

HAPLOTYPE DIVERSITY IN THE PARTIAL CYTOCRHOME B (CYTB) GENE OF WATER MONITOR LIZARDS (*Varanus salvator*) AT PENINSULAR MALAYSIA: A SHORT COMMUNICATION

Muhammad Kemal Ariq Naufal Ramadhan¹, Widya Pintaka Bayu Putra^{2*}

¹Department of Biology, Faculty of Education, Bursa Uludağ University, Bursa, Turkey
16059

²Research Center of Applied Zoology, National Research and Innovation Agency, Bogor,
Indonesia 16911

Email: widya.putra.lipi@gmail.com

ABSTRACT

Water Monitor (WM) lizard (*Varanus salvator*) are one of Varanidae family lizard that widely spread at Southeast Asia belonging to Peninsular Malaysia. This research was conducted to observe the halotype diversity in partial mitochondrial Cytochrome b (Cytb) gene (344 bp) of WM lizards at Peninsular Malaysia. A total of seventeen (17) Cytb sequences of WM lizard were collected from NCBI database. Research showed that five (5) haplotypes of Cytb gene were observed in the observed sequences. About 71% of WM lizards from Peninsular Malaysia were classified into Haplotype 3 (Hap.3). Hence, this haplotype was classified as the common ancestral of WM lizard at Peninsular Malaysia. The Neutrality test revealed that low allelic frequency in WM lizard can be caused by species expansion. In conclusion, the WM lizards at Peninsular Malaysia consisted of two (2) clades of Clade A (Hap.1 and Hap.2) and Clade B (Hap.3, Hap.4 and Hap.5).

Keywords: Cytb gene, GenBank, halpotype, mutation, *Varanus salvator*

Received 6 February 2022 *Accepted* 2 July 2022

INTRODUCTION

Water Monitor (WM) lizard (*Varanus salvator*) are large lizards distributed throughout South-East Asia [1]. They usually inhabit wetlands, agricultural zones, marshes, and canals [2,3], and play an ecological role as predators by controlling animal populations. Water monitors can attain sexual maturity within a few years, and females have clutches containing many eggs. It is likely that the resilience of water monitors comes from different aspects such as reproduction and adaptation to various habitats, as well as being a generalist predator [4] (Vitt, 1981). Water monitor meat and skins are used for dietary protein and leather goods [5]. International trade in water monitor skins has impacted on the wild population, with skins exported and fashioned into high-quality leather goods. The intense exploitation of their skins has adversely affected populations [6]. The squamate genus *Varanus* (family Varanidae) is comprised of a charismatic and diverse assemblage of more than 100 named lineages (73 species and 30 subspecies) and distributed throughout Africa, southern Asia, and the Indo-Australian regions [7].

Recently, the WM lizard was listed Not Extinction based on the IUCN Red List. However, over exploitation of WM lizard for skin and meat purpose will causing the

extinction in this species. An early effort to protect WM lizard can be assessed with a molecular genetic study of mitochondrial DNA [8]. The molecular study is important to predict the population size of this species. A previous studies has been worked to characterize WM lizard based on the mitochondrial 12s-rRNA region, 16s-rRNA region and Cytochrome b (Cytb) gene [9], control region (D-loop) [1,10] and ND1/ND2 regions [11]. This study was aimed to observe the haplotype diversity in the Cytb gene of WM lizard with a meta-analysis study. The results study can be used as the early information to explore the mitogenome of Varanidae lizard in the future.

MATERIALS AND METHODS

A total of seventeen (17) Cytb sequences (344 bp) of WM lizard were obtained from the NCBI database (<https://www.ncbi.nlm.nih.gov>). The obtained sequences in this study were collected from GenBank ID: EU145736; EU145737 and GU476579 - GU476593. Four (4) computer packages of BioEdit [12], MEGA-X [13], DNAsp [14] and NETWORK [15] were used in this study for the sequence analyses. A BioEdit package was used for sequence alignment analysis. A MEGA-X package was used to calculate Pairwise genetic distance and phylogenetic tree reconstruction. A DNAsp was used to obtain the haplotype diversity and Neutrality test calculation. A NETWORK package was used for obtaining Median-joining Network. All the observed sequences were originated from Peninsular Malaysia.

RESULTS AND DISCUSSIONS

The haloptype diversity of partial Cytb gene in WM lizards included of high category ($0.50 < H_d < 1.00$) with 5 haplotypes (Table 1). Haplotype 3 (Hap.3) was showed as the common haplotype in WM lizards at Peninsular with the haplotype frequency of 0.71. In addition, three reference sequences of EU145736, EU145737 and GU476584 were identified as Hap.1 Hap.2 and Hap.5, respectively. Two reference sequences of GU476582 and GU476586 were identified as Hap.4. Meanwhile, Hap.3 consisted of twelve (12) reference sequences of GU476579 - GU476581; GU476583; GU476585; GU476587 - GU476593. A previous study obtained 50 haplotypes from 72 heads of WM lizards based on D-loop region [1].

The Neutrality test of Fu's F_s statistic and Tajima's D test were showed in a negative value. The negative value in both parameters indicated that less of allelic frequency in was caused by species expansion. A similar finding was reported in WM lizards of Thailand (Bang Kachao peninsula / Kamphaeng Saen) that have a negative value of Fu's F_s (-26.422/-18.298) and Tajima's D (-2.537/-2.231) [1]. The pairwise genetic distance was ranged from 0.0029 to 0.0119 (Table 2). In the lizards, the genetic distance among individu can be affected by geographical distance factor [16]. In this study, the percentage of the geographical effect to the sequence variation was not calculated because of similar source in each sequence.

A total of five (5) mutation sites were detected in the partial Cytb gene of observed sequences (Figure 1). These mutation was occurred in the 49th, 144th, 222th, 262th and 279th positions. The phylogenetic tree revealed that WM lizards at Peninsular Malaysia separated into two clades of Clade A (Hap.1 and Hap.2) and Clade B (Hap.3; Hap.4 and Hap.5) as illustrated in Figure 2. In addition, about 88% of WM lizards at Peninsular Malaysia were classified into a Clade B. Therefore, The Median-joining network revealed that the Hap.3 lizards as the common ancestral in WM lizards at Peninsular Malaysia. Compared to the another species of Varanidae lizards, WM lizard (*V. salvator*) was clustered into Indo-Asian A group, together with *V. flavescens*, *V. dumerili*, *V. bengalensis* and *V. rudicollis* [8].

Table 1. The genetic diversity in partial COI gene in Water Monitor lizards (*Varanus salvator*) from Peninsular Malaysia

Parameter	Value
Number of observed sequence	17
Length of observed sequence (bp)	344
Number of mutation site	5
Number of haplotype	5
Haplotype diversity (H_d)	0.507
Nucleotide diversity (π)	0.003
Fu's F_s statistic test	-1.616
Tajima's D	-1.269

Table 2. Pairwise genetic distance among haplotype of Water Monitor lizards (*Varanus salvator*) from Peninsular Malaysia

Haplotype	1	2	3	4	5
1	1.0000				
2	0.0029	1.0000			
3	0.0089	0.0059	1.0000		
4	0.0119	0.0089	0.0029	1.0000	
5	0.0118	0.0088	0.0029	0.0059	1.0000

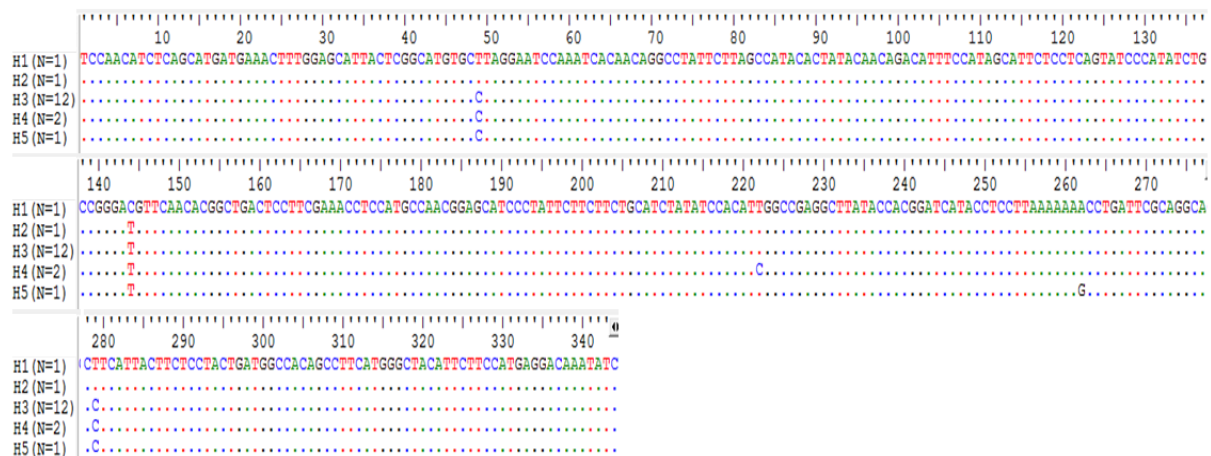


Figure 1. Five (5) haplotype (H) sequences of partial Cytb gene in Water Monitor lizard (*Varanus salvator*) from Peninsular Malaysia

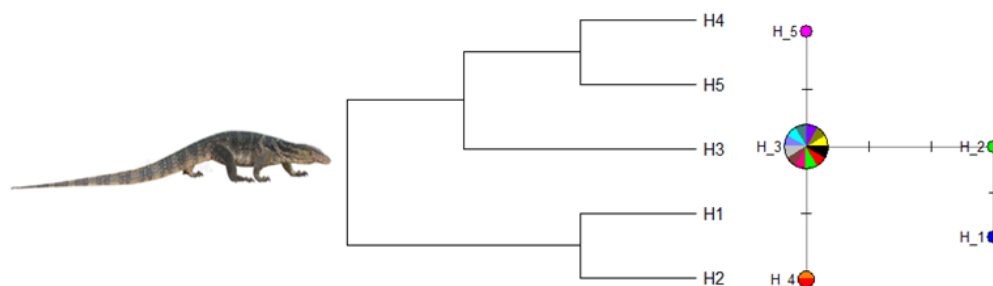


Figure 2. Neighbor-joining (NJ) tree and Median-joining network among haplotype of Water Monitor lizard (*Varanus salvator*) from Peninsular Malaysia

CONCLUSION

The WM lizards at Peninsular Malaysia consisted of 5 haplotypes. According to these haplotypes, the WM lizards at Peninsular Malaysia can be divided into 2 clades of Clade A (12%) and Clade B (88%). In the future, the depth study to observe the genetic diversity of WM lizards with large sample from another regions of Southeast Asia and mitogenome analysis are important to get the findings accurately.

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