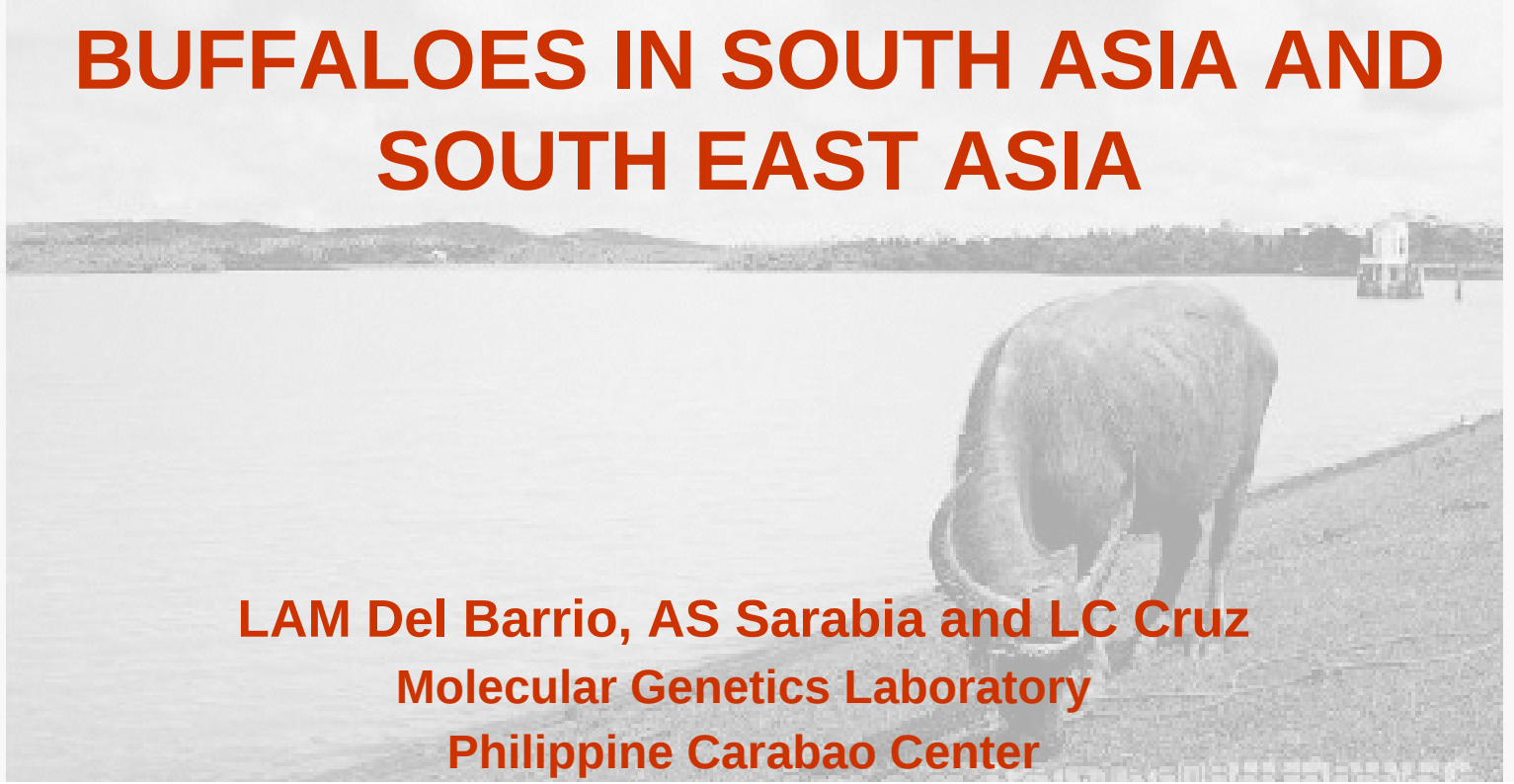
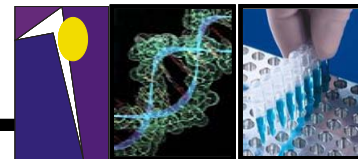


GENETIC RELATIONSHIP OF SWAMP BUFFALOES IN SOUTH ASIA AND SOUTH EAST ASIA



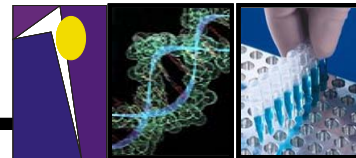
**LAM Del Barrio, AS Sarabia and LC Cruz
Molecular Genetics Laboratory
Philippine Carabao Center**

**National Headquarters and Genepool
Science City of Munoz, Nueva Ecija, Philippines**



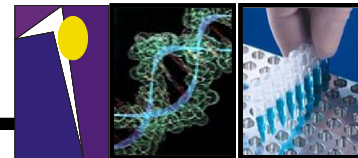
How to measure genetic relatedness?

1. Mitochondrial DNA (mtDNA)
2. Microsatellites
3. Protein coding loci

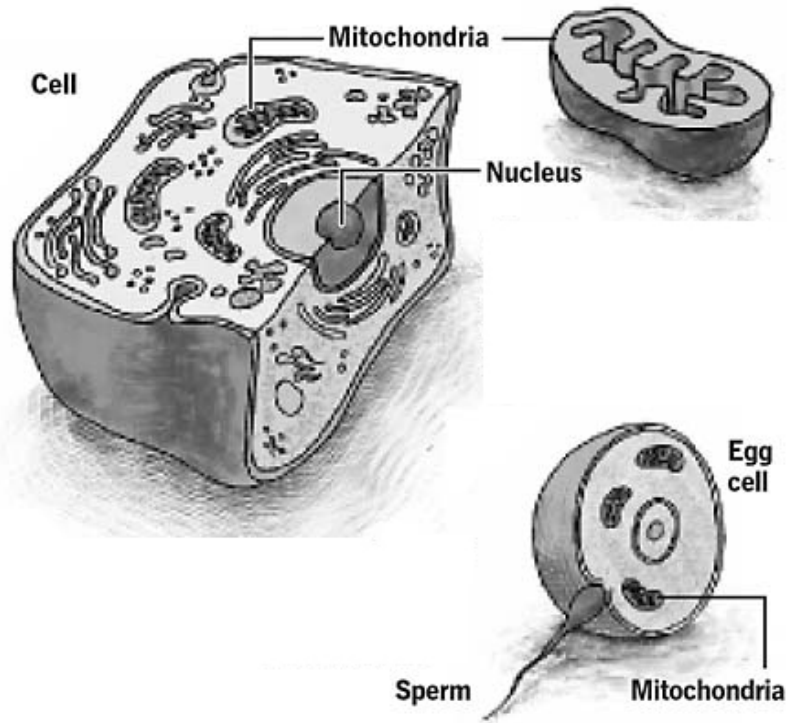


Molecular characterization of the Philippine carabao (*Bubalus bubalis* L.) from the major island groups of the Philippines using molecular cloning and sequence analysis of the D-loop of mitochondrial DNA

- LAM del Barrio, CB dela Vina, MGN Yebron Jr., CAS Estrella, JRV Herrera, AN del Barrio



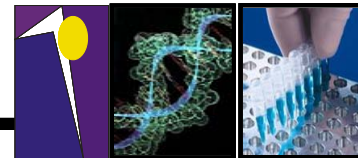
Mitochondrial DNA (mtDNA)



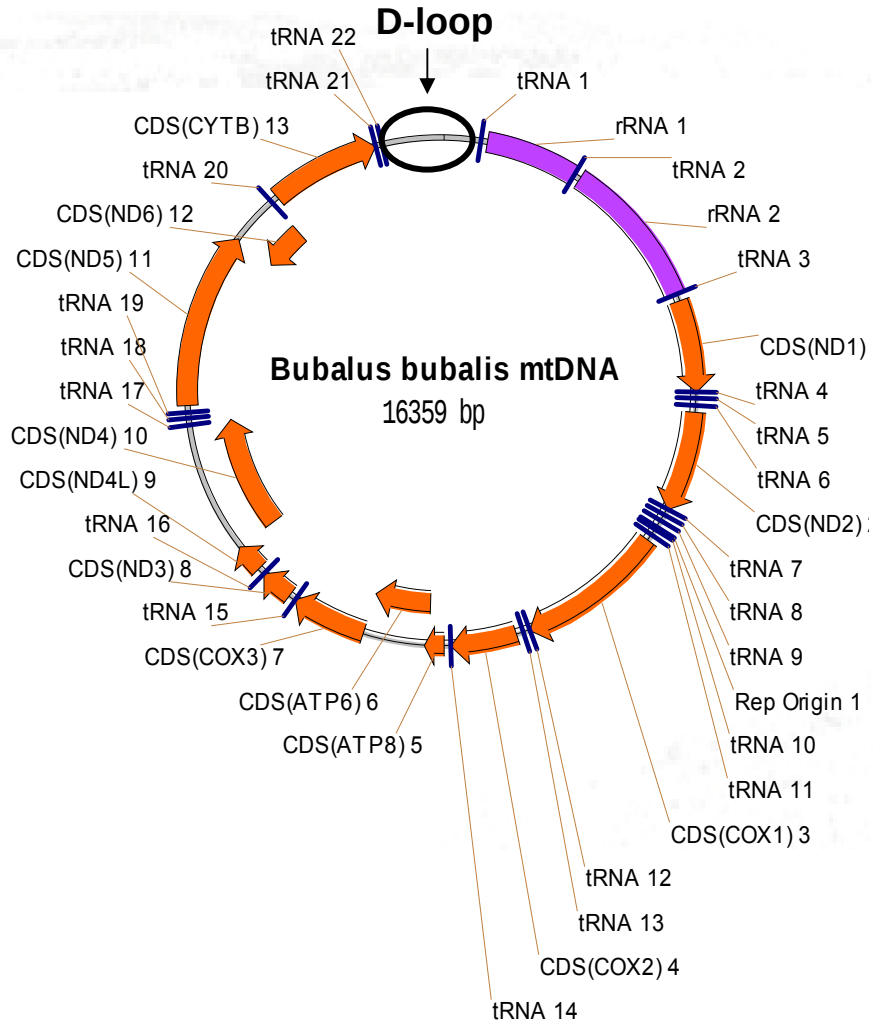
Source: AP

Associated Press Graphic

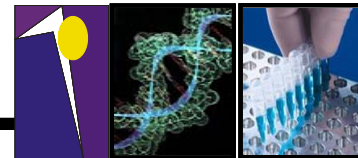
- Maternally inherited
- No recombination
- High rate of substitution
- Phylogenetic and relationship studies



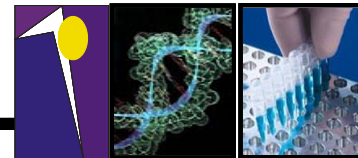
mtDNA of Water Buffalo



- ~ 16 kb
- D-loop
 - Non-coding
 - Most variable region
 - 910 bases (Qian *et al.*, 2004)

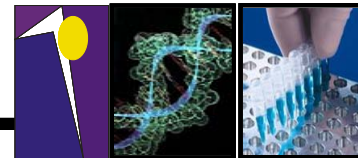


Philippine Carabao (*Bubalus bubalis* L.)



Objectives

- ▶ To characterize the Philippine carabao at the molecular level through mtDNA sequence analysis
- ▶ To analyze the D-loop DNA sequences and compare them to sequences from different parts of the country and from other countries



Methodology

Sample Collection



DNA Extraction



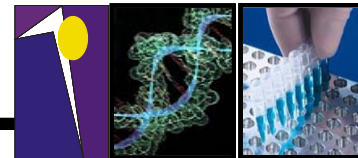
Optimization of Polymerase
Chain Reactions (PCR)

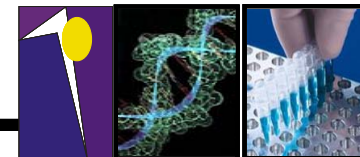
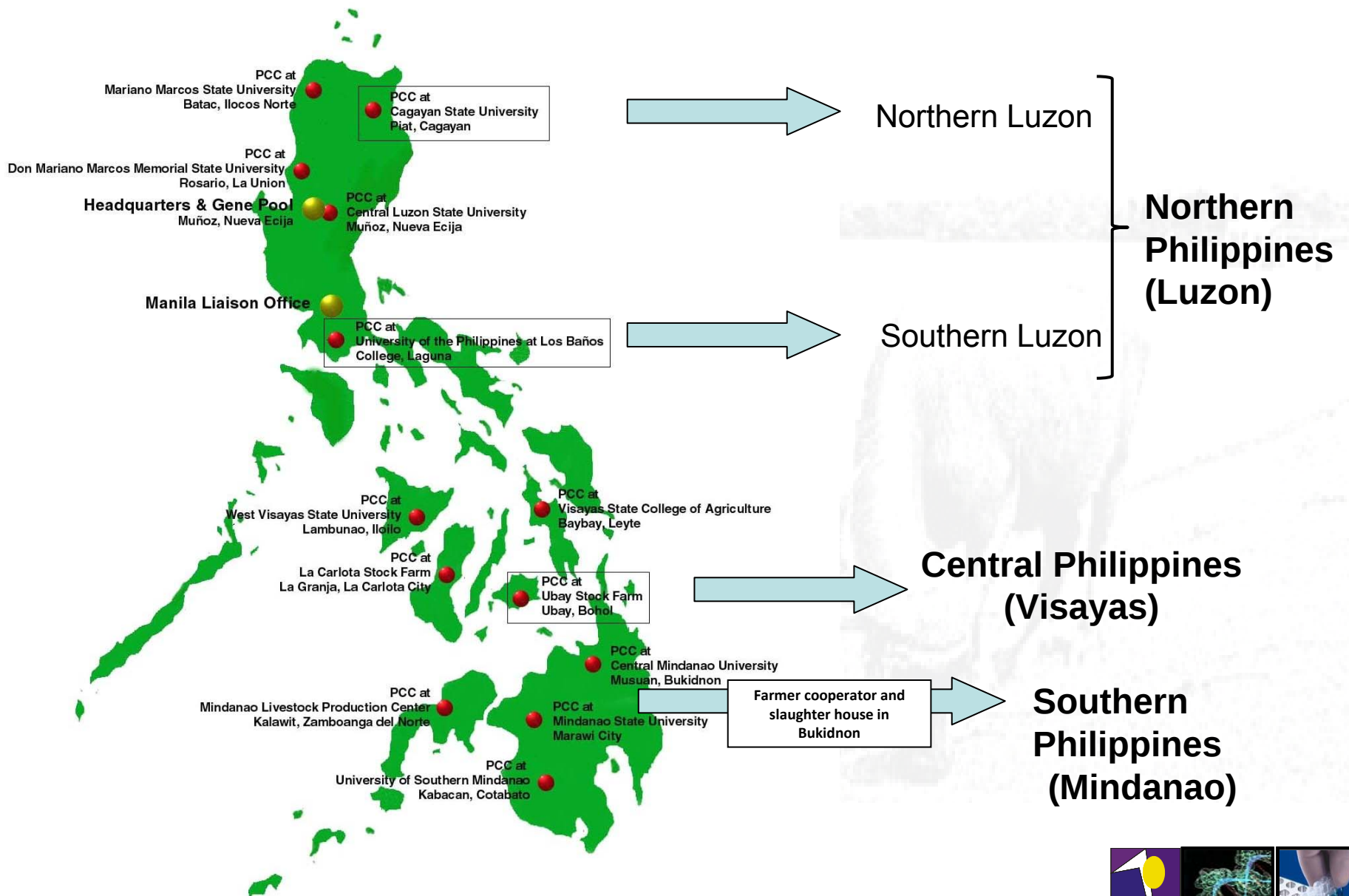


Molecular Cloning and
Sequencing



Sequence Analysis





Methodology

Sample Collection



DNA Extraction



Optimization of Polymerase
Chain Reactions (PCR)



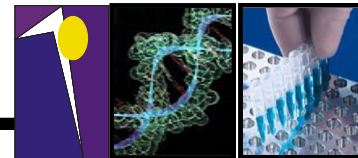
Molecular Cloning and
Sequencing



Sequence Analysis



MACROGEN
Advancing through Genomics



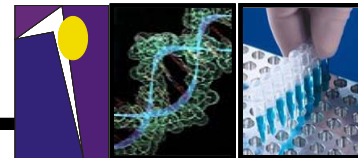
PCC Molecular Genetics Lab

Multiple Sequence Alignment Analysis

Island Group	Origin	Sample No.	GENBANK Accession No.
North Luzon	Cagayan	C1	FJ873680
		C9	FJ873681
South Luzon	Batangas	1	FJ873676
		5	FJ873677
Visayas	Bohol	B1	FJ873682
		B10	FJ873683
Mindanao	Bukidnon	A1	FJ873678
		A2	FJ873679

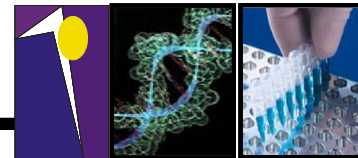


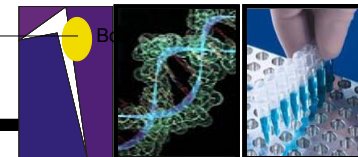
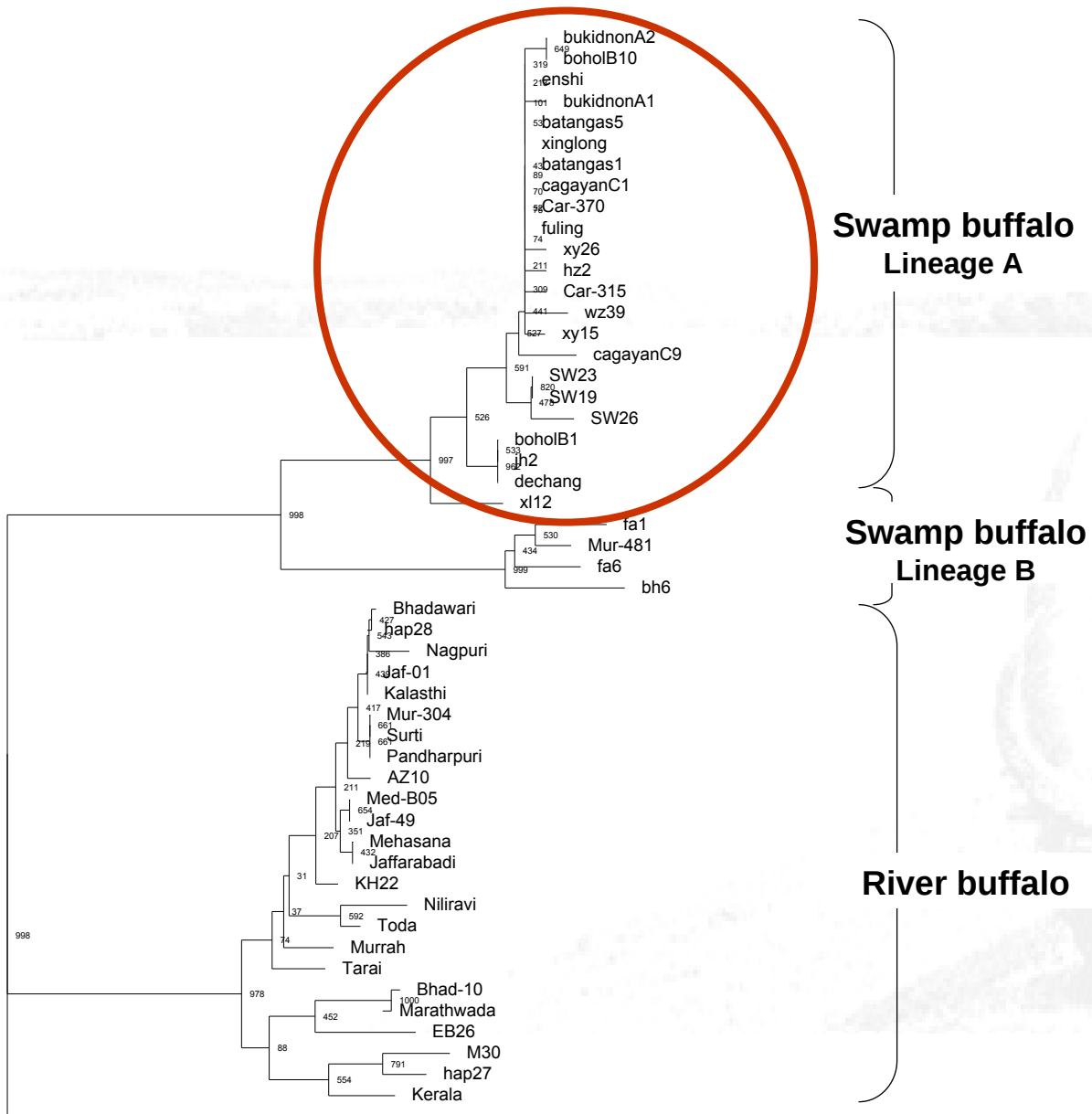
97.5 % homology



BLAST-N Analysis

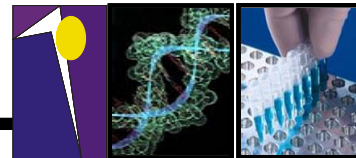
Island Group	Sample No.	BLAST Result	Origin	Homology
North Luzon	UPLB21	<i>Bubalus bubalis</i> isolate FuLing1213 D-loop, partial sequence	China	100 %
	UPLB26	<i>Bubalus bubalis</i> isolate FuLing1213 D-loop, partial sequence	China	99 %
South Luzon	UPLB1	<i>Bubalus bubalis</i> isolate FuLing1213 D-loop, partial sequence	China	100 %
	UPLB7	<i>Bubalus bubalis</i> isolate Xinglong-2105 control region, partial sequence	China	100 %
Visayas	UPLB31	<i>Bubalus bubalis</i> isolate Dechang-0163 control region, partial sequence	China	100 %
	UPLB36	<i>Bubalus bubalis</i> isolate Enshi-1803 control region, partial sequence	China	99 %
Mindanao	UPLB13	<i>Bubalus bubalis</i> isolate Xinglong-2105 control region, partial sequence	China	99 %
	UPLB16	<i>Bubalus bubalis</i> isolate FuLing1213 D-loop, partial sequence	China	99 %





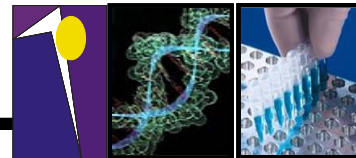
Findings

- Sequence analysis of Philippine carabao isolates from the major island groups presented a 97.5% homology.
- BLAST-N analysis showed the probable maternal line of the Philippine carabaos, which is the Chinese buffalo.



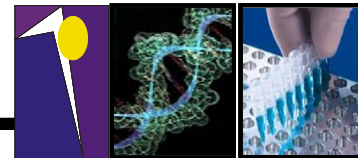
Findings

- Rooted phylogenetic tree confirmed that the Philippine carabao belongs to Lineage A of swamp buffaloes and that it is closely related to the Chinese buffaloes.



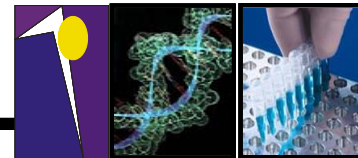
Genetic variation within and relationships among populations of Asian water buffalo (*Bubalus bubalis*)

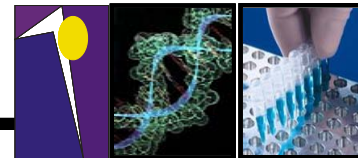
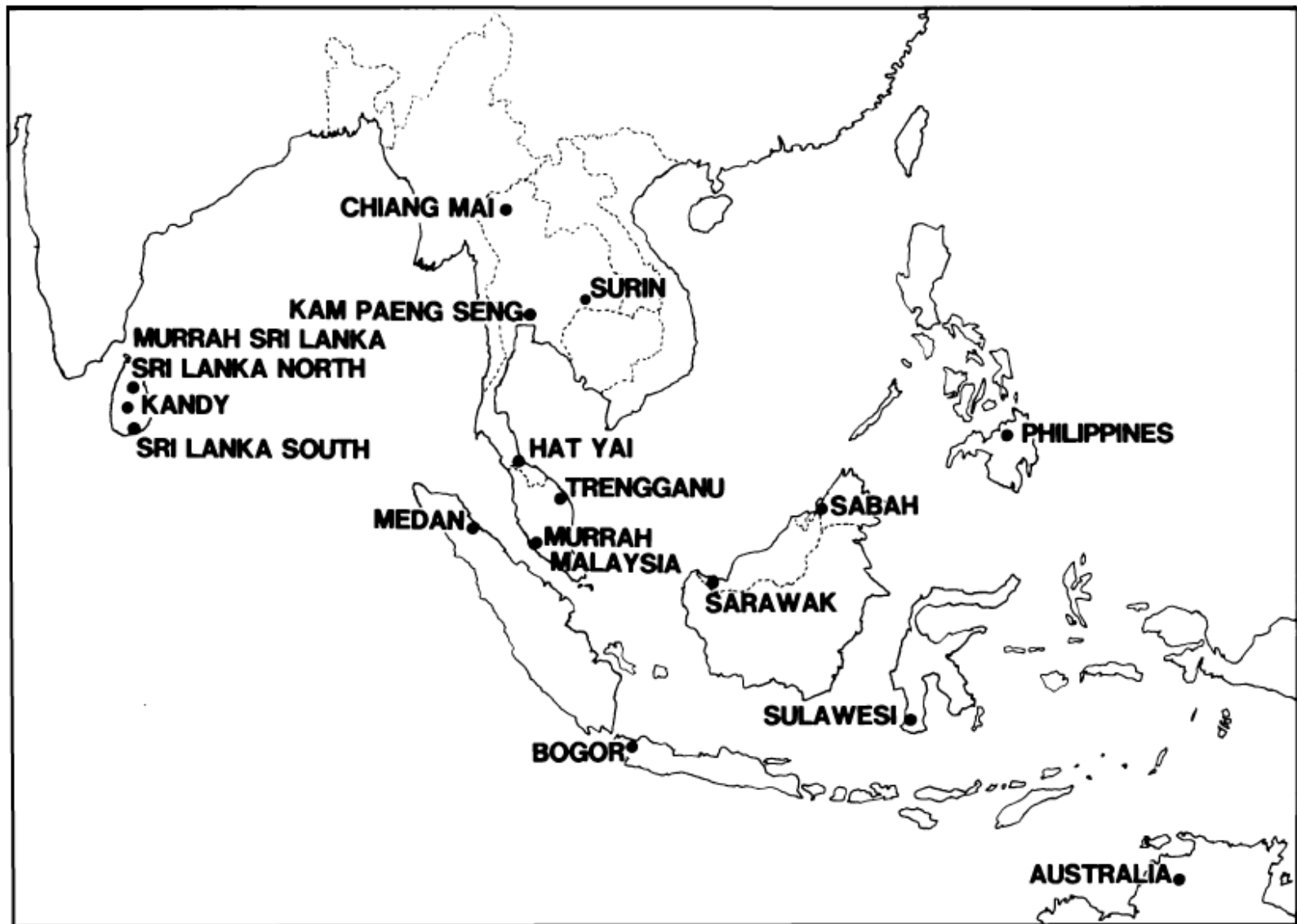
- JSF Barker, SG Tan, OS Selvaraj, TK Mukherjee
- *Animal Genetics* 1997, 28, 1-13



Background

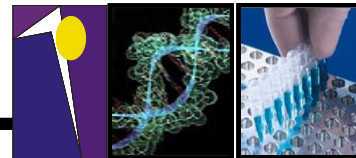
- 53 protein coding loci
- 12 swamp buffalo
 - Thailand, Malaysia, Philippines, Indonesia, Australia





Findings

- Significant genetic differentiation among populations of swamp types



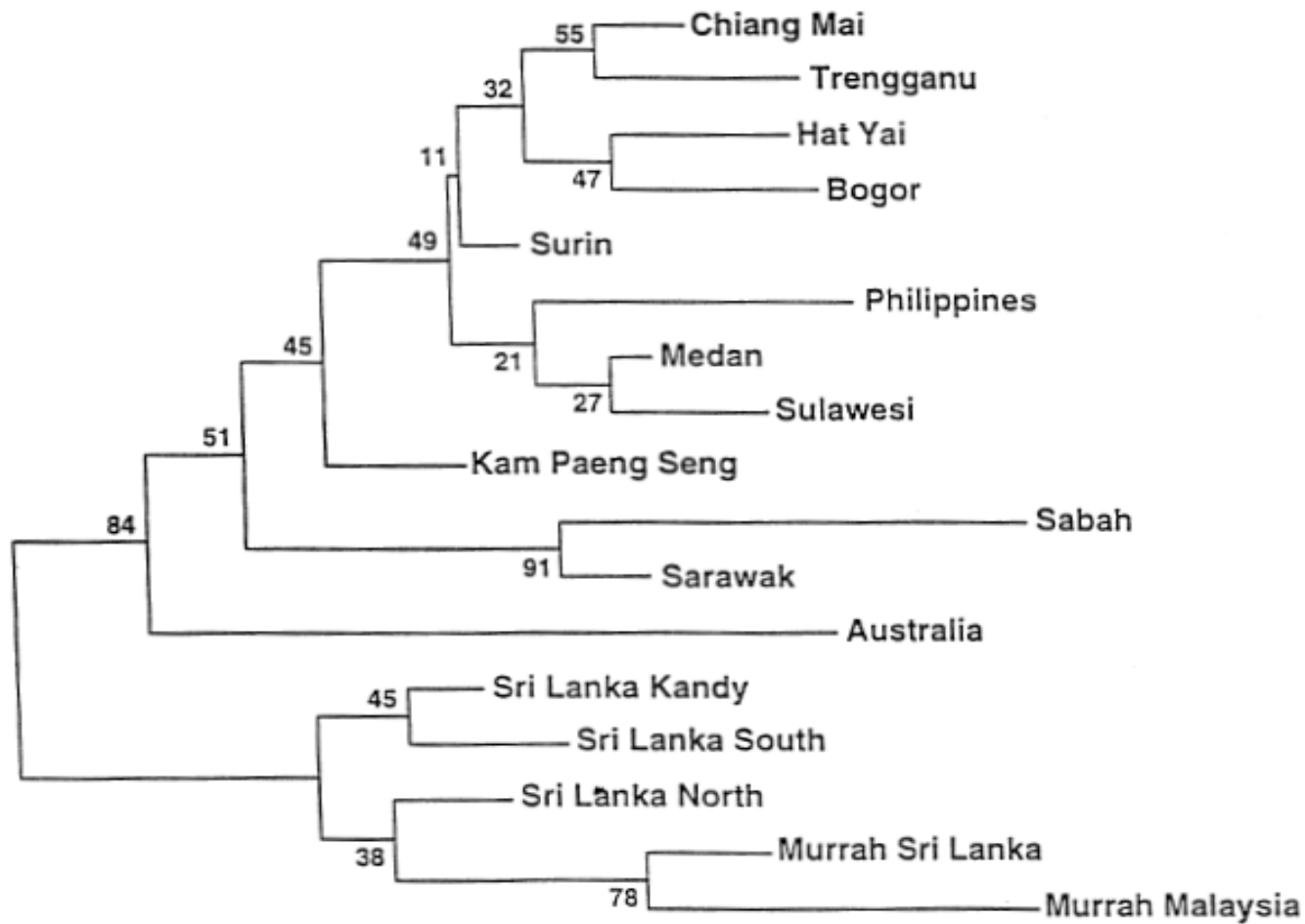
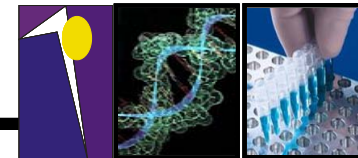
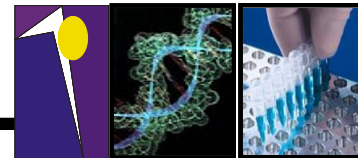


Fig. 2. Dendrogram of relationships among 17 water buffalo populations, using D_A genetic distances (Table 7) based on 53 protein coding loci, and the neighbour-joining method of clustering. Numbers on the nodes are percentage bootstrap values from 1000 replications of resampled loci.



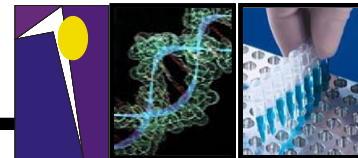
Genetic diversity of Asian water buffalo (*Bubalus bubalis*): microsatellite variation and a comparison with protein coding loci

- JSF Barker, SS Moore, DJS Hetzel, D Evans, SG Tan, K Byrne
- *Animal Genetics* 1997, 28, 103-115

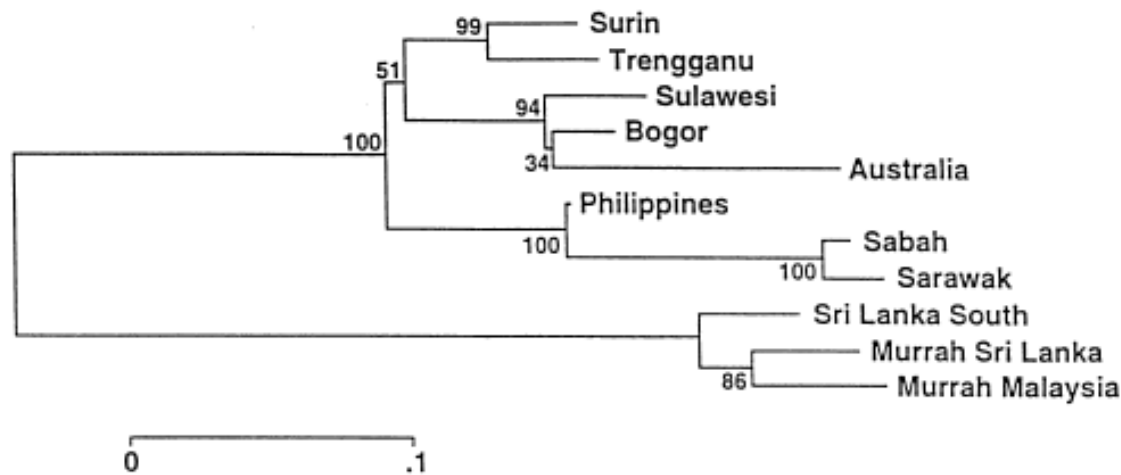


Background

- 25 polymorphic protein coding loci
- 21 microsatellite loci
- 8 swamp buffalo
 - Thailand, Malaysia, Philippines, Indonesia, Australia



(A) Microsatellites



(B) Protein coding loci

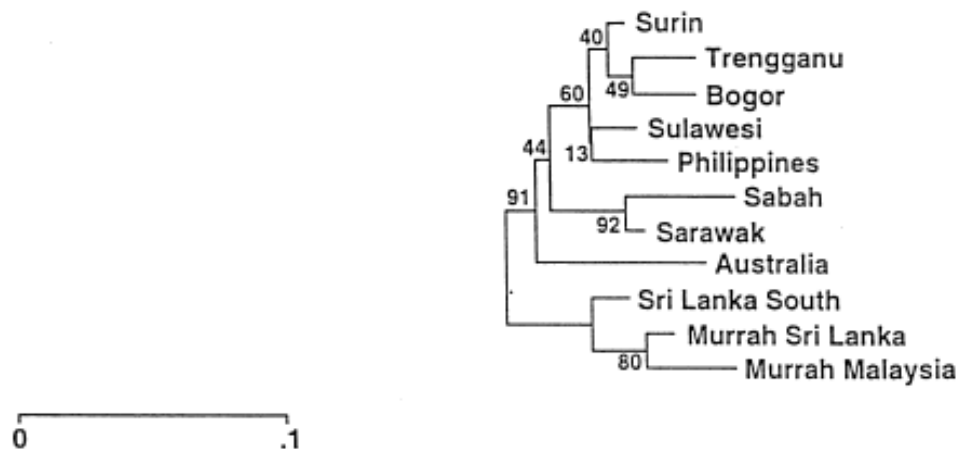
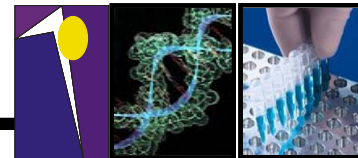


Fig. 3. Dendrograms of relationships among 11 water buffalo populations, using D_A genetic distances and the neighbour-joining method of clustering, which were based on (A) 21 polymorphic microsatellite loci, and (B) 25 polymorphic protein-coding loci. Numbers on the nodes are percentage bootstrap values from 1000 replications of resampled loci, and a scale bar for branch lengths is shown.

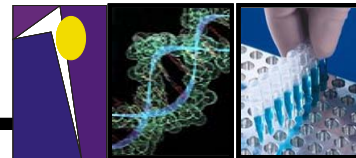
Findings

- Differentiation among swamp populations may reflect the geography of SE Asia and the presumed spread of swamp buffalo through this region and the relatively recent spread of water buffalo throughout the region.



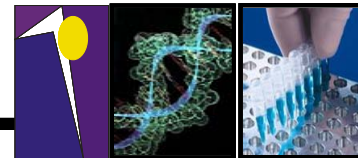
SUMMARY

- Genetic relationship of swamp buffaloes in Asia are determined through its evolution
- Differences among swamp populations may be due to geography and domestication



References

- Barker JSF, SS Moore, DJS Hetzel, D Evans, SG Tan and K Byrne. 1997. Genetic diversity of Asian water buffalo (*Bubalus bubalis*): microsatellite variation and a comparison with protein coding loci. *Animal Genetics* 28: 103-115.
- Barker JSF, SG Tan, OS Selvaraj and TK Mukherjee. 1997. Genetic variation within and relationships among populations of Asian water buffalo (*Bubalus bubalis*). *Animal Genetics*: 1-13.
- Del Barrio LAM, CB dela Vina, MGN Yebron Jr., CAS Estrella, JRV Herrera and AN del Barrio. 2009. Molecular characterization of the Philippine carabao (*Bubalus bubalis* L.) from the major island groups of the Philippines using molecular cloning and sequence analysis of the D-loop of mitochondrial DNA. Unpublished.



THANK YOU!

