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INITIAL ASSESSMENT OF THE GENETIC DIVERSITY OF THE LONG-TAILED MACAQUES (*MACACA FASCICULARIS*) AT KOSUMPEE FOREST PARK, THAILAND

T. Tanee[1], P. Thamsenanupap[1], P. Kyes[2], N. Pumipuntu[3], J. Teanma[1], B. Ferguson[4], R. Sudmoon[5 *], A. Chaveerach[6], and R. C. Kyes[7]

[1]Faculty of Environment and Resource Studies, Mahasarakham University, Maha Sarakham 44150, Thailand
[2]Department of Psychology, Center for Global Field Study, and Washington National Primate Research Center, University of Washington, USA
[3]One Health Research Unit, Faculty of Veterinary Sciences, Mahasarakham University, Maha Sarakham, 44000, Thailand
[4]Department of Molecular and Medical Genetics, Oregon Health & Sciences University, and Oregon National Primate Research Center, Oregon, USA
[5]Faculty of Law, Khon Kaen University, Khon Kaen 40002, Thailand
[6]Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[7]Departments of Psychology, Global Health, and Anthropology, Center for Global Field Study, and Washington National Primate Research Center, University of Washington, USA
[*]Corresponding Author's E-mail: rungla@kku.ac.th

ABSTRACT

Kosumpee Forest Park (KFP) is located in Northeast Thailand and is home to a resident population of long-tailed macaques (*Macaca fascicularis*). This study analyzed the genetic diversity of the KFP population based on random amplified polymorphic DNA technique by the Shannon-Weinner index of diversity (H') using the NTSYS program. Blood samples were collected from two of the social groups (RedDot group and HareLip group), and 16 successful primers produced 143 loci with 74.12% polymorphism. The H' value of the population was 3.30 and the genetic evenness was 0.97. A UPGMA dendrogram divided the samples into three distinct clusters base on pelage color: the first cluster consisted of individuals with gray pelage from both social groups, the second included individuals with yellow-gray and yellow pelage from both social groups, and the third included individuals with gray pelage from only the HareLip group. The H' values for the gray, yellow-gray, and yellow pelage groupings were 2.83, 2.30 and 1.10, respectively. The genetic similarity values ranged from 55 to 96%. Genetic diversity analysis indicated that yellow-gray macaques were genetically similar to yellow macaques. The results of this study indicate that the macaque population at KFP currently possesses substantial genetic diversity. To our knowledge, this paper represents the first report of genetic diversity in this population, and as such, provides an important baseline measure for future comparison.

Keywords: Long-tailed macaque, *Macaca fascicularis*, Genetic diversity, DNA fingerprint, Kosumpee Forest Park

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INTRODUCTION

The long-tailed macaque (*Macaca fascicularis*) is one of the most widely distributed non-human primate species in Southeast Asia, ranging throughout the Indochina Peninsula, Indonesia, Malaysia, Singapore, the Philippines, etc. (Malaivijitnond and Hamada, 2008; Liedigk *et al*., 2015). This species has recently been reclassified by the IUCN Red List as Vulnerable (Eudey *et al*., 2020). In Thailand, there are six recognized species of macaques, including the long-tailed macaque (*M. fascicularis*), stump-tailed macaque (*M. arctoides*), Assam macaque (*M. assamensis*), rhesus macaque (*M. mulatta*), northern pig-tailed macaque (*M. leonina*), and southern pig-tailed macaque (*M. nemestrina*) (Lekagul and McNeely, 1988; Roos *et al*., 2014; Kyes *et al*., 2018a). The long-tailed macaque is the most common species in Thailand and can be found living in a wide variety of habitats including natural forests, mangroves, disturbed forests, community forests, Buddhist temples, recreation parks, tourist sites, and in or nearby human settlements.

Malaivijitnond and Hamada (2008) recorded more than 70 populations of long-tailed macaques distributed around Thailand. The species has shown successful commensalism with humans in a number of locations and is considered sacred at many Buddhist temples. Despite the appreciation and reverence for these macaques, anthropogenic activities such as urban expansion and agricultural development have resulted in habitat loss and forest fragmentation. These pressures present serious challenges for the long-term genetic viability of many populations. Further, at many of the sites, especially those in close proximity to villages and towns (Malaivijitnond and Hamada, 2008), the macaque population size is increasing, due in part to provisioning

by humans. This situation, in turn, is resulting an increased human-primate interaction and conflict.

In Kosumpee Forest Park (KFP), located in Kosum Phisai district, Maha Sarakham province, Northeastern Thailand, the resident population of long-tailed macaques has shown a dramatic increase in size over the years. In 1989, the macaque population was estimated at 158 individuals (Aggimarangsee, 1992). This number increased to 287 individuals in 2003 (Hamada *et al*., 2005; Malaivijitnond and Hamada, 2008) and then to 734 individuals by year end 2016 (Kyes *et al*., 2018b). Our most recent population estimate indicates approximately 941 individuals as of 2019 (unpublished data). Given the relative isolation of this very small forest fragment representing the KFP (only 0.2 km^2), it has been noted that the rate of inbreeding may be high (Hamada *et al*., 2005). As such, the potential loss in genetic diversity presents a concern for the future of the KFP macaque population.

Traditionally, morphological features (Zhang *et al*., 2015) and karyotypic data (Fan *et al*., 2014; Sangpakdee *et al*., 2018) have been used to characterize patterns and levels of diversity or similarity indices. These indices, however, are limited in utility for describing the potentially complex genetic structure within and between populations. More recently, various molecular techniques have been used to study genetic diversity. Previous studies analyzed the genetic diversity of long-tailed macaques by protein and mitochondrial DNA (Perwitasari-Farajallah *et al*., 2001), short tandem repeats (STRs) (Kanthaswamy *et al*., 2008), mitochondrial DNA and Y-chromosome genes (Bunlungsup *et al*., 2016, 2017; Klegarth *et al*., 2017), and nuclear single nucleotide polymorphisms (SNPs) (Yao *et al*., 2020). Among these molecular techniques, study of the whole nuclear genome is still needed to provide a more detailed assessment of the genetic diversity of the population. Random amplified polymorphic DNA (RAPD) is one of the techniques commonly used for the whole genome analysis. This technique is relatively inexpensive and offers good efficiency in developing a large number of DNA markers in a short period of time. It has been successfully applied to describe genetic relationships and genetic diversity within and between populations in plants (Tsuda *et al*., 2004; Kaewdoungdee and Tanee, 2012; Tanee *et al*., 2012) and animals (Tanee *et al*., 2009; Noikotr *et al*., 2013; Kabir *et al*., 2017), including primates (Minhas *et al*., 2018).

Understanding the genetic diversity within and between populations of the same species is essential for establishing effective management and conservation strategies and represents an important component in our comprehensive approach to primate population assessment (i.e., abundance, habitat viability, genetic viability, pathogen transmission, anthropogenic threats, see Kyes *et al*., 2004, 2006). The present study is part of a larger, on-going project addressing human-primate conflict and coexistence in Thailand (Kyes *et al*., 2018a, 2018b; Grant *et al*., 2019; Schurer *et al*., 2019). Based on the limited genetic-related work that has been conducted at KFP to date (Kawamoto *et al*., 1994; Hamada *et al*., 2005), the purpose of this study was to provide baseline data on the genetic diversity of the KFP long-tailed macaque population using RAPD fingerprinting. To the best of our knowledge, this study provides the first measures of genetic diversity in the KFP population.

MATERIALS AND METHODS

Study site: Kosumpee Forest Park (KFP) is a wildlife conservation site located in Kosum Phisai district, Maha Sarakham province in northeastern Thailand at N 16° 15'; E 103° 04' (Figure 1). This small, deciduous, dipterocarp forest fragment comprises an area of only 0.2km^2 is home to a population of long-tailed macaques, which consisted of approximately 850 individuals at the time of this study (unpublished data). Hamada *et al*. (2005) and Kyes *et al*. (2018b) reported based on information from the local residents and park staff, KFP has been isolated for the past 45^+ years due to agricultural expansion and urbanization. The macaques were distributed among five social groups i.e., DroopLip, RedDot, StumpTail, HareLip, BigRed, (see Kyes *et al*., 2018b) with extensive overlapping home ranges. Group sizes ranged from 67 to 217 individuals (Kyes *et al*., 2018b). KFP is boarded to the south by the town of Kosum Phisai where conflict between the local residents and the monkeys (e.g., stealing food, destroying roofs, etc.) occurs daily. IBT

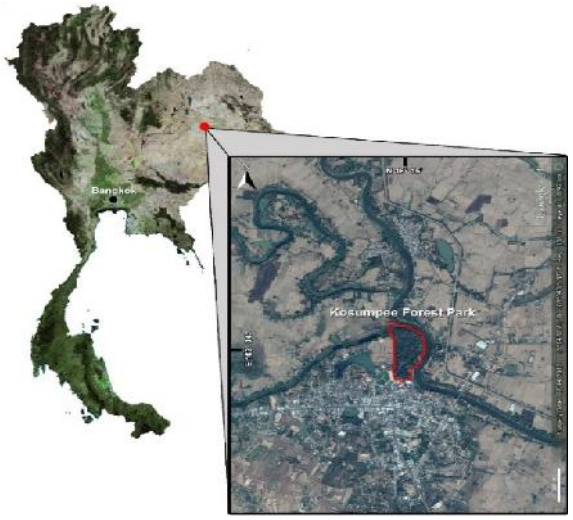

Figure 1. Location of Kosumpee Forest Park, Kosum Phisai District, Maha Sarakham Province, Northeastern Thailand (adapted from Kyes *et al.*, 2018b).

A unique feature of this KFP population is the variation in pelage color, ranging from the traditional or ordinary grayish color to a vivid yellow (Figure 2). This pelage color variation was noted as early as 1989 by Aggimarangsee (1992) and has been a topic of investigation in past studies of this population (Kawamoto *et al*., 1994; Hamada *et al*., 2005). Hamada *et al*. (2005) described the pelage coloration to include both "ordinary" and "yellow" types but noted that an "intermediate" coloration (ranging between the distinctly yellow and ordinary macaques) also was observed.

Figure 2. Examples of pelage color variation in the long-tailed macaques at Kosumpee Forest Park. Top left: adult female with typical grayish pelage and her infant with yellowish natal coat; top right: adult female with yellow pelage and her infant with typical black natal coat; bottom: several adults from one of the social groups with variations in pelage color including gray (not circled), yellow-gray (yellow-gray circles), and yellow (yellow circle). (photos by R. Kyes).

Sample collection and genomic DNA isolation: Trapping and blood sample collection were conducted between 10-11 November 2018 by a veterinary team from Mahasarakham University (MSU) following a protocol approved by the MSU Institutional Animal Care and Use Committee (see ethics statement below) and the Thai Department of National Parks. The monkeys were sedated with 3 mg/kg of Zoletil® (tiletamine HCl/zolazepam HCl). Following universal precautions, approximately 7–10 ml of blood was drawn from the femoral vein of each animal. The samples were collected only from adults, subadults and juveniles, no infants were sampled for the animals' safety. The monkeys were monitored closely throughout sedation and recovery, and subsequently returned to their groups. Blood samples were processed on site to separate the serum. Both whole blood and serum samples were then transported to the lab at MSU and stored at -20°C for subsequent analysis. Two human DNA samples were included as outgroup samples for phylogenetic analysis.

Total genomic DNA was extracted from blood samples using the Genomic DNA extraction kit (QIAGEN, USA) following the kit protocol. The quality and quantity of extracted DNA was assessed by 0.8% agarose gel electrophoresis. The DNA samples were then diluted to a final concentration of 20 ng/µl, and these dilutions were used as DNA templates in the PCR reactions.

DNA fingerprinting by RAPD marker and dendrogram construction: Genetic diversity and genetic relationships of the long-tailed macaque population in KFP were determined using RAPD technique. The RAPD reactions were carried out in 25 µl consisting of GoTaq Green Master Mix (Promega, USA), 0.5 µM primer (Invitrogen, USA) and 20 ng DNA template. The reaction mixture was denatured at 94°C for 3 min and amplification was performed with the following 35 thermal cycles: denaturation for 30 s at 94°C, annealing for 30 s at 40°C, and extension for 2 min at 72°C, followed by a 7 min final extension at 72°C. The DNA amplifications were performed using a Swift™ Maxi PCR Thermal Cycler (Esco Micro Pte. Ltd., Singapore). The amplified products were separated by 1.2% agarose gel electrophoresis in the TAE buffer and visualized using ethidium bromide staining (Noikotr *et al*., 2013).

The resulting RAPD bands were scored as present = 1 or absent = 0 and these 0-1 data were used to construct a dendrogram by NTSYSpc 2.10p using UPGMA cluster analysis (Rohlf 1998), and genetic similarity (S) values were obtained. The cophenetic correlation was computed to measure the conformity between the cluster analysis and the dataset. similarity values implied by the dendrogram and those of the original similarity matrix. The genetic diversity was calculated based on Shannon-Weinner index of diversity (H') for each social group, H'

= -∑*pi*ln*pi*, where *pi* is the frequency of a given RAPD bands. Genetic evenness (E) was calculated from the formulation, E = H'/*ln* individual richness (*ln* 30=3.40) (Nei, 1973, 1978).

RESULTS

With a random sampling, 30 monkeys were caught, representing two social groups: 16 individuals from the RedDot group (12 males/4 females; 9 adults/7 immatures); and 14 individuals from the HareLip group: (9 males/5 females; 5 adults/9 immatures). Of these, 17 individuals had gray pelage color, 10 had yellow-gray pelage, and 3 had yellow pelage. Pelage color designation (i.e., gray, yellow-gray, yellow) was determined based on concurrence between the two senior researchers (TT and RCK) at the time of sample collection.

Sixty-eight RAPD primers were screened, and 16 successful primers produced clarified fingerprints (Table 1). Figure 3 shows examples of the RAPD fingerprints among the long-tailed macaques. There was a total of 143 banding amplicons (loci) with sizes ranging from 200 to 3,000 bp and an average of 8.94 amplicons per primer. The percentage of polymorphism was 74.12%. The percentage of polymorphic bands (PPB) for each primer ranged from 20 to 100% (Table 1). For the outgroup, the two human samples showed a total of 56 loci with 41 monomorphic bands and percentage of polymorphism of 27%.

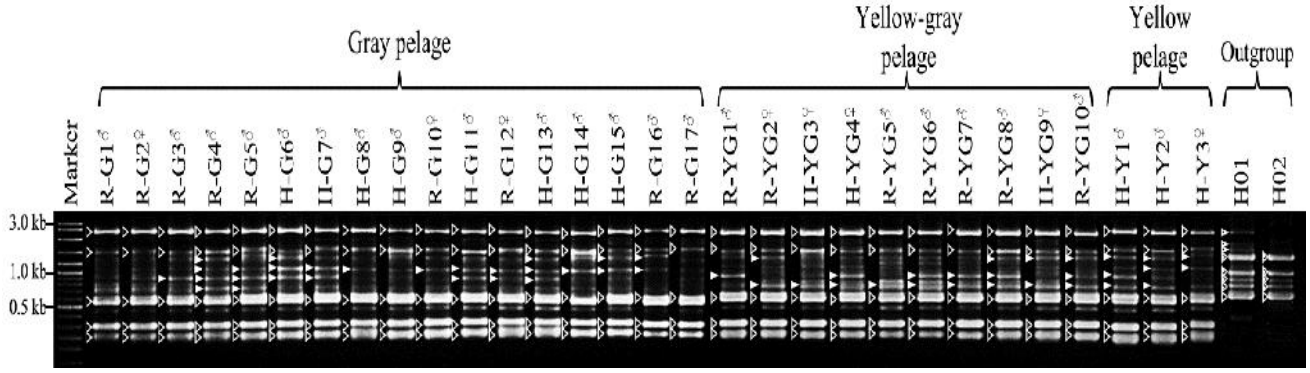

Figure 3. RAPD banding pattern of the long-tailed macaques at Kosumpee Forest Park with primer sequence AACGGGCAGC, triangle indicates monomorphic band, arrowhead indicates polymorphic band, whereas triangle with enclosed dot indicates monomorphic band and arrowhead with enclosed dot indicates polymorphic band of the outgroup.

Table 1. List of 16 Successful RAPD Primers Amplified for the 30 Long-tailed Macaques.

Primer sequence (5'-3')	Banding loci	Monomorphisms	Polymorphisms	Percentage of polymorphisms	H'	E
CAATCGCCGT	10	2	8	80.00	3.391	0.997
GGAGGGTGTT	13	3	10	76.92	3.393	0.998
TGGACCGGTG	11	0	11	100.00	3.265	0.960
AAGCCTCGTC	12	0	12	100.00	3.108	0.914
GGGCAATGAT	7	1	6	85.71	3.127	0.920
GTGCGTCCTC	10	2	8	80.00	3.377	0.993
CAGGCGGCGT	6	1	5	83.33	3.372	0.992
AACGGGCAGC	12	7	5	41.67	3.396	0.999
ATGACGACGG	2	0	2	100.00	3.080	0.906
CAAACGGCAC	14	0	14	100.00	3.314	0.975
AATCGGGCTG	7	2	5	71.43	3.381	0.994
GTATTGCCCT	5	0	5	100.00	3.226	0.949
CCGTCATTGG	8	5	3	37.50	3.397	0.999
GGGCCACGCT	10	8	2	20.00	3.400	1.000
AGAATCCGCC	7	1	6	85.71	3.333	0.980
AGGACGTGCC	9	5	4	44.44	3.386	0.996
Total	143	37	106	74.12	3.300	0.970

Note. Columns represent numbers of scored banding loci; monomorphisms; polymorphisms; percentage of polymorphisms; Shannon-Weinner index of diversity (H'); and genetic evenness (E) within population.

Based on Shannon-Weinner index of diversity, within the population of the macaques in KFP, the genetic diversity (H') was 3.30 and the genetic evenness (E) was 0.97 (Table 1). Within the social groups, RedDot and HareLip, the H' and E values were 2.77 and 2.63, and 0.999 and 0.997, respectively. When considering individuals of same pelage color, the yellow pelage monkeys showed the lowest H' value at 1.10 but the genetic evenness was still very high (1.00) (Table 2).

Regarding genetic relationships within the population, a dendrogram with UPGMA cluster analysis separated the monkeys into three main clusters; and the human outgroup separated from the monkey clusters (Figure 4). The cophenetic correlation of RAPD analysis was 0.912 implying robustness of the generated dendrogram and the conformity of the shown similarity values and those of the original similarity matrix. The first cluster consisted of individuals with gray pelage color (and included monkeys from both social groups). The second cluster included individuals with yellow-gray and yellow pelage (and included monkeys from both social groups). The third cluster included individuals with gray pelage all from the HareLip group.

Table 2. Values of Genetic Diversity (H') and Genetic Evenness (E) Within Social Groups and Pelage Colors of the Kosumpee Forest Park (KFP) Long-tailed Macaque.

Category (number of individuals)		H'	E
Social Group	RedDot (16)	2.77	0.99
	HareLip (14)	2.63	0.99
Pelage Color	Gray (17)	2.83	0.99
	Yellow-gray (10)	2.30	0.99
	Yellow (3)	1.10	1.00

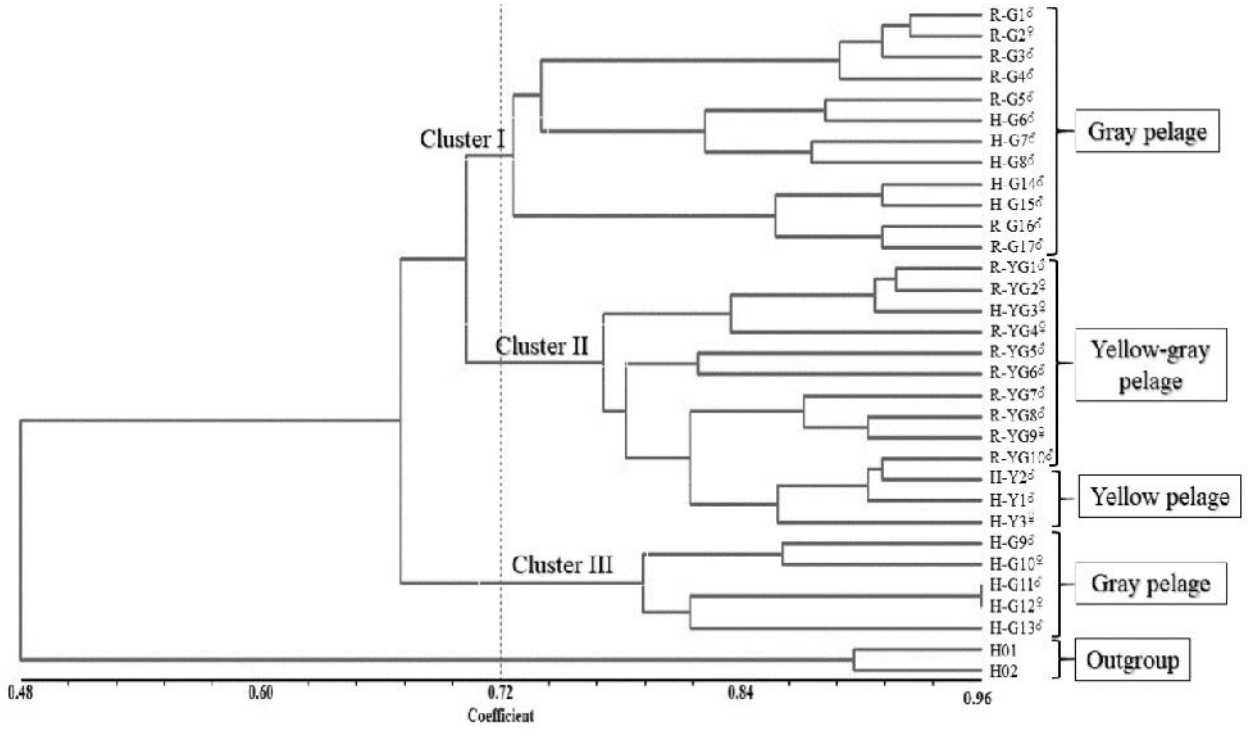

Figure 4. Dendrogram indicating genetic similarity and depicting the sixteen RAPD primers produced by UPGMA analysis that are used to classify the genetic relationships long-tailed macaque population at Kosumpee Forest Park.

The genetic similarity values (S) of this macaque population ranged from 57 to 96 % (Figure 5). The three yellow pelage individuals in the HareLip group had close genetic relationships with S values ranging from 86 to 91%. Further, they were more closely related to the yellow-gray and gray pelage individuals from the RedDot group than the gray pelage individuals within their own group. The same pelage color individuals showed higher S values even though the individuals are from different social groups. For example, in Cluster 1, the gray pelage individual numbers H-G14♂ and H-G15♂ from HareLip group are closely related with R-G16♂and R-G17♂ from the RedDot group with S values of 84 to 89%. Whereas, within the same group, HareLip, the gray pelage H-G9♂ and yellow pelage H-Y1♂ showed a lower S value of 61%.

	R-G1	R-G2	R-G3	R-G4	R-G5	II-G6	H-G7	H-G8	II-G9	R-G10	H-G11	R-G12	H-G13	H-G14	H-G15	R-G16	R-G17	R-YG1	R-YG2	H-YG3	H-YG4	R-YG5	R-YG6	R-YG7	R-YG8	H-YG9	R-YG10	II-Y1	H-Y2	II-Y3	H01	H02
R-G1	1.00																															
R-G2	0.93	1.00																														
R-G3	0.91	0.92	1.00																													
R-G4	0.87	0.91	0.89	1.00																												
R-G5	0.81	0.84	0.83	0.88	1.00																											
H-G6	0.72	0.75	0.76	0.76	0.89	1.00																										
H-G7	0.66	0.69	0.67	0.72	0.80	0.87	1.00																									
II-G8	0.67	0.70	0.68	0.74	0.81	0.82	0.88	1.00																								
H-G9	0.66	0.69	0.72	0.70	0.75	0.76	0.81	0.84	1.00																							
R-G10	0.68	0.69	0.69	0.69	0.69	0.70	0.74	0.76	0.86	1.00																						
H-G11	0.67	0.67	0.69	0.67	0.66	0.65	0.69	0.71	0.81	0.81	1.00																					
R-G12	0.68	0.69	0.70	0.69	0.70	0.69	0.71	0.74	0.84	0.81	0.96	1.00																				
H-G13	0.69	0.69	0.69	0.64	0.62	0.62	0.65	0.64	0.73	0.76	0.80	0.84	1.00																			
H-G14	0.69	0.71	0.73	0.69	0.73	0.71	0.76	0.72	0.71	0.67	0.75	0.77	0.78	1.00																		
H-G15	0.72	0.72	0.74	0.72	0.74	0.70	0.74	0.72	0.68	0.64	0.71	0.73	0.76	0.91	1.00																	
R-G16	0.70	0.73	0.79	0.76	0.79	0.75	0.75	0.74	0.71	0.64	0.67	0.69	0.66	0.84	0.88	1.00																
R-G17	0.69	0.69	0.74	0.71	0.74	0.71	0.74	0.74	0.70	0.64	0.67	0.69	0.70	0.84	0.89	0.91	1.00															
R-YG1	0.70	0.71	0.72	0.73	0.71	0.71	0.69	0.70	0.61	0.62	0.56	0.58	0.64	0.69	0.75	0.77	0.77	1.00														
R-YG2	0.72	0.74	0.73	0.75	0.71	0.66	0.67	0.69	0.59	0.61	0.55	0.57	0.66	0.70	0.76	0.76	0.76	0.92	1.00													
H-YG3	0.70	0.70	0.74	0.73	0.72	0.68	0.69	0.73	0.64	0.62	0.56	0.58	0.64	0.69	0.74	0.79	0.77	0.90	0.92	1.00												
H-YG4	0.72	0.72	0.79	0.71	0.69	0.64	0.66	0.66	0.71	0.69	0.61	0.61	0.66	0.70	0.74	0.78	0.75	0.82	0.86	0.84	1.00											
R-YG5	0.72	0.69	0.71	0.68	0.64	0.59	0.57	0.59	0.61	0.61	0.58	0.57	0.64	0.61	0.67	0.66	0.65	0.74	0.77	0.76	0.83	1.00										
R-YG6	0.76	0.74	0.74	0.71	0.69	0.61	0.64	0.61	0.64	0.62	0.66	0.68	0.69	0.62	0.68	0.67	0.66	0.70	0.72	0.73	0.75	0.82	1.00									
R-YG7	0.73	0.73	0.78	0.71	0.68	0.59	0.61	0.61	0.66	0.59	0.71	0.69	0.71	0.68	0.72	0.76	0.74	0.79	0.78	0.80	0.82	0.81	0.83	1.00								
R-YG8	0.75	0.76	0.77	0.75	0.70	0.61	0.63	0.61	0.62	0.66	0.66	0.67	0.72	0.69	0.74	0.75	0.72	0.84	0.84	0.82	0.81	0.81	0.82	0.91	1.00							
H-YG9	0.76	0.77	0.76	0.76	0.75	0.66	0.64	0.61	0.61	0.65	0.67	0.68	0.74	0.71	0.76	0.71	0.69	0.79	0.79	0.76	0.76	0.75	0.80	0.84	0.91	1.00						
R-YG10	0.76	0.78	0.74	0.76	0.74	0.66	0.70	0.65	0.68	0.71	0.69	0.71	0.71	0.69	0.74	0.71	0.71	0.79	0.79	0.75	0.74	0.73	0.84	0.81	0.89	0.89	1.00					
H-Y1	0.74	0.75	0.73	0.76	0.76	0.66	0.70	0.66	0.61	0.64	0.64	0.64	0.68	0.69	0.76	0.69	0.72	0.81	0.83	0.78	0.73	0.73	0.82	0.76	0.83	0.86	0.91	1.00				
H-Y2	0.74	0.76	0.71	0.74	0.73	0.63	0.70	0.69	0.66	0.69	0.71	0.71	0.71	0.69	0.74	0.71	0.71	0.75	0.79	0.75	0.73	0.74	0.84	0.79	0.84	0.84	0.91	0.90	1.00			
H-Y3	0.69	0.69	0.67	0.69	0.73	0.64	0.70	0.69	0.65	0.69	0.65	0.66	0.65	0.67	0.71	0.69	0.69	0.75	0.77	0.75	0.76	0.71	0.76	0.75	0.77	0.78	0.86	0.86	0.87	1.00		
II01	0.48	0.46	0.47	0.45	0.47	0.49	0.54	0.52	0.54	0.57	0.52	0.50	0.46	0.47	0.46	0.45	0.48	0.44	0.43	0.46	0.49	0.47	0.51	0.46	0.41	0.41	0.44	0.46	0.47	0.54	1.00	
H02	0.48	0.48	0.49	0.48	0.50	0.51	0.57	0.51	0.59	0.59	0.49	0.51	0.49	0.47	0.46	0.48	0.48	0.45	0.44	0.46	0.50	0.46	0.51	0.44	0.43	0.41	0.46	0.44	0.44	0.50	0.90	1.00

Figure 5. Genetic similarity values of the long-tailed macaque population at Kosumpee Forest Park.

DISCUSSION

The long-tailed macaques at KFP represent an expanding population existing in a small forest fragment that is geographically isolated and experiencing an increase in human-primate conflict. The KFP population is representative of an increasing number of primate populations in Thailand and throughout Asia that are in need of better characterization and management. This current study builds on our on-going assessment of the KFP macaque population (Kyes *et al.,* 2018b; Grant *et al.,* 2019; Schurer *et al.,* 2019) and provides, to the best of our knowledge, the first measures of genetic diversity in the KFP macaques. We believe these initial measures will be particularly important as baseline markers for future reference in the long-term management and conservation of this population.

The RAPD analysis revealed percentages of polymorphism of 74.12%, the higher values suggest that all markers used had high levels of efficiency for genetic analysis in this study. Further, the differences in the banding patterns and high percentages of polymorphism within the KFP population indicate the usefulness and applicability of RAPD markers in genetic analysis. This result is concordant with other studies that have successfully used RAPD markers to access genetic diversity within populations (Sharma *et al.,* 2001; Zhao *et al.,* 2006; Güneren *et al.,* 2010; Zarringhabaie *et al.,* 2012; Kabir *et al.,* 2017; Minhas *et al.,* 2017; Dwivedi *et al.,* 2018). Moving forward, we hope to utilize additional assessment methods (e.g., mitochondrial or SRR markers) to broaden our understanding of the genetic composition of this population.

As noted earlier, the yellow pelage color is a unique morphological character that has been observed in the KFP macaque population for at least 30 years (Aggimarangsee, 1992). Since that time, Hamada *et al.* (2005) reported that the frequency of individuals with yellow pelage had decreased by half compared to findings reported by Kawamoto *et al.* (1994) ten years earlier. The results of our study clearly indicate lower genetic diversity among the individuals of yellow pelage (H' = 1.10) compared to those of the yellow-gray pelage (H' = 2.30) and the gray pelage group (H' = 2.83). It is important to note that the small sample size of yellow pelage individuals (n = 3) may have contributed to the lower genetic diversity value, and thus, further assessment with larger samples sizes (for all pelage colors) will be needed to validate these initial findings.

According to the dendrogram shown in Figure 4, the genetic relationships are related to the pelage colors even though the individuals are from different social groups. The gray pelage individuals had S values of 84 to 89%, whereas, within the same group, the gray and the yellow ones had an S value of 61%. Moreover, the individuals of yellow pelage and yellow-gray pelage clustered together implying that the genetic relationships of the individuals of yellow pelage are more closely related with yellow-gray pelage than to the gray pelage. According to Hamada *et al.* (2005) that numbers of the

yellow pelage individuals has been decreasing. It would be of interest to examine the potential mechanisms responsible not only for the variation in pelage color but for any ongoing changes and are taking place. To elucidate such mechanisms, further studies are needed.

Our main finding, regarding overall genetic diversity of the KFP macaque population, indicated that there was still substantial diversity in the population (H' = 3.30). Given that this population has existed in a very small forest fragment, effectively isolated for at least 45 years, and with a population of monkeys that number about 158 individuals 30 years ago (Aggimarangsee, 1992), we were encouraged to find this level of genetic diversity. However, further study is needed to fully address and characterize the genetic diversity of this population.

If one considers more natural settings with large forest tracks where multiple groups or populations exist with minimal overlap, genetic diversity may be maintained via a number of mechanisms such as male migration, group fission, sex-biased philopatry (Di Fiore, 2003; Thierry *et al.,* 2004). In particular, Thierry *et al.* (2004) demonstrated that male dispersal serves as a mechanism for gene flow preventing inbreeding in macaque population. Migration between groups may well be the most important factor contributing to local changes in allele frequency.

KFP of course is not a large forest expanse and presumably would not allow for the more traditional means for gene flow - or so it would appear. But there may be a potential explanation that might account for the higher level of genetic diversity in the KFP macaque population. In addition to the five social groups confirmed in KFP during our extended field season in 2016 (September-December) (Kyes *et al.,* 2018b), we also identified a number of adult males that were often observed sitting/traveling alone, frequently outside of the KFP boundary and sometimes as much as a kilometer or more into the town. Notably, many of these males occasionally entered a social group (for minutes/hours) and interacted with the members, including foraging/eating in proximity, mating with the females, fighting with males, etc. Given their transient nature, these males were termed "extra-group males." We assumed these individuals were from the KFP population and choose a more solitary existence to avoid the high rates of aggression observed daily in this population.

During our 2016 field season, we also learned from the park staff of another isolated population of long-tailed macaques located in a small forest fragment near the village of Ban Muang Yai, about 16 km from KFP and approximately 27 km following the Chi River. The Park staff believed that the Ban Muang Yai macaque population originated from a small group monkey that left KFP sometime in the past. They further speculated that some of the monkeys in both populations (likely adult males) may occasionally travel between the two sites, following the river which provides some tree cover along the riverbank. This has led us to question whether some of the extra-group males observed in the KFP population might be from Ban Muang Yai. To date, we have no evidence to support any of these claims, but it does represent a plausible hypothesis to account for the level of genetic diversity found in the KFP macaque population, and as such, is worthy of further study. We have already sampled several monkeys from the Ban Muang Yai population as part of a recent study comparing the gut microbiome of the two populations (Grant *et al.,* 2019) and now plan to move forward with further study to assess the DNA from the Ban Muang Yai population, as well as the KFP extra-group males, to evaluate the genetic relatedness among these individuals/populations.

Conclusions: Maintaining sufficient genetic diversity is critical to the long-term survival of any animal species especially for those living in small, fragmented populations. The results of our study on the genetics of the long-tailed macaques in KFP indicate that this population currently possesses substantial genetic diversity. Given that this paper provides the first report of genetic diversity in this population, it serves as an important baseline measure for future comparison. These findings will assist in our our-going efforts to help with the management of this population and promote the healthy coexistence between the local residents and these macaques.

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P.T., P.K., and R.C.K. wrote the manuscript, and A.C., J.T., N.P. and B.F. assisted with editing and revision. All authors read and approved the final version of the manuscript.

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Ethics approval consent to participate: All applicable international, national, and institutional guidelines for the care and use of animals were strictly followed. All animal sample collection protocols complied with the current laws of Thailand. All animal procedures performed in this research were in accordance with the ethical standards of the Institutional Animal Care and Use Committee at Mahasarakham University (protocol number: 0009/2016). This study was part of a larger on-going project approved by the National Research Council of Thailand (NRCT project approval to RCK - Project ID: 2016/048; "Healthy Coexistence between Human and Non-human Primates: A One Health Approach").

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