA11
AF276923	China		Domestic	EA6
AF276925	China		Domestic	EA6
AF276927	China		Domestic	EA187
AF276928	China		Domestic	EA269
AF276935	South Korea	Cheju Island	Domestic	EA257
AF276934	South Korea	Cheju Island	Domestic	EA270
AF276933	South Korea	Cheju Island	Domestic	EA271
AF276929	China		Domestic	EA209
AF276922	China		Domestic	EA233
AF276930	China		Domestic	EA258
AF276931	China		Domestic	EA259
AF276924	China		Domestic	EA1
AF276926	China		Domestic	EA1
Okumura *et al.* 1996; Watanobe *et al.* 1999; Watanobe *et al*. 2001				
AF276932	China		Domestic	EA1
AF276921	Australia	Kangaroo Island	Feral	EA67
AB015091	Japan		Domestic	EA18
AB015092	Japan		Domestic	EA1
D42182	Japan		Wild and Dom	EA53
AB015088	Japan		Wild	EA75
AB015089	Japan		Wild	EA76
AB015090	Japan		Wild	EA77
D42174	Japan		Wild	EA69
D42178	Japan		Wild	EA71
D42171	Japan		Wild	EA68
D42176	Japan		Wild	EA70
D42173	Japan		Wild	EA70
AB015084	Japan		Wild	EA266
AB015085	Japan		Wild	EA72
AB015086	Japan		Wild	EA73
AB015087	Japan		Wild	EA74
D42184	Japan		Wild	EA74
D42181	Japan		Domestic	EA260
D42183	Japan/EuroAm		Wild and Dom	EA65
D42172	Japan		Wild	EA51
D42175	Japan		Wild	EA51
D42177	Japan		Wild	EA51
AB041475	East Asia		Domestic	EA144
AB041477	East Asia		Domestic	EA144
AB041480	East Asia		Domestic	EA9
AB041483	East Asia / EuroAm		Domestic	EA9
AB041478	East Asia		Domestic	EA10
AB041482	East Asia		Domestic	EA10
AB041467	Japan		Wild	EA261

Accession No.	Country	Location	Status	Haplotype
AB041468	Japan		Wild	EA262
AB041469	Japan		Wild	EA263
AB041470	Japan		Wild	EA51
AB041471	Japan		Wild	EA264
AB041472	Japan		Wild	EA265
AB041473	Japan		Wild	EA266
AB041476	East Asia		Domestic	EA267
AB041479	East Asia		Domestic	EA235
Larson *et al*. 2007				
AB041481	East Asia		Domestic	EA268
AB041466	China		Wild	EA37
DQ779432	Vietnam	Cao Bang, Mountainous region (North)	Domestic	EA6
DQ779433	Vietnam	Cao Bang, Mountainous region (North)	Domestic	EA6
DQ779434	Vietnam	Cao Bang, Mountainous region (North)	Domestic	EA70
DQ779435	Vietnam	Tay Nguyen province, highland area (south central)	Domestic	EA100
DQ779436	Vietnam	Tay Nguyen province, highland area (south central)	Domestic	EA101
DQ779437	Vietnam	Tay Nguyen province, highland area (south central)	Domestic	EA221
DQ779438	Vietnam	Lao Cai, Mountainous region, (North)	Domestic	EA14
DQ779439	Vietnam	Lao Cai, Mountainous region, (North)	Domestic	EA22
DQ779440	Vietnam	Lao Cai, Mountainous region, (North)	Domestic	EA29
DQ779441	Vietnam	Lao Cai, Mountainous region, (North)	Domestic	EA222
DQ779442	Vietnam	North Central Coast	Domestic	EA9
DQ779443	Vietnam	North Central Coast	Domestic	EA1
DQ779444	Vietnam	Tay Nguyen province, highland area (south central)	Domestic	EA1
DQ779445	Vietnam	Tay Nguyen province, highland area (south central)	Domestic	EA1
DQ779446	Vietnam	Nghe An province (north central)	Domestic	EA106
DQ779447	Vietnam	Nghe An province (north central)	Domestic	EA102
DQ779448	Vietnam	North Central Coast	Domestic	EA9
DQ779287	China	Modern restaurant, Daoqing	Domestic	EA231
DQ779420	China	Shanghei	Wild	EA6
DQ779421	North Korea		Wild	EA92
DQ779422	North Korea		Wild	EA82
DQ779399	Vietnam	Phouc Mon	Wild	EA97
DQ779410	Thailand		wild	EA275
DQ779403	Thailand	Klong Klung Camp	Wild	EA98
DQ779411	Burma	upper Burma, 26.49' N 98.10'E 6/4/39	Wild	EA99
DQ779415		Taiwan	Wild	EA6
DQ779417		Taiwan	Wild	EA219
DQ779418		Taiwan	Wild	EA220
DQ779521	South Korea		Wild	EA255
DQ779522	South Korea		Wild	EA255
DQ779523	South Korea		Wild	EA255
DQ779524	South Korea		Wild	EA255
DQ779525	South Korea		Wild	EA255
DQ779526	South Korea		Wild	EA255
DQ779527	South Korea		Wild	EA7
DQ779373	French Polynesia	Marquesas, Hiva Oa	Feral	EA39
DQ779375	French Polynesia	Marquesas, Hiva Oa	Feral	EA39
DQ779376	French	Marquesas, Hiva Oa	Feral	EA39

Accession No.	Country	Location	Status	Haplotype
	Polynesia			
DQ779409	Papua New Guinea	Admiralty islands, 'wild island'	Feral	EA39
DQ779425	Indonesia	Flores, Arkenas	Feral	EA39
DQ779429	French Polynesia	Marquesas, Tahuata (Hanatuuna)	Feral	EA39
DQ779430	French Polynesia	Marquesas, Hiva Oa (Hanaui)	Feral	EA39
DQ779312	Indonesia	Timor	Feral	EA276
DQ841948	Indonesia	Waingapoe, Soemba island	Domestic	EA276
DQ779337	Indonesia	Flores? Java?	Wild	EA276
DQ779320	Indonesia	Tandjong Morawa Deli (Sumatra)	Wild	EA277
DQ779328	Indonesia	Ceram (Selidewai)	Wild	EA278
DQ779326	Indonesia	Ceram (asaoede)	Wild	EA278
DQ779329	Indonesia	Bali (Sendang, W. Bali)	Wild	EA279
DQ779346	Indonesia	West New Guinea, Salawatti, Sagobos	Feral	EA279
DQ779431	French Polynesia	Marquesas, Hiva Oa (Hanaui)	Feral	EA279
DQ779330	Indonesia	Sendang, W. Bali	Wild	EA280
DQ779338	Indonesia	Flores? Java?	Wild	EA281
DQ779341	Indonesia	Morotai island, North of Halmahera	Feral	EA282
DQ779352	Indonesia	Ternate - small island west of Halmahera	Feral	EA282
DQ779343	Indonesia	Koerik (S.W. coast) bij Merauke, Ned. New Guinea	Feral	EA283
DQ779327	Indonesia	Ceram (Leciela)	Wild	EA284
DQ779349	Indonesia	Tobati, New Guinea	Feral	EA284
DQ779408	Indonesia	Flores, Arkenas	Feral	EA285
DQ779347	Indonesia	Omtrak van Bivak-Eiland, Central New Guinea	Feral	EA286
DQ779530	Solomon Islands	Lomlom Island (Reef Islands)		EA39
DQ779528	Cook Islands	Tangatatau Rock Shelter, Mangaia		EA288
DQ779350	Indonesia	Manikion-Gabied, on bird's head West N.G.	Feral	EA54
Zhang 2004 (unpublished)				
AY751460	China	Northeast	wild	EA36
Gongora *et al.* 2004				
AY486115	China		Domestic	EA93
AY486116	China		Domestic	EA2
AY486117	China		Domestic	EA94
AY486118	China		Domestic	EA95
AY463061	China		Domestic	EA6
AY463062	China		Domestic	EA96
Tanaka *et al.* 2008				
AB252826	Bhutan	Bhutan:Tsirang&Haa (3)	Domestic	EA38
AB252825	Bhutan	Bhutan wild :Jakar (3),Bhutan domestic:Tsirang (7)	Wild and Dom	EA40
AB252824	Cambodia	Kampong Cham	wild	EA124
AB252823	Cambodia	Mondul Kiri	wild	EA125
AB252822	Myanmar	Yangon	wild	EA126
AB252821	Myanmar	Shan State	Domestic	EA238
AB252820	Cambodia, Laos	Cambodia:Mondul Kiri& Ratanakiri(12),Laos:Champasak	Domestic	EA127
AB252819	Laos	Xieng Khuang Province	Domestic	EA128
AB252818	Laos	Borikamxai Province & Xiangkhoang (2) &Vientiane	Domestic	EA108
AB252817	Laos, Myanmar	Laos:Vientiane Province,Myanmar:Shan& Bago	Domestic	EA202
AB252816	Laos, Cambodia	Laos:Xieng KhuangProvince,Cambodia: Ratanakiri (5)	Domestic	EA100
AB252815	Myanmar	Bago division	Domestic	EA129

Accession No.	Country	Location	Status	Haplotype
AB252814	Cambodia	Mondul Kiri	Domestic	EA239
AB252813	Cambodia, Laos	Cambodia:Mondul Kiri (4) & Ratanakiri(3),Laos:Champasak (2)	Domestic	EA236
AB252812	Cambodia	Rattana Kiri	Domestic	EA240
AB252811	Bhutan	Mongar	Domestic	EA130
AB252810	Myanmar	Kachin State	Domestic	EA131
AB252809	Laos	Borikamxai Province	Domestic	EA22
AB252808	Myanmar	Shan State	Domestic	EA241
AB252807	Myanmar	Kachin State& Bago	Domestic	EA242
AB252806	Cambodia, Laos	Laos:Borikamxai Province & Vientiane,Cambodia:Mondulkiri (3)	Domestic	EA106
AB252805	Vietnam Cambodia	Viet Nam:Namha Province,Cambodia:Ratanakiri	Domestic	EA18
AB252804	Myanmar	Shan State	Domestic	EA96
AB252803	Myanmar	Kayin State	Domestic	EA65
AB252802	Laos	Xieng Khuang Province	Domestic	EA65
AB252801	Vietnam	Quang Ninhi Province	Domestic	EA14
AB252800	Myanmar	Kachin State	Domestic	EA32
AB252799	Cambodia	Rattana Kiri	Domestic	EA10
AB252798	Bhutan	Haa	Domestic	EA132
AB252797	Bhutan	Haa	Domestic	EA133
AB252796	Bhutan	Haa	Domestic	EA134
AB252795	Bhutan	Punakha	Domestic	EA243
AB252794	Myanmar, Bhutan	Myanmar:Kachin State,Bhutan:Punaka (2),Tsirang(4),Mongar(5)	Domestic	EA2
AB252793	Bhutan	Mongar	Domestic	EA244
AB252792	Vietnam. Cambodia	Viet Nam:Quang Ninhi Province,Cambodia:Mondulkiri(9)	Domestic	EA9
AB252791	Vietnam	Quang Ninhi Province	Domestic	EA135
AB252790	Laos, Cambodia, Myanmar	Laos:Borikamxai Province,Cambodia:Mondulkiri (5) Myanmar:Kachin(2)	Domestic	EA1
AB252789	Myanmar	Shan state	Domestic	EA188
AB252788	Myanmar	Sagaing division	Domestic	EA245
AB252787	Cambodia	Rattana Kiri	Domestic	EA25
AB252786	Myanmar	Kachin state	Domestic	EA228
AB252785	Myanmar	Bago division	Domestic	EA136
AB252784	Myanmar	Kayin state	Domestic	EA137
AB252783	Myanmar	Kachin state	Domestic	EA29
Ishiguro *et al*. 2008				
AB326952	Vietnam		wild	EA246
AB326951	Vietnam		wild	EA247
AB326950	Vietnam		wild	EA221
AB326949	Vietnam		wild	EA138
AB326948	Vietnam		wild	EA100
AB326947	Vietnam		wild	EA248
AB326946	Vietnam		wild	EA249
AB326945	Vietnam		wild	EA139
AB326944	Vietnam		wild	EA274
AB326943	Vietnam		wild	EA250
AB326942	Vietnam		wild	EA140
AB326941	Vietnam		wild	EA192
AB326940	Vietnam		wild	EA192
AB326939	Vietnam		wild	EA141

Accession No.	Country	Location	Status	Haplotype
AB326938	Vietnam		wild	EA142
AB326937	Vietnam		wild	EA251
AB326936	Vietnam		wild	EA252
AB326935	Vietnam		wild	EA253
AB326934	Vietnam		wild	EA143
AB326933	Vietnam		wild	EA254
AB306916	Vietnam		Domestic	EA272
Robins *et al.* 2006				
DQ444703	Laos	Ban Ni Giangi (18°19'N,104°44'E) Annamite Range	Wild	EA103
Hongo *et al.* 2002				
AB053622	Vietnam	Hunted in North Vietnam	wild	EA104
AB053621	Vietnam	Domestic collected near Hanoi	Domestic	EA29
AB053620	Vietnam	Domestic collected near Hanoi	Domestic	EA105
AB053619	Vietnam	Hunted in North Vietnam	wild	EA223
AB053618	Vietnam	Hunted in North Vietnam	Presumed wild	EA83
AB053617	Vietnam	Hunted in North Vietnam	wild	EA224
AB053616	Vietnam	Purchased 1997 Ba Vi Village, Ha Tay province, nr Hanoi	Domestic	EA9
AB053615	Vietnam	Domestic collected near Hanoi	Domestic	EA106
AB053614	Vietnam	Purchased 1997 Ba Vi Village, Ha Tay province, nr Hanoi	Domestic	EA22
AB053613	Vietnam	Purchased 1997 Ba Vi Village, Ha Tay province, nr Hanoi	Domestic	EA18
AB053612	Vietnam	Purchased 1997 Ba Vi Village, Ha Tay province, nr Hanoi	Domestic	EA18
AB053611	Vietnam	Purchased 1997 Ba Vi Village, Ha Tay province, nr Hanoi / Hunted in North Vietnam	Wild resumed wild	EA107
AB053610	Vietnam	Purchased nr Hanoi / Hunted in North Vietnam	Wild	EA46
AB053609	Vietnam	Purchased 1997 Ba Vi Village, Ha Tay province, nr Hanoi	Domestic	EA18
AB053608	Vietnam	Purchased 1997 Ba Vi Village, Ha Tay province, nr Hanoi	Domestic	EA65
AB053607	Vietnam	Purchased 1997 Ba Vi Village, Ha Tay province, nr Hanoi	Domestic	EA108
AB053606	Vietnam	Purchased 1997 Ba Vi Village, Ha Tay province, nr Hanoi	Domestic	EA6
AB053627		Taiwan	Wild	EA225
AB053626		Taiwan	Wild	EA109
AB053625		Taiwan	Wild	EA110
AB053624	South Korea		Wild	EA111
AB053623	South Korea		Wild	EA112
Cho *et al.* 2003, 2004 and 2005 (unpublished)				
AY879791	South Korea		Domestic	EA6
AY534282	South Korea		Wild	EA112
AY879775	South Korea		Wild	EA112
AY879779	South Korea		Wild	EA111
AY879784	South Korea		Wild	EA111
AY879783	South Korea		Wild	EA111
AY879778	South Korea		Wild	EA111
AY879777	South Korea		Wild	EA111
AY879771	South Korea		Wild	EA113
AY534288	South Korea		Wild	EA113
AY534284	South Korea		Wild	EA113
AY879772	South Korea		Wild	EA226
AY534287	South Korea		Wild	EA226

Accession No.	Country	Location	Status	Haplotype
AY879792	South Korea		Domestic	EA227
AY879787	South Korea		Domestic	EA228
AY879786	South Korea		Domestic	EA114
AY879782	South Korea		Wild	EA229
AY879781	South Korea		Wild	EA115
AY879780	South Korea		Wild	EA116
AY879776	South Korea		Wild	EA117
AY879774	South Korea		Wild	EA118
AY879773	South Korea		Wild	EA119
AY534286	South Korea		Wild	EA120
AY243480	South Korea		Domestic	EA230
Yue 2003 (unpulished)				
AY230827	China		Domestic	EA231
AY230826	China		Domestic	EA232
AY230825	China		Domestic	EA121
AY230824	China		Domestic	EA122
AY230821	China		Domestic	EA123
AY230818	China		Domestic	EA233
Yen 2005 (unpublished)				
AM040641		Taiwan	Domestic	EA1
AM040642		Taiwan	Domestic	EA1
AM040643		Taiwan	Domestic	EA1
AM040644		Taiwan	Domestic	EA1
AM040645		Taiwan	Domestic	EA1
AM040646		Taiwan	Domestic	EA1
AM040653		Taiwan	Domestic	EA1
Wu *et al.* 2007				
EF375877		Taiwan		
DQ972936		Taiwan	Domestic	EA287
Larson *et al.* 2010				
FJ601392	China	Hubei, jianli county	Domestic	EA1
FJ601393	China	Hubei, jianli county	Domestic	EA6
FJ601394	China	Zhejiang, Jinhua county	Domestic	EA144
FJ601395	China	Zhejiang, Jinhua county	Domestic	EA144
FJ601396	China	Zhejiang, Jiaxing county	Domestic	EA3
FJ601397	China	Zhejiang, Jiaxing county	Domestic	EA6
FJ601398	China	Jiangsu, Hai-an county	Domestic	EA1
FJ601399	China	Jiangsu, Hai-an county	Domestic	EA6
FJ601400	China	Jiangsu, Haimen county	Domestic	EA1
FJ601401	China	Jiangsu, Haimen county	Domestic	EA6
FJ601402	China	Yunnan, Kunming	Domestic	EA14
FJ601403	China	Yunnan, Kunming	Domestic	EA14
FJ601404	China	Inner Mongolia, Ordos	Domestic	EA11
FJ601405	China	Inner Mongolia, Ordos	Domestic	EA7
FJ601406	China	Sichuan, Hongya county	Domestic	EA2
FJ601407	China	Sichuan, Hongya county	Domestic	EA20
FJ601408	China	Sichuan, Neijiang city	Domestic	EA10
FJ601409	China	Sichuan, Neijiang city	Domestic	EA10
FJ601410	China	Jiangxi, Xingzi county	Domestic	EA14
FJ601411	China	Jiangxi, Xingzi county	Domestic	EA11
FJ601412	China	Jiangxi, Nancheng county	Domestic	EA6

Accession No.	Country	Location	Status	Haplotype
FJ601413	China	Jiangxi, Nancheng county	Domestic	EA6
FJ601414	China	Yunnan, Qujing county	Domestic	EA145
FJ601415	China	Yunnan, Qujing county	Domestic	EA146
FJ601416	China	Jiangxi, Leping county	Domestic	EA273
FJ601417	China	Jiangxi, Leping county	Domestic	EA273
FJ601418	China	Jiangsu, Taicang county	Domestic	EA2
FJ601419	China	Jiangsu, Taicang county	Domestic	EA2
FJ601420	China	Yunnan, Mengla county	Domestic	EA147
FJ601421	China	Yunnan, Mengla county	Domestic	EA65
FJ601422	China	Yunnan, Luquan county	Domestic	EA148
FJ601423	China	Yunnan, Luquan county	Domestic	EA14
FJ601424	China	Yunnan, Baoshan city	Domestic	EA149
FJ601425	China	Yunnan, Baoshan city	Domestic	EA149
FJ601426	China	Jiangxi, Dongxiang county	Domestic	EA6
FJ601427	China	Jiangxi, Dongxiang county	Domestic	EA6
FJ601428	China	Hunan, Xiangxi county	Domestic	EA1
FJ601429	China	Hunan, Xiangxi county	Domestic	EA1
FJ601430	China	Sichuan, Rongchang county	Domestic	EA2
FJ601431	China	Sichuan, Rongchang county	Domestic	EA14
FJ601432	China	Hainan, Wenchang city	Domestic	EA18
FJ601433	China	Hainan, Wenchang city	Domestic	EA9
FJ601434	China	Shandong, Jiaozhou city	Domestic	EA6
FJ601435	China	Shandong, Jiaozhou city	Domestic	EA6
FJ601436	China	Shaanxi, Lueyang county	Domestic	EA150
FJ601437	China	Shaanxi, Lueyang county	Domestic	EA14
FJ601438	China	Guizhou, Shibing county	Domestic	EA151
FJ601439	China	Guizhou, Shibing county	Domestic	EA151
FJ601440	China	Shandong, Linyi city	Domestic	EA144
FJ601441	China	Shandong, Linyi city	Domestic	EA144
FJ601442	China	Guizhou, Guanling county	Domestic	EA146
FJ601443	China	Guizhou, Guanling county	Domestic	EA14
FJ601444	China	Shandong, Laiwu city	Domestic	EA7
FJ601445	China	Shandong, Laiwu city	Domestic	EA7
FJ601446	China	Hainan, Lingao county	Domestic	EA10
FJ601447	China	Hainan, Lingao county	Domestic	EA1
FJ601448	China	Guizhou, Jiangkou county	Domestic	EA1
FJ601449	China	Guizhou, Jiangkou county	Domestic	EA1
FJ601450	China	Guangdong, Zijing county	Domestic	EA10
FJ601451	China	Guangdong, Zijing county	Domestic	EA10
FJ601452	China	Guizhou, Tongzi county	Domestic	EA152
FJ601453	China	Guizhou, Tongzi county	Domestic	EA146
FJ601454	China	Hubei, Dangyang city	Domestic	EA6
FJ601455	China	Hubei, Dangyang city	Domestic	EA6
FJ601456	China	Anhui, Xuancheng county	Domestic	EA1
FJ601457	China	Anhui, Xuancheng county	Domestic	EA1
FJ601458	China	Guizhou, Liping county	Domestic	EA9
FJ601459	China	Guizhou, Liping county	Domestic	EA9
FJ601460	China	Anhui, Xiuning county	Domestic	EA6
FJ601461	China	Anhui, Xiuning county	Domestic	EA6
FJ601462	China	Shanxi, Datong city	Domestic	EA2
FJ601463	China	Shanxi, Datong city	Domestic	EA2

Accession No.	Country	Location	Status	Haplotype
FJ601464	China	Hubei, Tongcheng county	Domestic	EA6
FJ601465	China	Hubei, Tongcheng county	Domestic	EA12
FJ601466	China	Hubei, Yangxin county	Domestic	EA1
FJ601467	China	Hubei, Yangxin county	Domestic	EA6
FJ601468	China	Heilongjiang, Fujin county	Domestic	EA7
FJ601469	China	Heilongjiang, Fujin county	Domestic	EA7
FJ601470	China	Jiangxi, Shanggao county	Domestic	EA18
FJ601471	China	Jiangxi, Shanggao county	Domestic	EA18
FJ601472	China	Guangdong, Huazhou city	Domestic	EA53
FJ601473	China	Guangdong, Huazhou city	Domestic	EA9
FJ601474	China	Jiangxi, Yushang county	Domestic	EA22
FJ601475	China	Jiangxi, Yushang county	Domestic	EA22
FJ601476	China	Qinghai, Huzhu county	Domestic	EA153
FJ601477	China	Qinghai, Huzhu county	Domestic	EA153
FJ601478	China	Hunan, Xiangtan county	Domestic	EA154
FJ601479	China	Fujian, Putian county	Domestic	EA6
FJ601480	China	Fujian, Putian county	Domestic	EA155
FJ601481	China	Fujian, Wuyishan city	Domestic	EA1
FJ601482	China	Fujian, Wuyishan city	Domestic	EA2
FJ601483	China	Fujian, Shanghang county	Domestic	EA156
FJ601484	China	Fujian, Shanghang county	Domestic	EA156
FJ601485	China	Jiangsu, Changshu city	Domestic	EA2
FJ601486	China	Jiangsu, Changshu city	Domestic	EA6
FJ601487	China	Hainan, Haikou city	Domestic	EA18
FJ601488	China	Hainan, Haikou city	Domestic	EA157
FJ601489	China	Shan-Dong	Domestic	EA6
FJ601490	China	Shan-Dong	Domestic	EA1
FJ601491	China	Hunan, Taoyuan county	Domestic	EA14
FJ601492	China	Hunan, Taoyuan county	Domestic	EA2
FJ601493	China	Guizhou, Hezhang county	Domestic	EA6
FJ601494	China	Guizhou, Hezhang county	Domestic	EA80
FJ601495	China	Sichuan, Chengdu city	Domestic	EA65
FJ601496	China	Sichuan, Chengdu city	Domestic	EA158
FJ601497	China	Guangxi, Longlin county	Domestic	EA70
FJ601498	China	Guangxi, Longlin county	Domestic	EA159
FJ601499	China	Guizhou, Guiyang city	Domestic	EA18
FJ601500	China	Guizhou, Guiyang city	Domestic	EA18
FJ601501	China	Guangxi, Donglan county	Domestic	EA1
FJ601502	China	Guangxi, Donglan county	Domestic	EA2
FJ601503	China	Hunan, Changsha city	Domestic	EA65
FJ601504	China	Hunan, Changsha city	Domestic	EA6
FJ601505	China	Guangxi, Luchuan county	Domestic	EA160
FJ601506	China	Guangxi, Luchuan county	Domestic	EA161
FJ601507	China	Guangxi, Quanzhou county	Domestic	EA10
FJ601508	China	Guangxi, Quanzhou county	Domestic	EA18
FJ601509	China	Zhejiang	Domestic	EA6
FJ601510	China	Zhejiang	Domestic	EA162
FJ601511	China	Guangxi, Liuzhou city	Domestic	EA160
FJ601512	China	Guangxi, Liuzhou city	Domestic	EA163
FJ601513	China	Hunan, Ningxiang county	Domestic	EA1
FJ601514	China	Hunan, Ningxiang county	Domestic	EA9

Accession No.	Country	Location	Status	Haplotype
FJ601515	China	Hubei, Gongan county	Domestic	EA164
FJ601516	China	Hubei, Gongan county	Domestic	EA2
FJ601517	China	Guangxi, Bama county	Domestic	EA18
FJ601518	China	Guangxi, Bama county	Domestic	EA10
FJ601519	China	Tibet, Linzhi city	Domestic	EA165
FJ601520	China	Tibet, Linzhi city	Domestic	EA22
FJ601390	China	Henan, Nanyang city	Domestic	EA259
FJ601391	China	Henan, Nanyang city	Domestic	EA270
FJ601521	China	Zhejiang, Jinhua county	wild	EA6
FJ601522	China	Zhejiang, Jinhua county	wild	EA14
FJ601523	China	Hainan, Sanya city	wild	EA166
FJ601524	China	Hainan, Sanya city	wild	EA167
FJ601525	China	Jiangxi, Jinan county	wild	EA20
FJ601526	China	Heilongjiang, Fujin county	wild	EA144
FJ601527	China	Heilongjiang, Fujin county	wild	EA144
FJ601528	China	Heilongjiang, Fujin county	wild	EA14
FJ601529	China	Heilongjiang, Fujin county	wild	EA14

APPENDIX 2: Survey questionnaire

Q1. Purpose of keeping indigenous pigs: *Tick any purpose considered. One or more boxes can be ticked. Then rank top three by writing 1 for primary, 2 for secondary, 3 for third.*

	Male		***Female***	
	Purpose	***Rank***	***Purpose***	***Rank***
Meat				
Hair				
Breeding				
Manure				
Savings				
Wealth status				
Gift for others				
Annual Pooja/Rimdo/				
Offering deity				
Send children to school				
Others (Specify)				

Q2. Origin / source of indigenous pigs: *Tick one or more boxes*

Own herd	
Inherited	
Government supplied	
Market	
Within village	
Neighbour	
Next village	
Within geog	
Within Dzongkhag	
Within Bhutan	
Outside Bhutan	

Q3. Number of indigenous pigs kept by the farmer:

Age	*Male*	*Female*
Piglets (<2 months)		
Growers (>2 – 8months)		
Adults (>8 monts)		

Q4. Population trend of indigenous pigs: *Click only one box from each column)*

Trend	
Increasing	
Stable	
Decreasing	
Unknown	
Other specify (below)	

Q5. Reasons for this trend:

Trend	*Tick only one box*
Decreased interest of the farmer	
Increased interest of the farmers	
Breed is widely available	
Breed has become very rare	
Competition with exotic breeds	
Competition with local breeds	
Others (specify)	

Q6. Important traits of indigenous pigs perceived by owner: *Use 1-5 to indicate importance of each trait.*

Traits	*Codes* Not important = 1; Poor = 2; Average = 3; Good = 4; No opinion =5
Size/shape	
Hair	
General appearance	
Compact body	
Feeding	
Heat/cold tolerant	
Temperament	
Meat taste	
Growth rate	
Fertility	
Mother ability	
Reproductive performance	
Scavenging ability	
Disease tolerance	
Longevity/durability	

Q7. Body measurement of indigenous pigs (please measure, record and count):

Measurement	Sex	Measurement
Heart girth (size - m)	*Sow*	
	Boar	
Body Length (m)	*Sow*	
	Boar	
Average no of teats (no)	*Sow*	
	Boar	
Adult live weight (kg)	*Sow*	
	Boar	

Q8. Physical description of indigenous pigs. Use column 2 as guide. But you can also describe according to your observation:

Physical Trait	*Guide*	*Physical Description of Rinchengang Indigenous pigs*
Bristles distribution	Only on the dorsal line,	
Hair	Curly, straight, short, long, dense, sparse	
Snout	Long thin, cylindrical snout, short, concave	
Head profile	Concave (dished), straight, convex	
Ear size	Large, medium, small	
Ear type	Droopy, prick, Semi-lop	
Ear Orientation	Project forwards, backwards, upwards	
Skin	Smooth or wrinkled	
Tail type	Straight or curly or kinked	
Udder symmetry	Symmetrical (equal number of teats on both sides), asymmetrical.	
State of domestication	Half wild (feral), domesticated, 75% wild	
Temperament	Placid and friendly, aggressive	

Q9. Production Performance of indigenous pigs:

Production parameters		Performance
Sexual maturity (months)	Boar	
	Gilt	
Oestrous length (days)		
Gestation period (days)		
live litters born (nos)		
Stillborn piglets (nos)		
Litter size (nos)		
Live litter birth weight (nos)		
No of farrowing per year (nos)		
Piglet mortality before weaning (nos)		
Weaning weight (kg)		
Mortality after weaning (nos.)		
Castration age (days)		
Live weight at slaughter (kg)		
Weight of dressed carcass (kg)		
Slaughtering age (months)		
Cost of pork/kg		

Q10. Source of indigenous boar? *Can tick more than one box box.*

Own herd	
Within the village	
Unknown boar	
Communal boar	
Breeding occurs without our knowledge	

Q11. Rearing/housing system:

Rearing/housing system	***Yes***	***No***
Always tethered		
Tethered & scavenging (combined)		
Scavenging with night shelter provided		
Scavenging without night shelter		
Backyard sty (confined)		
Semi-intensive type (>15 pigs)		
Any other form of housing/rearing system (Please mention below)		

Q12. Feeding

Feeds and feeding	***Yes***	***No***
Commercial feed		
Local feed		
No feeding due to scavenging		
Scavenging with local feed		
Frequency of feeding		
Once a day		
Twice a day		
Every other day		
Others (Specify)		

Q13. Watering

Water	***Yes***	***No***
Good clear water		
Muddy & smelly water		
Frequency of water		
One to twice a day		
Every other day		
Others (Specify)		

Q14. Health management

Health	***Yes***	***No***	***Remarks***
De-worming			If yes, who does it and how often?.....
Vaccination			If yes, who vaccinate and how often?.....
Do you clean your pig sty?			Who often do you clean the shed?.....
Any disease outbreak during past one year			If yes, how many times?.....

Q15. Any Other Additional Information

……

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