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**Research Article**

# The Connection of Distribution Location with Primate Kinship in Indonesia Based on Cytochrome B in Silico Analysis

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**ABSTRACT**

Primates are mammals in the animal kingdom and considered as terestrial creatures. Primates are arboreal creatures that spend most of their days hanging on trees for socializing, eating, and exploring. Those factors could be the reason why primates likes to migrate. Primates undergo progressive evolution which indicates its abilities to survive in different kinds of environment. The cause of their evolution varies from the earth geography, adaptability, natural selection, mating, and mutation. In-silico approach is being used to find out the connection between area distribution and primates' genetics in Indonesia using their cytochrome b sequence that were acquired from UniProtKB and NCBI site for analysis purposes. Based on gene analysis by MEGA and regional mapping by QGIS, we can conclude primates in Indonesia have genetic connection molecularly between one and another. Two of the first clades differentiate Haplorrhini and Strepirrhini. Haplorrhini divided into two infraorder, which are Similiformes and Tarsliformes. Simiiformes divided by two clades of superfamily which are Hominoidae and Cercopithecoidea. Cercopithecidae divided itself into two subfamily, which are Colobinae and Cercopithecinae. Furthermore, the differentiation between them is caused by geographical changes and wide immigration activity, which is not linier with their endemicity.

*Keywords:* Cytochrome b, evolution, in silico, phylogenetic, primate

## Introduction

Primates are mammals in the animal kingdom and they are terestrial creatures. Primates in Indonesia approximately has 59 species and 79 subspecies which has different sizes and characteristics [1]. Primates are arboreal creatures that spend most of their days hanging on trees for socializing, eating, and exploring. Those factors could be the reason why primates likes to migrate.

Moving from one location to another have made geographical isolation and evolutions possible. Evolution originated from the word evolve which means change. Evolution is the changes in the genes between the ancestor and its decendants in a long period of time. The cause of evolution varies from the earth geography, adaptability, natu ral selection, mating, dan mutation. Primates undergo progressive evolution which indicates its ability to survive in different kinds of environment [2].

This diversity is affected by the earth's geographical changes. Indonesia once had Sunda land and Sahul land. Sunda land or Sunda biogeography is land that has conjunction with Asia while Sahul land or Australia biogeography has cunjunction with Australia itself. Between those lands, there was a transitional zone consists of flora and fauna same as the previous lands called Wallacea biogeography zone [3]. Precisely because of those reasons, Indonesia has a wide variety of primates which most of them are endemic. To be able to see the evolution of an organism, two methods can be used which consists of fenetics and cladistics. Fenetic is used based on the similarities of its morphology meanwhile cladistics is used to see the

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connection of its character evolution or characteristics of each organism called phylogenetics. With the fast paced of technology development, Kinship can be processed through molecular analysis or more commonly known as molecular phylogenetics in Bioinformatics [4]. The making of molecular phylogenetics consists of protein chains and DNA but using cytochrome B will result in better molecular phylogenetics.

Cytochrome B genes in the eucaryote mitochondria genomes are one of the genes used as the molecular phylogenetics base. The advantages of cytochrome B genes usage are it can determine taxonomy, phylogeny and evolution connection as well as estimating the diversity range of each kind [5]. This gene is also used as the universal primer in the Polymerase Chain Reaction (PCR) and can also determine its nucleotides order through sequencing.

Based on the background above, this research aims to discover the connection between locations and the Kinship of primates in Indonesia based on in silico analysis of cytochrome B. The hypothesis of this research is there are kinship connection molecularly between kinds of species and its location on Indonesia.

## Methods

### *Distribution Map*

*Searching of Distribution Data*

Distribution data of each primates species was based on the National Center for Biotechnology Information site (NCBI) (https://www.ncbi.nlm.nih.gov/)[6]. Primates location data are saved in Excel format.

*Map Manufacturing*

The saved data is processed by digitation using Quantum Geographical Information System (QGIS) [7]. The steps for digitation the distribution map are open the base map of Indonesia. Then use spatialite layer to create dot for each location of the primate. Map manufacturing differentiate based on family and save as image format (.jpeg).

### *Phylogenetics Topology of Cytochrome B*

*Searching of Cytochrome B Gene Sequences*

Cytochrome B gene sequence of primate species are acquired from UniProtKB and NCBI. The primate cytochrome B accession number are: *Nycticebus coucang* (Q35131), *Nycticebus javanicus* (ALJ53333), *Nycticebus menagensis* (ALJ53338), *Tarsius fuscus* (ALN49334), *Tarsius lariang* (YP_009027692), *Tarsius wallacei* (YP_009027718), *Tarsius dentatus* (YP_009027705), *Cephalopachus bancanus* (NP_148750), *Macaca fascicularis* (AJO69445), *Macaca nemestrina* (AKC89451), *Macaca maura* (ARQ81320), *Macaca nigra* (YP_009115175), *Macaca nigrescens* (ADI44017), *Macaca hecki* (ADI44019), *Macaca tonkeana* (YP_009072475), *Macaca ochreata* (ADI44025), *Simias concolor* (YP_007625639), *Presbytis comate* (AEI52280), *Presbytis thomasi* (AEI52288), *Presbytis femoralis* (ANA91143), *Presbytis melalophos* (ABV44681), *Presbytis bicolor* (AEI52269), *Presbytis mitrata* (AEI52263), *Presbytis siberu* (AEI52283), *Presbytis potenziana* (AEI52285), *Presbytis frontata* (AEI52276), *Presbytis rubicunda* (AEI52274), *Presbytis chrysomelas* (AEI52275), *Presbytis hosei* (AEI522770, *Trachypithecus auratus* (ARC95544), *Trachypithecus mauritius* (ARC95557), *Trachypithecus cristatus* (YP_009025008), *Nasalis larvatus* (ABV44678), *Hylobates lar* (NP_007834), *Hylobates klossii* (ADC44543), *Hylobates agilis* (YP_003587226), *Hylobates moloch* (ADT82321), *Hylobates muelleri* (ADC44537), *Hylobates albibarbis* (ADC44535), *Hylobates abbotti* (ADC44542), *Hylobates funereus* (ADC44541), *Symphalangus syndactylus* (YP_003587317), *Pongo pygmaeus* (NP_008237), and *Pongo abelii* (NP_007847). The primate cytochrome B gene sequence is saved in FASTA format (.fas).

*Gene Sequence Alignment between Species*

The software that being used on this stage is Clustal W. Alignment is used to do the sequence alignment of two or more biological sequences, that resulted in the similarity of each sequence [8]. The steps of using Clustal W software are open the file and load the sequence of the cytochrome B gene. The primates gene sequence data which are going to be compared has to be inserted one by one using Append Sequence and do complete alignment to finish the alignment phase. Change the file name according to its species name and adjust by using the codes enlisted in GenBank.

### *Phylogenetics Topology*

The process of making the phylogenetics topology of its gene sequence from the compared species is possible by using Molecular Evolutionary Genetics Analysis (MEGA) [9]. Re-open the most recent alignment file that has been saved using the .fas format in MEGA. Alignment file consists of two visuals and choose the visual with the base nucleotides sequence. Click the Export Alignment in MEGA format before analyzing its filogenetics. This step is carried out to obtain the primates' filogenetics topology and to measure the distances between genetics and the primate species by using Pairwise Distances in the MEGA software.

## Results and Discussion

There are 44 species from 5 families of primates distribution locations in Indonesia from the NCBI site. The acquired data then changes into the distribution map through the QGIS software. As shown in Figure 1.

Based on the distribution map, Indonesian primates can only be located in the Sunda land which indicate primates in Indonesia originated from Asia (oriental). Sumatra island has 17 species, Kalimantan island has 11 species, Java island has 6 species, and Sulawesi island has 10 species.

This distribution happened because the primates undergo diferentiation that occured from the end of ice age. Differentiation process is linier with the biota distribution theory which define as the total of species in one island will be determined by even numbers between extinction average rate and imigration average rate [10]. Islands that are far from the continent have species smaller in numbers and big islands have species with high diversity rate. Small islands have a high isolation rate which enhanced the chances of endemicity.

On the Sunda land area, species numbers is big such as *Tarsius bancanus* and the big ape like *Pongo* sp. Primates that has wide range of distribution includes *Presbytis* genus, *Macaca, Hylobates, and Nycticebus* meanwhile *Pongo* genus, Tarsius, Trachypithecus, Symphalangus, *Nasalis, Simias,* and *Cephalopacus* don't have a wide range of distribution which makes it endemic. In the Wallacea area, species numbers is smaller than the Sunda area. This is because Wallacea area is farther from Asia. Species in this area has similarities with the ones in the Sunda area but because Wallacea area is a small island then the endemicity rate is higher than the Sunda area.

Every area has their own distinguishable traits on the body and behaviour as well as its genetics rate. To see the Kinship of species, cytochrome B data sequencing is being used from the UniProtKB and NCBI sites. Subsequently, align the acquired data between one and another species.

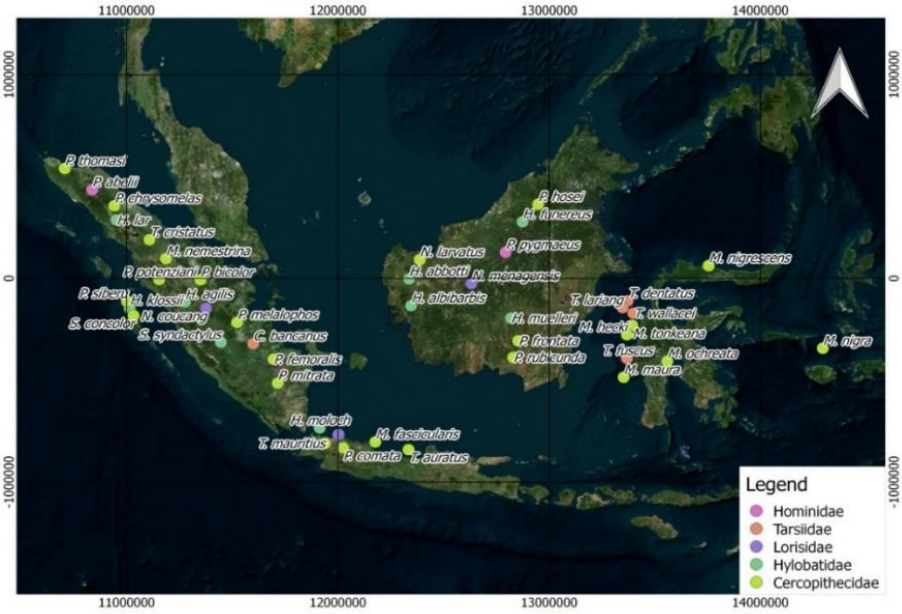


Figure 1. Map of Indonesian primate distribution

Analysis from the MEGA software used the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) as shown in Figure 2.

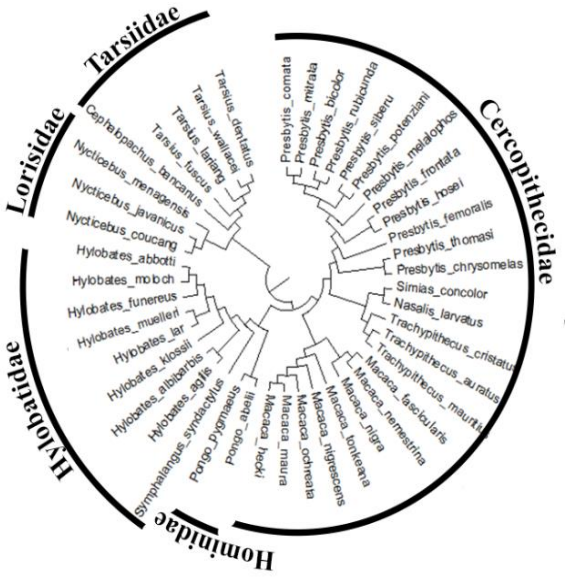


Figure 2. Molecular phylogenetic of primates in Indonesia

Based on the clade filogenetics results, the outcome are monophyletic. It indicates an ideal taxon because the results are based on close relatives [11]. Two of the first clades differentiate Haplorrhini suborder and Strepirrhini and also divide by biological time factors such as nocturnal clade and diurnal clade [12]. The dividing Haplorrhini suborder includes Simiiformes infraorder and Tarsiiformes. This difference can be seen from their body size. Tarsiiformes has small body while Simiiformes has big body shape. Tarsiiformes are often connected with ancient primates and cannot be divided between apes and monkeys [13]. Simiiformes suborder divided by two clades which are Hominoidae superfamily and Cercopithecoidea superfamily. The difference between Hominoidae and Cercopithecoidae can be seen from its body shape, tail, and brain volume as well as its behavior [14].

Hominoidae or the ape group has big bodies, long arms, and small brain volumes and also lives on top of trees (arboreal), meanwhile Cercopthecoidae or monkey group have small bodies, shorter arms, and smaller brain volume and also lives on trees and on land (semi terestrial). In the Cercopithecidae superfamily divided itself into the Colobinae subfamily and Cercopithecinae. The difference between these subfamilies are located in its foods. The main food for Colobinae are leaves while the Cercopithecinae are fruits [15].

Every type of primates in an area has different Kinships. Primates' migration routes can impact the distribution between populations and evenly spread in an area. After the creation of islands, differentiation happened and the population have to adapt to the environment. Tarsius is the primate in the Wallacea area that has small figures and big eyes and also lives in Sulawesi. *Nycticebus* genus has similar characteristic to *Tarsius* except for its body shape and habitat location. In accordance with the filogenetics point of view, *Nycticebus* genus has a close range to the *Tarsius* genus. This indicates that Wallacea area used to be the now Sunda. The same thing as the Macaca genus that generates different subspecies. *Macaca nigra* is a species that live in the Wallacea area, but has a closer kinship with the *M. fascicularis* and *M. nemestrina* in the Sunda area. This was caused by the macaca genus active immigration. Less active in migration primates will be differentiated based on geographical aspects only like the *Pongo* genus.

## Conclusion

Primates distribution in Indonesia has the connection with Kinship molecularly. Distribution was affected by the evolution of the earth's surface on the Sunda and Wallacea area. Differentiation is not only caused by geographical changes but also by wide imigration activity. Primates endemicity is not linier to the immigration activity.

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