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LIST OF CONTENTS

	Pages
WELCOMING SPEECH FROM CHAIR PERSON OF THE ORGANIZING COMMITTEE	iii
OPENING REMARKS FROM THE HEAD OF RESEARCH INSTITUTION AND COMMUNITY SERVICE, UNIVERSITY OF LAMPUNG	v
KEYNOTE SPEAKER: MINISTER OF ENVIRONMENT AND FORESTRY REPUBLIC OF INDONESIA	vi
SAFE SYSTEMS: HWC Safe Systems Approach and the HWC Rapid Assessment tool (Ashley Brooks, Ph.D.)	x
PROMOTING MULTI-STAKEHOLDER INTERNATIONAL COLLABORATIONS FOR ENDANGERED SPECIES RECOVERY (Barney Long)	xiv
INTEGRATING PLANTS INTO WILDLIFE CONSERVATION PROGRAMS (Siti Nur Hidayati, Ph.D.)	xvii
1. PREVENTION MODELS TOWARDS HUMAN - TIGER CONFLICT (HTC) IN BUKIT BARISAN SELATAN NATIONAL PARK (BBSNP), LAMPUNG (Firdaus Rahman Affandi, Tugiyono, G. Nugroho Susanto, Elly Lestari Rustiaty) ...	1 -- 10
2. IMPACT OF ANIMAL HOUSING TOWARDS WORMS INFECTION IN LOCAL BEEF CATTLE FARMS IN DUKUHBADAG VILLAGE, CIBINGBING, KUNINGAN, WEST JAVA, INDONESIA: AN ANALYSIS (Retno Widyani, Fitri Dian Perwitasari, Mus Nilamcaya, Ida Herawati)	11 -- 17
3. ESTABLISHING BASELINE DATA ON FISHERMAN AND FISH CAUGHT ON THE SERKAP RIVER, KAMPAR PENINSULA, RIAU (Sidiq Purwanto)	18--24
4. WALKING THROUGH CONVERSION: A MONITORING OF ELEPHANT MOVEMENT IN DEGRADED FOREST OF TESSO NILO LANDSCAPE (Febri Anggriawan Widodo, Wishnu Sukmantoro, Heri Irawan, Eka Septayuda, Yansen Gultom, Samsuardi, Sunarto, Nurchalis Fadhli)	25--29
5. EVALUATING THE INTERVENTION METHODS TO REDUCE HUMAN-ELEPHANT CONFLICT AROUND WAY KAMBAS NATIONAL PARK (Sugiyono, Ardiantiono, Agus Santo, William Marthy, Fahrul Amama)	30--36
6. JAVAN RHINO (<i>RHINOCEROS SONDAICUS</i>), BANTENG (<i>BOS JAVANICUS</i>) & OTHER MAMMALS COEXISTENCE IN UJUNG KULON NATIONAL PARK: SPATIAL AND TEMPORAL OVERLAP (Mahmud R, Rahmaningsih MD, Sunarto, Daryan, Firdaus AY, Muhtarom A, Setiawan R)	37--49
7. FILLING THE KNOWLEDGE GAP ON THE ENDANGERED ASIAN TAPIRS IN SOUTHERN PART OF TROPICAL RAINFOREST HERITAGE OF SUMATRA (Ardiantiono, Fahrudin Surahmat, Tri Sugiharti, Wulan Pusparini)	50--57
8. PEKON MUARA TEMBULIH, NGAMBUR, PESISIR BARAT: PRELIMINARY STUDY ON THE CHARACTERISTICS OF TURTLE HABITAT (Brina Wanda Pratiwi, Sugeng P. Harianto, Elly Lestari Rustiati)	58--65
9. SUMATRAN ELEPHANT (<i>ELEPHAS MAXIMUS SUMATRANUS</i> T) FOOD COMPOSITION AND ITS PREFERENCE IN TESSO NILO NATIONAL PARK (Defri Yoza and Yuliantony)	66--77
10. DIVERSITY AND ABUNDANCE OF AVIAN COMMUNITY AT COASTAL LAGOONS IN BUKIT BARISAN SELATAN NATIONAL PARK, INDONESIA: WHY WATERBIRD IS LACKING? (Ani Mardiasuti, Yeni A. Mulyani, Lina K. Dewi)	78--85

11.	HUMAN ELEPHANT CONFLICT STUDY BASED ON THE COMMUNITY INFORMATION IN RIAU – INDONESIA (Wishnu Sukmantoro, Yansen Gultom, Heri Irawan)	86--90
12.	STUDY ON HEALTH CARE MANAGEMENT SYSTEM OF CAPTIVE SUMATRAN ELEPHANT (<i>Elephas maximus sumateranus</i>) IN Prof. Dr. Ir. M. RUBINI ATMAWIDJAJA ELEPHANT HOSPITAL, WAY KAMBAS NATIONAL PARK (Firda Nur Islami, Dedi Candra, Diah Esti A, Priyambodo)	91--93
13.	A PRELIMINARY STUDY ON POPULATION ESTIMATION TECHNIQUE OF SIAMANG (<i>Sympalangus syndactylus</i>) in WAY CANGUK RESEARCH STATION, BUKIT BARISAN SELATAN NATIONAL PARK (Nafila Izazaya Idrus, Ryan Setiono, Fahrudin Surahmat)	94--98
14.	HELMINTHES PARASITIC (<i>PARAMPHISTOMUM SP</i>) INFECTION ON THE SUMATRAN ELEPHANTS IN ELEPHANT TRAINING CENTER WAY KAMBAS NATIONAL PARK LAMPUNG (Dedi Candra, Diah Esti, Elisabeth Devi, Catur Marsudi)	99--101
15.	TRAPPING FRUIT EATING BATS IN WAY CANGUK RESEARCH STATION, BUKIT BARISAN SELATAN NATIONAL PARK: MIST NET VS HARP TRAP (M. Khairul Ikhwani, Eka S. Ariyanti, Fahrudin Surahman, Janjiyanto)	102--105
16.	RESCUE SUMATRAN ELEPHANT BABY WITHOUT TRUNK IN WAY KAMBAS NATIONAL PARK LAMPUNG (Elisabeth Devi K, Dedi Candra, Diah Esti Angraini, Nazarudin, Mahfud Handoko)	106--108
17.	THE TABANID FLY BIODIVERSITY AND ITS POTENCY AS TRANSMISSION VECTOR OF TRYPANOSOMIASIS TO THE JAVAN RHINO POPULATION WITHIN THE UJUNG KULON NATIONAL PARK (Gita Alvernita, Kurnia O. Khairani, Dariyan, Dyah Lukitaningsih, Supriyono, Dedy S. Pahlawan, Zaenal Gesit Kalbuadi, Upik Kesumawati Hadi)	109--113
18.	ELEPHANT ENDOTHELIO-TROPIC HERPESVIRUS (EEHV) MANAGEMENT IN ELEPHANT CONSERVATION CENTER WAY KAMBAS NATIONAL PARK LAMPUNG (Diah Esti, Dedi Candra, Anhar Lubis, M. Wahyu, Elisabeth Devi)	114--116
19.	AN EXPERT SYSTEM TO DIAGNOSE CHICKEN DISEASES WITH CERTAINTY FACTOR BASED ON ANDROID (Aristoteles, Kusuma Adhianto, Puja Putri A)	117--126
20.	COMPARISON EFFECTIVENESS OF ANTIOXIDANT ACTIVITY EXTRACT HERBAL MIXTURE OF SOURSOP LEAF (<i>Annona muricata</i>), BAY LEAF (<i>Syzygium polyanthum</i>) AND PEGAGAN LEAF (<i>Centella asiatica</i>) (Khairun Nisa Berawi, Liana Shidarti, Samsu U. Nurdin)	127--132
21.	THE UTILIZATION OF ISOLATE <i>Bacillus thuringiensis</i> TO GRAYAK LARVAE PEST (<i>Spodoptera litura</i> Fab.) ON CABBAGE (<i>Brassica oleraceae</i> var. capitata Linn.) (Wibowo Nugroho Jati, Felicia Zahida, Sara Puspareni Prayitno)	133--137
22.	LEG AMPUTATION OF TIMOR DEER (Hastono, S.D)	138--140
23.	IDENTIFICATION OF THE SUMATRAN RHINO FOOD PLANTS IN WAY KAMBAS NATIONAL PARK LAMPUNG (Dedi Candra, Sumadi Hasmaran, Lamijo, Supriyono)	141--146
24.	SURVEILLANCE ANTHRAX (<i>Bacillus anthracis</i>) IN SURROUNDING WAY KAMBAS NATIONAL PARK LAMPUNG INDONESIA (Dedi Candra, Arie Khoiriyah, Diah Esti Angraini, Joko Siswanto)	147--151
25.	GENOMIC DNA ISOLATION OF GAJAH SUMATERA (<i>Elephas maximus sumatrensis</i>) IN ELEPHANT TRAINING CENTER, WAY KAMBAS NATIONAL PARK, EAST LAMPUNG (Elly L. Rustiati, Priyambodo)	152--155
26.	INDUCE RESISTANCE OF <i>SPATHOGLOTTIS PLICATA</i> BL. TOWARD TO	156--158

	<i>FUSARIUM OXYSPORUM</i> (Endang Nurcahyani, Rochmah Agustrina, Erdi Suroso)	
27.	THE EFFECTS OF A HEXANE FRACTION OF RED BETEL LEAF (<i>Piper cricatum</i>) ON LEARNING AND MEMORY IN MICE (Pratika Viogenta, Lilik Koernia Wahidah, Yudha Erlangga)	159--163
28.	THE LOCAL KNOWLEDGE OF COASTAL ETHNIC COMMUNITIES OF PLANTS THAT EFFICACIOUS AS MEDICINE IN 5 DISTRICTS OF SOUTH LAMPUNG REGENCY (Arum Asterini, Yulianty, Tundjung Tripeni Handayani) ..	164--169
29.	PHYTOTELMATA SPECIES AND ITS DISTRIBUTION IN SOUTH PRINGSEWU, LAMPUNG (Putri Minggar Oktaviani, Emantis Rosa, Yulianty) ...	170--174
30.	THE TOXICITY OF PURIFIED ISOLATE OF POLAR EXTRACT POWDER LEAFS <i>GLIRICIDIA MACULATA</i> HBR. TO CACAO MEALYBUG (<i>PLANOCOCCUS MINOR</i> MASKELL) (Ratih Andriyani, Nismah Nukmal, Emantis Rosa)	175--181
31.	SOCIAL BEHAVIOR OF SPOTTED DEER (<i>Axis axis</i>) IN GUNUNG MADU PLANTATIONS INC. SANCTUARY LAMPUNG TENGAH LAMPUNG PROVINCE INDONESIA (Rita Gusmalinda, Bainah Sari Dewi, Niskan Walid Masruri)	182--188
32.	THE COMPARISON OF TOXICITY PURIFIED ISOLATE OF WATER AND METHANOL EXTRACTS OF PAWDER LEAF <i>GLIRICIDIA MACULATA</i> ON MORTALITY SOURSOP MEALYBUG <i>PSEUDOCOCCUS CRYPTUS</i> (Fahrul Aksah, Nismah Nukmal, Emantis Rosa)	189--196
33.	DEVELOPMENT OF BOTANICAL INSECTICIDE FROM FLAVONOID OF COMPOUND LEAF EXTRACT <i>GLIRICIDIA MACULATA</i> TO CONTROL COFFEE MEALYBUG <i>PLANACOCCUS CITRI</i> (Apriliyani, Nismah Nukmal, Emantis Rosa)	197--204

GENOMIC DNA ISOLATION OF GAJAH SUMATERA (*Elephas maximus sumatrensis*) IN ELEPHANT TRAINING CENTER, WAY KAMBAS NATIONAL PARK, EAST LAMPUNG

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ABSTRACT

Elephant Training Center, Way Kambas National Park holds 44 captive sumatran elephants (*Elephas maximus sumatrensis*). This *critically endangered species* small population is in high risk of *inbreeding*. Its genomic DNA isolation was done to provide DNA amplification material for phylogenetic analysis. Individual blood samples were collected from 8 different individuals based on age and sex characteristics and stored at EDTA-anticoagulated blood tubes. Qualitative test by 1% agarose gel electrophoresis, visualized by UV transilluminator. Isolated genomic DNA was kept in -20°C elution buffer solution.

Keywords: sumatran elephant, blood, DNA isolation, Way Kambas National Park.

1. INTRODUCTION

Sumatran elephant (*Elephas maximus sumatrensis*) is endemic asian elephant that lives at seven provinces in Sumatera, included Lampung (Soeharsono, 2007). Since 2011, International Union for Conservation of Nature and Natural Resources (IUCN) classified the sumatran elephant as critically endangered species (Gopala *et al.*, 2011). Elephant Training Center in Way Kambas National Park was established to be one of solution of this problem.

Elephant Training Center in Way Kambas National Park holds 44 captive sumatran elephants. The small size of the population of the sumatran elephant allows to increased the inbreeding probability, then will made negative effect on gene flow in the sumatran elephant population. The bad gene flow of the population can adversely affect the viability of individual members of the population.

Research kinship patterns sumatran elephant has begun to do, especially regarding the relationship filogenik Asian elephants in Indonesia, Nepal, India and the elephants of the African continent. Fernando *et al* (2003) stated that the Sumatran elephants are related to elephants in Asia with diverse levels of phylogenetic closeness, while Sulandari and Zein (2012) states based on mitochondrial DNA genetic variation among populations of Sumatran elephants in Lampung, South Sumatra and Bengkulu is low.

Kinship patterns of captive sumatran elephants in Elephant Training Center in Way Kambas National Park has not been done. Potential high inbreeding and low genetic variation push for an immediate kinship between individual data collection of sumatran elephants in Elephant Training Center in Way Kambas National Park. Kinship patterns can be analyzed with DNA finger printing methods, for example methods Random Amplified Polymorphism DNA (RAPD) (Kumar and Gurusubramain, 2011). RAPD molecular markers are widely used in the analysis of genetic variation because it can be done without the need for data on the nucleotide sequence of the DNA template to be amplified (Yadav *et al.*, 2012). Therefore, research on the molecular genetics of the Sumatran elephant population is an important step that must be waged. Through genetics approach, expected to conservation efforts more focused and able to rescue survival of the Sumatran elephant. RAPD molecular markers require genomic DNA as a base material for the amplification and analysis of genetic kinship.

2. MATERIALS AND METHODS

Blood sampling

Venous blood samples were obtained from 8 individual sumatran elephants in Elephant Training

Center, Way Kambas National Park, based on age and sex characteristics (Table 1). Whole bloods were collected by syringe and stored at 3 mL EDTA-anticoagulated blood tube (Pic. 1). All samples were kept at 4°C before DNA isolation was performed.

Table 1. Sumatran elephants in Elephant Training Center, Way Kambas National Park

Number of Sample	Name of elephant	Sex	Age(years)
1	Agam	male	37
2	Daeng	male	29
3	Pangeran	male	2
4	Sugeng	male	6
5	Lingling	female	38
6	Suci	female	27
7	Yulia	female	3
8	Queen	female	5



Pic 1. Whole bloods were collected at 3 mL EDTA-anticoagulated blood tube

DNA Isolation

DNA was isolated from sumatran elephant's whole blood using Dneasy Blood & Tissue Protocol. Well mixed of 200 μ L EDTA-anticoagulant-treated blood, 20 μ L proteinase K and 200 μ L buffer AL in 1,5 mL microtube was incubate in 56°C for 10 minutes. Then 200 μ L ethanol 96% was added followed by centrifugation at 8000 rpm for 1 minute. Washing step was done by added AW1 and AW2 with high rise centrifugation. AE buffer was added to center of the spin column for ellution step than centrifuge at 8000 rpm for 1 minute.

DNA Qualitative Test

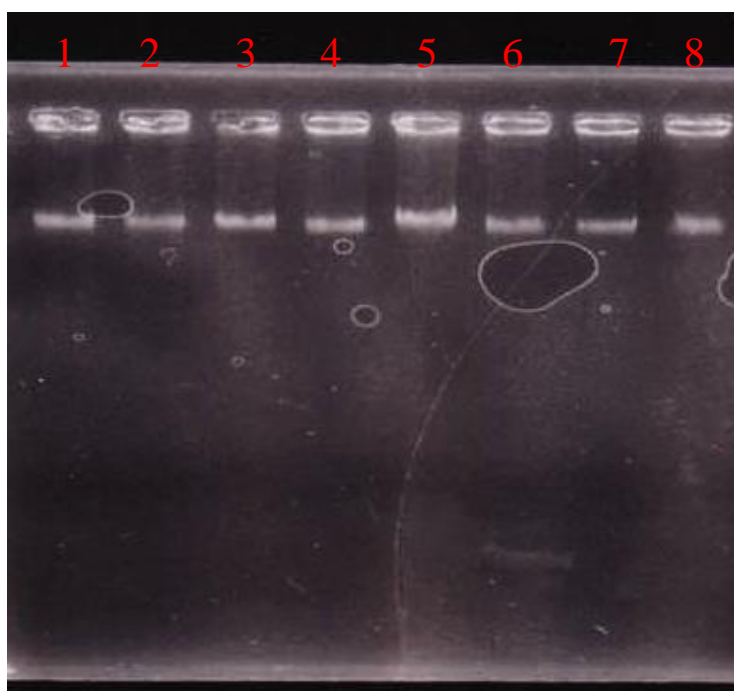
Isolated DNA was test by qualitative method, loaded in 1% agarose gell electrophoresis. DNA was visualized by good view staining and photographed under ultraviolet light. Isolated DNA kept at -20°C before DNA amplification and another reaction performed.

RESULTS

Genomic DNA from 8 individual sumatran elephant was eluted in AE buffer sollution than kept in -20°C (Pic 2). DNA was visualized by good view staining and photographed under ultraviolet light as shown at Pic 3.



Pic 2. Genomic DNA from elephant eluted in buffer solution



Pic 3. Photograph of qualitative test from genomic DNA under UV light
(1: Agam, 2: Daeng, 3: Pangeran, 4: Sugeng, 5: Lingling, 6: Suci, 7: Yulia, 8: Queen)

3. DISCUSSION

The type and condition of specimen and tissues, according to its origin, are key factors in selecting a DNA isolation method. The total genomic DNA from whole blood of sumatran elephant was isolated with Dneasy Blood & Tissue Protocol from QIAGEN®. The protocol consist of three step, lysis, washing, and elution, respectively. Lysis step using AL buffer, proteinase-K under incubating condition in 56°C. This step will destruct the cell wall and nucleus membrane. Second step was done by twice washing step, with AW1 and AW2 buffer with centrifugation condition. This step will removed the other materials besides DNA. Third step will eluted the DNA from the sillica gell in buffer solution with AE buffer.

Using the silica-coated gell in DNA isolation step allows to reversibly bind and purify DNA away from cell debris, proteins, and another materials released upon directed cell lysis. The binding of DNA to silica gell seems to be driven by dehydration and hydrogen bond formation, which competes against weak electro-static repulsion. Hence, a high concentration of slat will help drive DNA adsorption onto silica, and low concentration will help to release the DNA from the silica gell. The total genomic DNA will kept in -20°C condition before PCR analysis to construct the phylogenetic tree between sumatran elephant in Elephant Training Center, Way Kambas National Park.

4. CONCLUSION

The resulted quantity of genomic DNA is enough to conduct further PCR reactions. Using the above methode, good quality DNA samples from a sumatran elephant whole blood were isolated to study the phylogenetic analysis in sumatran elephant population.

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