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GENETIC DIVERSITY OF ALBINOID SWAMP BUFFALOES (*Bubalus kerabau*, Fitzinger, 1860) IN THE PHILIPPINES

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ABSTRACT

White swamp buffaloes or albinoids are having pink skin that is covered with white hair, black spots on the entire body, and freckles on the muzzle. The Lamac Cooperative, in Pinamungajan Municipality in Cebu, favors raising abinoids in the community for tourism as white buffalo producing milk. The study aimed to verify the species identification and population structure. Fifty-four blood samples from white water buffaloes from Lamac and Pinamungajan in Cebu and other populations in the country were included in the analysis. Twelve microsatellite markers were used for the population structure and breed categories for pure swamp, pure riverine, F1 (swamp x riverine), F2 (F1 crosses), backcrosses swamp, and riverine using Bayesian model-based clustering. Population structure analysis resulted in 69.8% membership coefficient to swamp populations in Carabao Island in Romblon and Pitogo Island in Bohol. The white buffaloes had also 92.2% posterior probability (PP) to the swamp group. However, one white buffalo fell in the F1 category at 77% PP. Research findings established the albionoids' baseline information on their DNA profiles, which is valuable information for developing rational conservation and management in the Philippines.

Keywords: albionoid, genetic diversity, microsatellites, population structure

INTRODUCTION

The coat color variants in the Philippine swamp buffaloes (*Bubalus kerabau*, Fitzinger, 1860) were usually dark, gray, brown, and sometimes completely white with white hair and pink skin (Kornel, 1989). The white coat color in buffaloes could be explained by a single gene that is entirely dominant to gray and is partially dominant or epistatic gray (Rife, 1962). Other studies reported that various terrains, such as mountain ranges, plains, and coastal areas, could result in diverse coat color patterns of swamp buffaloes. For example, the brown coat pattern could be linked to the adaptation of buffaloes to mountainous regions since mud holes and swamps were less abundant in these areas (Khan *et al.,* 2013). In Asia, white swamp buffaloes, with varying color pattern including the black-pied, red-pied, and red-black-pied and spotting, were previously reported with rare cases in the Eastern islands of Indonesia and Southern China in the Island of Hainan of about 30% were albino (Fischer, 1969; Levine, 1925).

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White coat color in humans and animals could be linked to a genetic type 1 occulocutaneous albinism (OCA). This condition was caused by an autosomal recessive hereditary pigmentation in which partial or total absence of melanin was observed in the hair, skin, and eyes. The reduction of melanin occurs due to the point of mutation that decodes the tyrosinase gene (*TYR*) enzyme that takes part in the melanin synthesis process (Oetting, 2000). In buffaloes, a nonsense mutation in the 1431 (G to A) was pointed to produce an inactive TYR protein, which resulted in the absence of pigmentation in the skin, hair, hooves, horns, iris, and mucous membranes and with some manifestation of photophobia (Damé *et al.,* 2012; Bernardino *et al.*, 2019).

The other study on the albinism test using the ASB Tyrosinase gene (ASB TYR) revealed no mutation from Guanine to Adenine and the absence of heterozygote gene in the Philippine white swamp buffaloes, implying that animals in the population have no presence and no carrier of OCA gene, respectively. The results suggested that white swamp buffaloes with pigmented periocular regions are not albinos and should be appropriately recognized as albinoids.

These animals are highly recommended for proper care and management to prevent susceptible skin and eye problems while utilizing them for draft power in farm cultivation in the Philippines. To date, established records of the current population of white buffaloes in the Philippines are lacking. In 1962, it was reported that white buffaloes were much less than 1 percent of the 3.5 million carabao in country. In the same year, the prevalence of albinoid was found in Marikina, San Mateo, and Montalban. However, failing records have yet to be accounted for (Singleton and Santos, 1964). The previous study on the genetic diversity of Philippine Carabao documented 25 white buffaloes across the Philippines with various phenotypic characteristics such as spotted, pure white, and pinkish coat color with white spots all over the body (Villamor *et al.*, 2018). In addition, Paraguas *et al.* (2018) reported a case of spotted coat pattern of swamp buffalo in the Island of Calayan, Cagayan which could a carrier of albinism gene in the population.

Since 2018, the Lamac Cooperative in Cebu and its nearby barangays favored raising white swamp buffaloes in the community. These individuals have inadequate pigmentation, with white hairs over the entire body and pinkish skin that sometimes has dark spots and eyes (Singleton and Santos, 1964). These initial efforts of the white swamp buffalo enthusiasts are supported by the Philippine Carabao Center (PCC) at Ubay Stock Farm. The fascinating question of propagating this buffalo breed leads to "How much do we know of the molecular characteristics of white swamp buffaloes?"

The fact is that little is known about the prevalence of their genes that make them easily grow and spread not only in Cebu but perhaps across the islands of Luzon, Visayas, and Mindanao. In support of conservation management and future utilization of this breed, concerted efforts by PCC at USF and Cryobank-Unit established the baseline information on the molecular characterization of white swamp buffaloes that could directly impact animal management and propagation. Thus, the study aimed to establish baseline information on albinoid genetics in the country.

MATERIALS AND METHODS

Twenty-nine blood samples were collected from white buffaloes in Brgy. Lamac in Pinamungajan, Cebu, and 25 samples from the other provinces in the country were included in the analysis (Table 1). Each animal owner voluntarily agreed with the consent on animal handling during the collection of blood samples, following the guidelines on animal care as implemented by the Philippine Carabao Center Ethics Committee.

Table 1. Number of white buffalo samples collected across Luzon, Visayas, and Mindanao.

Major Island	Province	No. of Samples
Luzon (n=3)	Occidental Mindoro	1
	Camarines Sur	1
	Zambales	1
Visayas (n=36)	Capiz	6
	Ilo Ilo	1
	Lamac, Cebu	29
Mindanao (n=15)	South Cotabato	4
	Saranggani	2
	Davao	7
	Bukidnon	1
	Sultan Kudarat	1
Total		54

Genomic DNA was extracted from the whole blood samples using the commercially available DNA extraction kit from RealiPrep™ Blood gDNA with some modifications (Villamor *et al.,*2021). The polymerase chain reaction (PCR) was composed with the final volume of 15µl and concentration of the following: 1X colorless Go Taq® reaction buffer, 1.33mM $MgCl_2$, 0.2mM dNTPs, 0.5µM of each forward and reverse primer, 1 Unit Go Taq® Polymerase, and at least 3.33ng of genomic DNA (gDNA). The PCR thermal cycling conditions were optimized for all 10 STR markers used in the study with initial denaturation at 95˚C for 2 min, followed by 35 cycles of 95˚C for 30 sec, 53-67˚C for 30 sec, 72˚C for 30 sec, and final extension of 72˚C for 5 min. The PCR products with various allele sizes were visualized using 2% agarose in gel electrophoresis by GelRedTM staining.

Genetic Diversity and Population Structure

The genotyping for each locus was scored using Geneious Prime® (v2021.1 Biomatters Ltd.). The Microsatellite plugin was downloaded to remove excess peaks following the internal size standard of GeneScan™ 500LIZ™. Allele scores and bins were manually checked and exported through the .csv file. The genetic diversity parameters for the number of alleles per locus (Na), observed (Ho), and expected heterozygosity (He) were computed using GenAlEx 6.503 software (Peakall and Smouse, 2012). *F*-statistics were computed for the overall deficit of heterozygotes (FIS), and genetic differentiation (FST) was analyzed using Arlequin 3.1 software (Excoffier and Heckel, 2006). Analysis of the genetic structure was carried out through an alternative model-based Bayesian clustering analysis using STRUCTURE (Pritchard *et al.,* 2000) and the optimum ΔK value was visualized and calculated using the STRUCTURE Harvester program (Earl and vonHoldt, 2012).

Estimating Blood Proportion

The blood proportions of white buffaloes were estimated using a seven and MCMC simulation. This computed the posterior probability of each individual that falls into distinct hybrid classes. The pure swamp types from Calayan Island, Cagayan (Swamp Cluster 1), and Pitogo Island, Bohol (Swamp Cluster 2) were considered swamp parents (Villamor *et al*., 2023). On the other hand, the Bulgarian Murrah buffaloes from the PCC-Genepool were the pure-type riverine references for the outgroup. These two parents were used as parental references for Pure Swamp (P0) and Pure Riverine (P1), which have a genetic pairwise FST of 0.272 value, following the recommendation of ≥0.12.FST value by Gagnaire *et al.* (2009).

RESULTS AND DISCUSSION

Genetic variability

Among the four selected albinoid populations, alleles range from 0.272 in Luzon to 0.536 in Lamac, Cebu, with a mean value of 0.375. These values were expected since Luzon has the lowest number of representative animals while Lamac in Cebu has the highest (Table 2). The heterozygosity observed in this study revealed a mean value of 0.564, which was noticeably higher than the expected heterozygosity with a mean value of 0.547. On the other hand, the genetic differentiation (FST) among white swamp populations included in the study revealed low population differentiation, which could be attributed to low genetic exchange among animals, specifically for the Mindanao and Lamac in Cebu albinoids. This can be further supported by the reduced number of observed (Ho) than the expected heterozygosity (He) in both populations. However, the negative FIS observed in Lamac, Cebu, revealed excess heterozygosity, which could reflect the widespread distribution and possible random mating of white buffalo animals with nearby locations. This also indicated non-inbreeding among the albinoid population. This could be explained by introducing other white buffalo semen as part of the organized artificial insemination activities facilitated by the PCC at USF.

Table 2. Genetic diversity and population differentiation among four white buffalo populations (P value<0.05).

Population	No. of Samples	No. of Alleles	Mean Obs. Het.	Mean Exp. Het.	F_{ST}	F_{IS}
Luzon	3	2.72	0.66	0.61	0.01	0.18
Visayas	7	3.27	0.50	0.48	0.02	0.12
Mindanao	17	3.81	0.51	0.52	0.02	0.01
Lamac, Cebu	29	5.36	0.55	0.56	0.01	-0.05
Mean		3.79	0.56	0.54		

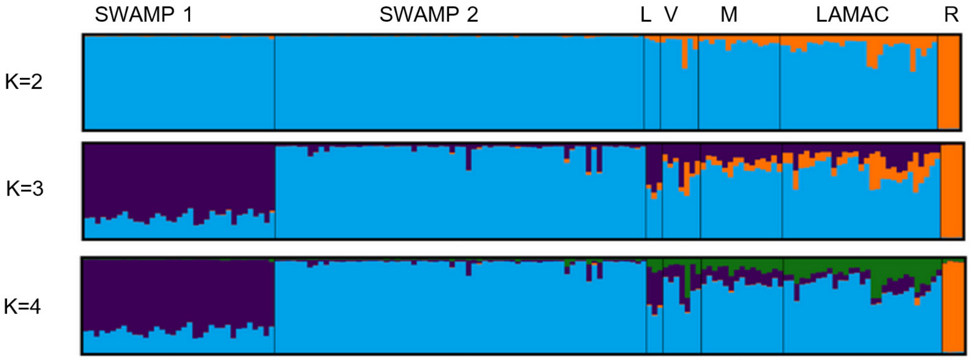
F_{ST}: genetic distance; F_{IS}: inbreeding coefficient

Population Structure and Blood Proportion

The increasing ΔK value from K=2 to K=5 revealed the separation of swamp and riverine buffaloes at K=2 while delineating three distinct clusters encompassing Swamp

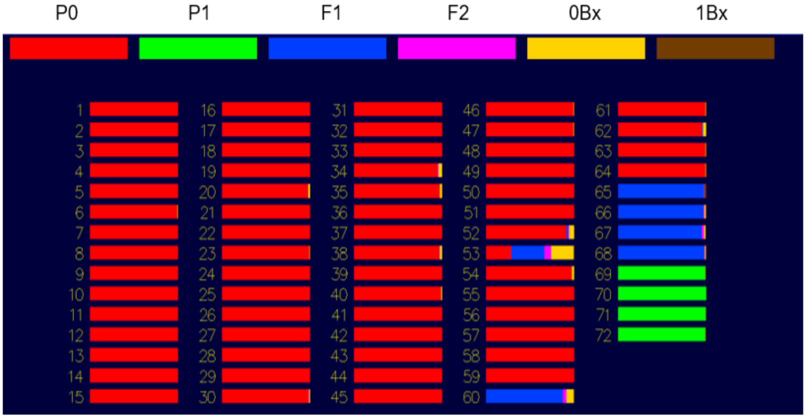
Cluster 1, Swamp Cluster 2, and riverine at an optimum K value of 3 (Figure 1). Interestingly, at K=4, the mean membership coefficient of the white buffalo populations revealed a closer affinity of 69.2% membership coefficient to the Swamp Cluster 2, which could be traced from swamp buffalo populations of Carabao Island in Southern Luzon and Pitogo Island in Visayas. This result supported the previous report from the DABIOTECH1506 project that most of the white buffalo populations could be traced from Visayas and Mindanao. Moreover, it could also be observed at K=4 the presence of white buffalo clustering with a membership coefficient ranging from 10.1% to 36.7%. This result could indicate the morphological difference between the albinoids and common swamp buffalo with brown coat color.

The Bayesian posterior probability analysis to determine the assigned category (at



L=LUZON, V=VISAYAS, M=MINDANAO, R=RIVERINE
SWAMP 1 – Calayan Is. Swamp buffaloes
SWAMP 2 – Carabao Is. and Pitogo Is. Swamp buffaloes

Figure 1. K=2 to K=5 plot estimate obtained by STRUCTURE analysis of albinoids included in the study. Swamp Clusters 1, 2, and riverine samples were used as references. Individual is presented by thin vertical line which was divided into colored segments that represent the proportional contribution of inferred clusters.



P0 – Swamp Parental; P1 – Riverine Parental; F1 – First Filial (50:50); F2 – Second Filial (25:25:25:25);
0Bx – Backcross Swamp (75:25); 1Bx – Backcross Riverine (75:25)

Figure 2. Posterior probability of individual presented by horizontal line which was divided into colored segments that represent breed categories.

Figure 3. Albinoid CEB25 was identified with 77% PP FI category which has a white coat color.

least 70% posterior probability (PP) of white buffaloes) revealed 92.2% PP to the swamp group (Figure 2). However, CEB25 (sample no. 60) from Lamac was the only sample that fell in the F1 category at 77% PP. This implied that CEB25 had a blood composition of 50% swamp and 50% riverine. CEB25 could be misidentified as swamp albinoid based on morphology (Figure 3). CEB18 (sample no. 53) showed assignment in the Backcross Swamp category at 51% PP, supported by the 22.1% membership co-ancestry to the riverine.

CONCLUSION

The overall research findings highlighted the understanding of the genetic diversity of albinoid swamp buffaloes that showed closer affinity to Swamp Cluster 2, from Carabao Island and Pitogo Island, than those in Swamp Cluster 1, Calayan Island, and riverine buffaloes. Moreover, most white buffalo samples in Lamac have an affinity to swamp buffaloes except for one white buffalo, which was detected to have a riverine bloodline. Lastly, this study established the baseline information on DNA profiles and genotypes of albinoid buffalo populations in the Philippines, which is valuable for developing rational production, conservation, and management in the country.

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