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Morphology, Morphometry and Analysis of the CO1 Gene in Silico Apis dorsata Binghami from Southeast Minahasa

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Abstrak

Tujuan penelitian ini adalah untuk mengetahui ciri fisik dan ukuran Apis dorsata Binghami asal Kabupaten Minahasa Tenggara. Selain itu, kami akan membuat pohon filogenetik untuk Apis dorsata menggunakan data komputasi yang diperoleh dari bank gen NCBI. Penelitian ini menggunakan metodologi deskriptif dengan pendekatan kualitatif, bertujuan untuk menggambarkan secara sistematis dan akurat fakta, kualitas, dan korelasi fenomena yang diteliti dengan menggunakan deskripsi, foto, atau penggambaran secara rinci. Penelitian dilakukan di Desa Maulit, Kecamatan Pasan, Kabupaten Minahasa Tenggara. Setelah menganalisis hasilnya, ditentukan bahwa analisis morfologi, morfometrik, dan molekuler menghasilkan identifikasi yang identik. Penelitian ini menggunakan gen mitokondria 16S rRNA dan CO1 untuk tujuan analisis hubungan. Pemanfaatan gen mitokondria 16S rRNA dan CO1 dalam metode analisis dibenarkan karena banyak keunggulannya dibandingkan gen lain. Hal ini menunjukkan bahwa ciri-ciri morfologi spesies A. dorsata Binghami sebanding dengan spesimen dari tempat lain, meskipun morfometrinya menunjukkan variasi ukuran dibandingkan dengan spesies yang sama yang tercatat di wilayah lain. Selain itu, ciri-ciri molekuler terlihat jelas dalam jarak filogenetik dan genetik yang ditetapkan.

Kata kunci: *Morfologi, Morfometri, Apis Dorsata Binghami.*

Abstract

The objective of this study is to examine the physical characteristics and measurements of Apis dorsata Binghami from Southeast Minahasa Regency. Additionally, we will create a phylogenetic tree for Apis dorsata using computational data obtained from the NCBI gene bank. The research employed a descriptive methodology with a qualitative approach, aiming to systematically and accurately depict the facts, qualities, and correlations of the phenomena under investigation using detailed descriptions, photographs, or depictions. The research was conducted in Maulit Village, Pasan District, Southeast Minahasa Regency. Upon analyzing the results, it was determined that the morphological, morphometric, and molecular analyses yielded identical identifications. The study employed the mitochondrial 16S rRNA and CO1 genes for the purpose of relationship analysis. The utilization of the

mitochondrial 16S rRNA and CO1 genes in the analysis method is justified due to their numerous benefits over other genes. This demonstrates that the morphological traits of A. dorsata Binghami species are comparable to specimens from other places, although the morphometry exhibits size variations in comparison to the same species documented in other regions. Additionally, molecular features are evident in the phylogenetic and genetic distances that are established.

Keywords: *Morphology, Morphometry, Apis dorsata Binghami.*

INTRODUCTION

As a tropical region, Indonesia is rich in plant and animal diversity. In Indonesia it is estimated that there are more than 7 species of endemic honey bees or the largest in the world. Two endemic species, including those found on the island of Sulawesi, are Apis dorsata binghami and Apis nigrocincta. Honey bees are a species from the insect class that is very beneficial ecologically for humans and has a very important role. The honey bee A. dorsata Binghami is an endemic honey bee to Indonesia, living naturally in the forests of Sulawesi. Bees have lived for around 125 million years and their evolution has succeeded in occupying almost all habitats on earth. However, until now the taxonomy of honey bees is still very confusing. This is due to the very wide distribution of honey bees (their biogeography), from temperate climates to tropical regions. Many honey bee species are protected geographically, among other things, influenced by the behavior of honey bees as social insects (Mokosuli, 2013).

Honey bees can be grouped into 4 types, namely giant honey bees (Apis Dorsata), western honey bees (Apis Mellifera), eastern honey bees (Apis Cerana), and dwarf honey bees (Apis Trigona/Flora). Apis dorsata binghami originating from Kombi has an average body length that is longer than Apis dorsata binghami which lives in the Plaslaten, Kaweruan and Wasian areas of North Minahasa (Mokosuli et.al. 2013). As with other members of the insect class, the body structure of honey bees consists of three parts, namely the head, thorax and abdomen. On the head there are compound eyes that are used to see long distances, directing the bee to fly towards the sun. Each compound eye consists of 3000 to 5000 omatidia units. In the rear condition, there is a special antenna cleaning structure, while in the front condition, there is a structure called the pollen box. The fore and hind wings are held together by approximately small hooks located along the edges and back of the wings. A bee's wings can move 200 times per second. On the stomach there are seven visible segments. The first segment is very small, the seventh segment of worker bees and queens has a stinger (stinger). In the stomach there are also glands that secrete wax to form honeycombs. The stinger is modified into an ovipositor, as it is only found in females.

The results of morphometric studies and genetic analysis, namely mitochondrial DNA polymorphism, prove that A. nigrocincta is a different species from A. cerana in Sulawesi. A. nigrocicnta has the greatest frequency of paternity compared to A. dorsata, A. laboriosa and A. nuluensis. Maa (1953). states that A. nigrocincta makes nests in the forests of Sulawesi but is also found in Sangihe and the Philippines. A. nigrocincta colonies are generally found at altitudes above 400 m, while A. cerana colonies are found at altitudes below 400 m. A.

cerana is found from coastal areas to forests adjacent to agricultural areas (Mokosuli, 2013; Mokosuli et al. 2019.)

Variations in size or morphometric characteristics of worker bees, whether large or small in size, are considered a form of morphological adaptation to different environmental conditions. These differences in environmental conditions cause differences in flying and foraging activities which affect the size of the hornbill. Changes in temperature or environmental conditions will cause living creatures to adapt morphologically as a form of adjustment to the environment and differences in flying and foraging activities. (Novita et al. 2013).

Phylogenetic analysis was carried out between 18 honey bee samples found in the study by comparing BLAST (Basic local alignment searching tools) data in GenBank. In a system using the CO1 gene (Cytochrome oxidase subunit 1), the genetic distance threshold for a species is 3% (Nur'aini. 2021). If the genetic distance of two individuals or groups of individuals exceeds this value, then they are not in the same species group (different species). Based on analysis using the mitochondrial CO1 gene, the first clade can be seen that samples of A. nigrocincta Central Sulawesi 1, A. nigrocincta Central Sulawesi 2, and A. nigrocincta Central Sulawesi 3 are in the same clade as the A. nigrocincta sample from Genbank with accession number MK880239 .1 which comes from North Sulawesi with a bootstrap value of 94%, compared to sample DQ020233.1 which comes from North Sulawesi with the same bootstrap value of 94%. Furthermore, it can be seen from the samples that A. cerana from Central Sulawesi has the closest genetic relationship to samples KY769042.1 which comes from West Kalimantan and KY769051.1 which comes from South Kalimantan with a bootstrap value of 70% (Nur'aini. 2021)).

This research needs to be carried out because until now there has been no research on morphology, morphometry and in silico CO1 gene analysis of A.dorsata Binghami from Southeast Minahasa, therefore the author is interested in adopting the title of this research. This study aims to analyze the morphology and morphometry of Apis dorsata binghami from Southeast Minahasa Regency and construct a phylogeny of Apis dorsata based on in silico data from the NCBI gene bank.

Morphometric analysis compared with in silico analysis based on the CO1 gene in Apis dorsata Binghami has never been reported. The Southeast Minahasa forest area is the habitat of Apis dorsata Bingham. Morphometric research on Apis dorsata Binghami from Minahasa and North Minahasa has been carried out (Mokosuli, 2013). Morphometric characterization is important to compare genetic data with phenotypic data.

Based on the results that have been analyzed, it was found that morphological, morphometric and molecular analyzes produced the same identification. Relationship analysis in this study used the mitochondrial 16S rRNA and CO1 genes. This study aims to analyze the morphology and morphometry of Apis Dorsata Binghami from Southeast Minahasa and construct a phylogeny of Apis Dorsata based on in silico data from the NCBI gene bank.

METHOD

This research uses a descriptive method with a qualitative approach, namely a research procedure that produces descriptive data in the form of words and images to describe the problem and research focus. This is in accordance with what Lexy J. Moleong stated that the data collected in qualitative research is an approach that does not use basic statistical work, but is based on qualitative evidence.

The method used to collect data is a descriptive analytical method designed to obtain information about morphology, morphometry and in silico CO1 gene analysis of Apis dorsata Binghami from Southeast Minahasa Regency. The aim of this descriptive research is to create a systematic, factual and accurate description, picture or painting of the facts, properties and relationships between the phenomena being investigated. This research will be carried out at the Unima Biology Laboratory, Tondano District, Minahasa Regency, North Sulawesi Province. The research was carried out from April 2023 to May 2023.

The tools used in this research were a Hirox microscope, biological microscope, temo cycle, clear plastic and XSZ-107 BN binocular microscope, bee catching net, bee collection container, surgical tools, petridis, micropipette and glass object. The materials used in this research were A. dorsata binghami bees from Southeast Minahasa Regency, (12 samples for morphological and morphometric analysis, 15 for molecular analysis), 96% ethanol, label paper, sample box, xylol, bionformatics application/software (Mega 11, Geneus, Biometra, Bioedit 7.0) credit/internet network.

This research used the honey bee Apis dorsata binghami from Southeast Minahasa. The method is to first take the honey bees by placing a clear plastic bag on the nest gate and the nest will come out trapped in the bag.

Identification of honey bee specimens based on morphological characters is based on characteristics such as body structure, overall, thorax, abdomen, head, antennae, wings and legs. Furthermore, five morphological characters of honey bees were used as parameters for measuring morphometric characters. Morphological and morphometric analyzes were carried out at the FMIPAK Biology Laboratory, Manado State University

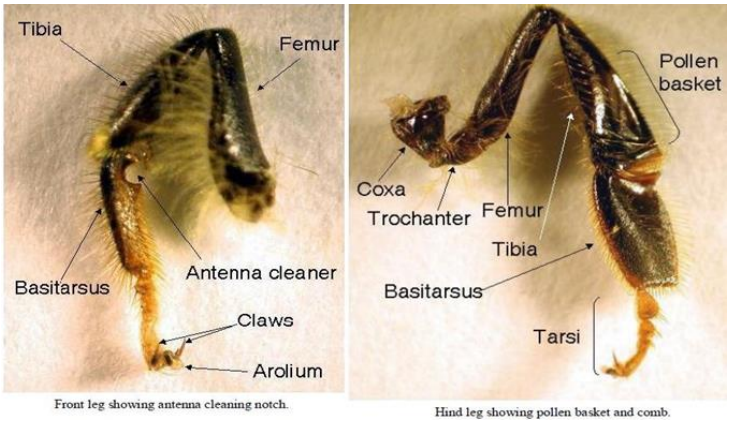

Figure 1. Morphology of bee limbs A. Morphology of forelimbs B. Morphology of hind limbs.

Figure 2. Comparison of worker bees (left), fertile male bees (middle) and queen bees (right) from Apis dorsata

Several specimens of each species were taken for morphological measurements and observations, especially color. Draw parts such as the overall body structure of the chest, abdomen, head, antennae, wings and legs for each species. Morphological and morphometric analysis was carried out using an XSZ-107 BN binocular microscope with an Optilab viewer and Image Raster software.

In silico analysis of the Apis dorsata Binghami CO1 gene using the Apis dorsata Binghami CO1 gene sequence from Southeast Minahasa author Prof. Dr. Yermia S. Mokosuli SSi, MSi. The CO1 gene sequence was then analyzed using the MEGA 12 program to determine the forward and reverse sequence consensus areas. Next, the consensus sequence is done in BLAST. One hundred BLAST sequences were then used to construct a phylogeny. The phylogeny formed was analyzed descriptively.

RESULT AND DISCUSSION

Samples were obtained from Maulit village. Maulit Village is one of the villages in Pasan subdistrict, Southeast Minahasa Regency. Maulit village is located at latitude 1.011400000000 and longitude 124.760800000000.

a b c

Figure 3. a. nesting location, b. Nest of A. dorsata Binghami, c. bee samples

Just like other members of the insect class, the body structure of the bee A.dorsata binghami consists of three parts, namely the head, thorax and abdomen. On the head there are compound eyes which are used to see long distances, directing the bee to fly towards the sun. The body of A. dorsata binghami is blackish. The clypeus has a black head covered with brownish hair. The dark black antenna consists of the scapula and pedicel. The mandibles are blackish on the lower half of the tip and yellow at the base near the malar area. The black thorax is covered with brownish yellow to black hairs on the mesonotum. The femurs of the hind legs are black covered with brownish hair. Tegula is dark black. The tibia on the leg is black, the rear tibia is slightly hairy. The propodeum (first abdominal segment) is yellow, hairless, smooth and shiny, figure 3.

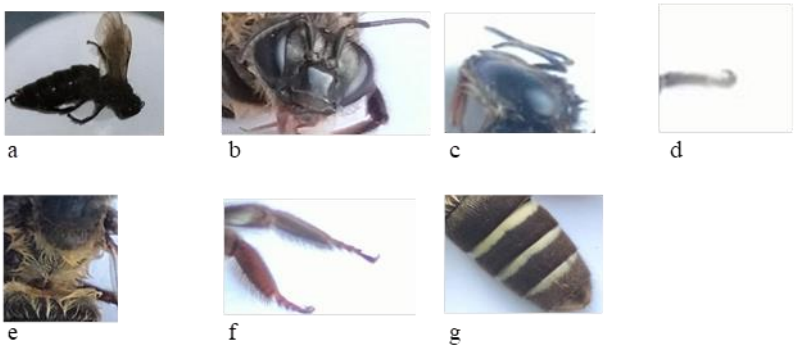

Figure 4. Morphological characters of the bee A. dorsata binghami; a. full body image, b. head, c. eyes, d. proboscis, e. thoracic, f. hind legs, g. abdomen.

Table 1. The morphometry of A. dorsata Binghami obtained varied in size

Individu	Karakter morfometri (milimeter)						
	PT	PSD	LSD	Pabd	PFTB	PTTB	Pprobosis
1	3.1	0.2767	0.0919	0.1836	0.2802	0.0873	0.3525
2	2.0	0.1227	0.1591	0.1895	0.1476	0.1013	0.3137
3	3.1	0.1545	0.0709	0.1695	0.0689	0.0959	0.4086
4	2.9	0.1005	0.0838	0.2449	0.0578	0.1344	0.4018
5	1.8	0.1733	0.0775	0.1483	0.146	0.0693	0.3942
6	3.2	0.1668	0.0763	0.2486	0.1353	0.0874	0.2267
7	4.3	0.0990	0.1518	0.1497	0.0742	0.1096	0.2972
8	3.1	0.0802	0.0954	0.1533	0.1019	0.1180	0.2492
9	3.8	0.0871	0.1681	0.1154	0.1679	0.0671	0.0685
10	3.5	0.1370	0.1626	0.2238	0.0769	0.0487	0.3562
11	2.9	0.1673	0.0915	0.1126	0.1774	0.0935	0.2060
12	4.3	0.1941	0.0959	0.1494	0.1623	0.1036	0.1285
13	3.2	0.1155	0.0925	0.2263	0.1453	0.1335	0.1169
14	2.6	0.0782	0.1596	0.1338	0.1529	0.1097	0.3649
15	3.2	0.1529	0.067	0.1228	0.1346	0.1151	0.3038

The body structure of the bee A. dorsata binghami consists of three parts, namely the head, thorax and abdomen. On the head there are compound eyes which are used to see long distances, directing the bee to fly towards the sun. The body of A. dorsata binghami is

blackish. The clypeus has a black head covered with brownish hair. The dark black antenna consists of the scapula and pedicel. The mandibles are blackish on the lower half of the tip and yellow at the base near the malar area. The black thorax is covered with brownish yellow to black hairs on the mesonotum. The femurs of the hind legs are black covered with brownish hair. Tegula is dark black. The tibia on the leg is black, the rear tibia is slightly hairy. The propodeum (first abdominal segment) is yellow, hairless, smooth and shiny.

A phylogenetic analysis was conducted using 18 honey bee samples obtained in the study, together with comparison data from BLAST results in GenBank. The phylogenetic analysis was conducted using the nucleotide sequence of the mitochondrial 16S rRNA and CO1 genes. Indicates the formation of many clades, with each species belonging to the same clade. This is corroborated by a substantial bootstrap value ranging from 98-100% and a minimal genetic distance, specifically less than 3.5%, which signifies that the individual remains within the same species. As stated by Zemlak et al. (2009). The threshold for genetic distance within a species is set at 3.5%.

The investigation conducted indicates that morphological, morphometric, and molecular analyses yield identical identification results. The study employed the mitochondrial 16S rRNA and CO1 genes for the purpose of relationship analysis. The utilization of the mitochondrial 16S rRNA and CO1 genes in the analysis method is justified due to their numerous benefits over other genes. Whitfield and Cameron (1998) stated that the mitochondrial 16S rRNA and CO1 genes provide the most valuable information for analyzing the evolutionary relationships of closely related species or populations, as well as between tribes, subfamilies, and families. In addition, the utilization of 16S rRNA and CO1 genes can offer resolutions to developing morphological challenges, such as cryptic species and sibling species. Furthermore, the choice of mitochondrial 16S rRNA and CO1 genes in this study was determined by the abundance of Apis bee databases accessible on the NCBI website (https://www.ncbi.nlm.nih.gov). The majority of Apis bee species sequences in the NCBI data predominantly employ the 16S rRNA and CO1 genes, making them suitable for sequence analysis or comparison in this study.

CONCLUSION

Morphologically, the character of the species A. dorsata Binghami is morphologically similar to similar specimens from other regions, while the morphometry has variations in size compared to the same species that have been reported in other regions. Meanwhile, molecular characters can be seen in the phylogenetic and genetic distances that are formed. Based on research to determine morphology, morphometry and analysis of the CO1 gene in Silico Apis dorsata Binghami from Southeast Minahasa, valid data was found.

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