InJAST
Volume 5, Number 2, October 2024
Pages: 118-121

P - ISSN: 2722 - 0133
E - ISSN: 2722 - 0141
DOI: 10.33751/injast.v5i2.11039

Genetic variation based on RAPD (Random Amplified Polymorphic DNA) markers in western tarsiers (*Cephalopachus bancanus*) from South Sumatra and Bangka Island

PUTRI RIZKI PRATIWI, MUHARNI, ARUM SETIAWAN, LAILA HANUM, INDRA YUSTIAN*

Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya QJJW+GFV, Indralaya Indah, Kec. Indralaya, Kabupaten Ogan Ilir, Sumatera Selatan 30862, Indonesia

*Corresponding author: *idr_yustian@unsri.ac.id*

Submitted 5 December 2024; Accepted 30 December 2024

ABSTRACT

The western tarsier (*Cephalopachus bancanus*) is a nocturnal primate classified as Vulnerable by the IUCN. Understanding the genetic variation of tarsier populations in South Sumatra and Bangka Island is crucial for developing effective conservation strategies to protect this unique species. The research aims to determine the genetic variations of tarsiers from South Sumatra and Bangka Island by using 10 primers of the Random Amplified Polymorphic DNA (RAPD) molecular marker, namely ILO 525, ILO 1212, ILO 1204, OPE 16, OPE 17, OPE 19, OPF 06, OPF 04, OPY 03 and OPY 13. The RAPD primers utilized in this study are universal and effectively detect genetic diversity at interspecies and intraspecies levels. These primers have demonstrated the ability to produce clear and distinct electrophoresis band patterns in primates and other mammals. A total of six tissue samples were collected from Bangka Island, specifically from Petaling Village, Mendo Barat, and two additional samples were obtained from South Sumatra, originating from Selangit, Musi Rawas, and Padang Bindu, Ogan Komering Ulu. The Bangka Island samples were derived from a single population inhabiting a secondary forest characterized by shrubs and old rubber plantations. In contrast, the South Sumatra samples were obtained from secondary forest environments. Eight of the ten RAPD primers successfully amplified 89 DNA fragments, exhibiting a high degree of polymorphism. The genetic distance analysis, based on Dice coefficient values ranging from 0.000 to 0.629, revealed varying levels of genetic divergence among the samples. The bootstrap analysis further demonstrated that the relationships among all Western Tarsier (*Cephalopachus bancanus*) samples had a confidence level exceeding 50%. The observed high polymorphism reflects substantial genetic variability among the samples. RAPD markers thus offer a valuable tool for studies focusing on the relationships within closely related populations.

ABSTRAK

Tarsius (*Cephalopachus bancanus*) merupakan primata nokturnal yang berstatus Rentan (Vulnerable) pada IUCN. Informasi mengenai keragaman genetik tarsius asal Sumatera Selatan dan Pulau Bangka sangat diperlukan untuk menerapkan strategi konservasi yang tepat dalam upaya perlindungan primata nokturnal yang menarik ini. Penelitian bertujuan untuk mengetahui keragaman genetik tarsius asal Sumatera Selatan dan Pulau Bangka dengan menggunakan 10 primer penanda molekuler Random Amplified Polymorphic DNA (RAPD) yaitu ILO 525, ILO 1212, ILO 1204, OPE 16, OPE 17, OPE 19, OPF 06, OPF 04, OPY 03 dan OPY 13. Primer RAPD yang digunakan merupakan primer universal yang dapat mengidentifikasi keragaman baik pada tingkat interspesies maupun intraspesies, serta terbukti memiliki visualisasi bentuk pita yang jelas dari hasil elektroforesis pada primata dan mamalia lainnya. Enam sampel jaringan dikumpulkan dari Pulau Bangka (Desa Petaling, Mendo Barat) dan dua sampel dari Sumatera Selatan (dari Selangit, Musi Rawas dan Padang Bindu, Ogan Komering Ulu). Sampel dari Bangka berasal dari populasi yang sama pada tipe habitat yang sama, yaitu hutan sekunder yang berisi semak belukar dan tanaman karet tua. Sementara sampel dari Sumatera Selatan berasal dari hutan sekunder. Delapan dari sepuluh primer RAPD yang digunakan menghasilkan amplifikasi DNA sebanyak 89 fragmen DNA dengan persentase polimorfisme yang tinggi. Hasil analisis nilai jarak genetik dengan nilai koefisien Dice sebesar 0,000 - 0,629 menunjukkan bahwa terdapat jarak yang berbeda pada setiap sampel. Berdasarkan analisis bootstrap, diketahui bahwa hubungan antar semua sampel tarsius (*Cephalopachus bancanus*) memiliki tingkat kepercayaan lebih dari 50%. Polimorfisme yang tinggi menunjukkan variasi genetik yang tinggi antar sampel. Marka RAPD mungkin lebih tepat untuk diaplikasikan dimana hubungan antara populasi yang terkait erat menjadi perhatian.

Keywords: *Cephalopachus bancanus, genetic variation, RAPD, tarsier*

INTRODUCTION

Indonesia ranks among the countries with the greatest primate species richness, hosting 60 of the approximately 250 known species worldwide (Supriatna, 2019; Priatna et al., 2023). Remarkably, at least 60% of these species are endemic to the country (Supriatna & Ramadhan, 2016).

Tarsier (*Cephalopachus bancanus*) is a small primate classified as an arboreal nocturnal carnivorous animals. They consume insects, live in trees, and are active at night. Tarsiers are categorized into three genera, distributed across distinct geographical regions: the genus *Cephalopachus* is found in Kalimantan, southern Sumatra, Bangka Belitung Islands, and the Natuna Islands; the genus *Tarsius* is native to Sulawesi and its surrounding islands; and the genus *Carlito* is located in the southern Philippines and nearby islands (Groves & Shekelle, 2010). Regarding conservation status, *Cephalopachus bancanus* is nationally protected under Government Regulation No. 7 of 1999 in Indonesia. Globally, the International Union for the Conservation of Nature (IUCN) classified its conservation status as "Vulnerable" in 2008 (Shekelle & Yustian et al., 2020).

Molecular genetic analysis enables the identification of genetic variations among individuals within a population. Tarsiers (*Cephalopachus bancanus*) inhabiting the islands of Bangka and South Sumatra are believed to share a common lineage, yet they may exhibit genetic differences driven by adaptations to distinct geographical environments. A key method for assessing such genetic variation involves using genetic or molecular markers (Yuliana & Enung, 2018).

The RAPD technique was selected due to its ability to detect high levels of polymorphism and generate high-quality genetic markers. This method requires only a small quantity of DNA, does not necessitate prior sequence information about the sample, and can reveal specific bands unique to the presence of genetic variation. (Annisa et al., 2021).

The permit for conducting the genetic access study was based on the decree of the Director General of Natural Resources and Ecosystem Conservation (Decree No.SK.83/KSDAE/SET.3/KSA.2/3/2022) concerning permission to access the genetic resources of wild animals. A prior study by Widayanti et al. (2014) investigated genetic variation in tarsiers using the RAPD-PCR method with two samples, *Cephalopachus bancanus bancanus* and *Cephalopachus bancanus borneanus*. However, the sample size in their study was limited, and of the ten RAPD primers tested, only four demonstrated polymorphism.

METHODS

Genetic Materials

This study analyzed genetic variations in Tarsiers (*Cephalopachus bancanus*) using genetic material obtained from eight individuals. Six individuals originated from Petaling and Kemuja villages in Bangka, one from Batu Gane village in South Sumatra, and one preserved specimen from Padang Bindu village in South Sumatra. Samples included non-destructively collected body materials such as blood, hair, tissue, and feces. All samples were stored in a cool box or insulated bag to ensure preservation for subsequent DNA extraction in the laboratory. All samples were put into a cool box or insulated bag for DNA extraction in the laboratory. DNA isolation from blood, hair, and tissue samples was carried out using the QIAmp Fast DNA Stool minikit, while the Zymo Quick DNA miniprep plus fecal/soil kit was used for faecal samples.

PCR amplification

The amplification process was performed using the Random Amplification of Polymorphic DNA (RAPD) method. Prior to amplification, all necessary materials were prepared and combined into a master mix. In this study, DNA amplification was conducted using the T100 Thermal Cycler with a set of 10 RAPD primers.

RAPD data analysis

The DNA bands visualized through PCR-RAPD are converted into binary data, where the presence of a band is assigned a value of 1, and its absence is assigned a value of 0. This binary data is then input into Microsoft Excel for organization. Once structured, the data is transferred to an NTedit table and analyzed using NTSYS software version 2.10. A dendrogram is generated employing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and the Jaccard Coefficient of Similarity. The Dice coefficient is utilized to calculate the similarity matrix or index. To assess the confidence level of the UPGMA-based dendrogram, bootstrap analysis is performed using the FreeTree program with 1,000 iterations.

RESULTS AND DISCUSSION

The successful amplification and visualization of DNA samples (Figure 1) indicate that the primers used are complementary to the nucleotide sequences of the Tarsier (*Cephalopachus bancanus*) genome. Conversely, the failure to amplify and visualize certain DNA samples suggests that the primer sequences were not complementary to the nucleotide sequences of the Tarsier genome (Williams et al., 1990). Zein (2013) highlighted that the visualization of monomorphic or polymorphic OPE 19 and OPF 04 bands generated through amplification is not consistently clear. This variability arises based on the abundance of DNA fragment copies. When the number of copies is high, the corresponding bands appear more fluorescent, thicker, and distinctly visible. Conversely, a lower number of DNA fragment copies results in bands that are less fluorescent, thinner, and less distinct.

The genetic variation is indicated by the percentage of polymorphisms presented in Table 1. Despite two primers failing to amplify any Tarsius DNA samples, the overall polymorphism percentage achieved was 80%,

reflecting a high degree of polymorphism. This aligns with the criteria established by Fajarudin et al. (2010), who defined a high level of polymorphism as having a percentage exceeding 50%. Furthermore, Welsh et al. (1996) highlighted that genetic variation is represented by the percentage of polymorphic bands or the proportion of polymorphic loci relative to the total loci identified.

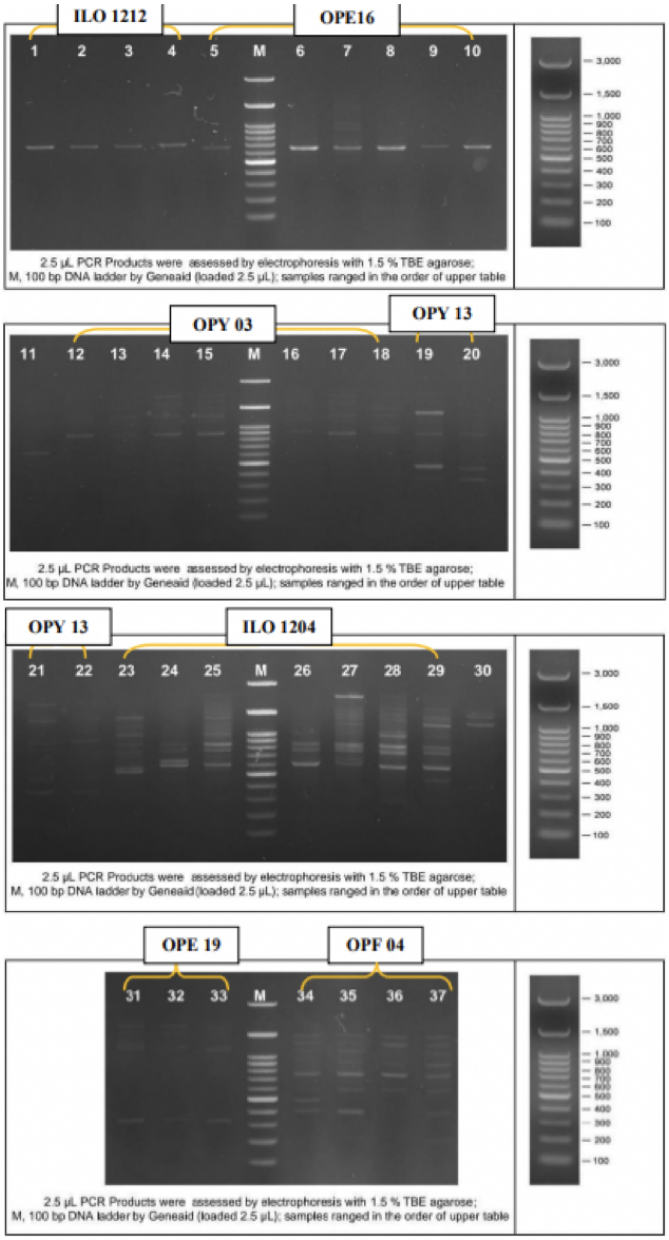

Figure 1. Result of PCR-RAPD.

The samples exhibiting the highest similarity index are Sample #3 and Sample #4, with a similarity index of 0.629 (Table 2). Conversely, the lowest similarity index is observed between Sample #5 and Sample #8, with a value of 0.000. A similarity index score approaching 1 or exceeding 0.5 indicates greater genetic similarity between species. In contrast, a score nearing 0 or below 0.5 reflects increasing genetic divergence among species (Wijayanto et al., 2013).

Table 1. Percentage of polymorphism.

No	Primer	N of Monomorphic Bands	N of Polymorphic Bands	N of Amplified Bands	Percentage
1	ILO 1212	0	2	2	100%
2	OPE 16	0	7	7	100%
3	OPY 03	0	11	11	100%
4	OPY 13	0	8	8	100%
5	ILO 1204	0	17	17	100%
6	OPE 19	0	9	9	100%
7	OPE 17	0	0	0	0%
8	OPF 06	0	0	0	0%
9	OPF 04	0	21	21	100%
10	ILO 525	0	14	14	100%

Table 2. Similarity index.

	Tarsius 1	Tarsius 2	Tarsius 3	Tarsius 4	Tarsius 5	Tarsius 6	Tarsius 7	Tarsius 8
Tarsius 1								
Tarsius 2	0.302							
Tarsius 3	0.245	0.439						
Tarsius 4	0.200	0.486	0.629					
Tarsius 5	0.140	0.196	0.154	0.205				
Tarsius 6	0.295	0.196	0.200	0.205	0.474			
Tarsius 7	0.193	0.138	0.109	0.123	0.314	0.396		
Tarsius 8	0.032	0.034	0.029	0.037	0.000	0.033	0.050	

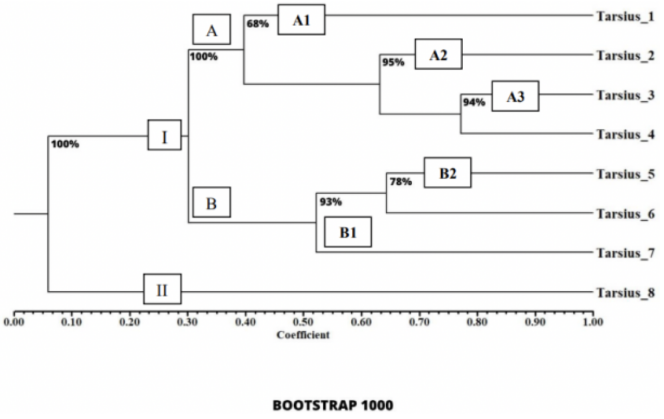

Figure 2. Dendogram relationship of the samples.

The relationship between Tarsiers (*Cephalopachus bancanus*) from South Sumatra and Bangka Island is depicted in Figure 2. The dendrogram indicates that cluster analysis separates the eight samples into two major groups. The first group is further divided into two sub-groups, designated as A and B. Sub-group A comprises Tarsiers #1, #2, #3, and #4, while sub-group B includes Tarsiers #5, #6, and #7. The second group contains only a single sample, identified as Tarsier #8.

The bootstrap analysis revealed a high range of values, spanning from 68% to 100%. The lowest bootstrap value, 68%, was observed at branch/node A1, which links sample #1 with the other samples. Conversely, the highest values, reaching 100%, were recorded at the branches/nodes associated with Group I, Group II, Group A, and Group B. According to Reddy et al. (2009), bootstrap values closer to the maximum of 100% indicate higher statistical confidence in the clustering. A cluster is generally considered statistically robust if its bootstrap value exceeds 50%.

CONCLUSION

The findings of this study reveal that the genetic variation of the tarsier (*Cephalopachus bancanus*) populations from South Sumatra and Bangka Island, analyzed using Random Amplified Polymorphic DNA (RAPD) molecular markers, is characterized by a 100% polymorphic band percentage. Cluster analysis indicates that the tarsiers from these regions form two primary clusters. Within the ingroup, individuals labeled Tarsier #1 through Tarsier #7 occupy distinct branches within the dendrogram, whereas Tarsier #8 is positioned in the outgroup. Among the analyzed samples, the closest genetic relationship is observed between sample #3 and sample #4, while the most genetically distant sample is Tarsier #8.

REFERENCES

Annisa, N., Akyuni, Q., Putri, F.R., Kanaya, O.N., Fauziah, N., Wahyuni, Y., Satria, R., & Achyar, A. (2021). Analisis variasi genetik Semut Ratu Pheidole sp. menggunakan teknik RAPD PCR. *Prosiding SEMNAS BIO 2021, 1(2): 1070-1076*. https://doi.org/10.24036/prosemnasbio/vol1/272

Fajarudin, A., & Poerba, Y.S. (2010). Penampilan random amplified polymorphic DNA pada *Azadirachta indica* A . Juss dari Taman Nasional Baluran. *J. Tek. Ling 11(1): 61–69*.

Fan H., & Gulley M.L. (2000). *Molecular Pathology Protocols: Methods in Molecular Medicine*. Totowa, NJ: Humana Press Inc.

Glasel J.A. (1994). Validity of nucleic acid purities monitored by 260 Nm/280 Nm absorbance ratio. *BioTechniques. 18(1): 62-62*.

Groves, C., & Shekelle, M. (2010). Genera and spesies of Tarsiidae. *International Journal of Primatology, 31(6): 1071- 1082*.

Priatna, D., Hidayat, A., Istiadi, Y., Nurwati, A., Yanuar, A., & Susilo, A. (2023). Daily behaviour and home range of adult rehabilitant Javan slow loris (*Nycticebus javanicus* É. Geoffroy, 1812) in Gunung Sawal Nature Reserve, Ciamis, West Java. *Indonesian Journal of Applied Environmental Studies, 4(1): 46-54*. DOI: 10.33751/injast.v4i1.3107

Reddy, C.S., Babu, A.P., Swamy, B.P.M., Kaladhar, K., & Sarla, N. (2009). ISSR markers based on GA and AG repeats reveal a genetic relationship among rice varieties tolerant to drought, flood, or salinity. *J. Zhejiang Univ. Sci. B., 10(2):133-141*.

Shekelle, M., &Yustian, I. (2020). *Cephalopachus bancanus* The IUCN Red List of Threatened Species 2020: e.T39764A1799 2286. https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS. T39764A17992286.en. Accessed on 21 July 2022.

Supriatna, J. (2019). *Field Guide to the Indonesia Primates*. Jakarta: Yayasan Pustaka Obor Indonesia.

Supriatna, J., & Ramadhan, R. (2016). *Pariwisata Primata Indonesia*. Jakarta: Yayasan Pustaka Obor Indonesia.

Thieman, W.J., and Michael, A.P. (2013). *Introduction to Biotechnology*. New York, USA: Pearson Publisher.

Welsh, J., David, R., & Michael, M. (1996). *DNA and RNA Fingerprinting Using Arbitrarily Primer PCR. In PCR Strategies*. New York: Academic Press: p. 247- 276.

Widayanti, R., Susmiati, T., Pramarta, A., Hardian, A.B., Budiariati, V., Istiqomah., dan Khesara, S.P.N. (2014). Kajian Keragaman Genetik *Tarsius bancanus bancanus* dan *Tarsius bamcanus borneanus* dengan Teknik PCR-RAPD. *Prosiding Seminar Nasional: Peran Rumah Sakit Hewan dalam Penanggulangan Penyakit Zoonosis. 105-117*.

Wijayanto T., Dirvamena., & La, E. (2013). Hubungan Kekerabatan Aksesi Pisang Kepok (*Musa paradisiaca formatypica*) di Kabupaten Muna berdasarkan karakter Morfologi dan Penanda RAPD. *Jurnal Agrotekno, 3(3): 163-170*.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalki, J. A., & Tinge, S. V. (1990). DNA Polymorphism Amplified by Arbitrary Primers is Useful as Genetic Markers. *Nucleic Acid Res., 18(22):6531–6535*. doi: 10.1093/nar/18.22.6531 186531-186535.

Yuliana G.D.A., & Eunung, S. M. (2018). Evaluasi keragaman genetik sembilan varietas rambutan (*Nephelium lappaceum*) dengan Marka RAPD. *Biopropal Industri, 9(1): 01-08*.

Zein, M. S. A., & Prawiradilaga, D. M. (2013). *DNA Barcode Fauna Indonesia*. Jakarta: Prenada Media.