BIOVALENTIA: BIOLOGICAL RESEARCH JOURNAL
A Sriwijaya Journal For International Biologist
E-ISSN : 2477-1392
Published Since November 2015
by Biology Department, Faculty of Mathematics and Natural Sciences
Sriwijaya University

VOL 5, NO 2 (2019)

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E-ISSN: 2477-1392

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Nomor: 10/E/KPT/2019
Tentang Hasil Akreditasi Jurnal Ilmiah Periode 2 Tahun 2019

BIOVALENTIA: Biological Research Journal

E-ISSN: 24771392

Penerbit: Biology Department, Faculty of Mathematics and Natural Sciences, Sriwijaya University

Ditetapkan sebagai Jurnal Ilmiah

TERAKREDITASI PERINGKAT 3

Akreditasi berlaku selama 5 (lima) tahun, yaitu
Volume 4 Nomor 2 Tahun 2018 sampai Volume 9 Nomor 1 Tahun 2023

Jakarta, 4 April 2019
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TERAKREDITASI

BIOVALENTIA: BIOLOGICAL RESEARCH JOURNAL
e-ISSN: 2477-1392
Vol. 5 No. 2, November 2019

DNA Extraction of Sumatran Striped Rabbit from Tissue Samples

Rio Firman Saputra[1], Arum Setiawan [2*], Indra Yustian[3], Enggar Patriono[4]
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Peer review under responsibility of Biology Department Sriwijaya University

Abstract *:*

The Sumatran Striped Rabbit (Nesolagus netscheri) is likely a naturally rare, which is a protected animal based on Government Regulation Number 7 of 1999 and renewal of Permen LHK No. P.106 which are categorized as Data Deficient by the IUCN (International Union of Conservation of Nature) since 2019. Samples were obtained from Pagar Alam, South Sumatra. Most commonly found at elevations above 600 m in montane and sub-montane primary habitat. Molecular genetic characterization of sumatran striped rabbit is to know genetic information and genetic identification of N. netscheri. Primers 12S rRNA were used in this study to characterize Sumatran striped rabbit. A 1 band were detected ranged from 900 to 1000 bp. Molecular markers represent reliable tools which may have a great impact in rabbit breeding and genetic improvement of rabbits. Molecular markers on Nesolagus netscheri is expected to tools in the identification of Sumatran striped rabbits from South Sumatra.

Keywords: Nesolagus netscheri, South Sumatra, Sumatran Striped Rabbit, PCR.

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1. Introduction

Sumatran striped rabbit (*Nesolagus netscheri*) is the endemic species of Sumatran [18] [6]. *Nesolagus netscheri* is found at an altitude between 600-1,600 m [3]. The observations have been reported since 1972, and most recently is on February 2017 in the area of Gunung Raya Wildlife Reserve [14]. Record from Gunung Leuser National Parks [3], Bukit Barisan Selatan National Parks [1] [7]. This species will be increasingly threatened if its natural habitat decreases due. This is interesting because information about *N. netscheri* very instrumental in conservation efforts [9].

Classification of the status of the Sumatran Striped Rabbit has been difficult. In 1994 the species was assessed as Endangered, in 1996 it was assessed as Critically Endangered, and in 2008 it was assessed as Vulnerable. These changes in status back and forth are reflective of the complete lack of information on the ecology of the species that has persisted over that time. Between 1996 when the species was assessed as Critically Endangered, and 2008 when the species was assessed as Vulnerable, there were only a handful of additional records of the species, all consisting of simple reports of occurrence [8].

Nesolagus netscheri is included in the list of protected animals based on Government Regulation No. 7 of 1999, Regulation of the Minister of Environment and Forestry Number P.106 of 2018 and includes animals in a Data Deficient status based on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species [8].

12S rRNA marker is a very good genetic marker for the study of kinship relationships between mammals [11]. Several studies have been carried out with genetic markers of 12S rRNA, such as in Lagomorpha [4] and in several other mammals [7] [16] [11], in addition in mammals, genetic markers of 12S rRNA have also been carried out on Insecta [12] [5]. Analysis of 12S rRNA gene sequences can be used for species determination and authentication of meat samples [13] and species identification [10] [19]

The Sumatran rabbits and Annamite rabbits are morphologically similar, but genetic data indicate that they have been isolated for millions of years. Assuming a steady

rate of divergence over time at this gene, the Sumatran and Annamite rabbits would have been diverging genetically for approximately 8 million years [15]. Molecular markers on *Nesolagus netscheri* is expected to help in the identification of Sumatran striped rabbits from South Sumatra.

2. Materials and Methods

2.1 Tissue Collection

The research sample is tissue (muscle) *N. netscheri* from South Sumatra, preserved with absolute ethanol. Samples were obtained from the local community, dead on February 23, 2018. The location of the *N. netscheri* sample was from the village of Rimba Candi, Pagar Alam, South Sumatra (Figure 1.), *N. netscheri's* specimens were stored in the Museum of Biology, Faculty of Biology, Gajah Mada University with the catalog number Musbio/Mam/Deposit/Coll. 1.281112018.

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The stages of DNA isolation are based on the procedure for using the Wizard® Genomic DNA purification Kit from Promega. A total of 20 mg of *N. netscheri* tissue was put into a 1.5 mL tube and 275 µL of Digestion Solution Master Mix was added to each tube.

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Next the Wizard®SV Minicolumn was transferred into a new sterile 1.5 mL tube and added with 250 µL Nuclease-Free Water which was previously incubated at 600 C for 30 minutes. After that, the solution was incubated at room temperature for 2 minutes and centrifuged at a speed of 13,000 rpm for 2 minutes. The results obtained in the form of DNA in a 1.5 mL tube that has been isolated, store it in the freezer at a temperature of -20° C to -70° C.

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Total DNA from isolation is used as printed DNA for the amplification process. Primers for amplifying 12S rRNA genes are 12SR and 12SL [17]. Primary attachment location for amplifying 12s rRNA genes (Table 2).

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The composition in a 25 µL PCR reagent mixture consists of:

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Primer (F)	: 1 µL
Primer (R)	: 1 µL
DNA Tamplate	: 2 µL
Nuklease Free Water	: 8,5 µL
Total	: 25 µL

DNA amplification by PCR in this study used the Bio-Rad T100 Thermal Cycler. Amplification of the 12S rRNA gene was carried out by the following procedure (Table 3):

Table 3. PCR Procedure for *Nesolagus netscheri* 12S rRNA gene amplification

Reaction	Temperature	Time	Cycle
Pre-denaturation	94°C	3 minute	1 Cycle
Denaturation	94°C	1 minute	35 Cycle
Annealing	59,9°C	1 minute	
Elongation	72°C	1,5 minute	
Post elongation	72°C	10 minute	1 Cycle

2.5 Analysis of Amplification Results using Electrophoresis

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As much as 40 grams of agarose was weighed, then put into a 300 ml erlenmeyer. Next, 200 ml of TAE 1x solution was added and dissolved by heating it using a Hot plate. After the agarose solution is not too hot, 5 µL of gel staining is added, and homogenized, then the agarose solution is poured into the mold provided. The comb is installed to make a a well in the gel, then allowed to stand

for 15 minutes so that the gel solids. After the gel solids the comb is removed and a well is formed in the gel. The gel is completely immersed in a TAE 1x solution on the electrophorator and is ready for use for electrophoresis.

2.5.1 PCR Product Electrophoresis

DNA samples from PCR, loading dye and markers were prepared. The agarose that has been made is inserted into the electrophorator. Then with a micropipette, as much as 2 µL of DNA sample and 1 µL of loading dye are mixed first on the parafilm paper and put in the 2nd well until the last well. The 1st well was filled with 1 µL marker. The electrofarator is closed, then the voltage is set at an 80 Volt voltage for 45 minutes, migration direction from pole - to +, and the ON button is pressed on the machine. After DNA migration is complete, the machine is turned off. Gel electrophoresis results were then observed and photographed using gel documentation.

3. Results and Discussion

Usage of biotechnology in rabbit's significantly to the development and enforcement of genetic improvement programs [2]. The study suggests that PCR can be successfully utilized for detecting molecular genetic markers for rabbit's such as sumatran rabbit. These markers (fingerprints) providing an easy and rapid tools for characterization, identification and sustainable use in breeding programs. Molecular markers were used in the present study to obtain fingerprints for Sumatran striped rabbit.

Figure 1. *Nesolagus netscheri* from South Sumatra.

12S rRNA primers were used in this study to characterize *N. netscheri*. Sample 1 DNA amount was too low to be detected, there is also multiple band observed, detected band ranging from 900 to 1000 bp, samples 2 and 3 did not have a band (Figure 2). The results of present studies can provide basic molecules information for future researchers. These results indicated efficiency of PCR techniques in the characterization of rabbit genotypes. Molecular markers on *N. netscheri* is expected to help in the identification.

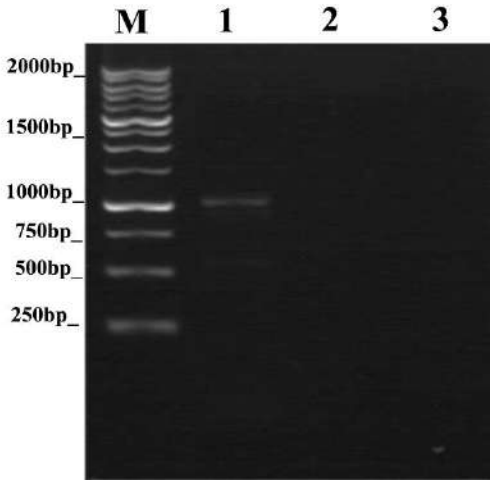

Figure 2. Fingerprint of *Nesolagus Netscheri* using 12S rRNA primers.

4. Conclusion

The use of methods for the identification and characterization of genotypes is essential for rabbit protection. This study supplies comprehensive approaches for studying the genetically molecular characterization of Sumatran striped rabbits which can help the genetic development of rabbits. PCR techniques are effective methods for detecting DNA markers in Sumatran striped rabbits. These markers are useful for estimating genetic distances and relationships among other rabbits.

5. Acknowledgement

We express our gratitude to the Sriwijaya University for any supports to this research. We also thank Muhammad Iqbal, Guntur Pragustiandi, Pormansyah, Amran Halim, Winda Indriati, Ina Aprillia all of field team member and colleagues who individually assisted in the research.

References

[1] Dinets, V. 2010. Observation of Sumatran Striped Rabbit (*Nesolagus netscheri*) in the Wild. *Mammalia*. 74, 1.

[2] El-Sabrout K., El-Seedy A., Shebl M. K., Soliman F. N. K., Azza El-Sebai, 2014 Molecular markers and productive performance relations for line V and Alexandria rabbits under Egyptian environmental conditions. *Int J Life Sci Biotechnol Pharma Res* 3(1):345-361.

[3] Flux, J. E. C. 1990. The Sumatran striped rabbit. in Rabbits, Hares and Pikas: Status Survey and

Conservation Action Plan (eds J.A. Chapman & J.E.C. Flux), Halaman. 137–139. IUCN, Gland, Switzerland.

[4] Ge, D., Yao, L., Xia, L., Zhang, L., dan Yang, Q. 2015. Geometric morphometric analysis of skull morphology reveals loss of phylogenetic signal at the generic level in extant lagomorphs (Mammalia: Lagomorpha). *Contributions to Zoology*, 84 (4) 267-284.

[5] Gillespie, J. J., Johnston, J. S., Cannone§, J. J., dan Gutell§, R. R. 2006. Characteristics of the nuclear (18S, 5.8S, 28S and 5S) and mitochondrial (12S and 16S) rRNA genes of (Insecta: Hymenoptera): structure, organization, and retrotransposable elements *Apis mellifera*. *Insect Molecular Biology* (2006) 15 (5), 657–686.

[6] Hoffmann, R.S. dan Smith, T.A. 2005. Lagomorpha: Leporidae. In: (D.E. Wilson and D.M. Reeder, eds.). Wilson, D.E and Reeder, D. M. (editors) (2005). Mammal Species of the World-A Taxonomic and Geographic Reference (Third ed.). Johns Hopkins University, Baltimore. Halaman. 185-211.

[7] McCarthy, J. L., Fuller, T. K., McCarthy, K. P., Wibisono, H. T., dan Livolsi, M. C. 2012. Using camera trap photos and direct sightings to identify possible refugia for the Vulnerable Sumatran striped rabbit *Nesolagus netscheri*. *Oryx* 46(3): 438–441.

[8] McCarthy, J., Holden, J., Martyr, D. & McCarthy, K. 2019. *Nesolagus netscheri*. The IUCN Red List of Threatened Species 2019: e.T14662A45178557. http://dx.doi.org/10.2305/IUCN.UK.2019-2.RL TS.T14662A45178557. en. Downloaded on 2 September 2019.

[9] Meijaard, E. dan Sugardjito, J. 2008. *Nesolagus netscheri*. Di IUCN Red List of Threatened. Http://www.iucnredlist.org [diakses tanggal 12 September 2017].

[10] Melton, T., dan Holland, C. 2007. Routine Forensic Use of the Mitochondrial 12S Ribosomal RNA Gene for Species Identification. *J Forensic Sci,* 52 (6).

[11] Olson, L. E., Sargis, E. J., dan Martin, R. D. 2005. Intraordinal phylogenetics of treeshrews (Mammalia: Scandentia) based on evidence from the mitochondrial 12S rRNA gene. *Molecular Phylogenetics and Evolution*, 35 (2005) 656–673.

[12] Page, R. D. M., Cruickshank R., dan Johnson†, K. P. 2002. Louse (Insecta: Phthiraptera) mitochondrial 12S rRNA secondary structure is highly variable. *Insect Molecular Biology* (2002) 11(4), 361–369.

[13] Rastogi, G., Dharne, M., Bharde, A., Pandav, V. S., Ghumatkar, S. V., Krishnamurthy, R., Patole, M. S., dan Shouche, S.Y. 2004. Species determination and authentication of meat samples by mitochondrial 12S rRNA gene sequence analysis and conformation sensitive gel electrophoresis. *Current Science*, 87 (9).

[14] Setiawan, A., Iqbal, M., Komarudin, Saputra, F. S., Setiawan, D., dan Yustian, I. 2018. New reports of the presence and ecology of the Sumatran Striped Rabbit (*Nesolagus netscheri*) in South Sumatra. *Mammalia,* 82(6): 589-591. Retrieved 16 Nov. 2018, doi:10.1515/mammalia-2017-0064.

[15] Surridge, A. K., Timmins, R. J., Hewitt, G. M. dan Bell, D. J. 1999. Striped rabbits in Southeast Asia. *Nature* 400**:** 726.

[16] Tougard, C., Delfosse, T., Hanni, C., dan Montgelard, C .2001. Phylogenetic Relationships of the Five Extant Rhinoceros Species (Rhinocerotidae, Perissodactyla) Based on Mitochondrial Cytochrome b and 12S rRNA Genes. *Molecular Phylogenetics and Evolution*. Vol. 19, No. 1. Halaman 34–44.

[17] Wang, H. Y., Tsai, M. P., Tu, M. C., dan Lee, S. C. 2000. Universal Primers for Amplification of the Complete Mitochondrial 12S rRNA Gene in Vertebrates. *Zoological Studies,* 39(1).Halaman 61-66.

[18] Wilson, D. E., dan Reeder, D. M. 2005. *Mammal species of the world : a taxonomic and geographic reference.* The Johns Hopkins University Press. United States of America.

[19] Yang, L., Tan,Z., Wang, D., Xue, L., Guan, M., Huang, T., dan Li, R. 2014. Species identification through mitochondrial rRNA genetic analysis. *Scientific Reports*, 4 : 4089.

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By Arum Setiawan

WORD COUNT 2415
TIME SUBMITTED 27-JAN-2020 09:05AM
PAPER ID 54164178

BIOVALENTIA: BIOLOGICAL RESEARCH JOURNAL
e-ISSN: 2477-1392
Vol. 5 No. 2, November 2019

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Figure 1. *Nesolagus netscheri* from South Sumatra.

12S rRNA primers were used in this study to characterize *N. netscheri*. Sample 1 DNA amount was too low to be detected, there is also multiple band observed, detected band ranging from 900 to 1000 bp, samples 2 and 3 did not have a band (Figure 2). The results of present studies can provide basic molecules information for future researchers. These results indicated efficiency of PCR techniques in the characterization of rabbit genotypes. Molecular markers on *N. netscheri* is expected to help in the identification.

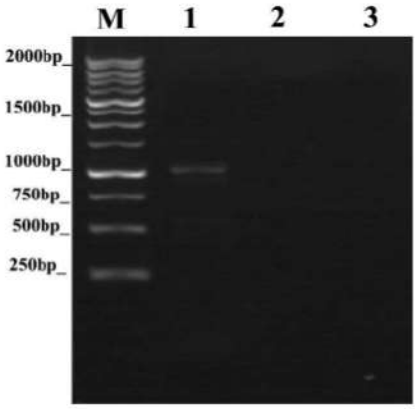

Figure 2. Fingerprint of *Nesolagus Netscheri* using 12S rRNA primers.

4. Conclusion

The use of methods for the identification and characterization of genotypes is essential for rabbit protection. This study supplies comprehensive approaches for studying the genetically molecular characterization of Sumatran striped rabbits which can help the genetic development of rabbits. PCR techniques are effective methods for detecting DNA markers in Sumatran striped rabbits. These markers are useful for estimating genetic distances and relationships among other rabbits.

5. Acknowledgement

We express our gratitude to the Sriwijaya University for any supports to this research. We also thank Muhammad Iqbal, Guntur Pragustiandi, Pormansyah, Amran Halim, Winda Indriati, Ina Aprillia all of field team member and colleagues who individually assisted in the research.

References

[1] Dinets, V. 2010. Observation of Sumatran Striped Rabbit (*Nesolagus netscheri*) in the Wild. *Mammalia*. 74, 1.

[2] El-Sabrout K., El-Seedy A., Shebl M. K., Soliman F. N. K., Azza El-Sebai, 2014 Molecular markers and productive performance relations for line V and Alexandria rabbits under Egyptian environmental conditions. *Int J Life Sci Biotechnol Pharma Res* 3(1):345-361.

[3] Flux, J. E. C. 1990. The Sumatran striped rabbit. in Rabbits, Hares and Pikas: Status Survey and

Conservation Action Plan (eds J.A. Chapman & J.E.C. Flux), Halaman. 137–139. IUCN, Gland, Switzerland.

[4] Ge, D., Yao, L., Xia, L., Zhang, L., dan Yang, Q. 2015. Geometric morphometric analysis of skull morphology reveals loss of phylogenetic signal at the generic level in extant lagomorphs (Mammalia: Lagomorpha). *Contributions to Zoology*, 84 (4) 267-284.

[5] Gillespie, J. J., Johnston, J. S., Cannone§, J. J., dan Gutell§, R. R. 2006. Characteristics of the nuclear (18S, 5.8S, 28S and 5S) and mitochondrial (12S and 16S) rRNA genes of (Insecta: Hymenoptera): structure, organization, and retrotransposable elements *Apis mellifera*. *Insect Molecular Biology* (2006) 15 (5), 657–686.

[6] Hoffmann, R.S. dan Smith, T.A. 2005. Lagomorpha: Leporidae. In: (D.E. Wilson and D.M. Reeder, eds.). Wilson, D.E and Reeder, D. M. (editors) (2005). Mammal Species of the World-A Taxonomic and Geographic Reference (Third ed.). Johns Hopkins University, Baltimore. Halaman. 185-211.

[7] McCarthy, J. L., Fuller, T. K., McCarthy, K. P., Wibisono, H. T., dan Livolsi, M. C. 2012. Using camera trap photos and direct sightings to identify possible refugia for the Vulnerable Sumatran striped rabbit *Nesolagus netscheri*. *Oryx* 46(3): 438–441.

[8] McCarthy, J., Holden, J., Martyr, D. & McCarthy, K. 2019. *Nesolagus netscheri*. The IUCN Red List of Threatened Species 2019: e.T14662A45178557. http://dx.doi.org/10.2305/IUCN.UK.2019-2.RL TS.T14662A45178557. en. Downloaded on 2 September 2019.

[9] Meijaard, E. dan Sugardjito, J. 2008. *Nesolagus netscheri*. Di IUCN Red List of Threatened. Http://www.iucnredlist.org [diakses tanggal 12 September 2017].

[10] Melton, T., dan Holland, C. 2007. Routine Forensic Use of the Mitochondrial 12S Ribosomal RNA Gene for Species Identification. *J Forensic Sci,* 52 (6).

[11] Olson, L. E., Sargis, E. J., dan Martin, R. D. 2005. Intraordinal phylogenetics of treeshrews (Mammalia: Scandentia) based on evidence from the mitochondrial 12S rRNA gene. *Molecular Phylogenetics and Evolution*, 35 (2005) 656–673.

[12] Page, R. D. M., Cruickshank R., dan Johnson†, K. P. 2002. Louse (Insecta: Phthiraptera) mitochondrial 12S rRNA secondary structure is highly variable. *Insect Molecular Biology* (2002) 11(4), 361–369.

[13] Rastogi, G., Dharne, M., Bharde, A., Pandav, V. S., Ghumatkar, S. V., Krishnamurthy, R., Patole, M. S., dan Shouche, S.Y. 2004. Species determination and authentication of meat samples by mitochondrial 12S rRNA gene sequence analysis and conformation sensitive gel electrophoresis. *Current Science*, 87 (9).

[14] Setiawan, A., Iqbal, M., Komarudin, Saputra, F. S., Setiawan, D., dan Yustian, I. 2018. New reports of the presence and ecology of the Sumatran Striped Rabbit (*Nesolagus netscheri*) in South Sumatra. *Mammalia,* 82(6): 589-591. Retrieved 16 Nov. 2018, doi:10.1515/mammalia-2017-0064.

[15] Surridge, A. K., Timmins, R. J., Hewitt, G. M. dan Bell, D. J. 1999. Striped rabbits in Southeast Asia. *Nature* 400**:** 726.

[16] Tougard, C., Delfosse, T., Hanni, C., dan Montgelard, C .2001. Phylogenetic Relationships of the Five Extant Rhinoceros Species (Rhinocerotidae, Perissodactyla) Based on Mitochondrial Cytochrome b and 12S rRNA Genes. *Molecular Phylogenetics and Evolution*. Vol. 19, No. 1. Halaman 34–44.

[17] Wang, H. Y., Tsai, M. P., Tu, M. C., dan Lee, S. C. 2000. Universal Primers for Amplification of the Complete Mitochondrial 12S rRNA Gene in Vertebrates. *Zoological Studies,* 39(1).Halaman 61-66.

[18] Wilson, D. E., dan Reeder, D. M. 2005. *Mammal species of the world : a taxonomic and geographic reference*. The Johns Hopkins University Press. United States of America.

[19] Yang, L., Tan,Z., Wang, D., Xue, L., Guan, M., Huang, T., dan Li, R. 2014. Species identification through mitochondrial rRNA genetic analysis. *Scientific Reports*, 4 : 4089.

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