



IDENTIFICATION OF JAVAN LANGUR (*Trachypithecus auratus*) IN JAVAN LANGUR CENTER (JLC) COBAN TALUN-BATU BASED ON *D*-LOOP SEQUENCES

Anisa Rizkyani¹, Elizabeth Marlynda Shierly Sai¹, Miftahul Muslih², Abdul Ghofur¹,
and Dwi Listyorini¹

¹Department of Biology, Faculty of Mathematic and Natural Science
State University of Malang, Jl. Semarang 5, Malang, Indonesia 65145,

²Faculty of Biology, Gadjah Mada University.

E-mail : anisaririzkyani@gmail.com; listyorini.alj@bio.um.ac.id

ABSTRACT

Javan Langur (*Trachypithecus auratus*) is one of endemic primates in Indonesia and classified into vulnerable level. Based on its morphological characteristic and geographic area, Javan Langur divided into 2 subspecies, those are *Trachypithecus auratus auratus* and *Trachypithecus auratus mauritius*. The identification of its morphological characteristic and genetic in species or subspecies level is important before releasing because it is correlated with inbreeding grouping and releasing place. The aim of this study is to identify the Javan Langur (*Trachypithecus auratus*) in Javan Langur Center (JLC) Coban Talun-Batu based on *D*-Loop sequence. The blood sample used in this study was taken from two adult female Javan Langur named Ijem and Embun, that have different morphological characteristics. Both (primates) are suggested to be *T.a.mauritius* and *T.a.auratus* respectively. The amplification of *D*-Loop sequence by mean of PCR technique was performed using specific designed primers forward D-LoopRs 5'-GTACTTAACTCCACCACCAA-3' and reverse D-LoopRs 5'-GTTGAGTTGGGTATGCTCGA-3'. The phylogenetic tree reconstruction based on MEGA 5 using Maximum Likelihood (ML) method and pairwise distance analysis with Kimura-2 parameter model shows that Ijem and Embun are placed in different clade. Pairwise distance from those samples value is 11,4%, it prove that they are interspecies. This result supports the morphological characteristic and previous research used *Cyt-b* sequence, Ijem is *Trachypithecus auratus mauritius* and Embun is *Trachypithecus auratus auratus*.

Key words : Identification, Javan Langur (*Trachypithecus auratus*), *D*-loop, Javan Langur Center

INTRODUCTION

Indonesia is one of countries which possesses the richest various species of primates in the world. One of endemic animals lives in Indonesia is Javan Langur (*Trachypithecus auratus*). Morphological differences and geographical distribution made Javan Langur separated into 2 distinct subspecies (Kurniawan: Personal Communication, 2013). Based on Rosenblum *et al.* (1997) and Douglas (2004), *Trachypithecus auratus* is group into 2 subspecies those are *Trachypithecus auratus mauritius* which distributed in West Java and *Trachypithecus auratus auratus* which distributed in Bali, Lombok, and East Java. The differences between *T. a. auratus* and *T. a. mauritius* are the fur color, male genital organ, and face shape (Figure 1). *International Union for Conservation of Nature (IUCN) Red List* version 2009.2 in 2009 included Javan Langur to *Vulnerable* category (IUCN, 2009). The importance of determination between *T. a. auratus* and *T. a. mauritius* is related to inbreeding grouping, mapping, and determination of natural habitat for releasing. Identification of *T. a. auratus* and *T. a. mauritius* in JLC is done based on morphological characteristics only, therefore it needs to know the phylogenetic position to support the morphological data. *D*-Loop sequence is a mitochondrial gene (11), which has mutation rate 4-5 time faster than

other sequences in mitochondrial DNA (Horai *et al.*, 1993). The length of *D-loop* sequence is around 1 kb, and it belongs to *non-coding* area (Arif & Khan, 2009). This research aimed to reveal the taxonomic position of Javan Langur (*Trachypitecus auratus*) in *Javan Langur Center* by *D-loop* sequence.

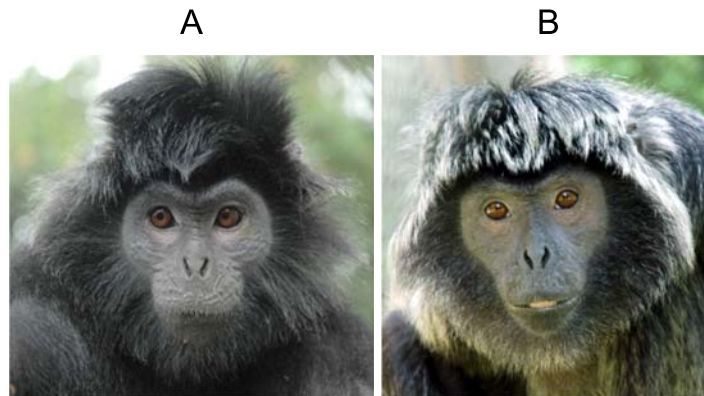


Figure 1. A. Male of *Trachypitecus auratus mauritius*, B. Male of *Trachypitecus auratus auratus* (Kurniawan, 2012)

MATERIALS AND METHODS

Material used in this research is blood sample of Javan Langur provided by *Javan Langur Center*, from adult females named Embun and Ijem. These samples were selected by reason of its morphological characteristic which are assumed as *T. a. auratus* for Embun, and *T. a. mauritius* for Ijem.

Blood Sampling

Total DNA isolation was conducted according to *Roche Isolation DNA Mini Kit* with some modification. *D-loop* amplification was done by mean of using *D-loopRs forward* 5'-GTA CTT AAC TCC ACC ACC AA -3' and *D-loopRs reverse* 5'-GTT GAG TTG GGT ATG CT GA-3' primers pair in cycle determined. DNA sequencing was performed in *Eijkman Institute for Molecular Biology*, Jakarta.

RESULTS AND DISCUSSION

PCR amplification resulted around 350 bp length fragment a 377 bp length fragment for Ijem and 370 bp for Embun. BLAST analysis result shows that *D-loop* fragment of Ijem's sample which has *query coverage* value 98-100% with *maximum identity* value 83-92% and Embun's sample has *query coverage* value 90-100% with *maximum identity* value 82-90% compared to *D-loop* sequence in references species. *Query coverage* and *maximum identity* value on both samples almost reach 100%, this shows that *D-loop* sequence obtained from both samples is indeed *D-loop* sequence (Golding *et al.*, 2012).

Clustal X analysis shows that *D-loop* sequence on both samples cannot be compared to *Trachypitecus auratus* since there is no reported in NCBI, therefore it can only be compared to other species, those are *Trachypitecus cristatus*, *Trachypitecus poliocephalus*, *Trachypitecus poliocephalus leucocephalus*, *Trachypitecus laotum*, and *Trachypitecus francoisi*.

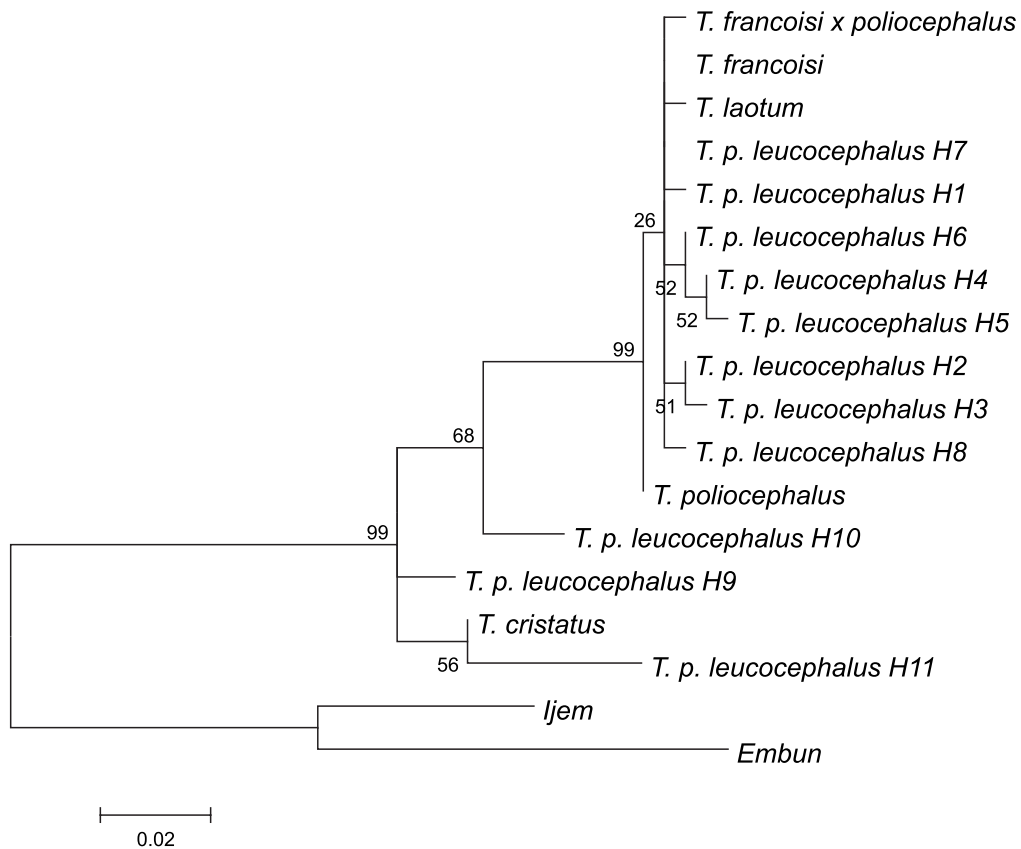


Figure 2. Phylogenetic Tree Utilizing *Maximum Likelihood* Method, *PairwiseDistance* Analysis with *Kimura-2 Parameter* Model

The reconstruction of phylogenetic tree using Maximum Likelihood in 1000 bootstrap resulted on separated clade of ljem and Embun from all references (Figure 2). It is supported the morphological determination that two samples are not belongs to references species (Aggarwal *et al.*, 2007). It is also supported a previous research which reported that ljem belongs to *Trachypithecus auratus mauritius* while Embun is belongs to *Trachypithecus auratus auratus* based on Cyt-b sequence (Sakti, 2011).

Genetic distance between ljem and Embun is 11.4% (Table 2). It is clarify that ljem and Embun belongs to since genetic distance higher than 1,9% (13) indicated the interspecies relationship between/among samples.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. ljem																		
2. Embun	0.114																	
3. T. cristatus	0.172	0.208																
4. T. francoisi x poliocephalus	0.220	0.225	0.056															
5. T. francoisi	0.215	0.220	0.052	0.004														
6. T. poliocephalus H1	0.210	0.221	0.056	0.008	0.004													
7. T. laotum	0.221	0.226	0.057	0.008	0.004	0.008												
8. T. poliocephalus H4	0.215	0.220	0.052	0.012	0.008	0.012	0.012											
9. T. poliocephalus H5	0.214	0.219	0.052	0.015	0.012	0.015	0.016	0.004										
10. T. poliocephalus H6	0.221	0.226	0.057	0.008	0.004	0.008	0.008	0.004	0.008									
11. T. poliocephalus H2	0.215	0.220	0.057	0.008	0.004	0.008	0.008	0.012	0.016	0.008								
12. T. poliocephalus H3	0.209	0.226	0.052	0.012	0.008	0.012	0.012	0.016	0.019	0.012	0.004							
13. T. poliocephalus H7	0.215	0.220	0.052	0.004	0.000	0.004	0.004	0.008	0.012	0.004	0.004	0.008						
14. T. poliocephalus H8	0.209	0.214	0.048	0.008	0.004	0.008	0.008	0.012	0.016	0.008	0.008	0.012	0.004					
15. T. poliocephalus H9	0.177	0.202	0.024	0.065	0.061	0.065	0.065	0.061	0.061	0.065	0.057	0.052	0.061	0.057				
16. T. poliocephalus H10	0.177	0.191	0.044	0.052	0.048	0.052	0.052	0.048	0.048	0.052	0.044	0.048	0.048	0.044	0.036			
17. T. poliocephalus H11	0.188	0.202	0.031	0.082	0.078	0.082	0.082	0.078	0.078	0.082	0.082	0.078	0.078	0.073	0.056	0.069		
18. T. poliocephalus	0.209	0.214	0.048	0.008	0.004	0.008	0.008	0.012	0.016	0.008	0.008	0.012	0.004	0.008	0.057	0.044	0.073	

Table 2. Table of Genetic Distance from Samples Used

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