

# Characteristics of the Genetic Variation of a Swamp Buffalo (*Bubalus bubalis*) of South Sumatra Based on Polymerase Chain Reaction-Random Amplified Polymorphic DNA (PCR-RAPD)

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**Abstract.** Swamp buffalo (*Bubalus bubalis*) is one of the endemic species that become a wealth of genetic resources of South Sumatra. This study aims to the genetic variation and relationships of kinship 6 variants of swamp buffalo South Sumatra. The methods used by the molecular approach using RAPD-PCR primer 5 i.e. ILO 1204, ILO 1212, ILO 525, OPW 03 and OPY 13. Data was analyzed using SPSS ver 16.0 and presented in dendrogram. The results of the amplification, all primary produce band with a total of 63 band of DNA (14.92%) with an average of every primary produce 12.6 band of DNA. The most primary produce DNA polymorphic bands namely OPW 03 (23.81%) and ILO 1204 (20.63%), while the primary ILO 525 (0.00%) do not generate polymorphic bands. Genetic variation of swamp buffalo has a low genetic variation with 14.92% percentage it generated polymorphic bands. The results of the dendrogram obtained two clusters namely cluster 1 included Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang and Kerbau Tanduk Dungkul, while the cluster 2 of them Kerbau Bule and Kerbau Rebah Belakang. Swamp buffalo variants that have the closest genetic distance. Kerbau Tanduk Langit and Kerbau Tanduk Bulat with 856 coefficient similarity, while the farthest Kerbau Tanduk Langit and Kerbau Bule with the coefficient similarity -972. Swamp buffalo (*Bubalus bubalis*) of South Sumatera, which consists of 6 variants of buffalo have low genetic variation and inbreeding of closekinship.

## 1 Introduction

The population of swamp buffalo Pampangan South Sumatera adapts to monotonous swamp areas that are not cultivated. Swamp buffalo Pampangan is a source of germplasm was endemic to South Sumatera with low productivity and limited distribution which is one of the genetic wealth of South Sumatera with Oganllir, Ogan Komering Ilir and Banyuasin district [1]. The important role of buffaloes are very strategic in certain regions in

Indonesia. Statistical data population in 2000 until 2008 shows buffalo livestock populations did not increase and even tended to decline by 8.85% with an average rate of decline of 1.03% per year. The influence of inbreeding on a large livestock on livestock productivity. Inbreeding depression or inbreeding pressure on buffalo can usually cause a decrease in the performance of livestock (growth), high mortality and low reproducibility. One of the economic impacts of the high level of inbreeding is inbreeding depression where there is a decrease in phenotypic averages, especially in traits that have high economic value. The development of biotechnology at this point give a positive impact against buffalo breeding and preservation in Indonesia are decrease each year. In line with the development of the technology of DNA levels, then weakness identification with morphological markers can be supported by a molecular approach. The use of molecular markers in the form of DNA is used along with the development of the science of molecular biology at this time[2].

Polymerase Chain Reaction (PCR) is a technique in molecular biology to multiply the amount of DNA in-vitro fertilization using the enzyme DNA polymerase and temperature [3]. The use of RAPD (Random Amplified Polymorphic DNA) is DNA analysis techniques are becoming very popular today. It is based on at the level of DNA [4]. The technique were generally faster, cheaper than any other method for detecting DNA sequence variation and does not require prior sequence information. The fact that RAPD's survey multiple loci in the genome makes the method attractive for analysis of genetic distance and phylogeny reconstruction [5]. Diversity or genetic variation can be used as a starting point for improving the quality and quantity of swamp buffalo. Thus, information about the genetic crossbreeding and kinship in cattle including swamp buffalo is crucial in the preservation of germplasm [6]. Genetic diversity became the important information in evaluating the genetic potential for the development, utilization, and conservation of species[7].

## 2 Materials and Methods

Blood sampling place the swamp Buffalo (*Bubalus bubalis*) in the area of TanjungSenai, Ogan Ilir Distrik, South Sumatera. Separation of serum is performed in the integrated laboratory Postgraduate Sriwijaya University. The research of DNA isolation, quantification of DNA and PCR-RAPD performed in the laboratory of Biochemistry at the Faculty of biology of the Gajah Mada University.



**Fig. 1.** Sampling location

A total of 300  $\mu$ l blood from 6 variants buffalo (Kerbau Bule, Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang, Kerbau Tanduk Dungkul, Kerbau Rebah Belakang) from 1 location were used in this study, with the number of samples for each variant were 2 individuals.

## 2.1 Procedures of Quantity and Quality Test Results DNA Isolation

Before being used in the PCR reaction, DNA quantification is done to determine the purity and concentration of DNA. Results of a DNA sample isolation, diluted as much as 10 in 0.2 ml tube with TE buffer, inserted into the as much as 1  $\mu$ l sample DNA and added 9  $\mu$ l of TE buffer. Then at homogeneous with spin down and spectrophotometer set at wavelength of 260 nm, and washing kuvet with aquadest sterile then conducted measurements of blanko solution first. According to [8], quantitative DNA test with spectrophotometry pure DNA can absorb ultraviolet light because of the existence of bases purin and pyrimidine. So the purity of DNA can be quantified by calculating the values of absorbance  $\lambda$  260 nm absorbance value divided by 280  $\lambda$  ( $\frac{A_{260}}{A_{280}}$ ) and the value of DNA purity ranges between 1.8-2.0.

## 2.2 Electrophoresis

The results of the DNA isolation obtained then in gel electrophoresis by using agarose 1.5%, by dissolving 1.5 gram agarose with 100 ml of TBE 1x, and put in the microvawe until dissolved. After a rather cold in the add 2  $\mu$ l EtBr. Then prepared parafilm, as much as 2  $\mu$ l DNA loading buffer (loading dye), then inserted 8  $\mu$ l sample and mixed with a loading buffer. Then, put in marker, DNA ladder 100 bp into wells. Electrode is then connected to a power supply with a voltage of 100 Volt, ampere 400 speed for 30 minutes. Then the agarose gel soaked in dye gel. Visualization is performed using the UV-transimulator to see the results of electrophoresis. Then photographed as documentation [8].

## 2.3 PCR-RAPD

Observation 6 variants molecular done with phase DNA templates preparation, DNA amplification, gel electrophoresis, gel electrophoresis quantification results, and data analysis. Before being used in the process of PCR-RAPD primer beforehand suspended using sterile aquabidest. Added with each primer 2x Master Mix PCR with 12,5  $\mu$ L, Primer RAPD (ILO- 1204, ILO-1212, ILO-525, OPW-03 and 2  $\mu$ L OPY-13, 10,5  $\mu$ L dH<sub>2</sub>O, totalvolume of 25  $\mu$ L to each microtube). The sample is then placed into the machine performed as many as 30 cycles of PCR with pre-denaturation at 95°C for 5 minute, denaturation at 95°C for 1 min, annealing at 55°C for 45 minutes, extention or elongation at 72°C for 1 minute, and final extention at 72°C for 10 minutes.

## 2.4 Visualization of Results Amplification

Visualization results of gel electrophoresis was conducted by using UV-Transilluminator and the results photographed as documentation.

## 2.5 Data Analysis

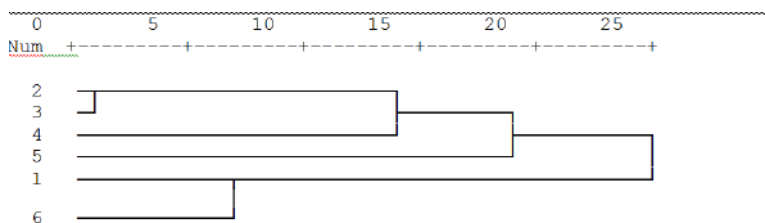
Data obtained from RAPD method in the form of a band or bands of amplification product was binary data represented by 'No (1) or no (0)'. DNA bands that have been subsequently measured distance migration. Determining the size of the amplification of DNA bands was done by measuring the migration distance of DNA standard (1 kb ladder)

ranging from pitting to place the DNA migration. DNA fragment size standards then created the algorithm, and the log is used as the Y axis while the migration distance of the DNA used as the X axis measurable standards After the X and Y values are known then made linear line equation  $Y = ax + b$  through linear regression line equation. The size of the DNA bands are amplified searched by entering the measured values in the equation of the line. Furthermore, the value of Y obtained and will be used as antilog long known bases are amplified by a primer. After lengthy bases are known and obtained binary data is expressed with 'No (1) or no (0)' [9], the data is then analyzed its genetic variation with the program SPSS (Statistical Package for the Social Sciences) ver 16.0 and presented with a form of cluster analysis, a dendrogram using the coefficients similaritas.

### 3 Results and Discussions

Based on the results amplification of the swamp Buffalo (*Bubalus bubalis*) South Sumatera based on PCR with RADP primary 5 (ILO 1204, ILO 1212, ILO 525, OPW 03 and OPY 13) using 6 variant of the Buffalo swamp. The resulting DNA bands is analyzed, then conducted by calculating the distance of migration of DNA. After that, if there is a DNA bands in the give a score of (1) and there is no DNA bands in the give the score (0). According with [10] that the DNA fragment pattern profiles of each primary based on give the score or not the fragment using the binary code. Based on the scores are then analyzed by using SPSS ver 16.0.

The results of the analysis kinship swamp buffalo South Sumatera by using SPSS 16.0 versi can be displayed in the form of a diagram called a dendrogram. According [11], that cluster formation process describes the dendrogram expressed in form of pictures. Horizontal lines above dendrogram indicates scale that describes the level of similarity, the smaller the scale value indicates the increasingly similar to that individual.



**Fig. 2.** Dendrogram of swamp buffalo (*Bubalus bubalis*) South Sumatra based on PCR- RAPD

Description : (1) Kerbau Bule, (2) Kerbau Tanduk Bulat, (3) Kerbau Tanduk Langit, (4) Kerbau Tanduk Melintang, (5) Kerbau Tanduk Dungkul, (6) Kerbau Rebah Belakang

The results of the analysis similarity matrix coefficients PCR-RAPD similarity between 6 variant of the swamp buffalo South Sumatera based on polymorphic DNA bands that 47 amplified similarity coefficients obtained with ranges of values -972 to 856. The value of the lowest similarity coefficients -972 are indicated with the variant number 3 (Kerbau Tanduk langit) with number 1 (Kerbau Bule) this indicates that due to the presence of the genetic distance is far enough, while the highest coefficients value i.e. 856 indicated by variant number 3 (Kerbau Tanduk Langit) and number 2 (Kerbau Tanduk Bulat), that is because the genetic distance is close enough.

The value of the coefficient of 1.000 (100%) indicates that the absence of genetic distance among the variants. It is in accordance with statement [12], that the value of the

coefficient similarity 100% indicate that it is not just the distance between genetic variants in one with the other variants. The smaller the distance its genetic then the higher its genetic similarities instead its genetic distance is so large it will lower its genetic difference also.

Based on the results of the analysis of the dendrogram can be seen that there are two clusters namely cluster 1 that included Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang and Kerbau Tanduk Dungkul, while the cluster 2 included Kerbau Bule and Kerbau Rebah Belakang. Swamp Buffalo variants that have the closest genetic distance is kerbau tanduk langit and kerbau tanduk bulat, while the farthest variants kerbau tanduk langit and kerbau bule. According [10] to the genetic distance is the degree of difference between the two populations and genes can be used in constructing phylogenetic.

It is supported by research [13], about the relationship of kinship based on morphological characteristics. Cluster 1 had the same character on the shape of the tip of the horn, the color of the nose, eye, eyebrows eyelashes, color necklace neck and color hair tail, while the cluster 2 has the same character on the color of the nose, ear, eye color, the color of the lashes, the color of the body and tail of the hair color.

According to [14], that the population had a close kinship This indicates the occurrence of inbreeding that can degrade the quality of genetic offspring, because it can increase homozygosity populations. The level of genetic similarity of a population can be described by the genetic distance of the individuals members of the population. The greater the genetic distance of individuals in a population, then the population has members who are increasingly diverse. Instead the smaller the genetic distance between individuals in a population, the more uniform the population.

Based on those results show that swamp buffalos South Sumatera have low genetic variation and genetic distance near or close relationship is suspected of inbreeding. It agreed with [7], which stated the inbreeding suspected of causing a decrease in the nature of phenotypes. Inbreeding among a variety of swamp Buffalo is certainly not only lowers quality but also genotip and phenotypes. According to [6], that inbreeding affects the productivity of the cattle. The low productivity of the herds of Buffalo are associated with genetic reduction of occurrence prediction.

This is coherent with the research conducted by kinship of swamp Buffalo had previously done by [15], that measures the of kinship based on morphology. The correlation coefficient is 0.57 shows more kinship between swamp Buffalo post enumerates the variance relatively close. Suspected cross between variants tend to be high. Advanced research by [7], about genetic characteristics based on protein profile showed an average value of results of all loci (H) 0.1286. Based on the average heterozygosity, swamp Buffalo (*Bubalus bubalis*) Pampangan have low genetic variation and genetic relationship of the nearest. Triwulaningsih (2005) in [16] states that traditional maintenance systems cause the quality of buffalo seedlings to decline and result in the development of a slow population. Indicators of the occurrence of inbreeding in buffalo livestock populations are characterized by symptoms of genetic disorders / defects such as downward curved horns, and the high incidence of albino.

[16] added that the influence of inbreeding on a large livestock on livestock productivity. Inbreeding depression or inbreeding pressure on buffalo can usually cause a decrease in the performance of livestock (growth), high mortality and low reproducibility. One of the economic impacts of the high level of inbreeding is inbreeding depression where there is a decrease in phenotypic averages, especially in traits that have high economic value.

A follow-up study on the relationship of kinship according to [15], about diversity and kinship swamp buffalo (*Bubalus bubalis*) Pampangan of South Sumatera based on

morphological characteristics. The result of the analysis of kinship based on morphological characteristics shows the correlation coefficient value of 0.85. Inbreeding and adaptation factor cause the difference in phenotypes and morphology. A high level of inbreeding can lead to inbreeding pressure which is characterized by a decrease in livestock production and reproduction performance [17] which results in a decrease in the profits of livestock businesses [18]. Some reports suspect the pressure of inbreeding in a population group that results in a decrease in productivity and a slow increase in the livestock population. According to [16] close relatives or inbreeding led to the emergence of recessive traits such as albino. The factors that led to the occurrence of inbreeding in this group of livestock due to closed populations, the inbreeding system was not directed, lack of knowledge about breeders and male limitations. Based on those results can be that they variants swamp buffalo Pampangan tend to be low due to inbreeding and adaptation to the environment that result in differences of phenotype.

## 4 Conclusion

Genetic variation of swamp buffalo (*Bubalus bubalis*) has a low variation with the percentage of polymorphic band 14.92 %. Swamp buffalo (*Bubalus bubalis*) South Sumatera there are two clusters namely cluster 1 that included Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang and Kerbau Tanduk Dungkul, while the cluster 2 included Kerbau Bule and Kerbau Rebah Belakang. Kerbau tanduk langit and kerbau tanduk bulat has a close kinship on coefficient of 856 while kerbau tanduk langit and kerbau bule have a kinship with the farthest distance coefficient-972. To study the characteristics of genetic diversity of swamp buffalo, it is necessary to use a different approach

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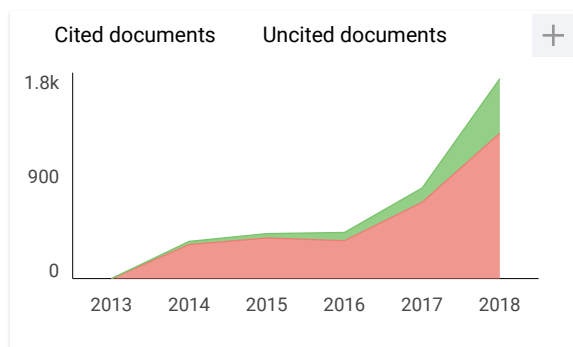
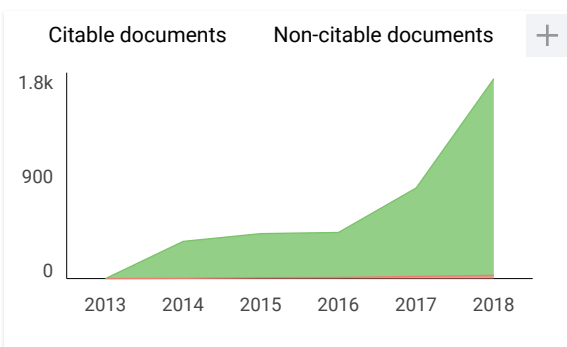
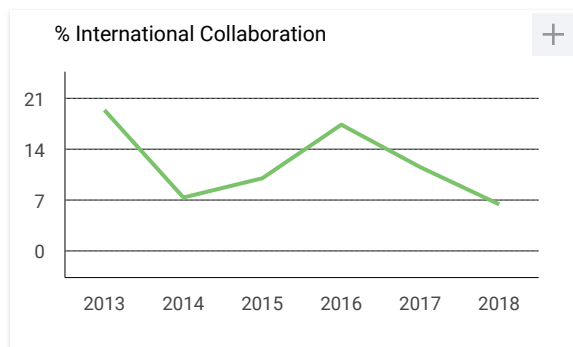
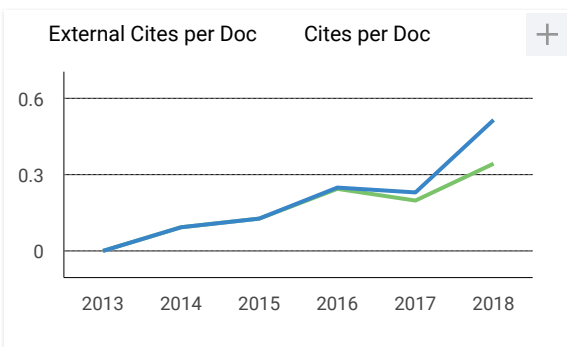
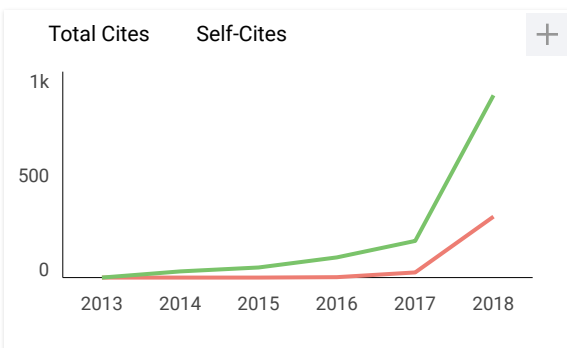
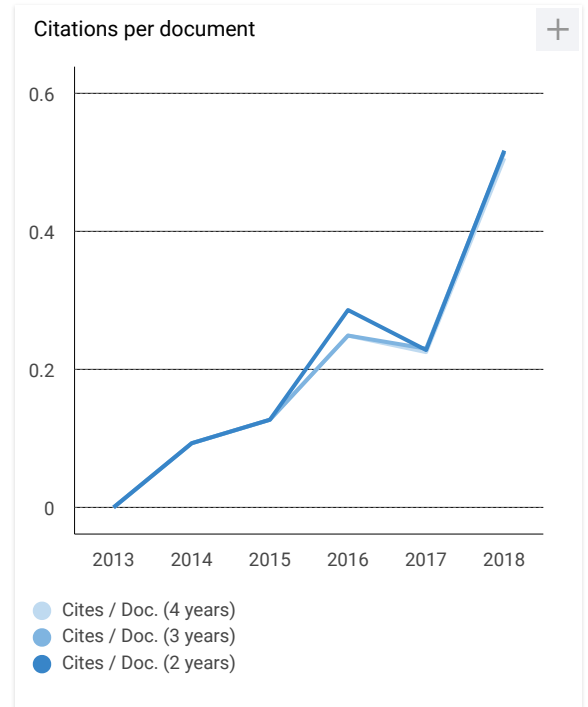
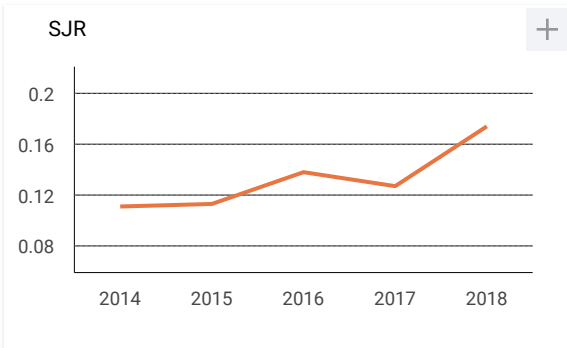


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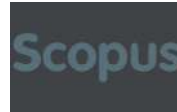
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## Preface

Environmental issues such as climate change or global warming is the greatest universal problem over the world. How we respond to this crisis will greatly impact both current and future generations.

The 1<sup>st</sup> Sriwijaya International Conference on Environmental Issues has been held to provide a vehicle the state of the art in research results and trends environmental and climate change topics, to offer interaction, discussion and possible collaboration among researchers in the future. This conference was held on September 26-27th, 2018 in Hotel Ultima Horison Palembang, South Sumatera, Indonesia hosted by Graduate School of Universitas Sriwijaya, Indonesia with co-organizer and Thai Nguyen University of Agriculture and Forestry Vietnam and Universiti Sultan Zainal Abidin Malaysia as Co-organizer. Participants of this conference was a combination of academics/researchers, development practitioners, community/civil society representatives, and national and local government units and non-government organizations.

This conference consists of 6 keynotes speakers from Germany, Malaysia, Vietnam and Indonesia, and 94 selected participants as oral presenters. For the selected articles will be published in Environment, Energy and Earth Sciences (E3S) Web of Conferences (Scopus and Thomson Reuters- indexed proceeding). Keynotes and participants presented topics related to following topics: 1) Promoting Environment System ; 2) Strengthening People-Environment Inter-Relationship; 3) Reducing Global Warming Effect; 4) Mitigation and Adaptation in Climate Change in Wetland. This conference was a great opportunity not only for sharing knowledge and experience in environmental research, but also for starting a long and fruitful cooperation and friendship among participants.



Hermansyah, PhD  
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[◀ Previous issue](#)

Table of Contents

[Next issue ▶](#)

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### The 1<sup>st</sup> Sriwijaya International Conference on Environmental Issues 2018 (1<sup>st</sup> SRICOENV 2018)

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[v Strengthening People-Environment Inter-Relationship](#)

[v Mitigation and Adaptation in Climate Change in Wetland](#)

## - Promoting Environment System

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### [Comparison of Blade Dimension Design of a Vertical Wind Turbine Applied in Low Wind Speed](#) 01001

Rizky Brillian Yuliandi, Rusdianasari and Tresna Dewi

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DOI: <https://doi.org/10.1051/e3sconf/20186801001>

[PDF \(1.760 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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### [Characteristics of the Genetic Variation of a Swamp Buffalo \(\*Bubalus bubalis\*\) of Sout. Sumatra Based on Polymerase Chain Reaction-Random Amplified Polymorphic DNA \(PCR-RAPD\)](#) 01002

Yuanita Windusari, Laila Hanum, Arum Setiawan and Veronika Larasati

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801002>

[PDF \(1.376 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

### [Promoting Industrial Symbiosis at Supply Chain](#) 01003

Yunita Ismail

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DOI: <https://doi.org/10.1051/e3sconf/20186801003>

[PDF \(1.327 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

### [Habitat Characterization of \*Mansoniaspp\* as Filariasis Vector in Banyuasin, South Sumatra, Indonesia](#) 01004

Rini Pratiwi, Chairil Anwar, Salni, Hermansyah, Novrikasari, Rachmat Hidayat and Ahmad Ghiffari

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801004>

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Mohammad Basyuni, Jayusman Jayusman and Rahmah Hayati

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[PDF \(1.359 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

[Bacterial Contamination of Food Handlers in X Hospital Palembang](#) 01006

Sri Utari, Irsan Saleh, Hermansyah and Rindit Pambayun

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[PDF \(1.503 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

[Family Burden of Narcotics Abusers Experiencing Relapse and Factors Exacerbating](#) 01007 OK

Rico Januar Sitorus, Novrikasari and Imelda G. Purba

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801007>

[PDF \(1.481 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

[Bioavailability of Silica on Paddy Soils with Various Land Aging in Musi Rawas South Sumatera of Indonesia](#) 01008

Jon. Bimasri, Dedi Budianta, Marsi and Umar Harun

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801008>

[PDF \(1.708 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

[The Quality of Life Chronic Renal Failure \(CRF\) Patients in Hemodialysis Unit at District General Hospital Pringsewu Regency Lampung Province in 2018](#) 01009

Virna Widora Saputri, Rico Januar Sitorus and H. M. Zulkarnain

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801009>

[PDF \(1.735 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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Herawati Idris, Tilla Safitri, Dian Safriantini and Inoy Trisnaini

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[PDF \(1.424 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

### [Understanding Abundance of Litter on Urban Lake in Developing Country: a Systems-thinking Approach](#) 01011

Muhammad Muhsin, Mahawan Karuniasa and Herr Soeryantono

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801011>

[PDF \(1.293 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

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### [The Philanthropy Culture in the Local Area: The Role Study of Philanthropy Institution after the Termination of PNPM in Boyolali Regency](#) 01012

Putut Suharso, Sarbini Sarbini and Dicky Sumarsono

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801012>

[PDF \(1.656 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

### [The Effectiveness of BCG \(\*Bacillus-Calmette Guerin\*\) Immunization to the Tuberculosis Incidence on Children at Banyuasin Regency](#) 01013

Ayu Febri Wulanda, Rico Januar Sitorus and Zulkarnain

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801013>

[PDF \(1.623 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

### [Optimization of Ensilage Total Mixed Fiber \(TMF\) with Additive and Incubation Periods Differences](#) 01014

A. Imsya, Yuanita Windusari and Riswandi

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801014>

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## Municipal Solid Waste Transport Operational Cost of Seberang Ulu Area, Palembang City 01015

Septi Rika Putri, Khalida Muda, Anis Saggaf and Dewi Astuti

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801015>

[PDF \(1.953 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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## Freshwater Molluscs as Bioindicator of Fe and Mn Contamination in Lematang River, South Sumatera, Indonesia 01016

Novin Teristiandi

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801016>

[PDF \(1.781 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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## The Characteristics and Potential of Lactic Acid Bacteria as Probiotics in Silage Made from *Hymenachne acutigluma* and *Neptunia oleracea* lour 01017

Sofia Sandi, Fitra Yosi, Meisji Liana Sari and Nuni Gofar

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801017>

[PDF \(1.490 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

## The Dominant Factors of Scabies Incidence in Two Islamic Boarding School Students, South Sumatera, Indonesia 01018

Yessi Arisandi, Chairil Anwar, Salni, Dadang Hikmah Purnama, Novrikasari and Ahmad Ghiffari

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DOI: <https://doi.org/10.1051/e3sconf/20186801018>

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Open Access

## The Effect of Degradation Time Variation on Porous Magnesium Implant Bone Scaffold 01019

Hasan Basri, Ardiansyah Syahrom, Amir Putra Md Saad, Adibah AR Rabiatal, Tri Satya Ramadhoni, Risky Utama Putra and Apreka Diansyah

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### [The Effect of Morphology on the Biodegradation Behavior of Porous Magnesium Bone Scaffold](#) 01020

Hasan Basri, Ardiansyah Syahrom, Amir Putra Md Saad, Adibah AR Rabiatal, Prakoso Akbar Teguh, Apreka Diansyah and Risky Utama Putra

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### [The Physical of Biscuit Complete Ration Based on Hymenacne Acutigluma Supplemented with Different Legumes](#) 01021

Riswandi, Basuni Hamzah, Agus Wijaya, Arfan Abrar, S Agus, Afnur Imsya, Fitra Yosi, Sofia Sandi and Eka Fitriani

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801021>

[PDF \(1.632 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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### [The Influence of Phytase Enzyme to Laying Performance and Quality of Egg Shell of Golden Arabian Chicken](#) 01022

Eli Sahara, Feni Despedia and Raden Ayu Aminah

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### [DNA Barcoding of Swamp Sediment Bacterial Isolates for Swamp Aquaculture Probiotic](#) 01023

Marini Wijayanti, Dade Jubaedah, Januar Ahlan Suhada, Siti Yuliani, Nabilah Saraswati, Tanbiyaskur, Mochamad Syaifudin and Hary Widjajanti

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Masayu Gemala Rabiah, Rini Mutahar and Rico Januar Sitorus

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[PDF \(1.526 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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### [Model of Demand Robust Counterpart Open Capacitated Vehicle Routing Problem \(DRC-OCVRP\) Simplification by Applying Preprocessing Techniques in Rubbish Controlling in Sematang Borang District, Palembang](#) 01025

Fitri Maya Puspita, Endro Setyo Cahyono, Siti Rahayu and Bauty Lisna Sintia

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801025>

[PDF \(1.981 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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### [Effect of Storage Duration and Butyric Acid Supplementation to Egg Quality of Laying Hens in The Third Phase of Production](#) 01026

Rizki Palupi, Fitri Novaliya Lubis and Demila Syukrima

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DOI: <https://doi.org/10.1051/e3sconf/20186801026>

[PDF \(1.506 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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### [Resilience Concept in Indonesian Small Town Development and Planning: a Case of Lasem, Central Java](#) 01027

Jawoto Sih Setyono, Wiwandari Handayani, Iwan Rudiarto and Landung Esariti

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801027>

[PDF \(1.611 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

### [Prevalence and Determinants Factors that Influence the Behaviour of People with Pediculosis Capitis in Orphanage](#) 01028

Ahmad Ghiffari, Anggun Nurul Fitria, Chairil Anwar and Mutiara Budi Azhar

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## Toxicity *Baginus trauingrensis*-based Bio Insecticide Enriched with Golden Snail Meat Flour Against Worker and Soldier Castes of *Coptotermes Curvignathus* (Isoptera: Termitidae) 01029

Yulia Pujiastuti

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186801029>

[PDF \(1.758 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

---

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## Study of Natural Dyes and Pineapple Leaf Fibres Growing Locations within Plant Stems on Dyeing Intensity. 01030

Amin Rejo, Rizky Tirta Adhiguna and Debora Geovanni Rajagukguk

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DOI: <https://doi.org/10.1051/e3sconf/20186801030>

[PDF \(1.423 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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## - *Strengthening People-Environment Inter-Relationship*

Open Access

## Surviving Strategies of Rural Livelihoods in South Sumatra Farming System, Indonesia 02001

Elisa Wildayana, Mustika Edi Armanto, Zulkifli Idrus, Iwan Adi Radiatmoko, Syuhada Adjiz Umar, Bella Syakina, Nursittah, Mubarika, Reszki Oktavia and Eka Sari

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## The Social Impact and Environmental Risks of Public Oil Mining in Musi Banyuasin Regency South Sumatera Province 02002

Mulyanto

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## Do Cultural Styles Predict Pro-Environment Behaviour among Slum-area Resident of Jakarta? 02003

Gumgum Gumelar, Ayuza Vania and Herdiyan Maulana

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## Naturalistic Intelligence and Environmental Awareness among Graduate Students

02004

Zarah Beby Ningrum, Tri Edhi Budhi Soesilo and Herdis Herdiansyah

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## Socio-Economic Strategy of Sustainability and Post-Mining Land Use in South Sumatra

02005

Rizkia Ayu Lestari, Mahawan Karuniasa, Tri Edhi Budhi Soesilo and Lana Saria

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## The Concept of Green Management in the Management of CSR: Implementation study of CSR Programs at Azana Hotel Group

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Dicky Sumarsono, Bani Sudardi, Wartyo Wartyo and Wakit Abdullah

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## Exploring Historical and Projection of Drought Periods in Cirebon Regency, Indonesia

02007

Nila Ardhyarini H. Pratiwi, Mahawan Karuniasa and Djoko Santoso Abi Suroso

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## Legal Aspects of PT. Gojek Indonesia in the Partnership Agreement Dealing with the Public Transport Standards

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### [Economic Valuation of Mangrove Forest at Taman Ayu Village, West Lombok Regency](#) 02009

Siti Dian Rosadi, Mufti Petala Patria and Nisyawati

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### [Developing Payment for Ecosystem Services Scheme on Pari Island Kepulauan Seribu](#) 02010

Nurul Hidayati, Mahawan Karuniasa, Mufti Petala Patria and M. Suparmoko

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Eni Murdiati, Sriati, Alfitri, Muhammad and Ridhah Taqwa

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---

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### [Analysis of Fire Control Hotel in Palembang City](#) 03001

Jimmy Tiarlina, Novrikasari and Rico Januar Sitorus

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### [Marketing Performance Evaluation of Purun Agroindustry as Peatland Friendly Commodities in South Sumatera](#) 03003

Dessy Adriani, Elisa Wildayana, Yulius, Nurilla E. Putri, Idham Alamsyah, Maryanah Hamzah, Maryadi and Melati Andarini

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DOI: <https://doi.org/10.1051/e3sconf/20186803003>

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### [Extraction of Uranium from Artificial Liquid Waste using Continuous Flow Emulsion Liquid Membrane Technique](#) 03004

Rusdianasari, Yohandri Bow, Tresna Dewi and Eka Sri Yusmartini

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DOI: <https://doi.org/10.1051/e3sconf/20186803004>

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### [Comparison Analysis of Anthocyanin Substances in various Plants for Testing Media of Formalin and Borax Content in Food](#) 03005

Neny Rochyani

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### [Pollutants of Fish Processing Industry and Assessment of its Waste Management by Wastewater Quality Standards](#) 03006

Setia Devi Kurniasih, Tri Edhi Budhi Soesilo and Roekmijati Soemantojo

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186803006>

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### [The New Renewable Energy Consumption Policy of Rare Earth Metals to Build Indonesia's National Energy Security](#) 03008

Muhamad Azhar, Solechan Solechan, Retno Saraswati, Putut Suharso, Suhartoyo Suhartoyo and Budi Ispriyarso

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DOI: <https://doi.org/10.1051/e3sconf/20186803008>

[PDF \(1.746 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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### [Building an Integrated Mining Licensing System in Order to Preserve the Environment in Indonesia](#) 03009

Muhamad Azhar, Putut Suharso, Budi Ispriyarso, Agus Purnomo, Suhartoyo Suhartoyo and Sukirno Sukirno

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DOI: <https://doi.org/10.1051/e3sconf/20186803009>

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### [Effects of Heat Treatment on the Color Change and Dimensional Stability of \*Gmelina arborea\* and \*Melia azedarach\* Woods](#) 03010

Wahyu Hidayat, Fauzi Febrianto, Byantara Darsan Purusatama and Nam Hun Kim

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DOI: <https://doi.org/10.1051/e3sconf/20186803010>

[PDF \(2.081 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

### [Control of Chromium Hexavalent \(Cr -VI\) Pollution on Waste Water in Nickel Ore Extraction Industry with Phytoremediation Technology](#) 03011

Erikha Maurizka Mayzarah, Setyo Sarwanto Moersidik and Lana Saria

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186803011>

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## [The Effects of Lead Contamination in Public Health Case: Pesarean Village, Tegal District, Indonesia](#) 03012

Indah Lestari, Tri Edhi Budhi Soesilo and Haruki Agustina

Published online: 27 November 2018

DOI: <https://doi.org/10.1051/e3sconf/20186803012>

[PDF \(1.482 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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## [Oil Spill Incidents and Their Impacts to Fisheries and Tourism Activities in Kepulauan Seribu](#) 03013

Mohammad Abdul Jabbar, Tri Edhi Budhi Soesilo and Udi Syahno Edi Hamzah

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DOI: <https://doi.org/10.1051/e3sconf/20186803013>

[PDF \(1.597 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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## [Optimisation of Phenol Removal from Palm Oil Mill Effluent \(POME\) Using Natural Bentonite](#) 03014

Muhammad Said, Tarmizi Taher, Addy Rachmat, Poedji Loekitowati Hariani and Aldes Lesbani

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DOI: <https://doi.org/10.1051/e3sconf/20186803014>

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## [Determinant Factors Effect Environmental Disclosure and Firm Value at Mining Companies listed Indonesia Stock Exchange](#) 03015

Luk Luk Fuadah, Kencana Dewi and Anton Arisman

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[PDF \(1.846 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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## [Unlicensed Mining as an Alternative Policy: Valuable Experiences in Southeast Sulawesi and East Java](#) 03016

Ahmad Sudiro, Ahmad Redi, Ade Adhari and Mardiana Rachman

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# Characteristics of the Genetic Variation of a Swamp Buffalo (*Bubalus bubalis*) of South Sumatra Based on Polymerase Chain Reaction- Random Amplified Polymorphic DNA (PCR- RAPD)

*By Arum Setiawan*

# Characteristics of the Genetic Variation of a Swamp Buffalo (*Bubalus bubalis*) of South Sumatra Based on Polymerase Chain Reaction-Random Amplified Polymorphic DNA (PCR-RAPD)

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**Abstract.** Swamp buffalo (*Bubalus bubalis*) is one of the endemic species that become a wealth of genetic resources of South Sumatra. This study aims to the genetic variation and relationships of kinship 6 variants of swamp buffalo South Sumatera. The methods used by the molecular approach using RAPD-PCR primer 5 i.e. ILO 1204, ILO 1212, ILO 525, OPW 03 and OPY 13. Data was analyzed using SPSS ver 16.0 and presented in dendrogram. The results of the amplification, all primary produce band with a total of 63 band of DNA (14.92%) with an average of every primary produce 12.6 band of DNA. The most primary produce DNA polymorphic bands namely OPW 03 (23.81%) and ILO 1204 (20.63%), while the primary ILO 525 (0.00%) do not generate polymorphic bands. Genetic variation of swamp buffalo has a low genetic variation with 14.92% percentage it generated polymorphic bands. The results of the dendrogram obtained two clusters namely cluster 1 included Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang and Kerbau Tanduk Dungkul, while the cluster 2 of them Kerbau Bule and Kerbau Rebah Belakang. Swamp buffalo variants that have the closest genetic distance. Kerbau Tanduk Langit and Kerbau Tanduk Bulat with 856 coefficient similarity, while the farthest Kerbau Tanduk Langit and Kerbau Bule with the coefficient similarity -972. Swamp buffalo (*Bubalus bubalis*) of South Sumatera, which consists of 6 variants of buffalo have low genetic variation and inbreeding of closekinship.

## 1 Introduction

The population of swamp buffalo Pampangan South Sumatera adapts to monotonous swamp areas that are not cultivated. Swamp buffalo Pampangan is a source of germplasm was endemic to South Sumatera with low productivity and limited distribution which is one of the genetic wealth of South Sumatera with Oganllir, Ogan Komering Ilir and Banyuasin district [1]. The important role of buffaloes are very strategic in certain regions in

Indonesia. Statistical data population in 2000 until 2008 shows buffalo livestock populations did not increase and even tended to decline by 8.85% with an average rate of decline of 1.03% per year. The influence of inbreeding on a large livestock on livestock productivity. Inbreeding depression or inbreeding pressure on buffalo can usually cause a decrease in the performance of livestock (growth), high mortality and low reproducibility. One of the economic impacts of the high level of inbreeding is inbreeding depression where there is a decrease in phenotypic averages, especially in traits that have high economic value. The development of biotechnology at this point give a positive impact against buffalo breeding and preservation in Indonesia are decrease each year. In line with the development of the technology of DNA levels, then weakness identification with morphological markers can be supported by a molecular approach. The use of molecular markers in the form of DNA is used along with the development of the science of molecular biology at this time[2].

Polymerase Chain Reaction (PCR) is a technique in molecular biology to multiply the amount of DNA in-vitro fertilization using the enzyme DNA polymerase and temperature [3]. The use of RAPD (Random Amplified Polymorphic DNA) is DNA analysis techniques are becoming very popular today. It is based on at the level of DNA [4]. The technique were generally faster, cheaper than any other method for detecting DNA sequence variation and does not require prior sequence information. The fact that RAPD's survey multiple loci in the genome makes the method attractive for analysis of genetic distance and phylogeny reconstruction [5]. Diversity or genetic variation can be used as a starting point for improving the quality and quantity of swamp buffalo. Thus, information about the genetic crossbreeding and kinship in cattle including swamp buffalo is crucial in the preservation of germplasm [6]. Genetic diversity became the important information in evaluating the genetic potential for the development, utilization, and conservation of species[7].

## 2 Materials and Methods

Blood sampling place the swamp Buffalo (*Bubalus bubalis*) in the area of TanjungSenai, Ogan Ilir Distrik, South Sumatera. Separation of serum is performed in the integrated laboratory Postgraduate Sriwijaya University. The research of DNA isolation, quantification of DNA and PCR-RAPD performed in the laboratory of Biochemistry at the Faculty of biology of the Gajah Mada University.



**Fig. 1.** Sampling location

A total of 300 1 blood from 6 variants buffalo (Kerbau Bule, Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang, Kerbau Tanduk Dungkul, Kerbau Rebah Belakang) from 1 location were used in this study, with the number of samples for each variant were 2 individuals.

## 2.1 Procedures of Quantity and Quality Test Results DNA Isolation

Before being used in the PCR reaction, DNA quantification is done to determine the purity and concentration of DNA. Results of a DNA sample isolation, diluted as much as 10 in 0.2 ml tube with TE buffer, inserted into the as much as 1 sample DNA and added 9 of TE buffer. Then at homogeneous with spin down and spectrophotometer set at wavelength of 260 nm, and washing kuvet with aquadest sterile then conducted measurements of blanko solution first. According to [8], quantitative DNA test with spectrophotometry pure DNA can absorb ultraviolet light because of the existence of bases purin and pyrimidine. So the purity of DNA can be quantified by calculating the values of absorbance  $\lambda$  260 nm absorbance value divided by 280  $\lambda$  ( $\text{Å}260/\text{Å}280$ ) and the value of DNA purity ranges between 1.8-2.0.

## 2.2 Electrophoresis

The results of the DNA isolation obtained then in gel electrophoresis by using agarose 1.5%, by dissolving 1.5 gram agarose with 100 ml of TBE 1x, and put in the microvawe until dissolved. After a rather cold in the add 2 EtBr. Then prepared parafilm, as much as 2 DNA loading buffer (loading dye), then inserted 8 sample and mixed with a loading buffer. Then, put in marker, DNA ladder 100 bp into wells. Electrode is then connected to a power supply with a voltage of 100 Volt, ampere 400 speed for 30 minutes. Then the agarose gel soaked in dye gel. Visualization is performed using the UV-transimulator to see the results of electrophoresis. Then photographed as documentation [8].

## 2.3 PCR-RAPD

Observation 6 variants molecular done with phase DNA templates preparation, DNA amplification, gel electrophoresis, gel electrophoresis quantification results, and data analysis. Before being used in the process of PCR-RAPD primer beforehand suspended using sterile aquabidest. Added with each primer 2x Master Mix PCR with 12,5 L, Primer RAPD (ILO- 1204, ILO-1212, ILO-525, OPW-03 and 2 L OPY-13, 10,5 L dH<sub>2</sub>O, totalvolume of 25 L to each microtube). The sample is then placed into the machine performed as many as 30 cycles of PCR with pre-denaturation at 95°C for 5 minute, denaturation at 95°C for 1 min, annealing at 55°C for 45 minutes, extention or elongation at 72°C for 1 minute, and final extention at 72°C for 10 minutes.

## 2.4 Visualization of Results Amplification

Visualization results of gel electrophoresis was conducted by using UV-Transilluminator and the results photographed as documentation.

## 2.5 Data Analysis

Data obtained from RAPD method in the form of a band or bands of amplification product was binary data represented by 'No (1) or no (0)'. DNA bands that have been subsequently measured distance migration. Determining the size of the amplification of DNA bands was done by measuring the migration distance of DNA standard (1 kb ladder)

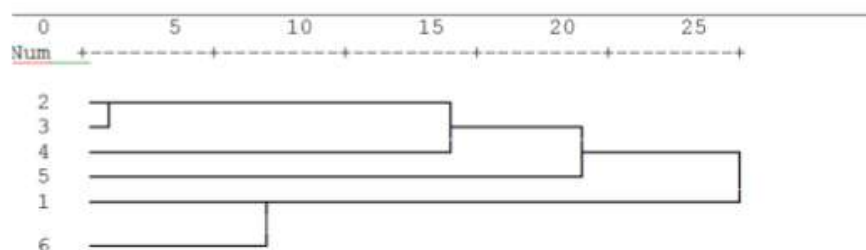


ranging from pitting to place the DNA migration. DNA fragment size standards then created the algorithm, and the log is used as the Y axis while the migration distance of the DNA used as the X axis measurable standards After the X and Y values are known then made linear line equation  $Y = ax + b$  through linear regression line equation. The size of the DNA bands are amplified searched by entering the measured values in the equation of the line. Furthermore, the value of Y obtained and will be used as antilog long known bases are amplified by a primer. After lengthy bases are known and obtained binary data is expressed with 'No (1) or no (0)' [9], the data is then analyzed its genetic variation with the program SPSS (Statistical Package for the Social Sciences) ver 16.0 and presented with a form of cluster analysis, a dendrogram using the coefficients similaritas.

### 3 Results and Discussions

Based on the results amplification of the swamp Buffalo (*Bubalus bubalis*) South Sumatera based on PCR with RADP primary 5 (ILO 1204, ILO 1212, ILO 525, OPW 03 and OPY 13) using 6 variant of the Buffalo swamp. The resulting DNA bands is analyzed, then conducted by calculating the distance of migration of DNA. After that, if there is a DNA bands in the give a score of (1) and there is no DNA bands in the give the score (0). According with [10] that the DNA fragment pattern profiles of each primary based on give the score or not the fragment using the binary code. Based on the scores are then analyzed by using SPSS ver 16.0.

The results of the analysis kinship swamp buffalo South Sumatera by using SPSS 16.0 versi can be displayed in the form of a diagram called a dendrogram. According [11], that cluster formation process describes the dendrogram expressed in form of pictures. Horizontal lines above dendrogram indicates scale that describes the level of similarity, the smaller the scale value indicates the increasingly similar to that individual.



**Fig. 2.** Dendrogram of swamp buffalo (*Bubalus bubalis*) South Sumatra based on PCR- RAPD

Description : (1) Kerbau Bule, (2) Kerbau Tanduk Bulat, (3) Kerbau Tanduk Langit, (4) Kerbau Tanduk Melintang, (5) Kerbau Tanduk Dungkul, (6) Kerbau Rebah Belakang

The results of the analysis similarity matrix coefficients PCR-RAPD similarity between 6 variant of the swamp buffalo South Sumatera based on polymorphic DNA bands that 47 amplified similarity coefficients obtained with ranges of values -972 to 856. The value of the lowest similarity coefficients -972 are indicated with the variant number 3 (Kerbau Tanduk langit) with number 1 (Kerbau Bule) this indicates that due to the presence of the genetic distance is far enough, while the highest coefficients value i.e. 856 indicated by variant number 3 (Kerbau Tanduk Langit) and number 2 (Kerbau Tanduk Bulat), that is because the genetic distance is close enough.

The value of the coefficient of 1.000 (100%) indicates that the absence of genetic distance among the variants. It is in accordance with statement [12], that the value of the

coefficient similarity 100% indicate that it is not just the distance between genetic variants in one with the other variants. The smaller the distance its genetic then the higher its genetic similarities instead its genetic distance is so large it will lower its genetic difference also.

Based on the results of the analysis of the dendrogram can be seen that there are two clusters namely cluster 1 that included Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang and Kerbau Tanduk Dungkul, while the cluster 2 included Kerbau Bule and Kerbau Rebah Belakang. Swamp Buffalo variants that have the closest genetic distance is kerbau tanduk langit and kerbau tanduk bulat, while the farthest variants kerbau tanduk langit and kerbau bule. According [10] to the genetic distance is the degree of difference between the two populations and genes can be used in constructing phylogenetic.

It is supported by research [13], about the relationship of kinship based on morphological characteristics. Cluster 1 had the same character on the shape of the tip of the horn, the color of the nose, eye, eyebrows eyelashes, color necklace neck and color hair tail, while the cluster 2 has the same character on the color of the nose, ear, eye color, the color of the lashes, the color of the body and tail of the hair color.

According to [14], that the population had a close kinship This indicates the occurrence of inbreeding that can degrade the quality of genetic offspring, because it can increase homozygosity populations. The level of genetic similarity of a population can be described by the genetic distance of the individuals members of the population. The greater the genetic distance of individuals in a population, then the population has members who are increasingly diverse. Instead the smaller the genetic distance between individuals in a population, the more uniform the population.

Based on those results show that swamp buffalos South Sumatera have low genetic variation and genetic distance near or close relationship is suspected of inbreeding. It agreed with [7], which stated the inbreeding suspected of causing a decrease in the nature of phenotypes. Inbreeding among a variety of swamp Buffalo is certainly not only lowers quality but also genotip and phenotypes. According to [6], that inbreeding affects the productivity of the cattle. The low productivity of the herds of Buffalo are associated with genetic reduction of occurrence prediction.

This is coherent with the research conducted by kinship of swamp Buffalo had previously done by [15], that measures the of kinship based on morphology. The correlation coefficient is 0.57 shows more kinship between swamp Buffalo post enumerates the variance relatively close. Suspected cross between variants tend to be high. Advanced research by [7], about genetic characteristics based on protein profile showed an average value of results of all loci (H) 0.1286. Based on the average heterozygosity, swamp Buffalo (*Bubalus bubalis*) Pampangan have low genetic variation and genetic relationship of the nearest. Triwulaningsih (2005) in [16] states that traditional maintenance systems cause the quality of buffalo seedlings to decline and result in the development of a slow population. Indicators of the occurrence of inbreeding in buffalo livestock populations are characterized by symptoms of genetic disorders / defects such as downward curved horns, and the high incidence of albino.

[16] added that the influence of inbreeding on a large livestock on livestock productivity. Inbreeding depression or inbreeding pressure on buffalo can usually cause a decrease in the performance of livestock (growth), high mortality and low reproducibility. One of the economic impacts of the high level of inbreeding is inbreeding depression where there is a decrease in phenotypic averages, especially in traits that have high economic value.

A follow-up study on the relationship of kinship according to [15], about diversity and kinship swamp buffalo (*Bubalus bubalis*) Pampangan of South Sumatera based on

morphological characteristics. The result of the analysis of kinship based on morphological characteristics shows the correlation coefficient value of 0.85. Inbreeding and adaptation factor cause the difference in phenotypes and morphology. A high level of inbreeding can lead to inbreeding pressure which is characterized by a decrease in livestock production and reproduction performance [17] which results in a decrease in the profits of livestock businesses [18]. Some reports suspect the pressure of inbreeding in a population group that results in a decrease in productivity and a slow increase in the livestock population. According to [16] close relatives or inbreeding led to the emergence of recessive traits such as albino. The factors that led to the occurrence of inbreeding in this group of livestock due to closed populations, the inbreeding system was not directed, lack of knowledge about breeders and male limitations. Based on those results can be that they variants swamp buffalo Pampangan tend to be low due to inbreeding and adaptation to the environment that result in differences of phenotype.

#### 4 Conclusion

Genetic variation of swamp buffalo (*Bubalus bubalis*) has a low variation with the percentage of polymorphic band 14.92 %. Swamp buffalo (*Bubalus bubalis*) South Sumatera there are two clusters namely cluster 1 that included Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang and Kerbau Tanduk Dungkul, while the cluster 2 included Kerbau Bule and Kerbau Rebah Belakang. Kerbau tanduk langit and kerbau tanduk bulat has a close kinship on coefficient of 856 while kerbau tanduk langit and kerbau bule have a kinship with the farthest distance coefficient-972. To study the characteristics of genetic diversity of swamp buffalo, it is necessary to use a different approach

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**LEMBAR  
HASIL PENILAIAN SEJAWAT SEBIDANG (PEER REVIEW)  
KARYA ILMIAH: PROSIDING**

Judul Karya Ilmiah : Characteristics of the Genetic Variation of a Swamp Buffalo (*Bubalus bubalis*) of South Sumatra Based on Polymerase Chain Reaction- Random Amplified Polymorphic DNA (PCR- RAPD)  
 Jumlah Penulis : Yuanita Windusari, Laila Hanum, **Arum Setiawan** and Veronika Larasati  
 Identitas Prosiding : a. Nama Prosiding : E3S Web of Conferences (**Scopus**), The 1<sup>st</sup> Sriwijaya International Conference On Environmental Issues 2018 (1<sup>ST</sup> SRICOENV)  
 b. ISBN/ISSN : 2267-1242  
 c. Nomor/Volume/Hal : 1/68/1002-1009  
 d. Penerbit : Pascasarjana Universitas Sriwijaya  
 e. Jumlah Halaman : 8

Kategori Publikasi Jurnal Ilmiah :  Prosiding Forum Ilmiah Internasional  
 (Beri  $\surd$  pada kategori yang tepat)  Prosiding Forum Ilmiah Nasional  
 Makalah tidak disajikan dalam seminar/symposium.lokakarya, tetapi dimuat dalam prosiding internasional  
 Makalah tidak disajikan dalam seminar/symposium.lokakarya, tetapi dimuat dalam prosiding nasional  
 Makalah disajikan dalam seminar internasional (Tetapi tidak dimuat dalam prosiding)  
 Makalah disajikan dalam seminar nasional (Tetapi tidak dimuat dalam prosiding)

## I. Hasil Penilaian Validasi:

No.	ASPEK	URAIAN/KOMENTAR PENILAIAN
1	Indikasi Plagiasi	7 %
2	Linieritas	Sudah linier dengan bidang biologi konservasi

II. Hasil Penilaian *Peer Review*:

Komponen Yang Dinilai	Nilai Maksimal Jurnal Ilmiah (Isikan di kolom yang sesuai)						Nilai Akhir Yang Diperoleh
	Prosiding Forum Ilmiah Internasional (Maks. 30)	Prosiding Forum Ilmiah Nasional (Maks. 10)	Makalah Tidak diseminarkan tetapi dimuat dalam prosiding internasional (Maks. 10)	Makalah Tidak diseminarkan tetapi dimuat dalam prosiding nasional (Maks. 5)	Makalah disajikan dalam seminar internasional (Tetapi tidak dimuat dalam prosiding) (Maks. 5)	Makalah disajikan dalam seminar nasional (Tetapi tidak dimuat dalam prosiding) (Maks. 3)	
Kelengkapan dan Kesesuaian unsur isi paper (10 %)	3						3
Ruang Lingkup dan kedalaman pembahasan (30 %)	9						8
Kecukupan dan Kemutakhiran data/Informasi dan metodologi (30 %)	9						9
Kelengkapan unsur dan Kualitas penerbit / prosiding (30 %)	9						9
Total = (100 %)	30						29
Kontribusi Pengusul (Penulis Pertama/Anggota Utama)	Anggota Utama=(0,4x29)/3= <b>3,87</b>						

**KOMENTAR/ULASAN PEER REVIEW**

Kelengkapan dan Kesesuaian Unsur	Paper terkait karakteristik variasi genetic kerbau rawa di Sumatera Selatan. Isi paper sudah memenuhi kaidah-kaidah karya ilmiah dan sudah sesuai dengan bidang biologi konservasi
Ruang Lingkup dan Kedalaman Pembahasan	Hasil penelitian dibahas cukup komprehensif dengan penyampaian pembandingan dari temuan-temuan penelitian lainnya dan teori terkait. Referensi yang diacu dalam pembahasan sudah cukup update untuk bidang kajian ini.
Kecukupan & Kemutakhiran Data & Metodologi	Data-data hasil penelitian sudah baik dan didukung peta lokasi sampling, tabel dan gambar yang ditampilkan menarik. Data didapatkan dengan menggunakan metode yang sudah standard.
Kelengkapan Unsur & Kualitas Penerbit	Penerbit Pascasarjana Universitas Sriwijaya berkualitas baik, tidak termasuk predatory publisher, dan prosiding terindeks di scopus

Surabaya, 15 Mei 2020  
Penilai 1



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Bidang Ilmu : Biologi  
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Kelengkapan unsur dan Kualitas penerbit / prosiding (30 %)	9						9
Total = (100 %)	30						30
Kontribusi Pengusul (Penulis Pertama/Anggota Utama)	E3S Web of Conferences ( <b>Scopus</b> ), The 1 <sup>st</sup> Sriwijaya International Conference On Environmental Issues 2018 (1 <sup>ST</sup> SRICOENV) ISBN/ISSN 2267-1242. Vol. 68(1): 1002-1009 Nilai maksimal 100%. Nilai Pengusul: (0,4 x 1 x 30)/3 : 4						
<b>KOMENTAR/ULASAN PEER REVIEW</b>							
- Kelengkapan dan Kesesuaian Unsur	Sangat lengkap semua unsur						
- Ruang Lingkup dan Kedalaman Pembahasan	Masih dalam ruang lingkup bidang ilmu.						
- Kecukupan & Kemutakhiran Data & Metodologi	Data sangat cukup.						
- Kelengkapan Unsur & Kualitas Penerbit	Penerbit Pascasarjana Unsri berkualitas.						

Yogyakarta, 6 Juli 2020

tanda tangan: 

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Bidang Ilmu : Biologi/Ekologi

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