

IDENTIFICATION OF JAVAN LANGUR (*Trachypithecus auratus*) IN JAVAN LANGUR CENTER (JLC) COBAN TALUN, BATU BASED ON *Cytochrome-b* SEQUENCE

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ABSTRACT

Javan Langur (*Trachypithecus auratus*) is an endemic langur in Indonesia which classified as vulnerable primate. Some researches divided Javan Langur species into some subspecies, yet was not described properly. *Cytochrome-b* sequence from mitochondrial DNA is able to determine samples between and intra-species as well. This study aimed to identify the Javan Langur (*Trachypithecus auratus*) samples named Andin (@&) and Bobby (B&), both are rehabilitated in Javan Langur Center (JLC). The amplification of *Cytochrome-b* gene from whole blood DNA with PCR technique using forward primer L151625'-CTTCCATGAGGACAAATATC-3' and reverse primer Rmuc15'-GTGGAGTATAGGTATGATTGC-3'. The phylogenetic tree reconstruction based on MEGA 5 using maximum likelihood (ML) method and pairwise distance analysis with Kimura-2 parameter model resulted that Andin and Bobby are in the same group with *T. a. auratus*. Andin is in the same clade with *T. a. auratus haplotype aaF*, while Bobby in the same clade *T. a. auratus* and *T. a. auratus haplotype aaC*. We concluded that both of them belong to *T. a. auratus* subspecies.

Key words: Javan Langur (*Trachypithecus auratus*), Javan Langur Center (JLC), *Cytochrome-b*

INTRODUCTION

Javan Langur (*Trachypithecus auratus*) is an Indonesian endemic Langur, lives as group of 6 to 30 (13; 10). Groves (1985) divides *T. auratus* into two subspecies, they are *T. auratus auratus* and *T. auratus mauritius*. *T. a. auratus* subspecies living in East Java, Sempu Island, Bali, and Lombok, whereas *T. auratus mauritius* subspecies is limited in West Java and Banten. Both display difference morphological characters.

Javan Langur (*T. auratus*) are listed as vulnerable on the IUCN Red List. Populations are decreasing due to human activities, such as habitat loss, hunting, and the illegal pet trade. NGO confiscate number of Javan Langur (*T. auratus*) as their effort for fighting the illegal trading and domesticating of Javan Langur (*T. auratus*). These Javan Langur (*T. auratus*) then being rehabilitated before release to its origin habitat. One of the rehabilitation center is *Javan Langur Center* (JLC), an ex-situ institution which administratively is part of *Javan Primate Project–The Aspinall Foundation* (TAF) *Indonesia Program* (10). This institution located in Coban Talun Batu of East Java, focus on rehab of eastern Javan Langur. So far *Javan Langur Center* (JLC) only recording morphometric data from each individual of Javan Langur (*T. auratus*) besides data of confiscation area. The release of rehabilitated Javan Langur (*T. auratus*) requires a certified data of the origin. Since behavior and morphology characters could not give enough answer to fully resolve their evolutionary relationships or devise a stable phylogeny-based taxonomy (8). The phylogenetic data becomes

important tool to identify each Langur.

Phylogenetic analysis can be performed using Barcoding DNA, one of it is a mitochondrial *Cytochrome-b* (16). *Cyt-b* has been found as a strong indicator to indentify species (14). *Cyt-b* has been proven to be more accurate in solving problem about the interaction of mammal phylogeny and it can differ in species level, between species and intra-species, as well (5; 6; 4; 15; 12).

MATERIALS AND METHODS

Material including blood sample of 2 individu Javan Langur (*T. auratus*) named Andin (♀) and Bobby (♂) morphologically belongs to *T. a. auratus*. The isolation of whole DNA using *Roche Isolation Mini Kit* modified. Total DNA yield was measured using *NanoDrop Spechtrphotometer* (ND-2000).

Cyt-b was amplified using *Forward* primer L151625'CTTCCATGAGGACAAATATC3' and *Reverse* primer Rmuc15'GTGGAGTATAGGTATGATTGC3' (Muslih, 2011). Sequencing was done at *Eijkman Institute for Molecular Biology*, Jakarta. *Peak Trace* software was used to optimize the chromatogram. *DNA Baser* was used to get consensus sequence of *Cyt-b*. The consensus sequence then confirmed using BLAST program. The phylogenetic tree reconstruction based on MEGA 5 in Maximum Likelihood (ML) method and pairwise distance analysis using Kimura-2 parameter model.

RESULT AND DISCUSSION

PCR amplification of *Cyt-b* from Andin and Bobby resulted consensus sequences of 488 bp and 503 bp, respectively. BLAST analysis showed that the position of *Cyt-b* fragments of Andin and Bobby spend bases 1 to 450 and 1 to 500, respectively, compared to *Cyt-b* sequences of reference species. Andin and Bobby's query value are 100% which indicates that the fragments obtained from both samples are *Cyt-b*. Alignment result using *Clustal X* software showed that *Cyt-b* sequences from both samples have a high similarity with the references from NCBI, there are *T. a. auratus*, *T. a. auratus haplotype AAC*, and *T. a. auratus haplotype AAF*. Based on the analysis of phylogenetic trees, it is known that Andin and Bobby sharing the same group with *T. a. auratus* and separated from *T. a. mauritius*, *T. germani*, *T. cristatus*, and *T. johnii* (out-group) (Figure 1). Andin is in the same clade with *T. a. auratus haplotype AAF*, while Bobby in the same clade with *T. a. auratus* and *T. a. auratus haplotype AAC*. The pairwise distance of the two samples show very low values 0.5% (<2%) which indicates that the samples included in one species (intra-species) (Figure 2). Pairwise distance values between Andin, Bobby, and *T. a. auratus* group are 0% to 1.7% (less than 2%), indicated that both samples have a very close relationship with *T. a. auratus* (Vun et al., 2011). If each individual has a genetic distance (pairwise distance) based on *Cyt-b* sequence variation less than 2%, it indicates intra-species (Bradley and Baker, 2001). We concluded that both of them belong to *T. a. auratus* subspecies. This is supported by the morphological features of the two samples: Andin and Bobby have a silvery black hair colour, wide facial features, and not too clear angle facial curve (Brandon-Jones, 1995; Brandon-Jones, 2004; Kurniawan, 2012a).

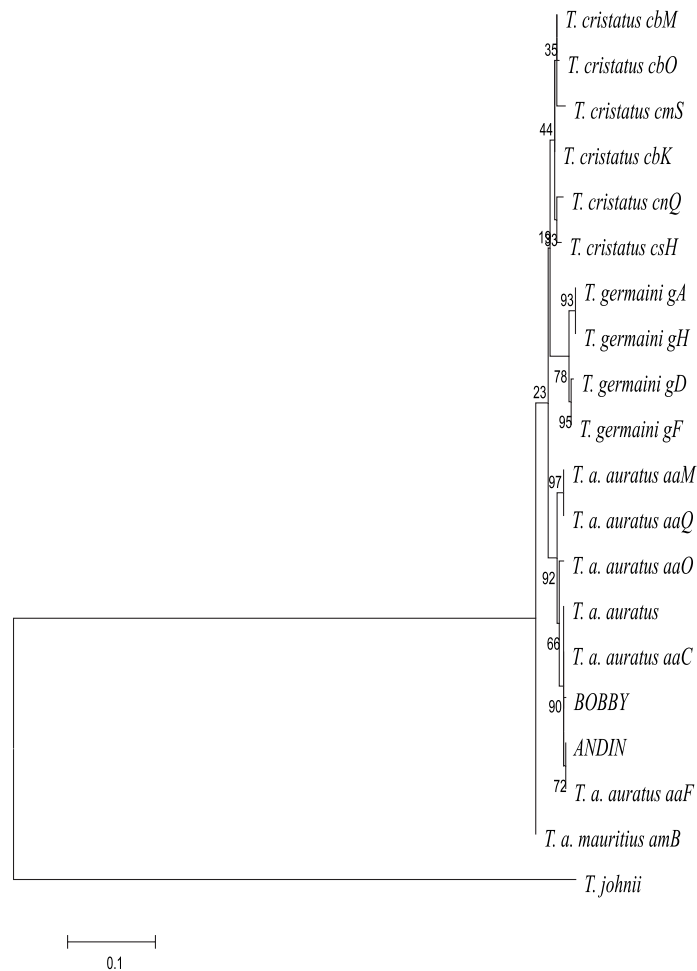


Figure 1. *Cyt-b* Sequence Analysis in Andin, Bobby, and Other Group of *Trachypithecus*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. T. germaini gA																				
2. T. germaini gH	0.000																			
3. T. germaini gD	0.012	0.012																		
4. T. germaini gF	0.010	0.010	0.002																	
5. ANDIN	0.048	0.048	0.050	0.048																
6. T. a. auratus aaf	0.048	0.048	0.050	0.048	0.000															
7. BOBBY	0.048	0.048	0.050	0.048	0.005	0.005														
8. T. a. auratus	0.045	0.045	0.047	0.045	0.002	0.002	0.002													
9. T. a. auratus aaC	0.045	0.045	0.047	0.045	0.002	0.002	0.002	0.000												
10. T. a. auratus aaO	0.040	0.040	0.042	0.040	0.012	0.012	0.012	0.010	0.010											
11. T. a. auratus aaM	0.045	0.045	0.045	0.045	0.017	0.017	0.017	0.015	0.015	0.015										
12. T. a. auratus aaQ	0.045	0.045	0.045	0.045	0.017	0.017	0.017	0.015	0.015	0.015	0.000									
13. T. cristatus cnQ	0.040	0.040	0.042	0.040	0.037	0.037	0.037	0.035	0.035	0.035	0.035	0.035								
14. T. cristatus csH	0.037	0.037	0.040	0.037	0.035	0.035	0.035	0.032	0.032	0.032	0.032	0.032	0.012							
15. T. cristatus cmS	0.037	0.037	0.040	0.037	0.040	0.040	0.040	0.037	0.037	0.032	0.037	0.037	0.017	0.015						
16. T. cristatus cbM	0.032	0.032	0.029	0.027	0.029	0.029	0.029	0.027	0.027	0.022	0.027	0.027	0.012	0.010	0.010					
17. T. cristatus cbO	0.035	0.035	0.032	0.030	0.032	0.032	0.032	0.029	0.029	0.025	0.030	0.030	0.015	0.012	0.012	0.002				
18. T. cristatus cbK	0.035	0.035	0.032	0.030	0.027	0.027	0.027	0.024	0.024	0.025	0.025	0.025	0.010	0.007	0.012	0.002	0.005			
19. T. a. mauritius amB	0.037	0.037	0.040	0.037	0.035	0.035	0.035	0.032	0.032	0.032	0.032	0.032	0.027	0.025	0.029	0.025	0.027	0.022		
20. T. johnii	1.015	1.015	1.049	1.040	1.024	1.024	1.037	1.037	1.037	1.040	1.040	1.040	1.003	1.003	1.037	1.028	1.040	1.015	1.003	

Figure 2. Pairwise Distance in Andin, Bobby, and Other Group of *Trachypithecus*

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