BIODIVERSITAS
Volume 19, Number 5, September 2018
Pages: 1601-1606

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190501

Sequence variation of captive Malayan Gaur (*Bos gaurus hubbacki*) based on mitochondrial D-loop region DNA sequences

BADRUL MUNIR MD-ZAIN[1,♥], AQILAH ABDUL-AZIZ[1], NOR RAHMAN AIFAT[1], NUR SYAFIKA MOHD-YUSUF[1], ROSLI NORSYAMIMI[2], JEFFRINE JAPNING ROVIE-RYAN[2], KAYAL VIZI KARUPPANNAN[1,2], NADIATUR AKMAR ZULKIFLI[1], SALMAH YAAKOP[1]

[1]School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. 43600 Bangi, Selangor, Malaysia. Tel.: +60-389-213200, ♥email: abgbadd@ukm.edu.my, abgbadd1966@yahoo.com
[2]National Wildlife Forensic Laboratory, Ex-Situ Conservation Division, Department of Wildlife and National Park (DWNP) Peninsular Malaysia, KM 10 Jalan Cheras, 56100 Kuala Lumpur, Malaysia

Manuscript received: 2 July 2018. Revision accepted: 1 August 2018.

Abstract. *Md-Zain BM, Abdul-Aziz A, Aifat NR, Mohd-Yusof NS, Norsyamimi R, Rovie-Ryan JJ, Karuppannan KV, Zulkifli NA*, *Yaakop S. 2018. Sequence variation of captive Malayan Gaur (*Bos gaurus hubbacki*) based on mitochondrial D-loop region DNA sequences. Biodiversitas 19: 1601-1606.* Malayan gaur (*Bos gaurus hubbacki*) can only be found in Peninsular Malaysia and southern Thailand. The International Union for Conservation of Nature (IUCN) has listed Malayan gaur in the Red List as vulnerable. The main objective of this study was to investigate sequence variation in the mitochondrial D-loop region of *B. g. hubbacki* from two captive centers. We collected 30 DNA samples of Malayan gaur from Jenderak Selatan Wildlife Conservation Center in Pahang and the Sungkai Wildlife Reserve in Perak. Polymerase chain reactions were performed to amplify all the samples. DNA sequences were analyzed using Neighbor-Joining (NJ) and Maximum Parsimony (MP) methods. Based on the 652 base pairs obtained, we found only seven variable characters with a value of 1% and a genetic distance between the two captive centers of 0.001. Haplotype analyses using DnaSP software detected only four haplotypes between these two captive centers. Both NJ and MP trees portrayed all Malayan gaur individuals in Jenderak Selatan and Sungkai captive centers as belonging to the same clade. Genetic variation of Malayan gaur in these centers is considered low due to individuals possibly sharing the same common parent. This sequence variation information is of paramount importance for the proper breeding and conservation management program of Malayan gaur in the future.

Keywords: Malayan gaur, *Bos gaurus hubbacki*, Genetic variation, DNA Sequence, Seladang

INTRODUCTION

Bos gaurus, locally known as seladang, is the largest extant bovine species in the *Bos* genus (Rosli et al. 2016). Malayan gaur has wide shoulders and are brown in color while the lower parts of their legs are pure white as though they are wearing stockings (Figure 1). The three subspecies of *B. gaurus*—namely *B. g. hubbacki*, *B. g. gaurus*, and *B. g. laosiensis* (Rosli et al. 2011) have different distributions: *B. g. hubbacki* is found in Peninsular Malaysia and southern Thailand, *B. g. gaurus* is found in India, southern Nepal, and Bhutan, while *B. g. laosiensis* is found in Myanmar, Laos, Vietnam, and Cambodia (Duckworth et al. 2016).

The International Union for Conservation of Nature (IUCN) has listed *B. gaurus* as vulnerable in its red list (Duckworth et al. 2016). About 500 individuals of Malayan gaur were estimated to inhabit Peninsular Malaysia (Sahir 2001); however, this number has decreased to between 273 and 333 individuals based on statistics provided by the Department of Wildlife and National Parks (DWNP) in 2005. The decreasing number of individuals has put this mammal under the Protected Wildlife Animals Act 76/72 by the DWNP, and conservation effort is needed. Therefore, *B. gaurus* has been placed in captivity as an *ex-situ* conservation program. In Malaysia, a few captive centers are home for *B. gaurus* including Jenderak Selatan Wildlife Conservation Center (Pahang), Sungkai Wildlife Reserve (Perak), the National Zoo, the Taiping Zoo and the Malacca Zoo (Rosli 2012).

Previous studies on Malayan gaur focused on nutrition aspects (Yusof 2009), chromosome evolution (Mamat-Hamidi et al. 2012), semen analysis (Iswadi et al. 2012), phylogenetic relationships (Rosli et al. 2011, 2016), inbreeding (Norsyamimi et al. 2016), and species identification of Malayan gaur, Bali cattle, and Zebu cattle (Romaino et al. 2014). However, little has been done to study the molecular sequence variation of Malayan gaur in captivity. Information gained about the genetic structure within the population can help determine the breeding program and genetic conservation of the species (Xuan et al. 2010). This study of sequence variation in Malayan gaur was conducted to improve conservation efforts and help prevent species extinction. Mitochondrial DNA (mtDNA) of the displacement loop (D-loop) region was chosen because this locus has a potential for molecular studies at the population level (Ang et al. 2011; Ang et al. 2012; Abdul-Latiff et al. 2014a; Md-Zain et al. 2017). The D-loop region has a high mutation rate compared to other mtDNA regions and has the ability to provide high resolution data on phylogenetic relationships (Syed-Shabthar et al. 2013). Thus, the main objective of this study is to infer the sequence variation in the mtDNA D-loop region of *B. g.*

hubbacki in captivity with a focus on the two captive centers.

MATERIALS AND METHODS

Thirty samples of Malayan gaur were obtained from two captive centers in Jenderak Selatan Wildlife Conservation Center, Pahang (14 samples) and Wildlife Reserve Sungkai, Perak (16 samples) (Abdul-Latiff et al. 2017). These noninvasive genetic samples were obtained either from feces or hair follicles. DNA was extracted from these samples using QIAGEN QIAamp DNA Micro Kit and amplified with Polymerase Chain Reactions (PCRs) using an Eppendorf Mastercycler. One pair of specific primers were used to amplify the D-loop region: Walid F (TCA CCG TCA ACT CCC AAA GCT GA) and Walid R (AGG GGG AAG TTT TAT GGA AGG GGG) (Syed-Shabthar et al. 2013). PCR components used in this study were Taq DNA polymerase (5 U/µL), 10X PCR Buffer, 50 mM $MgCl_2$ and 10 mM dNTP. Table 1 lists the PCR components, concentrations, and chemical volumes and Table 2 shows the PCR profile. After amplification, purification was performed using the purification kit GF-1 PCR CLEAN-UP Kit (Vivantis) to remove excessive dNTPs, primers, and buffer. Final purification products were sent for DNA sequencing to First Base Sdn. Bhd. (Shah Alam, Malaysia).

DNA sequences were checked using BLAST software while DNA alignment was completed using BioEdit Sequence Alignment Editor 7.2.0 (Aifat et al. 2016). Using Bioedit, DNA sequences were viewed as chromatograms with different colors for base peaks, and sequences were then compared using ClustalW software (Bakar et al. 2017). All DNA sequences were aligned using MEGA 4.0 (Md-Zain et al. 2018a). Phylogeny trees were constructed based on distance and character approaches using Neighbor-Joining (NJ) and Maximum Parsimony (MP) methods. Bison (*Bison bison*) and water buffalo (*Bubalus bubalis*) were chosen as the outgroup (Syed-Shabthar et al. 2013). The NJ tree was created using the Kimura-2-parameter algorithm (Kimura 1980) supported with 1,000 bootstrap replications (Md-Zain et al. 2018b). MP was executed using a heuristic search of Tree Bisection and Reconnection (TBR) with 1000 random stepwise additions and a 50% consensus majority rule. DnaSP software was used to analyze haplotypes (Rozas et al. 2003). The population genetic structure of Malayan gaur was revealed based on haplotype forms generated from the Arlequin Haplotype List.

RESULTS AND DISCUSSION

Total genomic DNA was isolated from 30 samples of Malayan gaur and three other samples representing the outgroup. The results from DNA sequencing showed that 520 characters (80%) of the 652 characters in the sequences were constant, leaving the remaining 20% as variable characters. Of these 132 variable characters, 63 sites were parsimony informative characters (48%), while the other 52% were parsimony uninformative. However, after the outgroup was removed from the analysis, there were only seven (1%) variable characters. Table 3 shows that there are no parsimony informative characters among the seven variable characters. The nucleotide composition for all the Malayan gaur was also identified. Nucleotide A has the highest frequency of 31.8% while nucleotide G has the lowest frequency of 15.6% only. Nucleotides T and C have a frequency of 28.2% and 24.4% respectively. A genetic distance of 0.001was also calculated between the Jenderak Selatan and Sungkai groups based on the Kimura 2-parameter.

Figure 1. Male Malayan gaur (*Bos gaurus hubbacki*)

Table 1. Final concentrations of the PCR mixture component

PCR component	Final concentration	Volume (µL)
ddH_2O	-	18.8
10X PCR buffer	1X	2.5
dNTP mix (10 mM)	0.28 mM	0.7
$MgCl_2$ (50 mM)	2.4 mM	1.2
Forward primer (10 µM)	0.12 uM	0.3
Reverse primer (10µM)	0.12 uM	0.3
Taq Polymerase (5U/ µL)	1 U	0.2
DNA template	50 ng/uL	1.0
Total	-	25.0

Table 2. PCR cycle profile

Parameter	Temperature (°C)	Duration (sec)	Cycle
Pre-Denaturation	94	180	-
Denaturation	94	60	35
Annealing	58	30	
Extension	72	90	
Post Extension	72	420	-
Cooling	4	∞	-

Table 3. Summary of sequence analyzed

Characters	Base pair (bp)	Percentage (%)
Total characters examined	652	100
Constant characters	520	80
Variation characters	132	20
Parsimony uninformative characters	69	53
Parsimony informative characters	63	47
Variation characters excluding outgroup	7	1

Table 4. Summary of haplotype analysis

Haplotype	Haplotype Sequence	Individual	Location
Hap_1	CTCCCCC	27	14-Jenderak Selatan, 13-Sungkai
Hap_2	TTCCTCC	1	Sungkai (Seladang 3)
Hap_3	CTATCAT	1	Sungkai (Seladang 5)
Hap_4	CACCCCC	1	Sungkai (Seladang 9)

Haplotype analyses were performed using DnaSP version 5 software. The purpose of this analysis was to reveal sequence variation between the two captive centers. Among the 30 individuals, there were only four haplotypes, and each haplotype had only seven base pairs. Haplotype 1 was found in the highest number of individuals (27 individuals) consisting of 14 individuals from Jenderak Selatan and 13 individuals from Sungkai. Haplotypes 2, 3, and 4 were from individuals located in Sungkai. These are Seladang 3, Seladang 5, and Seladang 9 (Table 4). The haplotype diversity value was 0.1931.

The NJ tree (Figure 2) shows the divergence between the ingroup (Malayan gaur) and the outgroup (bison and water buffalo). Malayan gaur from the two captive centers was grouped into the same clade (clade A) with a 100% bootstrap value. The sister clade to the Malayan gaur, composed of bison, was clustered into a different group. Within the Malayan gaur group, Seladang 9 was separated from the other 29 individuals and supported with a 65% bootstrap value. The MP (Figure 3) and NJ tree topologies show little difference: All individuals in the same group in the MP tree are also supported by a 100% bootstrap value. The number of trees produced through the MP analysis was 151. The MP tree analysis has a consistency index (CI) of 0.9747, a homoplasy index (HI) of 0.0253, a retention index (RI) of 0.9773, and a rescaled consistency index (RC) of 0.9525.

Discussion

We conducted a study on the sequence variation within Malayan gaur from two captive centers using the mtDNA D-loop region. The D-loop region is often used for variation studies in either wild animals or animals in captivity (Abdul-Latiff et al. 2014a; Bruford et al. 2003). In this study, only seven sequence variations were observed from a total of 652 base pairs, carrying a value of 1%. This shows that there is low genetic variation among the 30 individuals between the Jenderak Selatan and Sungkai captive centers. This variation is lower than that found by Rosli et al. (2011), who observed a variation of 13.25% by comparing Malayan gaur with other *Bos* genus members using the cytochrome *b* gene. Furthermore, we detected no parsimony informative characters among our seven sequence variations. This suggests that inbreeding has occurred among the individuals of our study and that it is possible that the same common parent has been shared (Norsyamimi et al. 2016). This finding is not surprising as a previous phylogenetic study on Orang Asli and Iban also found similar results with close ties based on a common shared ancestor (Ang et al. 2011).

Our preliminary assumption was that all the Malayan gaur originated from wild populations, captured and protected in these two captive centers. However, an interview with the management officer at Jenderak Selatan Wildlife Conservation Center informed us that only six individuals were originally wild-caught. These six animals consisted of four males (Ahad, Mikael, Isnin, and Jadi) and two females (Biak and Mak Edan) (Abdul-Aziz 2014). These Malayan gaur obtained from wild habitat were used as pioneers for a breeding program (Abdul-Aziz 2014). The reproduction effort started as early as 1982 when the male parent (Ahad) and female parent (Biak) were engaged for a captive breeding program (Sahir 2001). This supports our main finding that a common parent was shared by individuals.

The conservation program of Malayan gaur in the Sungkai Wildlife Reserve was begun in 1998. A few individuals from the Jenderak Selatan Wildlife Conservation Center were brought to Sungkai for a breeding program (Sahir 2001). However, the early lack of experience from the Sungkai Wildlife Reserve management to detect inbreeding in the early stages has led to the genetic consequence that the two captive centers share the same gene pool. Our DNA sequence analysis of Malayan gaur clearly demonstrates that individuals from Jenderak Selatan and Sungkai have low sequence variation with only a 1% difference between them as a result of sharing the same common ancestor.

Haplotype analysis

Previous Malaysian mammal studies also conducted haplotype analyses of the mtDNA D-loop region using the DnaSP software. These haplotype analyses were conducted in the Asian elephant of Peninsular Malaysia (Elliza et al. 2015) and in a population study of otters in Peninsular Malaysia in which a new subspecies of an otter was identified (Rosli et al. 2014). Other loci, such as cytochrome *b*, have also been used, but these results are considered less effective. For example, in a study conducted by Rovie-Ryan et al. (2008), the use of the Cyt *b* region in the haplotype analysis of the Malayan Tapir showed very low haplotype diversity. However, this low variation may be due to the short sequences of the Cyt *b* region.

Based on our haplotype analysis of the D-loop region, only four haplotypes were obtained from the 30 DNA samples. All the Malayan gaur at the Jenderak Selatan Wildlife Conservation Center had one similar haplotype, Hap 1 (5'-CTCCCCC-3'), while the Sungkai captive center had four different haplotypes. Of the 27 individuals with haplotype 1, 13 were from Sungkai. According to the manager at the Sungkai Wildlife Reserve, several individuals of Malayan gaur in this center were taken from Jenderak Selatan Wildlife Conservation Center (Abdul-Aziz 2014), and our haplotype analysis supported this fact.

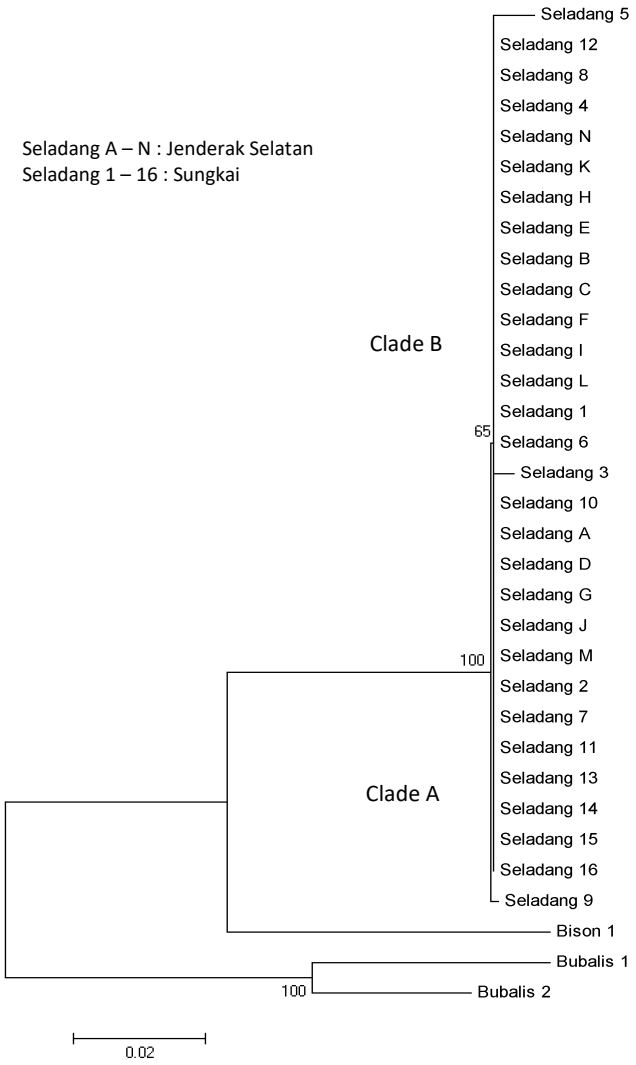

Figure 2. The Neighbor-Joining (NJ) tree of D-loop region

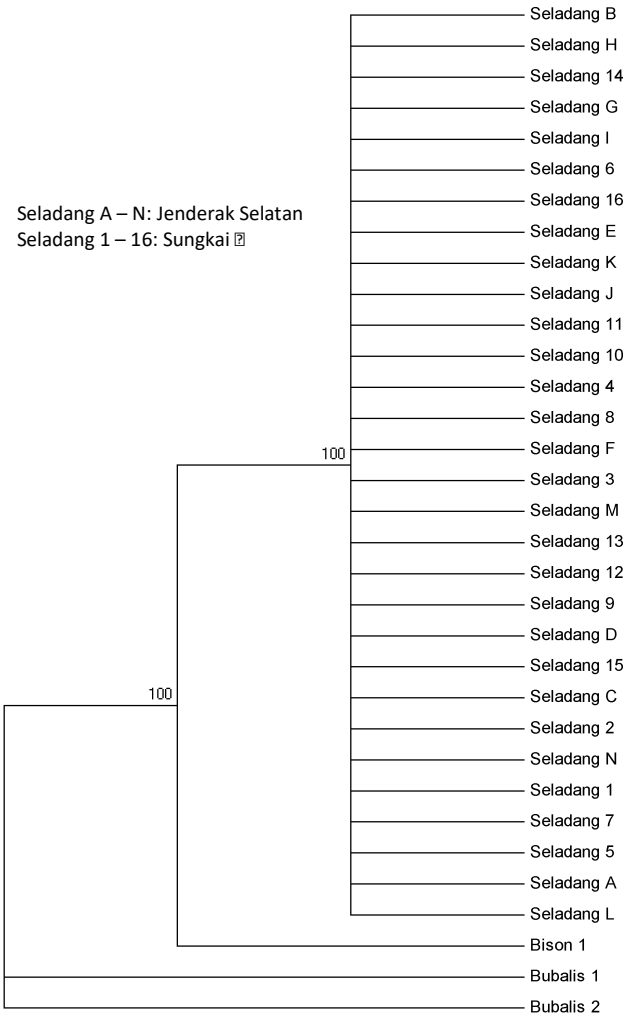

Figure 3. The Maximum Parsimony (MP) phylogenetic tree of D-loop region

Each of the other haplotypes, Hap 2 (5'-TTCCTCC-3'), Hap 3 (5'-CTATCAT-3'), and Hap 4 (5'-CACCCCC -3 '), were found in only one individual gaur each at Sungkai Wildlife Reserve, namely Seladang 3, Seladang 5, and Seladang 9 respectively. Based on the pedigree data at the Sungkai Wildlife Reserve, the breeding program was less managed compared to that at Jenderak Selatan (Abdul-Aziz 2014). Thus, the Malayan gaur population in Sungkai exhibit higher haplotype diversity compared to the gaur in Jenderak Selatan with its single haplotype. However, the haplotype diversity of all samples recorded a low value of 0.1931. According to Hassan et al. (2009), sequence variations characterized the haplotype in their studied sequences. The higher the haplotype diversity, the higher the sequence variation in a population (Liu et al. 2006). The low haplotype diversity value in this study corresponds to the number of haplotypes obtained and the low sequence variation of 1%. This may be due to the samples originating from a captive population in which inbreeding occurred, thereby reducing the variation in the gene pool.

NJ and MP Tree Analysis

All the phylogenetic tree topologies showed a significant separation between the outgroup (bison and water buffalo) and the ingroup (Malayan gaur). The outgroup was selected based on previous studies that also used the *Bos* genus in their studies (Rosli et al. 2011, 2016; Syed-Shabthar et al. 2013). For the NJ tree, clade A was formed by the separation of bison from Malayan gaur, supported with a 100% bootstrap value. Within the Malayan gaur clade A, Seladang 9 is not in the same group as the others (clade B), supported by a 65% bootstrap value, as Seladang 9 is a different haplotype from the other individuals. Seladang 3 and Seladang 5, both individuals in clade B, have a tree length that differs slightly from the other Malayan gaur. The resulting tree is thus equivalent to the results from the haplotype analysis. High haplotype diversity will produce a tree that can distinguish between the populations (Liu et al. 2006).

The MP tree was constructed based on the character method. We produced 151 trees and selected the MP tree

with the least number of changes. The MP tree topology shows a clear separation between Malayan gaur and the outgroup (Rosli et al. 2011, 2016; Syed-Shabthar et al. 2013). The MP tree is slightly different to the NJ tree: All the Malayan gaur are clustered within the same group since there are no parsimony informative characters among the seven variations in the mtDNA D-loop sequence. Parsimony informative characters are important in the formation of MP tree topology (Abdul-latiff et al., 2014a, b; Md-Zain et al. 2014). Thus, the lack of separation between the 30 individuals of Malayan gaur shows that the two captive centers cannot be genetically distinguished from each other.

Both NJ and MP trees show that there is no significant genetic difference in Malayan gaur between Jenderak Selatan and Sungkai. Both captive centers have low genetic variation. The genetic distance analysis, with its value of 0.001, also supports the lack of significant genetic distance between the two captive centers, with all individuals grouped into a single clade. The results of this study support the findings from previous studies of a similar phenomenon of Elliot bird species in captivity (*Syrmaticus ellioti*; Jiang et al. 2005) and the Kangaroo of New Guinea Island (*Dendrolagus matschiei*; McGreevy et al. 2009).

In conclusion, sequence variation of the Malayan gaur was studied to determine the genetic relationships between two Malayan gaur captive centers. The genetic distance among individuals approached zero while haplotype analysis clearly showed no significant genetic differences between Jenderak Selatan and Sungkai captive centers. The findings of this research are of paramount importance for Malayan gaur conservation efforts and its breeding program. Low genetic variation will have a negative effect on this Malayan gaur population which is vulnerable to extinction. Conservation management should avoid the practice of inbreeding among individuals, and the DWNP should increase the number of wild-caught gaur at both captive centers.

ACKNOWLEDGMENTS

The authors are deeply indebted to the Department of Wildlife and National Park (DWNP) Peninsular Malaysia and Agro-Biotechnology Institute (MOSTI) provided us with the necessary assistance during our sample collection. The authors acknowledge Cytogenetic Laboratory members, Universiti Kebangsaan Malaysia, and Ministry of Natural Resources and Environment for providing the necessary funding, facilities, and assistance. This research was supported by Grants AP-2015-004, ST-2015-018, ST-2016-012, 02-01-02-SF0762, and FRGS/1/2015/SG05/NRE//2.

REFERENCES

Abdul-Aziz A. 2014. Variasi jujukan Seladang Malaya (*Bos gaurus hubbacki*) dalam kurungan berdasarkan jujukan DNA mitokondria kawasan D-loop. Tesis Prasiswazah, Pusat Pengajian Sains Sekitaran dan Sumber Alam, Universiti Kebangsaan Malaysia, Selangor DE.

Abdul-Latiff MAB, Ruslin F, Faiq H, Hairul MS, Rovie-Ryan JJ, Abdul-Patah P, Yaakop S, Md-Zain BM. 2014a. Continental monophyly and molecular divergence of Peninsular Malaysia's *Macaca fascicularis fascicularis*. BioMed Res Intl 2014: 897682. DOI: 10.1002/ajp.22596.

Abdul-Latiff MAB, Ruslin F, Vun VF, Mohd-Hashim A, Rovie-Ryan JJ, Abdul-Patah P, Lakim M, Roos C, Yaakop S, Md-Zain BM. 2014b. Phylogenetic relationships of Malaysia's long-tailed macaques, *Macaca fascicularis*, based on cytochrome b sequences. ZooKeys 407: 121-139.

Abdul-Latiff MAB, Aifat NR, Yaakop S, Md-Zain BM. 2017. A noninvasive molecular approach: Exploiting species-locus-specific PCR primers in defeating numts and DNA cross-contamination of Cercopithecidae. J Anim Plant Sci 27: 1015-1023.

Aifat NR, Yaakop S, Md-Zain BM. 2016. Optimization of partial Cyt *b* gene sequence from selected ancient *Presbytis* museum skin specimens. Malays Appl Biol 45: 93-96.

Ang KC, Leow JWH, Yeap WK, Hood S, Mahani MC, Md-Zain BM. 2011. Phylogenetic relationships of the Orang Asli and Iban of Malaysia based on maternal markers. Genet Mol Res 10: 640-649.

Ang A, Srivasthan A, Md-Zain BM, Ismail MRB, Meier R. 2012. Low genetic variability in the recovering urban banded leaf monkey population of Singapore. Raffles Bull Zool 60: 589-594.

Bakar M-AAA, Rovie-Ryan JJ, Ampeng A, Yaakop S, Nor SM, Md-Zain BM 2017. Optimisation of polymerase chain reaction conditions to amplify D-LOOP region in the Malaysian mousedeer genomic DNA. Malays Appl Biol 46: 63-71.

Bruford MW, Bradley DG, Luikart G. 2003. DNA markers reveal the complexity of livestock domestication. Nat Rev Genet 4: 900-910.

Duckworth JW, Sankar K, Williams AC, Samba Kumar N, Timmins RJ. 2016. *Bos gaurus*. The IUCN Red List of Threatened Species 2016: e.T2891A46363646. DOI: 10.2305/IUCN.UK.2016-2.RLTS.T2891A46363646.en. www.iucnredlist.org

Elliza MN, Shukor MN, Othman N, Md-Zain BM. 2015. Haplotype distribution among endangered Asian elephants (*Elephas maximus*) in Peninsular Malaysia. Malays Appl Biol 44: 129-135.

Hassan AA, El Nahas SM, Kumar S, Godithala PS, Roushdy KH. 2009. Mitochondrial D-loop nucleotide sequences of Egyptian river buffalo: Variation and phylogeny studies. Livest Sci 125: 37-42.

Iswadi MI, Ann ZF, Hafiz MM, Hafiz MD, Fahrul FJ, Hajarian H, Wahid H, Zawawi I, Khairiah MS, Mazni OA. 2012. Collection, analysis, and cryopreservation of semen from Malayan gaur (*Bos gaurus hubbacki*): A preliminary study. Open Vet J 2: 109-114.

Jiang P-P, Lang Q-L, Fang S-G, Ding P, Chen L-M. 2005. A genetic diversity comparison between captive individuals and wild individuals of Elliot's Pheasant (*Syrmaticus ellioti*) using mitochondrial DNA. J Zhejiang Univ Sci B 6: 413-417.

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

Liu R-Y, Yang G-S, Lei C-Z. 2006. The genetic diversity of mtDNA D-loop and the origin of Chinese goats. Acta Genetica Sinica 33: 420-428.

Mamat-Hamidi K, Hilmi M, Idris I, Di Berardino D, Iannuzzi L. 2012. Chromosome evolution of the Malayan gaur (*Bos gaurus hubbacki*). Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics 65: 34-39.

McGreevy TJ Jr, Dabek L, Gomez-Chiarri M, Husband TP. 2009. Genetic diversity in captive and wild Matschie's tree kangaroo (*Dendrolagus matschiei*) from Huon Peninsula, Papua New Guinea, based on mtDNA control region sequences. Zoo Biol 28: 183-96.

Md-Zain BM, Majid S, Rosli MKA, Yaakop S. 2014. Phylogenetic position of selembu and other cattle via paternal marker comparison. Life Sci J 11: 42-46.

Md-Zain BM, Teh MS, Ang KC, Lim LS. Yaakop S. 2017. Phylogenetic relationships of the orang asli (Proto Malay) tribe based on the HVS II gene sequences. Malays Appl Biol 46: 35-42.

Md-Zain BM, Abid-Kamal SNA, Aifat NR, Abdul-Latiff MAB, Mohd-Hashim A, Ampeng A, Yaakop S, Samat A. 2018a. Molecular identification of shark fins in Malaysian Borneo's local markets. Biodiversitas 19: 1035-1043.

Md-Zain BM, Abdul-Mutalib SA, Aifat NR, Masstor NH, Mohd-Yusof NS, Mohd-Hashim A, Abdul-Latiff MAB, Yaakop S, Samat A. 2018b. Molecular phylogenetic inference of White-Spotted Guitarfish (*Rhynchobatus australiae*) collected from local Malaysian fish markets. Biodiversitas 19: 1382-1386.

Norsyamimi R, Ithnin H, Rovie-Ryan JJ 2016. Inbreeding depression of captive Malayan gaur (*Bos gaurus hubbacki*) at Jenderak Selatan wildlife conservation center, Pahang. Journal of Wildlife and Parks 31: 71-79.

Romaino SMN, Fazly-Ann ZA, Loo S-S, Hafiz MM, Hafiz MD, Iswadi MI, Kashiani P, Rosli MKA, Syed-Shabthar SMF, Md-Zain BM, Abas-Mazni O. 2014. Species identification of Malaysian cattle using PCR-RFLP method. Genet Mol Res 13: 406-414.

Rosli MKA, Syed-Shabthar SMF, Rovie-Ryan JJ, Zainuddin R, Shukor MN, Yaakop S. Md-Zain BM. 2016. Phylogenetic relationships of the vulnerable wild cattle, Malayan gaur (*Bos gaurus hubbacki*), and its hybrid, the selembu, based on maternal markers. Turk J Zool 40: 369-378.

Rosli MKA, Syed-Shabthar SMF, Abdul-Patah P, Abdul-Samad Z, Abdul SN, Burhanuddin MN, Zulkifli NA, Shukor MN, Budsabong K, Changtragoon S, Sekiguchi T, Sasaki H, Md-Zain BM 2014. A new subspecies identification and population study of the Asian Small-Clawed Otter (*Aonyx cinereus*) in Malay Peninsula and Southern Thailand based on fecal DNA method. Sci World J 2014: 457350. DOI: 10.1155/2014/457350

Rosli MKA, Zakaria SS, Syed-Shabthar SMF, Zainal ZZ, Shukor MN, Mahani MC, Abas-Mazni, O, Md-Zain BM. 2011. Phylogenetic relationships of Malayan gaur with other species of the genus *Bos* based on cytochrome *b* gene DNA sequences. Genet Mol Res 10: 482-493.

Rosli MKA. 2012. Kajian filogenetik Seladang Malaya (*Bos gaurus hubbacki*) dan lembu-lembu lain berdasarkan data jujukan DNA. Tesis Sarjana, Pusat Pengajian Sains Sekitaran dan Sumber Alam, Universiti Kebangsaan Malaysia, Selangor DE.

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19: 2496-2497.

Rovie-Ryan JJ, Traeholt C, Marilyn-Jaoi E, Zainuddin ZZ, Mohd Shariff K, Elagupilly S, Sekiguchi T, Abdullah AA, Cornelia CS. 2008. Sequence variation in Malayan tapir (*Tapirus indicus*) inferred using partial sequence of the Cytochrome B segment of the mitochondrial DNA. J Wildl Parks 25: 16-18.

Sahir O 2001. Breeding performance of the Malaysian captive gaur. Journal of Wildlife and Parks 19: 25-32.

Syed-Shabthar SMF, Rosli MKA, Mohd-Zin NAA, Romaino SMN, Fazly-Ann ZA, Mahani MC, Abas-Mazni O, Zainuddin R, Yaakop S, Md-Zain BM. 2013. The molecular phylogenetic signature of Bali cattle revealed by maternal and paternal markers. Mol Biol Rep 40: 5165-5176.

Xuan TP, Georgescu SE, Manea MA, Hermenean AO, Costache M. 2010. Genetic diversity and phylogenetic relationships of Romanian cattle breeds inferred from cytochrome *b* gene partial sequences. Rom Biotechnol Lett 15: 5154-5158.