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Low genetic diversity and no genetic differentiation between maleo hatched at coastal and inland nesting grounds in North Sulawesi, Indonesia

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Abstract. *Saputra AW, Yuda P. 2020. Low genetic diversity and no genetic differentiation between maleo hatched at coastal and inland nesting grounds in North Sulawesi, Indonesia. Biodiversitas 21: 4772-4777.* Maleo Senkawor (*Macrocephalon maleo*), an endemic and endangered bird of Sulawesi (Indonesia), is burrow-nesting megapodes that incubate its eggs in communal nesting sites in soils heated by sun on beaches and by volcanic activity in inland. The aims of this study were to assess genetic diversity of the Maleo and examine whether those which have different nesting sites have become genetically differentiated. In total, 24 eggshell membranes of Maleo were collected from Tanjung Binerean (coastal nesting ground) and Tambun (inland nesting ground), and the DNA was extracted using silica spin-column kit. PCR was applied to amplify the hypervariable region 1 (HV1) and partial mtDNA control region of HV2 using a specific primer set designed for Maleo. The PCR products were sequenced, resulted in 612 bp, and showed 9 polymorphic sites and 9 haplotypes (H). Further sequences analysis suggested that there was no genetic differentiation between coastal nesting population and inland nesting population (Fst = 0.0009; P = 0.431). As expected, the genetic diversity of Maleo was relatively low (coastal nesting population, Hd: 0.727270, π: 0.002377 and inland nesting population Hd: 0.848480, π: 0.002203).

Keywords: Differentiation, genetic diversity, maleo, mtDNA control region

INTRODUCTION

Maleo (*Macrocephalon maleo*) is an endemic megapode (family Megapodiidae) of Sulawesi, Indonesia. Most megapodes build mounds of rooting leaves for their egg incubation. However, Maleo is the only megapode that burrows the eggs into soils both at volcanic heated soils and at sun-exposed beaches (Dekker and Brom 1992). The other megapodes which incubate their eggs at beaches are the Moluccan Scrubfowl (*Eulipoa wallacei*) (Harris et al. 2014) and Philippine Scrubfowl (*Megapodius cumingii*) (Bashari et al. 2017). Meanwhile, Tongan Scrubfowl (*Megapodius pritchardii*) also incubates in geothermal sites (Harris et al. 2014).

It was assumed that the incubation strategies in megapodes were correlated with their phylogeny. Burrow nesting megapode was believed to have derived from mound-building species, in which the latter represents the plesiomorphic condition in Galliformes (Dekker and Brom 1992). Furthermore, burrow-nesting at sun-exposed beaches were believed to have evolved from burrow-nesting in volcanic heated soils (Dekker and Brom 1992; Mayr 2017). This hypothesis was supported by previous biogeography analyses based on molecular dating (Harris et al. 2014). However, further analysis of Maleo which has two different incubation strategies were not included in the previous study. Do the different incubation strategies influence the population genetics of Maleo? Are they two different or separated populations?

The genetic variation of small population was assumed to reduce (Fraser 2017; Linløkken 2018). Maleo was listed in IUCN's Red List as endangered species, due to its small populations, severe fragmented, and continual rapid declines (BirdLife International 2020). Does Maleo also have low genetic variation? Based on nuclear DNA (*rhodopsin RDP1*) and mitochondrial DNA (dehydrogenase sub-unit2, ND2), previous studies revealed that Maleo has low genetic diversity (Budiarsa et al. 2009a; 2009b). Their nucleotide diversity of ND2 was 0.01-0.02 and 0.0037-0.013 of RDP1. However, the study was limited in samples, having only 3-4 samples in each of the four study sites, and there was no analysis on genetic differentiation among the four populations studied (Budiarsa et al. 2009a; 2009b). Thus, in this study, the researcher reported more samples and used mitochondrial DNA control region (mtDNA CR) or D-loop to assess whether the different nesting strategies in Maleo could cause genetic differentiation. mtDNA CR has been considered as standard for this kind of study due to its rapid mutation compared to other parts of mtDNA (Smith et al. 2017; Song et al. 2017). The aims of this study were to assess the genetic variation of endangered Maleo in North Sulawesi and to explore the genetic differentiation between chicks of Maleo incubated in two different incubation strategies.

MATERIALS AND METHODS

Study area

We used post-hatched egg-shell membranes of Maleo as DNA materials. They were collected from two different semi-natural hatcheries (captive housing in in-situ habitat) at two different nesting grounds at Tambun (Bogani Nani Wartabone National Park) and Tanjung Binerean, North Sulawesi, Indonesia. The first site is the representative of inland geothermal heated nesting grounds and the latter is a sun-heated sand beach-nesting ground (Figure 1).

Procedures

Genetic sampling

We collected the post-hatched eggshells of Maleo in the soil/sand surface around or in the hole-nest. The cleanest membrane eggshells were selected, 12 samples were taken from different post-hatched eggs for each sampling site. Each sample was stored in silica gels inside a separate zip-lock plastic bags. Sample collecting was conducted from 4th April until 1st May 2018. In order to prevent the degradation of DNA, all samples were placed at -40°C until DNA extraction was completed.

DNA extraction

DNA was isolated from post-hatched eggshell membranes using gSYNCTM DNA Extraction Kit (Genaid). Detailed DNA extraction protocol and its quantification were described in our previous work (Yuda and Saputra 2020). The average DNA concentration extracted from Tanjung Binerean: 213±179 ng/µL,) was significantly less than Tambun: 322±153 ng/µL, p=0.004). DNA visualization by agarose gel electrophoreses indicated some degradation. However, based on absorbance ratio quantification (using NanoVue Plus™, Biochrom, Harvard Bioscience, Inc), at A260 nm and 280 nm, the ratio of all samples ranged from 1.81 to 1.89. This result suggested good purity of DNA extracted from eggshell membrane samples. All samples were also successfully amplified for CHD genes to determine the sex of Maleo (Yuda and Saputra 2020).

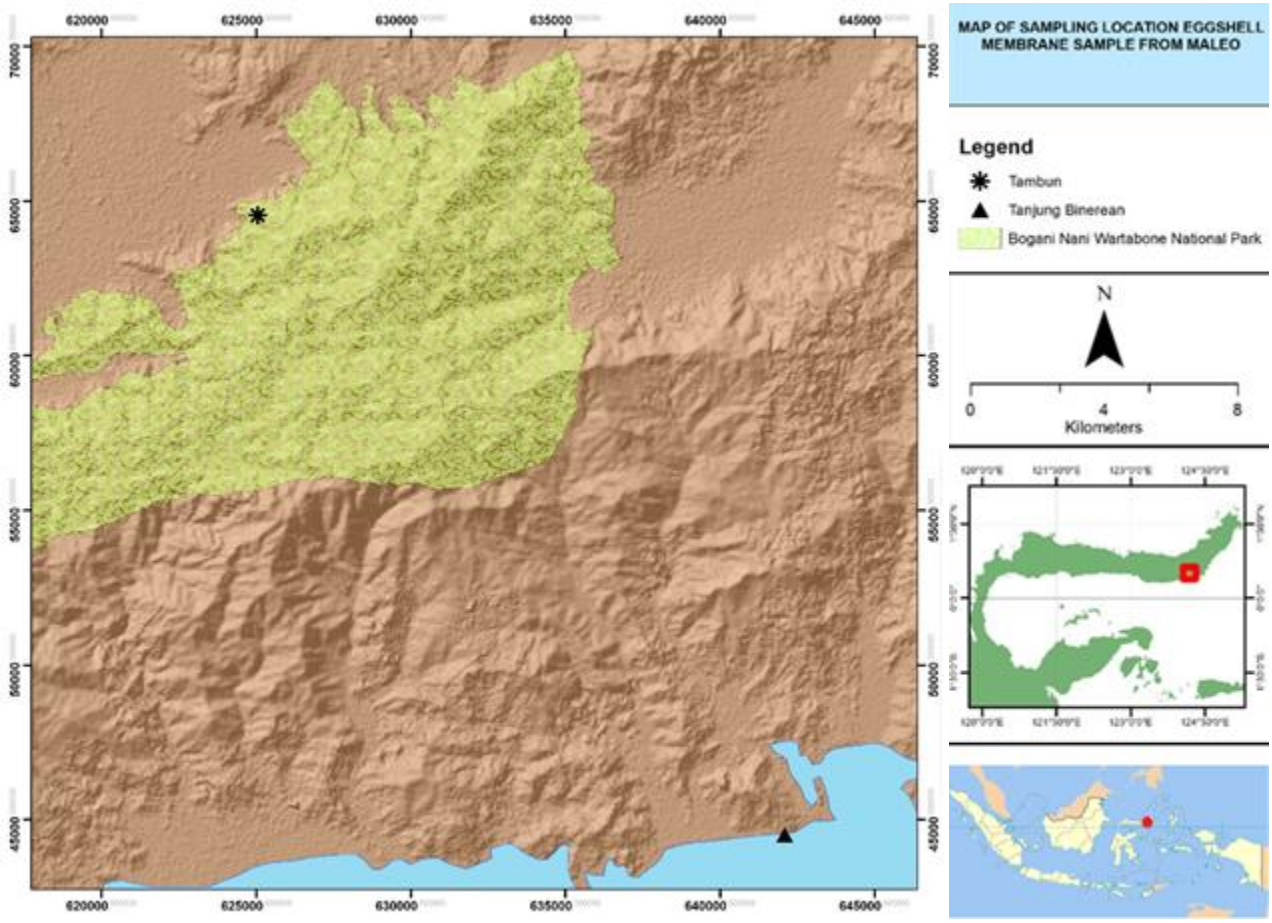

Figure 1. Location of sampling sites at Tambun and Tanjung Binerean nesting grounds, North Sulawesi, Indonesia. Source: Indonesia Topographic Map (www.tanahair.indonesia.go.id), Distribution Map of Maleo Nesting Ground. WCS-IP. 2018.

mtDNA amplification and sequencing

We applied several primer sets designed for mtDNA-CR of domestic chicken, which was L16750/ H547 (Lee et al. 2007); L16750/H1255 (Huang and Ke 2017), and L16750/CR1b (Zein and Sulandari 2012) to amplify the CR mtDNA of Maleo. Unfortunately, none of those primers sets successfully amplified the CR mtDNA of Maleo. For that reason, we designed a specific primer set for Maleo. We used the sequence of mtDNA to complete genome of closely related to megapode species, *Alectura lathami* (AY346091) (Slack et al. 2007), and applied a primer designer program Primer3Plus (https://primer3plus.com/cgi-bin/dev/primer3plus.cgi) (Untergasser et al. 2012). The result was a specific primer set to amplify mtDNA CR of Maleo: MalCRa(f) (5'-TGG CTA CAC TCC AAG GAC TAT GGC T-3') and MalCRa(r) (5'- CTG GAA GGG CAA TCT GTG AAG ACG G-3'). The primer set was expected to amplify ~600 bp of the mtDNA CR.

The PCR was run in a 25 µL reaction, containing 1.7 µL DNA template (30 ng/µL), 3.8 µL free DNAse H20, 12.5 µL 2x PCR buffer KOD FX Neo, 5 µL dNTPs (2mM), 0.75 µL 10 µM Primer MalCRa(f), 0.75 µL 10 µM Primer MalCRa(r), and 0.5 µL 1U/µL KOD FX (DNA Polymerase). PCR was performed using a Veriti™ 96-well thermal cycler (Applied Biosystems™), with the following cycles condition: Pre-denaturation 94ºC, 2 minutes; 30× cycles of denaturation (98ºC 10 sec), annealing (62ºC, 30 sec), extension 62ºC, 45 sec: and final extension at 68ºC, 7 minutes. The PCR products (1 µL) were resolved on an agarose gel (0.8%), in TBE (0.5×) buffer, stained with Ethidium bromide (1%), 100 V for 20 minutes. The gel was then visualized on Gel Logic 200 Imaging System dan Kodak Molecular Imaging Software. Further sequencing reaction was applied for all good PCR products, using the services of 1stBASE Laboratories (Apical Scientific Sdn Bhd), both for forward and reverse primers.

Data analysis

We checked manually the quality of all DNA sequences on Chromas ver. 2.6.5 (Technelysium Pty Ltd). Furthermore, DNA sequences editing and alignment were performed on Bioedit version 7.0.5.3 (Hall 1999; Alzohairy 2011). To confirm the sequence and to know the similarity, we used the database on BLAST online https://blast.ncbi.nlm.nih.gov/ (Zhang et al. 2000).

In order to assess the genetic diversity of mtDNA CR of Maleo, we measured some parameters including nucleotide diversity (π), number of polymorphic or segregating sites (S), haplotype number (h), and haplotype diversity (Hd). All parameters were assessed using DnaSP version 6.12.01 (Rozas et al. 2017) and Arlequin ver. 3.5.2.2 (Excoffier and Lischer 2010). Other parameters revealed were haplotype identity, haplotype frequency, and nucleotide composition.

To reveal the phylogenetic relationships among samples or haplotypes, we conducted haplotype network analysis based on Median-Joining Networks on PopART version 1.7 (Leigh and Bryant 2015). Furthermore, we also measured fixation index (Fst) - the proportion of the total genetic variance in subpopulation relative to the total genetic variance, and analysis of molecular variance (AMOVA), in order to reveal the genetic divergence, and differentiation between the studied populations (Allendorf et al. 2013). Fst and AMOVA were performed in Arlequin ver. 3.5.2.2 (Excoffier and Lischer 2010), in 1000 times permutation.

RESULTS AND DISCUSSION

mtDNA control region of Maleo

All 24 samples were successfully amplified using the primer set of MalCRa(f) and MalCRa(r) (Figure 1). The size of bands was about 600 bp, as expected, when it was designed on Primer3Plus (Untergasser et al. 2012). Further, sequencing reaction resulted in good mtDNA CR sequences of Maleo on the size of 612 bp. All of the sequences were deposited in the GenBank under accession numbers MT899445 to MT899468. Sequence similarity searching in GenBank database showed that the sequences resulting from this study have similarity with other Megapodes species: 94% with partial mtDNA CR of *Megapodius freycinet* (Crowe et al. 2006), 86%-88% partial mtDNA CR or mtDNA genome of *Alectura lathami* (Martins et al. 2014). The DNA sequences of Maleo revealed from this study were hypervariable region 1 (HV1) or domain 1 and partial part of domain 2 of mtDNA CR of bird species (Huang et al. 2017; Zhang et al. 2017). The nucleotides composition consisted of T (28.4%); C (30.8%); A (24.7%); and G (16.2%).

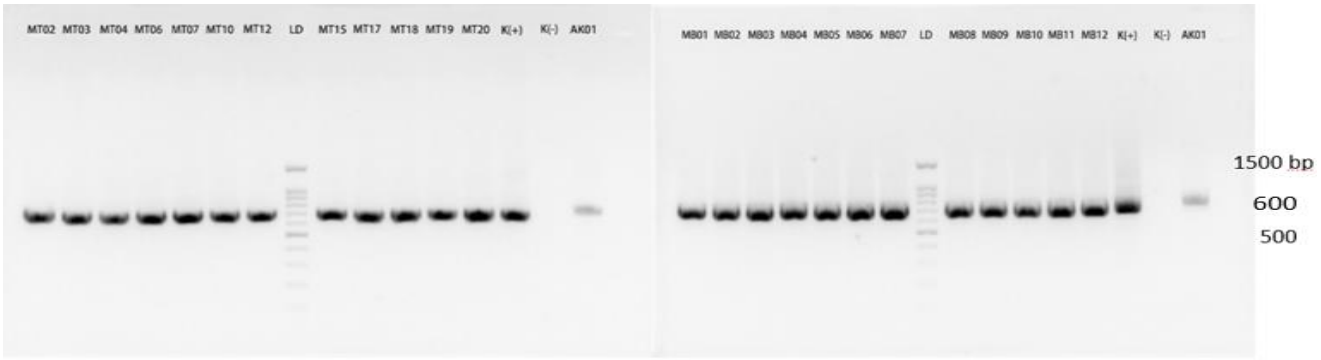

Figure 2. Visualization of electrophoresis gel of PCR products of Maleo's mtDNA control region of in North Sulawesi, Indonesia

Genetic diversity and differentiation

The analysis using 24 sequences of mtDNA CR of Maleo was collected from two different nesting grounds in North Sulawesi revealed relatively low genetic diversity of Maleo. The haplotype diversity (Hd) was 0.79, nucleotide diversity (π) 0.002, and 9 segregating/polymorphic sites (S). Nine haplotypes were observed across all sequences, two haplotypes were found in both sites. Meanwhile, novel haplotypes were also found in both sites, respectively 4 haplotypes in Tambun (inland nesting grounds) and three haplotypes in Tanjung Binerean (coastal nesting grounds (Tables 1 and 2). The polymorphic sites of each haplotype and its frequency are described in Tables 1 and 2.

The species with small population size were expected to have low genetic diversity. Threatened species, including Critically Endangered (CR), Endangered (EN), and Vulnerable (VU), typically have small population sizes and/or declining populations (IUCN 2020). Hence, it was expected that threatened species experience low genetic diversity. Previous studies (Willoughby et al. 2015; Kleinhans and Willows-Munro 2019) agreed with this hypothesis, that endangered species have lower genetic diversity compared to their non-endangered close-relative species. The low genetic diversity of Maleo provided additional field evidence in accordance with the hypothesis. The results based on all samples revealed that genetic diversity of Maleo, both on haplotype diversity (Hd) and nucleotide diversity (π) (respectively 0.7899 and 0.0023) was lower than other non-endangered species of Galliformes. The previous studies on non-endangered species reported the mtDNA CR genetic diversity was higher than genetic diversity of Maleo including Domestic chicken (*Gallus gallus domesticus*) (Phasianidae, Hd: 0.825 and π: 0.0060) (Zein and Sulandari 2012), Grey francolin (*Francolinus pondicerianus interpositus*) (Phasianidae, Hd: 0.818 and π: 0.308) (Khaliq et al. 2011), and Chinese bamboo partridge (*Bambusicola thoracica thoracica*) (Phasianidae, Hd: 0.942 and π: 0.0052) (Huang et al. 2010). Furthermore, compared to other endangered species, Maleo genetic diversity was relatively lower than Cabot's tragopan *Tragopan caboti* (Phasianidae, Galliformes) (Hd: 0.97 and π: 0.0193) (Dong et al. 2010); Green peafowl (*Pavo muticus*) (π: 0.043), but its haplotype diversity was relatively the same (Hd: 0.784) (Sawangtham and Wiwegweaw 2018).

Table 1. Haplotypes and polymorphic sites founded in control region mtDNA of Maleo from North Sulawesi, Indonesia

Haplotype	Nucleotide site									Sample per nesting ground		
	175	225	239	248	254	376	538	604	609	Tambun	Tanjung Binerean	Total
1	A	A	T	C	C	C	G	C	C	4	3	7
2	G	•	•	•	•	•	•	T	•	2	0	2
3	•	•	•	•	•	•	A	•	•	1	0	1
4	•	•	•	•	•	•	•	T	•	3	6	9
5	•	•	C	•	•	•	•	T	•	1	0	1
6	•	•	•	•	•	•	•	•	T	1	0	1
7	•	•	•	T	•	•	•	•	T	0	1	1
8	•	G	•	•	•	T	•	T	•	0	1	1
9	•	•	•	•	T	T	•	T	•	0	1	1

Table 2. Genetic diversity of Maleo in two different nesting grounds in North Sulawesi, Indonesia

Nesting ground	n	H	Haplotype frequency									Hd	π
			1	2	3	4	5	6	7	8	9		
Tambun (in land)	12	6	0.33	0.17	0.08	0.25	0.08	0.08	-	-	-	0.848	0.002
Tanjung Binerean (coastal)	12	5	0.25	-	-	0.50	-	-	0.08	0.08	0.08	0.727	0.002

Note: n: sample size; H: haplotype number; Hd: haplotype diversity; π: nucleotide diversity

Table 3. Analysis of molecular variance results for mtDNA control region of Maleo

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variance	*Fst*	*P*
Among population	1	0.708	0.00063	0.09	0.0009	043011
Within population	22	15..417	0..70076	99..91		
Total	23	16.125	0.70139			

Note: d.f.: degree of freedom (derajat kebebasan: dk)

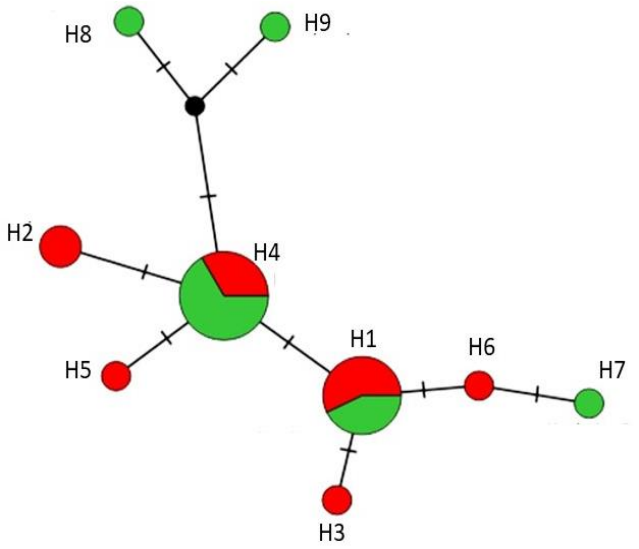

Figure 3. Haplotype networks of mtDNA CR Maleo from Tambun and Tanjung Binerean North Sulawesi using median-joining networks. H: Haplotype; color red and green indicate the origin of samples, red: Tambun; and green: Tanjung Binerean. The size of circle indicates the number of samples

The haplotype network displayed a pattern in the spatial distribution of the genetic lineages of Maleo at Tambun and Tanjung Binerean (Figure 3). The network revealed that all haplotypes were still in one clade. Even though there were three novel haplotypes of Tanjung Binerean (H7, H8 and H9) and four novel haplotypes of Tambun (H2, H3, H5, and H6), they were only one or two site differences in the two main haplotypes (H1 and H5). In addition, the major haplotypes occurred in both study sites. This finding suggested that there is no population structuring between Maleo which hatched at coastal nesting ground (Tanjung Binerean) and inland nesting ground (Tambun). The remnant forest patch surrounding Tanjung Binerean (Hunowu and Patandung 2015) may act as corridor, connecting the two Maleo populations.

The analysis of molecular variants (AMOVA) for Maleo chicks collected from Tambun and Tanjung Binerean nesting grounds revealed that there was no significant genetic differentiation between the two populations (Table 3). The genetic variation between populations was very small (0.09%). Furthermore, the Fst value was also very small (0.0009), compared to the minimum value (0.2) for populations, and considered to have experienced strong structuring or differentiation (Allendorf et al. 2013). The P-value (0.43) was more than 0.05, suggested there were no statistically significant genetic differences. These results suggested that based on mtDNA sequences data the Maleo in Bogani Nani Wartabone Landscape was still a single panmictic (random mating) population. In concordance with the haplotype network, the AMOVA revealed that the different reproduction strategies did not lead to population structuring in Maleo.

The direct distance between the two nesting grounds was about 27 km. Between the two sites, forest patch still exists and provides a good corridor, allowing gene flow between the two populations. This situation may explain the lack of structuring in the Maleo populations. In order to maintain the connectivity between the two populations from two different nesting grounds, it is necessary to protect the remnant forest patch.

In Megapodidae, temporal and spatial variation in available heat sources for nesting may have led to the lability on evolution of nesting behavior. Some species of *Megapodius* can change their reproduction strategy when encountering sudden environmental changes (Harris et al. 2014). This situation may happen with Maleo Senkawor, which nests on two different heat sources for egg incubation:i.e. sun radiation at the coastal nesting grounds, and geothermal heat at inland nesting grounds (Gorog et al. 2005). Even though mtDNA-CR is considered a standard molecular marker for phylogeographic study (Barker et al. 2012), to get more robust analyses, further genetic study of Maleo is still necessary. This study is still limited in sample size and sites, and also only used a single locus molecular marker. For conservation of Maleo, which encounters habitat fragmentation, broader molecular landscape study across Sulawesi and using multi loci markers, such as microsatellite or SNPs are recommended. Next-generation sequencing technology and analysis tools are now available for population genetics study. Among them is a restriction-site associated DNA sequencing, (RadSeq), which has been applied and provides high-resolution population genomic data for any organism at reasonable costs (Davey and Blaxter 2010; Peterson et al. 2012; Ravindran et al. 2019).

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REFERENCES

Allendorf FW, Luikart G, Aitken SN. 2013. Conservation and the Genetics of Populations. 2nd ed. Wiley-Blackwell, USA.

Alzohairy AM. 2011. BioEdit: An important software for molecular biology. GERF Bull Biosci 2 (1): 60-61.

Barker FK, Benesh MK, Vandergon AJ, Lanyon SM. 2012. Contrasting evolutionary dynamics and information content of the avian mitochondrial control region and ND2 gene. PLoS ONE 7 (10): e46403. DOI: 10.1371/journal.pone.0046403.

Bashari H, Mangangue B, Mangangue A. 2017. Incubation strategy of Philippine Scrubfowl *Megapodius cumingii* on Manumpitaeng islet, North Sulawesi, Indonesia. BirdingASIA 27: 82-85.

BirdLife International. 2020. Species Factsheet: Macrocephalon Maleo. BirdLife International, UK. www.birdlife.org.

Budiarsa IM, Artama IWT, Sembiring L, Situmorang J. 2009a. Diversitas Genetik Burung Maleo (*Macrocephalon maleo*) berdasarkan Intron Satu Gen Rhodopsin Nukleus (RDP1). Seminar Nasional Penelitian, Pendidikan dan Penerapan MIPA. Fakultas MIPA, Universitas Negeri Yogyakarta, Yogyakarta. [Indonesian]

Budiarsa IM, Artama IWT, Sembiring L, Situmorang J. 2009b. Diversitas genetik burung Maleo (*Macrocephalon maleo*) berdasarkan sekuen gen dehydrogenase sub-unit 2 (ND2) mitokondria. Berkala Penelitian Hayati Edisi Khusus 3B: 11-15. [Indonesian]

Crowe TM, Bowie RCK, Bloomer P, Mandiwana TG, Hedderson TaJ, Randi E, Pereira SL, Wakeling J. 2006. Phylogenetics, biogeography and classification of, and character evolution in, gamebirds (Aves: Galliformes): Effects of character exclusion, data partitioning and missing data. Cladistics 22 (6): 495-532. DOI: 10.1111/j.1096-0031.2006.00120.x.

Davey JL, Blaxter MW. 2010. RADSeq: Next-generation population genetics. Brief Funct Genom 9 (5-6): 416-423.

Dekker R, Brom, T. 1992. Megapode phylogeny and the interpretation of incubation strategies. Zoologische Verhandelingen 278: 19-31.

Dong L, Zhang J, Sun Y, Liu Y, Zhang Y, Zheng G. 2010. Phylogeographic patterns and conservation units of a vulnerable species, Cabot's Tragopan (*Tragopan caboti*), endemic to Southeast China. Conserv Genet 11 (6): 2231-2242. DOI: 10.1007/s10592-010-0108-6.

Excoffier L, Lischer HEL. 2010. Arlequin Suite Ver 3.5 : A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10 (3): 564-567. DOI: 10.1111/j.1755-0998.2010.02847.x.

Fraser DJ. 2017. Genetic diversity of small populations: Not always "doom and gloom"? Mol Ecol 26 (23): 6499-6501. DOI: 10.1111/mec.14371.

Gorog AJ, Pamungkas B, Lee RJ. 2005. Nesting ground abandonment by the maleo (*Macrocephalon maleo*) in North Sulawesi: Identifying conservation priorities for Indonesia's endemic megapode. Biol Conserv 126 (4): 548-555.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. DOI: 10.14601/Phytopathol_Mediterr-14998u1.29.

Harris RB, Birks SM, Leaché AD. 2014. Incubator birds: Biogeographical origins and evolution of underground nesting in megapodes (Galliformes: Megapodiidae). J Biogeogr 41 (11): 2045-2056.

Huang Z, Ke D. 2017. Organization and variation of the Tetraonidae (Aves: Galliformes) mitochondrial DNA control region. Mitochondrial DNA Part B: Resour 2 (2): 568-570.

Huang Z, Liu N, Liang W, Zhang Y, Liao X, Ruan L, Yang Z. 2010. Phylogeography of Chinese bamboo partridge, *Bambusicola thoracica thoracica* (Aves: Galliformes) in South China: Inference from mitochondrial DNA control region sequences. Mol Phylogenet Evol 56 (1): 273-280.

Huang Z, Shen Y, Ma Y. 2017. Structure and variation of the Fringillidae (Aves: Passeriformes) mitochondrial DNA control region and their phylogenetic relationship. Mitochondrial DNA Part A 28 (6): 867-871. DOI: 10.1080/24701394.2016.1199023.

Hunowu I, Patandung A. 2015. Laporan Program Konservasi Maleo dan Sumber Daya Pesisir di Tanjung Binerean, Manado. [Indonesian]

IUCN 2020. The IUCN Red List of Threatened Species. Version 2019-3.

Khaliq I, Tejedor MT, Monteagudo LV, Riaz M, Khan AA. 2011. Mitochondrial DNA diversity in Francolinus *Pondicerianus interpositus* (Grey Francolin, Galliformes) from Pakistan. Hereditas 148 (2): 70-76. DOI: 10.1111/j.1601-5223.2010.02206.x.

Kleinhans C, Willows-Munro S. 2019. Low genetic diversity and shallow population structure in the endangered vulture, *Gyps coprotheres*. Sci Rep 9 (1): 5536. DOI: 10.1038/s41598-019-41755-4.

Lee YJ, Bhuiyan MSA, Chung HJ, Jung WY, Choi KD, Jang BG, Paek WK, Jeon JT, Park CS, Lee JH. 2007. Mitochondrial DNA diversity of Korean Ogol chicken. Asian-Australasian J Animal Sci 20 (4): 477-481.

Leigh JW, Bryant D. 2015. POPART : Full-feature software for haplotype network construction. Methods Ecol Evol 6 (9): 1110-1116. DOI: 10.1111/2041-210X.12410.

Linløkken AN. 2018. Genetic diversity in small populations. In: Liu Y (eds.). Genetic Diversity and Disease Susceptibility. IntechOpen, United Kingdom.

Martins FMS, Dawson DA, Goth A, Wells DA, Eiby YA, Wilmer JW, Horsburgh GH, Booth DT, Jones DN, Burke T. 2014. Phylogeographical population structure in a mound-building megapode, the Australian Brush-Turkey (*Alectura lathami lathami*). Mol Ecol Laboratory, Dept of Animal Plant Sci, NERC-BAF, UK.

Mayr G. 2017. Evolution of avian breeding strategies and its relation to the habitat preferences of Mesozoic birds. Evol Ecol 31 (1): 131-141. DOI: 10.1007/s10682-016-9872-1.

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. 2012. Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS ONE 7 (5): e37135. DOI: 10.1371/journal.pone.0037135.

Ravindran PN, Bentzen P, Bradbury IR. Beiko RG. 2019. RADProc: A computationally efficient de novo locus assembler for population studies using RADseq data. Mol Ecol Resour 19 (1): 272-282.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 34 (12): 3299-3302.

Sawangtham T, Wiwegweaw A. 2018. Genetic diversity of green peafowl (*Pavo muticus*) in northern Thailand based on mitochondrial DNA control region sequences. Trop Nat Hist 18 (1): 11-23.

Slack KE, Delsuc F, Mclenachan PA, Arnason U, Penny D. 2007. Resolving the root of the avian mitogenomic tree by breaking up long branches. Mol Phylogenet Evol 42 (1): 1-13.

Smith BT, Seeholzer GF, Harvey MG, Cuervo AM, Brumfield RT. 2017. A latitudinal phylogeographic diversity gradient in birds. PLoS Biol 15 (7): e1002610. DOI: 10.1371/journal.pbio.1002610.

Song S, Jiang B, Liu N-F. 2017. Phylogeography of chukar partridge (*Alectoris chukar*) in China based on mtDNA control region. Mitochondrial DNA Part A 28 (4): 473-481. DOI: 10.3109/19401736.2015.1137901.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. 2012. Primer3-New capabilities and interfaces. Nucleic Acids Res 40 (15): 1-12.

Willoughby JR, Sundaram M, Wijayawardena BK, Kimble SJA, Ji Y, Fernandez NB, Antonides JD, Lamb MC, Marra NJ, DeWoody JA. 2015. The reduction of genetic diversity in threatened vertebrates and new recommendations regarding IUCN conservation rankings. Biol Conserv 191: 495-503. DOI: 10.1016/j.biocon.2015.07.025.

Yuda P, Saputra AW. 2020. Eggshell membrane for DNA sexing of the endangered Maleo (*Macrocephalon maleo*). F1000 Res 9: 599. DOI: 10.12688/f1000research.23712.1.

Zein M, Sulandari S. 2012. Keragaman genetik dan distribusi haplogrup ayam kampung dengan menggunakan Hipervariabel-I daerah kontrol DNA mitokondria. Jurnal Ilmu Ternak dan Veteriner 17 (2): 120-131. [Indonesian]

Zhang Y, Xie Z, Deng X, Xie Z, Xie L, Fan Q, Luo S, Liu J, Huang L, Huang J, Zeng T, Wang S. 2017. Complete sequence cloning and bioinformatics analysis of the chukar partridge (*Alectoris chukar*, Aves, Galliformes) in Guangxi base on mitochondrial genome. Mitochondrial DNA Part B 2 (1): 361-362. DOI: 10.1080/23802359.2017.1339215.

Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. J Comput Biol 7 (1/2): 203-214.