Exploring Environmental DNA for Barcoding Analysis of Sumatran Rhino in Way Kambas National Park

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Abstract. The Sumatran rhino (*Dicerorhinus sumatrensis*) is one of five species of rhino in the world. The existence of the Sumatran rhino is also considered very vulnerable to habitat degradation, internal cross-breeding, disease, and hunting. The Sumatran rhino population will experience extinction if there are no mature management measures for the long term. It is assumed that the dynamics of the natural ecosystem in the Sumatran rhino's natural habitat will harm the existence of its population. Sumatran rhino wallow water is one of the remaining sources of environmental genetic material. Extraction of eDNA in the wallow water of individual Sumatran rhinos in Sumatra Rhino Sanctuary, Way Kambas National Park aims to determine the results of testing the quality of genetic material from Sumatran rhino wallow water in the Sumatran Rhino Reserve, Way Kambas National Park using simple and molecular methods. DNA extraction using a simple method detects 0/12 samples, whereas using molecular methods can detect 11/12 samples. These results should be followed by detection using specific primers to ensure that the eDNA extracted is Sumatran rhino eDNA.

INTRODUCTION

In the world there are five species of rhinoceros, according to their distribution, two species are found on the African continent and three species are found on the Asian continent. Indonesia has two rhino species, namely the Javan rhino (*Rhinoceros sondaicus*) and the Sumatran rhino (*Dicerorhinus sumatrensis*) [1]. The Sumatran rhinoceros was once widespread in Southeast Asia, currently, it is estimated that only about 30 individuals in Sumatra and Kalimantan [2] are critically endangered and are included in the CITES Appendix I list [3]. Threats facing the Sumatran rhino include poaching, habitat destruction, and extremely low reproductive rates. Many efforts have been made to conserve the Sumatran rhinoceros to avoid extinction. The Sumatran rhino population will experience extinction if there is no long-term rescue action. The dynamics of natural ecosystems in the natural habitat of the Sumatran rhino are expected to have an impact on the existence of its population [4].

Sampling for DNA analysis can be done using invasive methods, namely sampling that is in direct contact with individuals, through taking blood or body parts from animals. This method requires capturing or coming into direct contact with the animal, which can result in a risk of physiological stress or dangerous side effects from the overuse

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of the drug. Currently, DNA sampling is starting to develop, one of which is using non-invasive methods [5]. This method utilizes feces, urine, scrapes, and wallows of water on these animals [3].

METHOD

The tools and materials needed for collecting wallow samples are 10 ml vacutainer tubes, large syringes, labels, plastic, dippers, and iceboxes. A sampling of wallow water in the area at 08.00-10.00 WIB. The Sumatran rhinoceros wallow was found in an area ±100 m from the captive location, the active Sumatran rhino wallow sample was a wallow that was still routinely used to wallow the Sumatran rhino, while the inactive sample was a wallow that was no longer used for the Sumatran rhino wallow.

A sampling of wallow water was carried out at two points, namely the edge point and the middle point. At the edge point, sufficient water samples were taken using a large syringe, then put into a 10 ml vacutainer tube. Taking at the midpoint, a sufficient sample of wallow water was taken using a dipper that had been tied with wood, then the sample was taken using a large syringe and put into a 10 ml vacutainer tube. Each sample taken was taken by taking pictures and recording important data related to individual names, conditions, sampling points, and information about the time of the Sumatran rhino wallow into the survey datasheet. All of the wallow samples that had been put into a 10 ml vacutainer tube were stored in the refrigerator.

Extraction of eDNA from Sumatran rhinoceros wallows samples was carried out concerning the QIAmp® Fast DNA Stool extraction protocol Mini Kit (50) catalog number: 51504 from Genecraft. Testing the quality of DNA extracted from Sumatran rhino wallows with a simple technique using 1% agarose gel electrophoresis. 1 g of agarose powder was added with 100 ml of Tris Acetate-EDTA (TAE) buffer which was used as a buffer solution, then heated in the microwave for 3 minutes, then 1.5 l of safe SYBR was added until homogeneous which served as a dye to see the extracted DNA after it's done electrophoresis.

The molecular method used in this test is to see the accuracy of the DNA quality test results extracted on the method simple. The technique used is Polymerase Chain Reaction (PCR) on Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) primers which have three stages, namely the master mix and template stages, amplification, and electrophoresis stages. The master mix and template stage aims to mix all the reaction components, including DNA templates, reagents, and other materials. The amplification stage aims to double the amount of sample DNA, and the electrophoresis step agarose gel aims to check the results of the PCR reaction.

RESULT AND DISCUSSION

Water samples were collected from six of the seven Sumatran rhinos at SRS, TNWK (Table 1). Sumatran rhino Rosa could not be sampled as a source of genetic material, this is because the Sumatran rhino Rosa is more sensitive than the other six Sumatran rhinos to human presence in the area, which will cause stress to the Sumatran rhino. Water samples were taken when the Sumatran rhino went to the captivity, which was led by the keeper with the condition of the Sumatran rhino being muddy, indicating that the Sumatran rhino had just finished wallowing in the morning and dry muddy conditions indicating that the Sumatran rhino was wallowing at night.

TABLE 1. Identity of Samples

Name of Rhino	Age	Source of Genetic Material	
		Active wallow	Nonactive wallow
Andatu	8	1	1
Andalas	19	1	1
Harapan	13	1	1
Ratu	15	1	1
Bina	37	1	1
Delilah	4	1	1

The characteristics of active wallows are that the wallows that are often used by the Sumatran rhino for wallowing have more soft mud and less water, while inactive wallows are wallows that have not been used for wallowing by the Sumatran rhino for about 1-3 months, in inactive wallows more water is collected. pooled because the wallow had never been used for wallowing and around the wallow it looked neat, there were no footprints or traces of the Sumatran

rhino's feet (Figure 1). The sampling point of the wallow water is at the center point which is the wallowing axis of the Sumatran rhinoceros, and the edge point is the place to scavenge the Sumatran rhino. The wallow water sample at the midpoint was taken using a dipper and the edge point was taken using a large syringe and then put into a non-EDTA vacutainer tube which was made in airtight conditions.

FIGURE 1. (a) active wallow, (b) inactive wallow

The vacutainer tube is labeled with the individual name of the Sumatran rhino, type of wallow, collection point, date of sampling, then the vacutainer tube is put into an icebox for transportation to Bandar Lampung to the Biotechnology Laboratory, Lampung Veterinary Center, the wallow samples are stored in the freezer (-20^{o}C) until the next process.

Qualitative test results using a simple method of water samples Sumatran rhinoceros wallow showing no visible luminescence DNA bands, so that treatment is carried out using the method Another, a more sensitive method is the molecular method. This is done to ascertain whether the results of the electrophoresis of water samples Sumatran rhinoceros wallow that does not show DNA band luminescence caused by the absence of a remaining source of genetic material in the Sumatran rhino wallow or caused by the amount of DNA which is too little so that it is not detected in qualitative tests using a simple method. Visualization of agarose gel electrophoresis results was carried out using digital documents (digidoc). The results were obtained from the gel visualization agarose using Digidoc visible as DNA band luminescence (Figure 2).

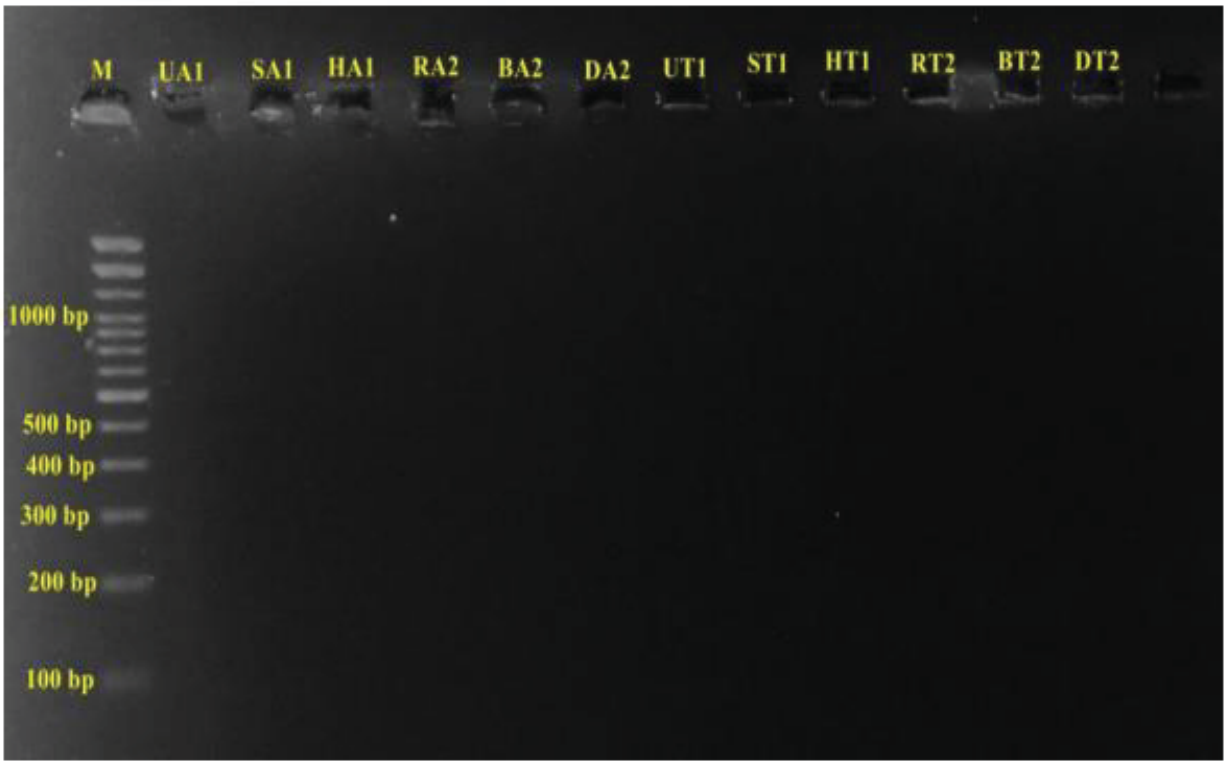

FIGURE. 2 Qualitative test results using a simple method of water samples Sumatran rhinoceros wallow

The amplification process is carried out by setting a certain temperature and time which will produce 12 DNA amplicons. Electrophoresis of DNA results from the amplification process is visualized using digidoc. Visualization results of 12 pool water samples of individual Sumatran rhinoceros showed 11 positive samples and 1 negative sample (Figure 3).

FIGURE. 3. Visualization results of 12 amplicons of GAPDH fragment

Delilah Sumatran rhino wallow water sample at point collection center that gives a negative result or does not show luminescence DNA bands on the molecular method. The sample is also not showing the presence of luminescence of DNA bands in a simple method. Thing This can be caused by the absence of genetic material left behind in an inactive pond individual Sumatran rhino Delilah or genetic material damage. The results of the quality test of the simple method and the molecular method shows there is a difference, that the PCR reaction can increase the number of Extracted DNA so that during electrophoresis it can capture the DNA and produces DNA bands glow [6]. The use of GAPDH primers can detect enzymes found in Sumatran rhinoceros wallows so that the method.

Molecular GAPDH is more sensitive in detecting the presence or absence of genetic material in the Sumatran rhinoceros wallow The quality of DNA and the amount of DNA extracted affect the thickness thin luminescence of DNA bands during electrophoresis. Luminance thickness DNA bands on the visualization results are directly proportional to the quality of DNA. The luminescence of the DNA bands that are getting thicker indicates a higher quality of DNA getting better [7]. The age of use of the wallow to affects the thickness of the luminescence of the DNA band, the thicker the luminescence DNA bands obtained, the wallow is actively used for a wallow. The thinner the luminescence of the DNA bands obtained, the age the use of wallows is not being actively used by rhinos Sumatra.

This initial study was conducted for the manufacture of DNA banks to support the Sumatran rhinoceros phylogenetic tracing source in SRS, TNWK [8]. Sumatran rhino feces samples that were isolated DNA [3]. eDNA extraction was carried out to determine the presence of genetic material left in rhino wallows Sumatra in SRS, TNWK using a simple method and method molecular. Of the 12 DNA extracted from the wallows of the Sumatran rhinoceros in SRS, TNWK showed 11 samples were suitable for testing further steps such as the DNA sequencing stage so that this research can support molecular-based conservation efforts. While 1 sample cannot be used for further tests due to the possibility of the presence of defects in the sample or the absence of a source of genetic material what is left is like in an inactive wallow of the Sumatran rhino Delilah.

CONCLUSION

This study concludes that the results of the DNA quality test shows there are differences in the results of quality tests using a simple method and molecular method. Quality test results using molecular methods are more accurate in detecting the presence or absence of a source of genetic material that left in the wallows of the Sumatran rhino, while the results of the quality test using a simple method is less specific if it is used for determining the presence of environmental genetic material sources left behind in the Sumatran rhino wallow.

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