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Identification of Visual Character of Leaves with Sumatran Rhino's Urine in the Sumatran Rhino Sanctuary Way Kambas National Park as Potential Source of eDNA

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Abstract

The Sumatran rhinoceros is one of the large endemic mammals to the island of Sumatra, with critically endangered status by the IUCN. Currently, the existence of the Sumatran rhino is challenging to find in its natural habitat. Therefore, it is necessary to develop indirect monitoring to maintain and increase the stability of the population in nature. Monitoring through camera traps has been conducted in several locations. Another method is carried out by identifying traces and remains of the Sumatran rhino in natural habitats, for example, through urine samples. The Sumatran rhinoceros has the behavior of urinating, namely through urine spray. Urine sprayed by the Sumatran rhino will hit plant parts in its habitat, including leaves. This study was aimed to identify the visual characteristics of leaves containing samples of Sumatran rhino urination as a potential source of eDNA material. The results showed the leaves characteristics containing the Sumatran rhino's urine on the 0, 1, and 2 days after the Sumatran rhino urination. The difference in visual observations of the Sumatran rhino's urine spray attached to the leaves can be seen through the change in the color of the urine fluid, which is getting more and more concentrated. Visualization of leaves containing Sumatran rhino urine can be used to recommend visual leaf characteristics that can be taken as similar samples in natural habitats.

1. INTRODUCTION

There are five species of rhinos in the world, two species are found in Africa continent, namely the black rhino (*Diceros bicornis*) and the white rhino (*Ceratotherium simun*), while three species are found on the Asian continent, one species living in India is the Indian rhino (*Rhinoceros unicornis*), and two species live in Indonesia, namely the Javan rhino (*Rhinoceros sondaicus*) and the Sumatran rhino (*Dicerorhinus sumatrensis*) (Pusparini, 2006). According to the International Union for the Conservation of Nature and Natural Resources (IUCN), the Sumatran rhino is listed as critically endangered. Sumatran rhinos have been listed on Appendix I since 1975 in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) which means that Sumatran rhinos are internationally protected from all forms of trade (Putra, 2014).

Sumatran rhinos are one of the main animals with critical status due to poaching, natural habitat destruction, and very low reproduction rates (Pusparini, 2006). One of the Sumatran rhino conservation efforts carried out by Way Kambas National Park (WKNP) is by establishing a semi-in-situ conservation area in collaboration with Yayasan Badak Indonesia (YABI/the Indonesian Rhino Foundation) under the name Sumatran Rhino Sanctuary (SRS). The SRS WKNP was established in 1996 with the aim of breeding and providing a natural habitat for Sumatran rhinos. The SRS WKNP was established in the core zone, covering 100 ha of the 10,000ha area between Way Kanan and Way Negara Batin (YABI, 2015).

Currently, SRS WKNP is building an expansion area or often called SRS II with an area of 150 ha which has been built since 2016 and inaugurated in November 2019 (Darlina, 2019). Currently, SRS WKNP is inhabited by eight Sumatran rhino individuals, namely Bina (female), Ratu (female), Andalas (male), Rosa (female), Harapan (male), Andatu (male), and

Delilah (female), and a rhino calf resulting from the mating of a male rhino named Andatu and a female rhino named Rosa on March 29, 2022 (KemenLHK, 2022).

Sumatran rhino conservation efforts have been carried out thoroughly from the regulatory, ecological and molecular fields (Priyambodo et al., 2022) examined how to extract DNA through a non-invasive method through fresh water. Sample of DNA can be acquired by noninvasive methods, which are sampling methods that do not come into direct contact with animals, such as feces, friction marks, water, and animal urine (Savira, 2012). Non-invasive sampling avoids the risk of stress due to the side effects of excessive anesthetic use in invasive sampling.

The importance of genetic studies, especially molecular DNA, requires a large number of samples, so it is necessary to explore more potential material as a source of environmental DNA from Sumatran rhinos. This source can be obtained by paying attention to the behavior of Sumatran rhinos in their daily lives.

Urination behavior is one of the ways Sumatran rhinos mark territories and communicate with other individuals or animals. Sumatran rhinos have two ways of urinating, namely by ordinary urination and spray urination. Urine spray is urine sprayed by Sumatran rhinos on leaves or shrubs. Sumatran rhinos can do urine spray when they are stressed, lustful, feel disturbed, attracting mates and marking territory areas. According to several observations, it is known that the urine spray of male rhinos is higher than female rhinos (Zain, 2018).

Sumatran rhinoceros urine spray behavior is preceded by sniffing and grunting into the bush area, twisting the bush with its hind legs, then turning around, scratching its hind legs on the ground, then dragging it into the bush accompanied by spraying its urine (Kurniawanto, 2007). A distinctive feature that can be found from the Sumatran rhinoceros urination behavior is scratching along 30 to 50cm on the ground in front of

the bush. The height of the Sumatran rhino's urine spout can reach 2 m and as far as 3 m. Urine spray behavior is more dominantly carried out by male Sumatran rhinos. Female Sumatran rhinos perform urination in the usual urine method (Alikodra, 2013).

There is no data related to the visual characteristics of leaves stained with Sumatran rhino urine up to date, even though this data can be used as a basis for sampling wild Sumatran rhinos as part of comprehensive conservation efforts. Therefore, in this study, research was carried out related to the observation of visual characteristics of leaves stained with Sumatran rhino urine at SRS WKNP. Data on the visual characteristics of leaves with Sumatran rhino urine can be used as a standard for similar sampling in native habitats. This will be very valuable in efforts to update data on the presence of Sumatran rhinos in the wild.

2. MATERIALS AND METHODS

The research was conducted at the Sumatran Rhino Sanctuary (SRS), Way Kambas National Park (WKNP), East Lampung Regency, Lampung Province, Indonesia (Figure 1). The preliminary survey was carried out by conducting a survey of the research location at SRS, WKNP and coordination with YABI regarding sampling techniques at the preliminary survey stage.

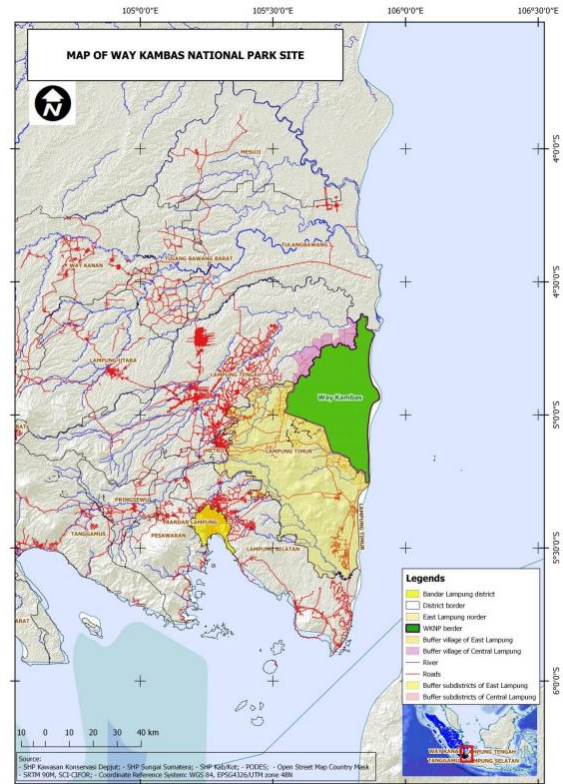


Figure 1. Maps of Way Kambas National Park (Indraswati et al., 2018)

The survey stage to data collection was carried out from July to September 2020.

3. Results and Discussion

The results of the analysis of variance (The results of observations that have been obtained on the visual characteristics of leaves attached to Sumatran rhino urine on day 0, day 1, and day 2 show some differences (Figure 2). The part of the leaf with Sumatran rhinoceros urine is depicted with a yellow circle. The differences observed were in the form of the quantity of Sumatran rhinoceros urine attached and the color of the leaves attached by the Sumatran rhinoceros urine.

Based on the picture, it appears that the quantity of Sumatran rhino urine attached to the leaves is decreasing day by day. Meanwhile, the color of the leaves covered with Sumatran rhino urine gradually becomes darker.

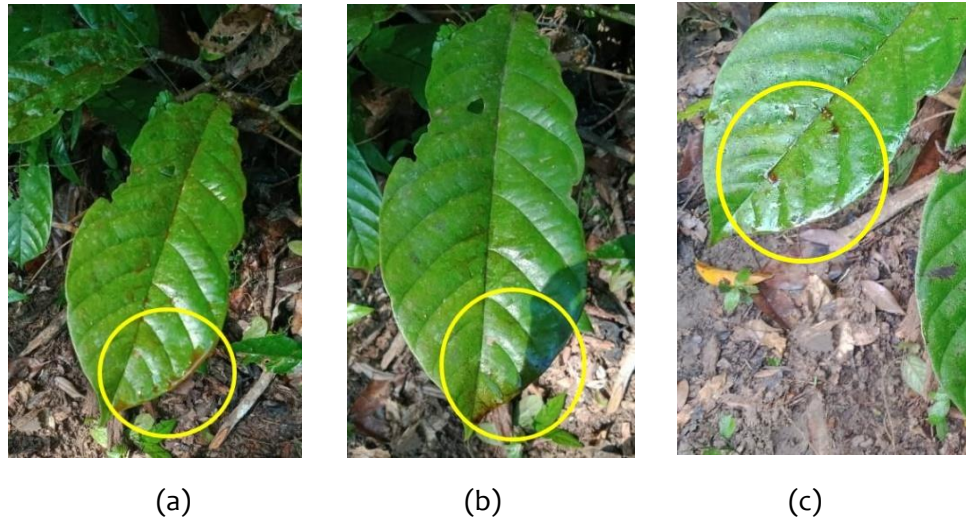





Figure 1. Visualization of leaves stained with Sumatran rhinoceros urine on (a) day -0, (b) day 1 and (c) day 2 posturination.

Schematically, the colour change of Sumatran rhino urine attached to the leaves from day 0 to day 2 appears to gradually get a lot darker (Table 1).

Table 1. Discolouration of Sumatran rhinoceros urine attached to leaves

Number	Day	The colour shown	Description
1	0		Bright brown colour, leaves are still wet.
2	1		The colour of the urine is darker, and the urine on the leaves has dried up.
3	2		The urine colour is deep brown, with only a few spots left on the leaves.

4. Discussion

Important habitat components to support the development of Sumatran rhinos are food, water, clear areas, and shade. Water sources are very important for the life of Sumatran rhinos, because water is an important component of the body of living things (Putra, 2014). Home range is the area of animal movement in carrying out their daily activities (Sudrajat, 2017). To find out the Sumatran rhino's home range, direct and indirect observation methods can be used. Direct observation can be done by direct encounter with animals. Indirect encounters can be done with indirect signs of animal presence such as traces left by animals (Djalil, 2018). Sumatran

rhino behavior to mark the home range includes defecation, paw pawing, twisting, and urination (Pusparini, 2006).

Urination marks can stick to leaves or other parts of plants in Sumatran rhino habitat. Urine fluid consists of water and metabolic waste in the body such as urea, dissolved salts, and organic matter. Urine contains red blood cells, white blood cells, and epithelial cells. Epithelial cells function to protect organisms or living things from microbial diseases. Cells contained in urine have the potential as a source of eDNA material from Sumatran rhinos.

In previous studies, urine DNA was used to gain further knowledge on parasite fitness and the ecological and evolutionary complexity of

these host-parasite interfaces (Duval et al., 2021).

The eDNA molecule is a source of genetic material left by animals through indirect signs. In obtaining information about the population of the species is done by taking samples that have eDNA potential. This eDNA sample comes from cells or tissues left behind such as feces, hair, skin and urine. One of the Sumatran rhino home range markings is done by urination. Urine is the residual metabolic fluid resulting from kidney excretion, and is excreted by the body which is called the urination process. The behavior of marking the home range by Sumatran rhinos by making a mound of soil from footprints, making twists in the stem, making sapling breaks, and spraying urine on plant leaves and stems (Van Strein, 1985).

The characteristics of Sumatran rhino urine are not too pungent and the color of Sumatran rhino urine turns thick overtime. The odor of urine is influenced by its dietary (Haffey et al., 2008).

The difference in visual observation of Sumatran rhinoceros urine spray attached to the leaves can be seen through changes in the color of the urine liquid which is getting thicker as the day goes on. The urine spray on day 0 showed that the color of the urine produced is brown and the condition of the urine looks wet on the surface of the leaves.

The urine spray on day 1 post-urination, The urine adhering to the leaves getting more concentrated brown urine and the condition of the urine dries up but if the surface of the leaf is touched the urine liquid still feels wet. The condition of urine spray on the second day after urination, the urine remained dark brown in color and dried up (Figure 2).

The reduction in the quantity of Sumatran rhinoceros urine attached to the leaves is due to evaporation caused by sunlight. Visual color changes in urine are caused by environmental factors such as humidity and temperature. The presence of high temperatures during the day causes evaporation, so that the water contained in the remaining Sumatran rhino urine decreases. This reduction in urine

content allows the color of the urine spots to become more intense. Changes in the visualization of Sumatran rhino urine spray can be used as the basis of information for the field team when sampling animals non-invasively in their natural habitat

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6. CONCLUSION

There are differences in visual observations of Sumatran rhinoceros urine spray attached to the leaves that can be seen through changes in the color of the urine liquid which is getting thicker overtime.

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