Diversity of pollen collected by *kelulut* bee (*Heterotrigona itama*) from South Kalimantan

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**Abstract.** Pollen is an essential source of proteins and vitamins for the bee diet. In addition, the diversity of pollen found in honey provides information about plant species that illustrate the bees foraging activity. This study aims to identify pollen in *kelulut* (*Heterotrigona itama*) beehives and identify its botanical origins. This study was conducted in two bee farming locations which are Layuh Village, Hulu Sungai Tengah Regency, and Gambah Luar Village, Hulu Sungai Selatan Regency, South Kalimantan Province. Pollen was collected from the beehives and stored in an air-tight container. Collected pollen was identified using the acetolysis method. According to this study, there were 14 pollen types found, where only 9 of them can be identified. In the first locations (Layuh Village), most pollen came from Asteraceae (38.8%) and Arecaceae (31.2%) families. While at the Gambah Luar village, pollen was dominated by those who originated from the Rubiaceae family (31.2%). This result also revealed that *Heterotrigona itama* is a multi-floral bee and has various diets consisting of fruit, ornamental, and herbaceous plants. The information generated from this study can be used as plant enrichment recommendations in the honey bee farming areas.

1. Introduction

The honey bee is one of the important Non-Timber Forest Products (NTFPs) in South Kalimantan. The availability of forest areas and plantations in South Kalimantan could support the development of honey bee farming by providing its food resources. There are three different species of stingless bee in the South cultivated by the local farmers in Kalimantan, namely *Heterotrigona (Trigona) itama*, *H. levicaps*, and *Geniotrigona thorasica*. The demand for honey produced by the stingless bee is high. Hence this NTFPs is very potential to be developed by farmers. In addition, the stingless bee is easier to cultivate and more resistant to disease than *Apis melifera* bee.

Pollen is an essential protein source for the bee that supports the bee’s colony life. In addition, pollen is also a source of amino acids, various vitamins (A, B, C, D, and E), carbohydrates, lipids, and other micronutrients [1]. A bee collects pollen from various plants and stores them in the beehives. In their foraging activity, stingless bees prefer to gain pollen and food sources near their beehives to reduce the energy used during the foraging activity and avoid the risk of environmental disturbances.
such as predator and high-temperature conditions [2, 3]. The study of pollen contained in the honey is known as Melissopalinogy [2, 4, 5]. This study aims to identify pollen in stingless bee honey and its botanical origin [6]. Indirectly, the study of Melissopalinology could provide information about plant species enrichment for honey bee farmers and ensure honey quality.

2. Materials and Methods
The research was carried out from September to December 2019. Sampling was conducted in two honey bee farming locations (Table 1), and the samples were collected during the dry season in September 2019. Pollen was collected from the beehives and stored in an airtight container. In addition, vegetation surveys were done to identify the plant species located near to bee farming location.

Table 1. Study location.

| Location | Village | District | Regency | Geographical information | Altitude (m asl) | Vegetation condition | Total area (Ha) |
|----------|---------|----------|---------|--------------------------|-----------------|---------------------|----------------|
| A        | Layuh   | Batu     | Hulu    | 115°28’ 37.738” E; 2°36’ 30.054” S | 27              | rubber plantation, agroforestry area | 10             |
|          |         | Benawa   | Sungai  |                           |                 |                      |                |
|          |         |          | Tengah  |                           |                 |                      |                |
| B        | Gambah  | Kandangan| Hulu    | 115°16’ 18.237” E; 2°45’ 2.618” S | 22              | Rice field back yard garden, settlement | 0.18           |
|          |         | Luar     | Sungai  |                           |                 |                      |                |
|          |         |          | Selatan |                           |                 |                      |                |

Pollen samples were sent and identified using the acetolysis method in the Palynology Laboratory of Jambi University, Jambi province [4, 6]. Acetolysis is a method of purifying and staining pollen through strong acids to identify the pollen (Figure 1). The sample in the container was diluted with warm destile water, transferred into a water bath, and heated for about 20-30 minutes up to 90°C then sieved. Next, samples were centrifuged for 4 minutes at 3500 rpm to obtain supernatant. The supernatant was added with 4 ml of acetic acid and centrifuged for 5 minutes at 3500 rpm, and then the sample was put in a beaker glass. Afterward, the supernatant was removed and then added with a mixture of acetic anhydride ((CH₃CO)₂O) and concentrated sulphuric acid (H₂SO₄) (9:1). Subsequently, the sample is shifted into the water bath with a temperature of 60°C for up to 10 minutes. Next, the tube is centrifuged again at 12,000 rpm for up to 3 minutes. Finally, the supernatant is discarded and then added aquades. A drop of this mixture was placed onto a microscope slide. Pollen microscopic slides were observed using a binocular microscope at 40x magnification.
3. Results and Discussion

Information on pollen gathered by a stingless bee could benefit farmers since it could provide information on plant species frequently visited and utilized by bee colonies. This information is needed when a farmer initiates plant enrichment to boost honey productivity. The foraging activity and plant selection were determined by several variables: season, food source availability, colony condition, food, and food source location. Furthermore, pollen preference by bees depends on color, shapes, odor, flower shapes, and size [3, 7, 8].

The Stingless bee is well known for its role as a general forager [9]. Based on the research result, it is known that stingless bee’s honey is categorized as multi-floral honey. In the first location (Layuh village), nine types of pollen were identified to family, four pollen types were identified to species level, and one type of pollen was unidentified (Table 2). Furthermore, pollen from the Asteraceae family (38.8%) dominated the pollen of stingless bees in the location, followed by pollen from Arecaceae (29.4%) and Macaranga sp. (8.4%). There are several categories of pollen frequency found in the bee honey which are categorized as very frequent pollen (>45%), frequent pollen (16-45%), isolated pollen (4-15%), and rare pollen (< 3%) [4]. Therefore, the pollen of the Asteraceae plant has become frequent pollen in the honey collected in Layuh Village. However, pollen from Dipterocarpaceae (3.6%), Avicenniaceae (2.4%), Myrtaceae (1.8%), and Malphigiaceae (2.4%) are classified as rare pollen. Moreover, this result revealed that Heterotrigona itama has various diets consisting of fruit, ornamental, and herbaceous plants.

Table 2. Pollen types found in the honey from Layuh village honey-bee farm.

| Pollen type | Family      | Genus/Species | Total pollen found | Percentage (%) |
|-------------|-------------|---------------|--------------------|---------------|
| Type 1      | Asteraceae  | Unidentified  | 194                | 38.8          |
| Type 2      | Arecaceae   | Arenga sp     | 147                | 29.4          |
| Type 3      | Unidentified| Unidentified  | 66                 | 13.2          |
| Type 4      | Avicenniaceae| Unidentified | 12                 | 2.4           |
| Type 5      | Arecaceae   | Cocos nucifera| 8                 | 1.6           |
| Type 6      | Euphorbiaceae| Macaranga sp | 42                 | 8.4           |
| Type 7      | Malphigiaceae| Lophantera longifolia | 4 | 0.8 |
| Type 8      | Myrtaceae   | Unidentified  | 9                  | 1.8           |
| Type 9      | Dipterocarpaceae | Unidentified | 18                | 3.6           |
Unlike the first location, fewer pollen types [5] were identified in the second location. One type was identified to species level, two types to family, and two types were unidentified (Table 3). Pollen of *Oldenlia* sp. (31.2%), unidentified pollen (22.4%), and Rubiaceae (22.2%) were considered frequent pollen. The figure of pollen is present in Figure 2.

**Table 3.** Pollen types found in the honey from Gambah Luar honey-bee farm.

| Pollen type | Family      | Genus/Species | Total pollen found | Percentage (%) |
|-------------|-------------|---------------|--------------------|----------------|
| Type 1      | Dipterocarpaceae | Probably Hopea sp. | 21                | 4.2            |
| Type 2      | Rubiaceae   | *Oldenlia* sp.  | 156               | 31.2           |
| Type 3      | Unidentified | Unidentified   | 70                 | 14.0           |
| Type 4      | Unidentified | Unidentified   | 30                 | 6.0            |
| Type 5      | Unidentified | Unidentified   | 112                | 22.4           |
| Type 6      | Rubiaceae   | Unidentified   | 111                | 22.2           |

**Figure 2.** Pollen types found in a sample of honey produced by the stingless bee in South Kalimantan: (a) Asteraceae, (b) Arecaceae/Arenga sp. (c) Avicenaceae (d) *Cocos nucifera* (e) *Macaranga* sp (f) Malphigiaeae (*Lophantera longifolia*) (f) Myrtaceae (g) Dipterocarpaceae/Hopea sp. (h) Rubiaceae/ *Oldenlia* sp.
| No | Layuh Village | Gambah Luar Village |
|----|---------------|---------------------|
|    | Local name   | Scientific name     | Family | Local name | Scientific name | Family |
| 1  | Rumput bambu | Pogonatherum paniceum | Poaceae | Jotang     | Synedrella nodiflora | Asteraceae |
| 2  | Rumput israel| Asystasia intrusa   | Acanthaceae | Rumput Paitan | Conjugatum | Poaceae |
| 3  | Gewor        | Commelina benghalensis | Commelinaeae | Padi      | Oryza sativa | Poaceae |
| 4  | Rumput Keladingan | Scleria purpurascens | Cyperaceae | Rumput israel | Asystasia intrusa | Acanthaceae |
| 5  | Saveg        | Amorphophallus paeonifolius | Araceae | Rumput teki | Cyperus rotundus | Cyperaceae |
| 6  | Sirih hutan  | Piper aduncum L.    | Piperaceae | Matahari kecil | Melampodium divaricatum | Compositae |
| 7  | Karet        | Hevea brasiliensis  | Euphorbiaceae | Aren | Arenga pinnata | Arecaleae |
| 8  | Bidara       | Ziziphus mauritiana | Rhamnaceae | Sirih hutan | Piper aduncum L. | Piperaceae |
| 9  | Bunilan      | Ageratum conyzoides | Asteraceae | Pacing tawar | Costus speciosus | Costaceae |
| 10 | Meniran      | Phyllanthus urinaria | Euphorbiaceae | Rumput Bantak | Leersia hexandra S. | Poaceae |
| 11 | Sungkai      | Peronema canescens | Verbenaceae | Pisang | Muta sp. | Musaceae |
| 12 | Mahang gajah | Macaranga gigantea | Euphorbiaceae | Nyawa | Ficus variegata | Moraceae |
| 13 | Mahang damar | Macaranga trifolia  | Euphorbiaceae | Ramania | Bouea macrophylla | Anacardiaceae |
| 14 | Jualing      | Micromelum minutum | Rutaceae | Sungkai | Peronema canescens | Verbenaceae |
| 15 | Pisang       | Musa spp.           | Musaceae | Kelapa | Cocos nucifera | Arecaleae |
| 16 | Jambu-jambu  | Syzygium           | Myrtaceae | Mangga | Mangifera indica | Anacardiaceae |
| 17 | Bunga kupu-kupu | Bauhinia purpurea | Fabaceae | Pinang | Areca atechu | Arecaleae |
| 18 | Tarap        | Artocarpus odoratissimus | Moraceae | Sirsak | Annona muricata | Anonaceae |
| 19 | Alaban       | Vitex pubescens    | Verbenaceae | Tarap | Artocarpus odoratissimus | Moraceae |
| 20 | Langsat      | Lansium domesticum | Meliaceae | Anggung | Trema orientalis | Urticaceae |
| 21 | Cempekak     | Artocarpus integer | Moraceae | Rambutan | Nephelium lappaceum | Sapindaceae |
| 22 | Sirih hutan  | Piper aduncum L.   | Piperaceae | Langsat | Lansium domesticum | Meliaceae |
| 23 | Aren         | Arenga pinnata     | Arecaleae | Durian | Durio zibethinus | Malvaceae |
| 24 | Durian       | Durio zibethinus   | Malvaceae | Karet | Hevea brasiliensis | Euphorbiaceae |
| 25 | Sirak        | Annona muricata    | Anonaceae | Kendong | Spondias dulcis | Anacardiaceae |
| 26 | Rambai       | Baccaraea motleyana | Phyllanthaceae | Nanga | Artocarpus heterophyllus | Moraceae |
| 27 | Asam         | Tamarindus indica  | Fabaceae | Jeruk | Citrus sp. | Rutaceae |
| 28 | Birik        | Albizia procera    | Fabaceae | Jengkol | Archidendron pauciflorum | Fabaceae |
| 29 | Tarap        | Artocarpus integer | Moraceae | Hambawang | Mangifera foetida | Magnoliophyta |
| 30 | Kelapa       | Cocos nufera       | Arecaleae | Belimbing | Averrhoa carambola | Oxalidaceae |
Research result reveals that two sampling locations had different vegetation composition (Table 4). The first location (Layuh village) was dominated by agroforestry plantation with several dominant plant species, namely karet (Hevea brasiliensis), sungkai (Peronema canescens), asam (Tamarindus indicus), ramania (Bouea macrophylla), meranti (Dypterocarpaceae), laban (Vitex sp.), tarap (Artocarpus odoratissimus), mahang (Macaranga gigantea), and cempedak (Artocarpus integer). While, the second location (Gambah Luar Village) consisted of a backyard plantation, rice field, and river that only drains water flow during the rainy season. The dominant plant species in the area are kelapa (Cocos nucifera), langsat (Lansium parasiticum), karet (Hevea brasiliensis), pinang (Areca catechu), and rambutan (Nepptelium lappaceum).

Subsequently, Table 4 shows that the bee farming location has diverse plant species. However, based on the pollen analysis, it is known that stingless bees only used certain plant species as food sources. Accordingly, stingless bee determines their food source plant by its distance to their nest [1]. Commonly, they will choose the closest food source to reduce the energy used during the foraging activity. In addition, choosing a close food source will reduce the risk of predators and other environmental disturbances such as heat or rain. Another study revealed that stingless bees only used less than ten plant types as their food source [8]. Those findings confirm our research results where only nine plant sources were found in the honey sample of Layuh Village and six pollen types found in the pollen sample of Gambah Luar Village. However, it needs to be underlined that the study result depends on various factors, i.e., season and duration of observation.

The Stingless bee is an important pollinator agent. It is reported that the stingless bee is a pollinator for at least 215 different families and 1434 genera of plant species in the world [8]. In addition, about 30,000–50,000 tropical plant species are visited by this insect. Plant from Asteraceae and Rubiaceae families is considered the most utilized by the stingless bee and categorized as the most frequent pollen in the honey (Table 1 and 2). Asteraceae is a plant family commonly known as an ornamental plant or herbaceous that is potentially a food source. Several plant families reported to be the most widely used by a stingless bee are Fabaceae, Asteraceae, Rubiaceae, Poaceae, Euphorbiaceae, Myrtaceae, Malvaceae, Arecaceae, Solanaceae, and Anacardiaceae [8, 10]. Besides being beneficial for the environment, stingless bees also benefited farmers since their role as pollinators for plants that provide food for people worldwide [11].

Conclusion
The Stingless bee is a multi-floral bee and uses the various plant as a pollen source. Pollen from Asteraceae, Arecaceae and Rubiaceae was considered as the most frequent pollen found during this study. Thus, this study reveals several important plants that can be used as enrichment in bee-keeping farming.

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**Authors’ Contributions**

All authors contributed equally to this work as the main contributor. SSH, SS, RSW designed the study, collected the samples, and together writing the manuscript. WH, BR, FL, HAB, MSA, AS, DCB, and ES conducted fieldwork. AA performed the pollen analysis and identification.