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Original article

Biodiversity and in silico phylogenetic analysis of Tapanuli Orangutan food in the Batang Toru Forest, North Sumatra

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Abstract

Tapanuli orangutan is one of the Critically Endangered endemics in North Sumatra because there is a population decline due to habitat loss. Conservation efforts are needed to survival. One possible way is through the availability of food for orangutans. This research aims to determine the diversity and environmental factors that influence food diversity and phylogenetic analysis of Tapanuli orangutan food trees in Indonesia. This research used the line transect method with 3 transect lines (transects I, II, III) which were used to observe the Tapanuli orangutan food trees at a distance of 1000 m and a width of 100 meters. Sequence data was obtained from GenBank, an NCBI sequence data provider. Phylogenetic relationships were analyzed using the maximum likelihood method in MEGA 11 software. The results found were that five species of food for Tapanuli orangutan were dominated by *Campnosperma auriculatum*. Low diversity index category in the diet of the Tapanuli orangutan in the Batang Toru Forest. Phylogenetic analysis showed that the closest relationship is to the food trees of the Tapanuli orangutan in Indonesia, such as *Aglaia tomentosa* with Brunei Darussalam, *Campnosperma auriculatum* with Singapore, and *Artocarpus heterophyllus* with Sri Lanka. The novelty of this research will be useful for providing recommendations for food management strategies as initial data for Tapanuli orangutan conservation efforts.

Keywords: food preference, in silico phylogenetic, plant diversity, tapanuli orangutan
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Introduction

Orangutans are the only living species of Asian ape. This makes orangutans one of the protected animals, both species and habitat which are increasingly approaching extinction (Nasution et al., 2018). The new orangutan species discovered in 2017 was the Tapanuli orangutan (Nater et al., 2017). The Tapanuli orangutan was previously considered the southernmost population of the Tapanuli orangutan. However, based on in-depth research conducted by domestic and international research groups in the fields of genetics, morphology, ecology, and behavior, it turns out that the Tapanuli Orangutan is taxonomically closer to the Bornean Orangutan (*Pongo pygmaeus*) and should be separated into its species (Kuswanda et al., 2020).

The Tapanuli orangutan is very sensitive to environmental changes or other disturbances, so it is feared that extinction will occur quickly. To overcome this problem, all remaining Tapanuli orangutan habitats must be protected, not only the animals but also the biodiversity of their food sources. Currently, the number of Tapanuli orangutans is around 800. The Tapanuli orangutan has a role as an ecosystem balancer (Wich et al., 2019). The habitat of the Tapanuli orangutan is only found in the Batang Toru forest, also called Harangan Tapanuli, which covers an area of approximately 150,000 hectares. Batang Toru Forest is located in three districts in North Tapanuli Province. Of this area, nearly 142,000 hectares are primary forest, which appears dark green on satellite images. Some of these forest areas have been degraded, requiring smart rehabilitation measures and the creation of corridors between separate forest blocks (Sianipar et al., 2021). Around 61% of the primary forest is in the North Tapanuli Regency, 29.7% in South Tapanuli, and 9.3% in Central Tapanuli (Nater et al., 2017).

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Various efforts have been conducted to conserve orangutans. One way is to restore a new environment that suits the orangutans' needs, including the Tapanuli orangutan food trees. The food eaten by Tapanuli orangutans in the Batang Toru Forest consists of *Aglaia tomentosa, Agathis borneensis, Artocarpus heterophylus, Campnosperma auriculatum* and *Castaopsis argentea* (Khakim 2015). There is competition between Tapanuli orangutans and humans because food trees are used, for example several known types of Aglaia include: the wood is used as building materials, the fruit can be eaten, while the flowers are used as tea and perfume because of their fragrant aroma (Praptiwi et al., 2006). This use causes the number of *Aglaia tomentosa* to decrease, resulting in a reduction in orangutan food (Meijaard et al., 2018).

The diet diversity of Tapanuli orangutans is closely related to soil content. Soil content is influenced by the availability of organic matter, water purification, carbon sequestration, nutrient cycling, and providing habitat diversity for organisms. Macro-nutrients in soil are nutrients that plants need in large quantities, such as Nitrogen, Phosphate, and Sulphur which play an important role in plant protein, which is the required amount of energy for the active absorption of plant nutrients. Meanwhile, microelements such as iron (Fe) are essential because they are part of certain enzymes and part of proteins that carry electrons in photosynthesis and respiration (Heldt and Piechulla, 2011). Another way to conserve the Tapanuli orangutan is through in silico phylogenetic analysis.

Tapanuli orangutan conservation efforts through species identification based on DNA sequences are a method that is considered fast, reliable, and consistent, making it important in conservation research (Stevanus & Pharmawati, 2021). Therefore, it is important to carry out phylogenetic characterization of Tapanuli orangutan food in silico to provide scientific information to the public regarding the results of the analysis of phylogenetic relationships which will influence the correctness of its use as Tapanuli orangutan food.

There has been no research regarding genetic diversity in the diet of the Tapanuli orangutan. To provide this information, it is necessary to carry out research consisting of studying genetic variations using molecular markers such as the tRNA-Leu (*trnL*), trnL-trnF, *matK*, and *rbcL* gene introns from the NCBI database (Basendahl, 2000). It is desired that the known phylogenetic analysis can become basic data on the food kinship of the Tapanuli orangutan in Indonesia which can be used as a conservation strategy for food management.

Methods

Study area

This research was conducted in October – December 2023, namely at the Research Station of the Sustainable Ecosystem Foundation Orangutan Conservation Program in the Batang Toru Forest Area (Camp Mayang) North Sumatra Province. Geographically located between 98° 53' - 99° 26' East Longitude and 02° 03' - 01° 27' North Latitude, Nitrogen and Fe laboratory tests were conducted at Research and Standardization Center, Medan.

Soil sampling

Tapanuli orangutan food trees were found using the line transect method with a length of 1000 m and a width of 100 m with a total area of 100,000 m² then the number of trees found was tabulated. This research has three transects, each of which measures environmental factors such as temperature and pH, while nitrogen and Fe are measured using soil tested in the laboratory.

Testing for nitrogen and iron in the soil

The nitrogen laboratory test consists of taking a soil sample of 500 grams at each point, drying it placing it in a Kjeldahl flask, then adding 25 mL of concentrated sulfuric acid (H_2SO_4) with a Kjeldahl tablet heated at 400ºC for 2 hours, then diluting it with distilled water and then titrating (Patti et al. 2013). Fe laboratory test, namely 1 g of sample is put into a volumetric flask then solvent is added such as HCL and HNO_3 then the solution is cooled for 10 minutes, after it has cooled, 25 mL of distilled water is added. The solution is measured using an Atomic Absorption Spectrophotometer with a wavelength for iron of 248.3 nm (Sahrawat, 2010).

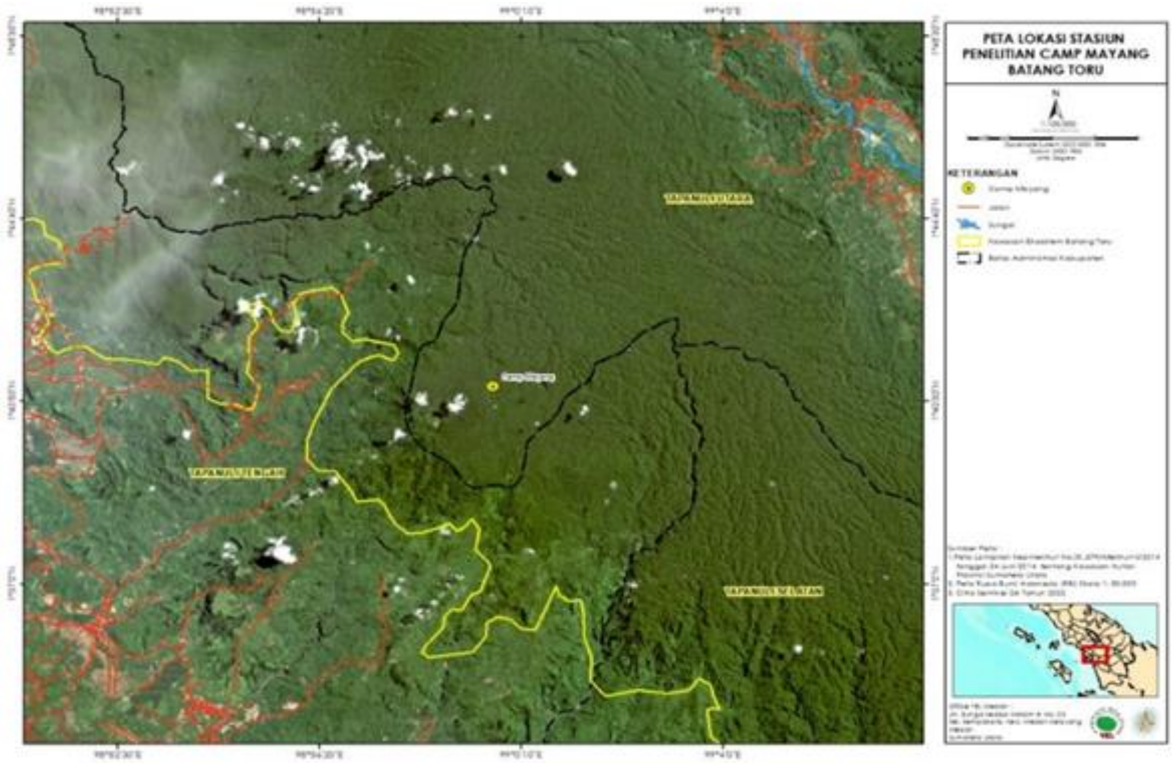

Figure 1. Map of research locations

Diversity index

The diversity index (H') according to Mazawin and Subiakto (2013) is calculated using the Shannon-Wiener formula as follows:

$$H' = -\sum_{i=1}^{s} (pi \ln pi)$$

$$pi = ni/N$$

Description:

H' = Diversity index value
N = Number of individuals of all species
ni = Number of individuals of type i
ln = Natural logarithm
s = Number of species in the community

The value of the species diversity index (H') according to Shannon-Wiener is defined as follows: According to Barbour et al., (1987) the diversity index value can range between 0-7, with the criteria: 0-2 (low), 2-3 (medium), and > 3 (high).

Research Sample In Silico

In silico phylogenetic studies using a database taken from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) using a random sampling method to ensure variability, there are three species of Tapanuli orangutan food in NCBI which are sourced from Indonesia, namely *Aglaia tomentosa*, *Artocarpus heterophyllus*, and *Campnosperma auriculatum*, while the other two species *Castanopsis argantea* and *Agathis borneensis* have no data in NCBI.

Phylogenetic analysis

The sequences were aligned using the ClustalW method in Mega 11.0 software. The phylogenetic tree was built using the Maximum Likelihood method with mega (Stamatakis, 2014). Phylogenetic analysis for Maximum Likelihood uses the GTRGAMMA+I algorithm with a bootstrap of 1000, the higher the bootstrap value, the more accurate the phylogenetics. Bootstrap results were visualized using FigTree v1.4.4 phylogenetic tree software and saved in jpg format (Stamatakis, 2014).

Table 1. Origin of *Aglaia tomentosa* the tRNA-Leu (*trnL*) and trnL-trnF genes found in the gene bank

No	Accession Code	Country
1	KF211803.1 *Aglaia tomentosa* voucher Greger HG543 (WU)	Thailand
2	KF211804.1 *Aglaia tomentosa* voucher Geesink et al. 7201 (K)	Thailand
3	KF211805.1 *Aglaia tomentosa* voucher Sinclair and Edano 9552 (K)	Philippines
4	KF211807.1 *Aglaia tomentosa* voucher Niyandhan et al. 297 (K)	Thailand
5	KF211808.1 *Aglaia tomentosa* voucher Greger and Vajirodya HG698 (WU)	Thailand
6	KF211809.1 *Aglaia tomentosa* voucher Greger and Vajirodya HG818 (WU)	Thailand
7	KF211810.1 *Aglaia tomentosa* subsp. cordata voucher Muellner et al. 2040 (K, BRUN)	Brunei Darussalam
8	KF211811.1 *Aglaia tomentosa* subsp. cordata voucher Burley et al. 396 (FHO)	Indonesia
9	KF211812.1 *Aglaia tomentosa* subsp. cordata voucher Pannell 2036 (FHO)	Indonesia
10	KF211813.1 *Aglaia tomentosa* subsp. tomentosa voucher Muellner et al. 2000 (K, BRUN)	Brunei Darussalam
11	KF211814.1 *Aglaia tomentosa* subsp. tomentosa voucher Muellner et al. 2011 (K, BRUN)	Brunei Darussalam
12	KF211815.1 *Aglaia tomentosa* subsp. tomentosa voucher Muellner et al. 2020 (K, BRUN)	Brunei Darussalam
13	KF211816.1 *Aglaia tomentosa* subsp. tomentosa voucher Muellner et al. 2036 (K, BRUN)	Brunei Darussalam
14	KF211817.1 *Aglaia tomentosa* subsp. tomentosa voucher Muellner et al. 2037 (K, BRUN)	Brunei Darussalam
15	KF211818.1 *Aglaia tomentosa* subsp. tomentosa voucher Muellner et al. 2043 (K, BRUN)	Brunei Darussalam
16	KF211840.1 *Aglaia lawii* subsp. oligocarpa voucher Muellner et al. 2003 (K, BRUN)	Brunei Darussalam

Table 2. Origin of *Artocarpus heterophyllus* rbcL genes found in the gene bank

No	Accession Code	Country
1	MK264364.1 *Artocarpus heterophyllus* voucher DMB 28	Sri Lanka
2	OK052735.1 *Artocarpus heterophyllus* voucher A4696	Malaysia
3	MH748842.1 *Artocarpus heterophyllus* voucher MO:6857146	United States of America
4	AB981765.1 *Artocarpus heterophyllus* chloroplast rbcL gene	Indonesia
5	OK052736.1 *Artocarpus integer* voucher A4698	Malaysia

Table 3. Origin of *Campnosperma auriculatum* matK genes found in the gene bank

No	Accession Code	Country
1	KJ708852.1 *Campnosperma auriculatum* voucher BT_0067704387	Indonesia
2	KJ708853.1 *Campnosperma auriculatum* voucher BT_0070234239	Singapore
3	KJ708854.1 *Campnosperma auriculatum* voucher BT_0070234201	Singapore
4	MH095572.1 *Campnosperma auriculatum* voucher KR2617	Singapore
5	JQ586473.1 *Mangifera indica* voucher BioBot06618	Costa Rica

Results and Discussion

There are five species of Tapanuli orangutan food trees found, namely *Aglaia tomentosa*, *Artocarpus heterophyllus*, *Campnosperma auriculatum*, *Castanopsis argantea* and *Agathis borneensis* which can be seen in Figure 2.

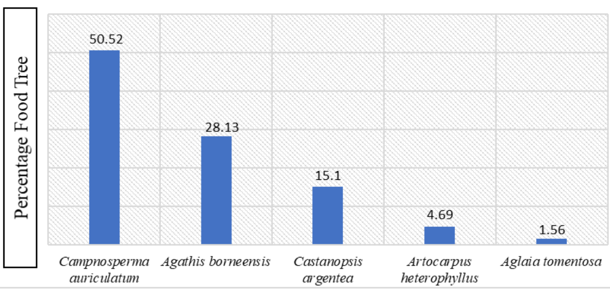

Figure 2. Percentage of Tapanuli orangutan food trees

Figure 2 shows that the most common food type found in Tapanuli orangutans was *C. auriculatum*

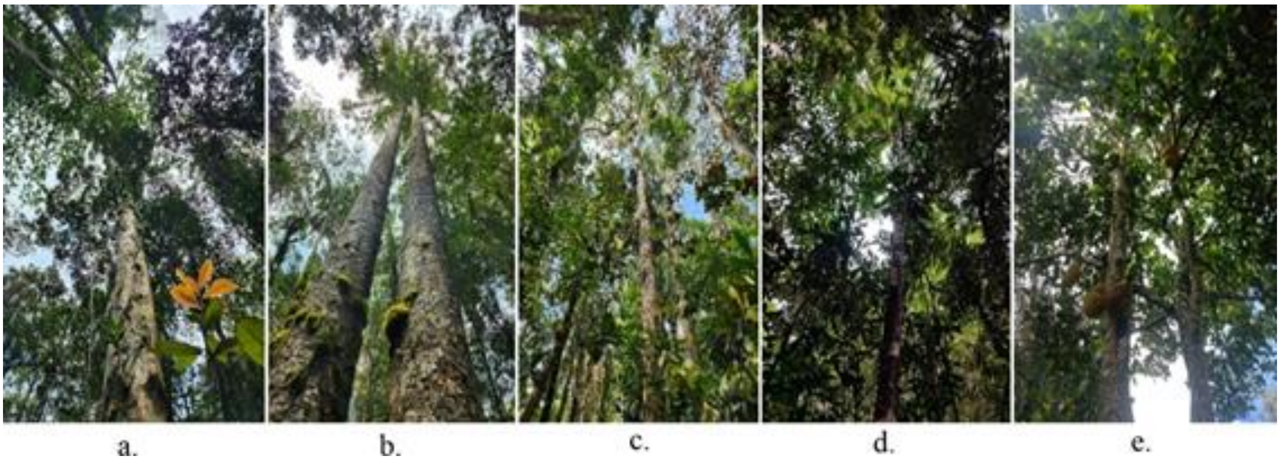

Figure 3. Tree of a. *Castanopsis argantea* b. *Agathis borneensis* c. *Campnosperma auriculatum* d. *Aglaia tomentosa* e. *Artocarpus heterophyllus*

(50.52%) and the least was *A. tomentosa* (1.56%). *Campnosperma auriculatum* grows and develops in Tapanuli forests at altitudes around 500-1000 meters above sea level. The *C. auriculatum* plant is up to 40 m tall and 90 cm in trunk diameter, with a wood-specific gravity ranging from 310-600 kg/m3 (average 435 kg/m3). The growing area of this plant is below the altitude of the Batang Toru forest, which is 850-1,100 m above sea level (Soerianegara and Lemmens, 1994).

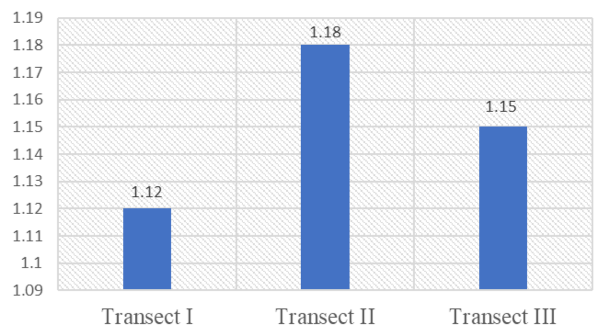

Figure 4. Diversity index of Tapanuli orangutan food trees

Tapanuli orangutan food tree diversity index is in the low category (1.12 - 1.18) because the value is below 2 as shown in Figure 4. According to Barbour et al., (1987) the diversity index value can range between 0-7, with the criteria: 0-2 (low), 2-3 (medium), and > 3 (high). One of the reasons why the diversity of food trees is low is that humans use it, for example, jackfruit (*Artocarpus heterophyllus*), which is known to include: wood is used as a building material and dye for silk fabrics, the fruit can be eaten, the seeds are made into flour while the leaves are used as medicine. This use causes the number of jackfruit trees in the forest to decrease, resulting in less food for orangutans (Rosyida and Subiyati, 2018).

The abundance of Tapanuli orangutan food trees also depends on environmental factors such as temperature, pH, and nutrients (Nitrogen and Fe) that fulfill plant growth. Measurements of field conditions can be seen in Table 4.

Table 4. Environmental parameters of the Batang Toru Forest

Parameter	Transect I	Transect II	Transect III
Temperature (°C)	25,8	25,2	24,9
pH	6,6	6,8	6,7
Nitrogen (%)	0,27	0,3	0,34
Fe (%)	2,12	2,1	0,23

Based on the temperature measurements above, there are no significant differences between the three transects, the above temperatures were taken during the day, including optimal conditions (24-27°C) for plant growth. Soil temperature in the root area is important for plant growth and development because it influences physiological processes in plant roots, such as water and mineral nutrients from the soil. Therefore, the temperature in the Batang Toru Forest helps the growth of Tapanuli orangutan food trees (Li et al., 2017).

The soil pH of the Batang Toru forest (6.6-6.8) is in neutral conditions, namely around 6.5-7.8, where the soil pH greatly influences plant growth, such as the availability of nutrients. The availability of nitrogen levels in the soil is essential, especially for plant growth, the nitrogen obtained is around 0.27-0.34%, which is included in the low category because it is below 1%, causing low diversity Tapanuli orangutan food tree. Some of the functions of the nitrogen element for plants are to increase plant growth and healthy leaf growth where the leaves as the organ that makes up the plant function to receive and absorb light and are the part of the plant that functions as a place for photosynthesis so that it becomes a place for the production of photosynthesis for all parts of the plant and increases protein levels, in the plant body (Fathi, 2022).

Fe levels from the research results are known to be in the range of 0.23-2.12%, if the Fe levels are in the range 1-9 then the plant will experience Fe poisoning. Excess Fe in the soil can cause tree roots to be covered in Fe so that they cannot absorb nutrients, so the quality of the tree will be compromised (El-temsah et al., 2014). Tapanuli orangutan conservation strategies can be carried out through in silico phylogenetic studies on Tapanuli orangutan food tree species as shown in the image below.

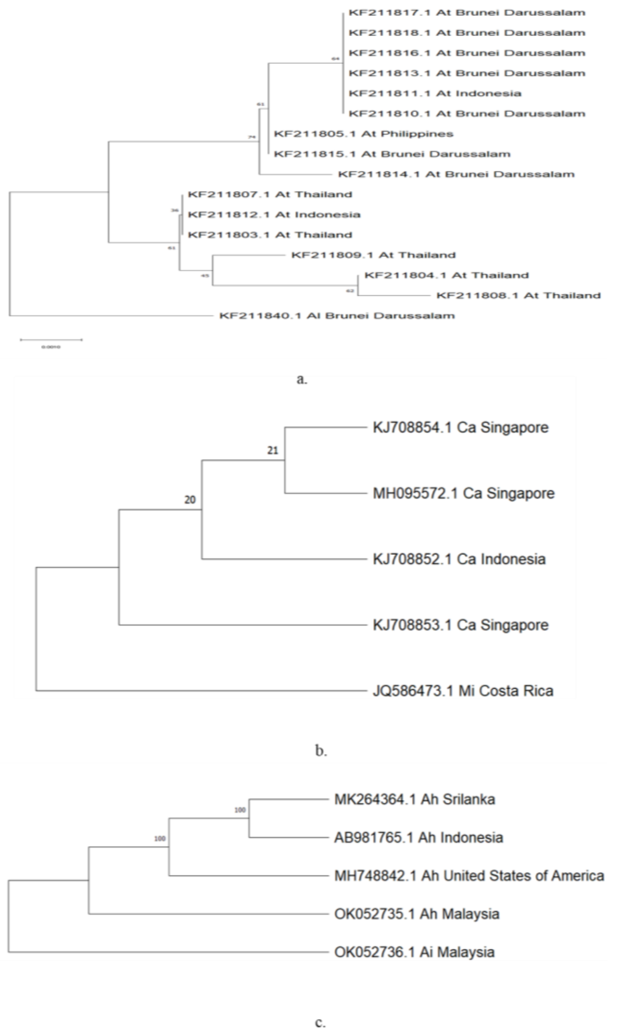

Figure 5. Phylogenetic tree of a. *Aglaia tomentosa* (At). b. *Campnosperma auriculatum* (Ca). c. *Artocarpus heterophyllus* (Ah). *Aglaia lawi* (Al), *Mangifera indica* (Mi), and *Artocarpus interger* (Ai) were used as outgroups.

According to Figure 5, the phylogenetic tree shows the relationship between DNA sequences in the studied plants compared to other plants from the GenBank database. What is produced is monophyletic, which means that the group has one ancestor who passed on genetic, morphological, and biochemical characteristics to all its descendants. This makes monophyletic members very closely related to each other (Ningrum and Chasani, 2021). This statement is under the phylogenetic tree formed because all the members in the phylogenetic tree (except the out-group).

Relationship between *Aglaia tomentosa*, *Campnosperma auriculatum*, *Artocarpus heterophyllus* with a bootstrap value of 100%. The bootstrap value is considered high because according to li et al (2020), it can be trusted with a bootstrap value of 90% and is not trusted with a bootstrap value of 25%. Bootstrap analysis with values of 70% or higher indicates a reliable grouping.

Maximum likelihood method analysis using the Kimura-2 model reconstructs relationships between species based on the length of branch lines (Tamura et al., 2021), and different line lengths indicate the level of evolution of each species (Nikmah et al., 2016). A longer line shows a further evolutionary distance, while a shorter line shows the closer the evolutionary distance of a species.

Based on the reconstruction of the *Aglaia tomentosa* phylogenetic tree sample, the highest branching value is 74 and the lowest is 36. The higher the branching value, the closer the relationship, and vice versa, the lower the branching value, the further the relationship (Talley et al., 2016). From the research results, it is known that *Aglaia tomentosa* originating from Brunei Darussalam has a very close relationship with Indonesia.

The phylogenetic tree of the *Campnosperma auriculatum* sample has the highest branching value with a value of 21 and the lowest with a value of 20. From the results of this research, it is known that *Campnosperma auriculatum* which originates from Singapore has a very close relationship with Indonesia.

The phylogenetic tree reconstruction of the *Artocarpus heterophyllus* sample is an ingroup, with the highest branching value with a value of 100. From the results of this research, it is known that *Artocarpus heterophyllus* which originates from Sri Lanka has a very close relationship with Indonesia. The results of this research will be useful for providing recommendations for food management conservation strategies as initial data for Tapanuli orangutan conservation efforts.

The in silico approach to habitat management refers to the use of computer simulations, mathematical models, and data analysis to understand and manage ecosystems. Utilizing big data to analyze patterns and trends in species distribution, throughout the country so that we can evaluate risks to the Tapanuli orangutan's habitat due to human activities, such as illegal logging of trees so that we can plan restoration efforts, including selecting species that are most closely related to the Tapanuli orangutan's food trees. is in Indonesia to be planted.

Conclusion

Five species of food for Tapanuli orangutans in Batang Toru consist of *Aglaia tomentosa, Artocarpus heterophyllus, Campnosperma auriculatum, Castanopsis argantea* and *Agathis borneensis* with the highest percentage found being *Campnosperma auriculatum*. The diversity index is included in the low category due to environmental factors such as Nitrogen and Fe. The results of in silico phylogenetic studies show that the closest relationship is to the food trees of the Tapanuli orangutan in Indonesia, such as *Aglaia tomentosa* with Brunei Darussalam, *Campnosperma auriculatum* with Singapore, and *Artocarpus heterophyllus* with Sri Lanka.

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