A PRELIMINARY STUDY ON THE DIVERSITY OF POLLEN COLLECTED BY STINGLESS BEE (*Tetragonula* aff. *minor*) IN UNIVERSITY OF INDONESIA CAMPUS AREA

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ABSTRACT

One of the stingless bee species that can be found in the University of Indonesia campus area is *Tetragonula* aff. *minor*. Therefore, research about *T.* aff. *minor* must be done to support conservation of campus' natural ecosystem. Research was conducted to gather information about the diversity of pollen collected by *T.* aff. *minor* in the University of Indonesia, campus Depok area. Samples were collected in November 2019 from 0930 until 1330. Pollen samples were collected from the corbicula of stingless bees that traveling back to the hives. Pollen preparation was carried out by acetolysis method. After preparation, pollens were observed and counted under a light microscope in 100x and 400x magnifications. Result shows that *T.* aff. *minor* collected pollens from 10 taxa of plants; *Asystasia gangetica* (chinese violet), *Caesalpinia pulcherrima* (peacock flower), *Carica papaya* (papaya), *Cocos nucifera* (coconut), *Dendrophthoe pentandra* (mistletoe), *Mimosa* sp. (mimosa), *Morinda citrifolia* (noni), *Lagerstroemia* sp. (crape-myrtle), family of *Cyperaceae*, and *Poaceae*. Shannon-Wiener diversity index value is 1.431 and evenness value is 62.1%, therefore pollen collected by *T.* aff. *minor* has moderate diversity and evenness. Based on this study, it is known that *T.* aff. *minor* has polylecty diet spectrum and shows individual floral constancy during a foraging trip.

Keywords: Pollen, Shannon-Wiener diversity index, stingless bees, *Tetragonula minor*

ABSTRAK

Salah satu spesies kelulut yang dapat dijumpai di kawasan kampus Universitas Indonesia adalah *Tetragonula* aff. *minor*. Oleh itu, buat kajian mengenai *T.* aff. *minor* mesti dilakukan untuk menyokong pemuliharaan ekosistem di kampus. Penyelidikan dilakukan untuk mengumpulkan maklumat mengenai kepelbagaian debunga yang dikumpulkan oleh *T.* aff. *minor* di Universiti Indonesia, kawasan kampus Depok. Sampel dikumpulkan pada bulan November 2019 dari 0930 hingga 1330. Sampel debunga dikumpulkan dari korbicula kelulut yang kembali ke sarang. Penyediaan debunga dilakukan dengan kaedah asetolisis. Selepas penyediaan, serbuk sari diperhatikan dan dikira di bawah mikroskop cahaya dalam pembesaran 100x dan 400x. Hasil menunjukkan bahawa debunga dari *T.* aff. *minor* terkumpul dari 10 jenis tanaman; *Asystasia gangetica* (chinese violet), *Caesalpinia pulcherrima* (bunga merak),

Carica papaya (betik), *Cocos nucifera* (kelapa), *Dendrophthoe pentandra* (mistletoe), *Mimosa* sp. (mimosa), *Morinda citrifolia* (noni), *Lagerstroemia* sp. (*crape-myrtle*), famili Cyperaceae, dan Poaceae. Nilai indeks kepelbagaian Shannon-Wiener adalah 1.431 dan nilai kesamaan adalah 62.1%, oleh itu debunga dikumpulkan oleh *T.* aff. *minor* mempunyai kepelbagaian dan keseimbangan yang sederhana. Berdasarkan kajian ini, diketahui bahawa *T.* aff. *minor* mempunyai spektrum diet polilekti dan menunjukkan *floral constancy* individu semasa proses mencari makan.

Kata kunci: Debunga, indeks kepelbagian Shannon-Wiener, kelulut, *Tetragonula minor*

INTRODUCTION

Insects have various ecological and economic functions, therefore the decline of insect populations can have an adverse impact on the environment and humans. One of the insects that have an important role in the environment and human's life is stingless bees (Kwapong et al. 2010; Quezada-Euan 2018). Stingless bees can act as a pollinator for the flowers and produce various beneficial products (Gupta 2008; Kwapong et al. 2010). One of the stingless bees that can be found in the University of Indonesia campus area is *Tetragonula* aff. *minor* (Siauwanda 2019).

Stingless bees are a type of bee that belong to the Meliponini tribe. It has more than 500 species in tropical regions (Michener 2013). Stingless bee can act as a pollinator for the flowers and produce various products, such as honey, beebread, propolis, and wax (Kwapong et al. 2010). The high benefits that stingless bees provided make them popular to be cultivated in Indonesia. However, there are still few researches that have discussed the ecology and behaviour of stingless bees in Indonesia.

Tetragonula minor is characterized by its black body and relatively small size. The body length of the worker bee of *T. minor* ranges from 3.2-3.4 mm. It has five hamuli(s) and part of their mesoscutum is covered with 6 hairbands, a collection of hair that is continuously arranged in rows. The hair in this section consists of red and dark hair. Each hairband is bounded by a glabrous area, which is a part that is not covered with hair. *T. minor* has narrower glabrous areas than other stingless bees (Sakagami 1978). The presence of hairbands made *Tetragonula* different from other genera of stingless bee (Samsudin et al. 2018).

Tetragonula aff. *minor* requires pollen as one of their nutrition sources (Quezada-Euan 2018). Bees need pollen for protein source and primary food source for its larvae. Without pollen, bee colonies cannot regenerate (Jalil & Shuib 2014). Bees' pollen preference can vary from specialists to generalists (Cane & Sipes 2006). Bees gather pollen from foraging process carried out by worker bees. Pollens are gathered by worker bees from flower's anthers and then stored in the corbicles (pollen baskets) that are located at the hind legs (Quezada-Euan 2018). Pollens are mixed with nectar so that it is easy to stick to the corbicles (Thorp 2000). Saliva fluid is also secreted by bees to mix with pollens so that a pre-digestion process can occur (Kleinert et al. 2012). Bees then carry those pollen to the hive and store it in pollen storage pots (Quezada-Euan 2018).

During the collection process, stingless bees generally exhibit flower constancy behaviour (Grant 1950). Individual worker bees consistently collect pollen from one type of plant species for a certain period. The flower constancy behaviour was first described by Aristotle & Thompson (1910) as the behaviour of bees that land on the same type of flower

during a single foraging trip (Grant 1950). The term has since expanded because the constant selection of flowers by individual bees does not occur only in one foraging trip but can occur over several days (Grant 1950).

This study was conducted by identifying pollen type and counting the number of pollen grains collected by *Tetragonula* aff. *minor*. The purpose of this study is to determine the diversity of pollen gathered by domesticated *Tetargonula* aff. *minor* that was bred at the University of Indonesia Campus, Depok.

MATERIALS AND METHODS

This study was conducted at the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok, West Java. The scope of this study is to determine the diversity of pollen that gathered by workers from the same colony, therefore the samples came from a single colony. The colony selected is considered active as its foraging activities happened continuously. They were taken from the front side of the *Tetragonula* aff. *minor*'s hive that was bred in the faculty area. Research was conducted in September – December 2019.

Insect Sampling

The sampling unit used in this study was individual worker bees (n=8) which returned to the hive while carrying pollen. Bees were caught using insect nets at 0930 until 1330, when workers from the colony chosen were most active (Siauwanda 2019). The collected bees were put into a sample tube and taken to the laboratory. After that, bees were inactivated by the cooling method. They were transferred to a glass tube and cooled in the freezer at a temperature of -27^0C for 8 minutes.

After bees were inactivated by cooling method (-27^0C for 8 minutes), they were removed from the sample tube into a slide. The slide was then placed under a portable digital microscope that was connected to the laptop. The magnification and focus of the portable digital microscope lens were adjusted so that the bee image can be seen clearly in the DinoCapture 2.0 program. After the bee image was seen, the pollen clumps were removed from the bee's corbicles. The remaining pollen in the bee corbicles was then released by dropping distilled water on the hind legs of the bees so that the pollen dissolved. Bees that have been used were then released in front of their hive. The sampling activity was repeated after all pollen from one pair of corbicles were counted.

Pollen Preparation

Pollen samples were heated on the hot plate at a temperature of 100°C. The heating process occurred until the distilled water was evaporated. After that, pollens were prepared using $\pm$3mL acetolysis solution (Erdtman 1960). Then samples were immersed with 10-15 drops of alcohol. The acetolysis solution and debris were absorbed by using tissue. Pollens were stained and hydrated by a mixture of $\pm$1-2 mL of safranin solution and 1-3 drops of glycerin. Pollen that has been stained is then covered with a cover glass.

Pollen Count and Identification

Pollens were observed using light microscopes with magnifications of 40x, 100x, and 400x. Photos of each type of pollens were taken and the morphological characters of pollen were recorded. The number of pollens of a similar form was counted manually. Pollen count are done at a magnification of 100x. The observed pollen characters and photos were then matched and compared with the pollen characters and images from the identification key and literature.

Pollen identification was carried out based on references Erdtman (1943), Halbritter et al. (2018), and the Australasian Pollen and Spore Atlas database (APSA 2019). Pollen was identified to the family level or the level of taxa below the family. The results of pollen identification were then compared with the types of plants around the hive of *Tetragonula* aff. *minor*.

RESULTS AND DISCUSSION

Results show that pollens that were collected by *Tetragonula* aff. *minor* came from 10 groups of plant species. Six types of pollen were identified at the species level: *Asystasia gangetica* (chinese violet), *Caesalpinia pulcherrima* (peacock flower), *Carica papaya* (papaya), *Cocos nucifera* (coconut), *Dendrophthoe pentandra* (mistletoe), and *Morinda citrifolia* (noni). Two types of pollen up to the genus level: *Lagerstroemia* sp. (Crepe-myrtle) and *Mimosa* sp. (mimosa), while the two types of pollen are up to family level, Cyperaceae and Poaceae (Table 1, Figure 1).

Table 1. Types and Number of Pollens Collected by *Tetragonula* aff. *minor*

No.	Types of pollen (species/genus/family)	No. of sample unit (individual)								Average number of pollen (±SD)
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
1	*Asystasia gangetica*	1	0	1	0	0	0	0	0	0.29±0.353
2	*Caesalpinia pulcherrima*	0	0	0	1	6	**932**	2	0	134.43±329.06
3	*Carica papaya*	6	0	**17,996**	163	1	0	5	0	2.596±6.35.96
4	*Cocos nucifera*	**10,750**	27	46	**7,172**	**6,841**	53	**7,149**	46	4,576±4,411.71
5	*Dendrophthoe pentandra*	0	0	16	0	25	0	91	**31,074**	3,900.75 ±10,979.69
6	*Lagerstroemia* sp.	4	**21,451**	1	3	1	0	4	0	3,066.29±7,583.41
7	*Mimosa* sp.	0	0	0	1	0	0	3	0	0.57±1.07
8	*Morinda citrifolia*	0	0	0	1	0	0	0	0	0.14±0.35
9	Cyperaceae	3	5	2	135	12	0	72	0	32.71±49.33
10	Poaceae	367	7	1	26	1	2	2	0	58±128.07
	Total	11,132	21,492	18,066	7,506	6,892	993	7,335	31,128	

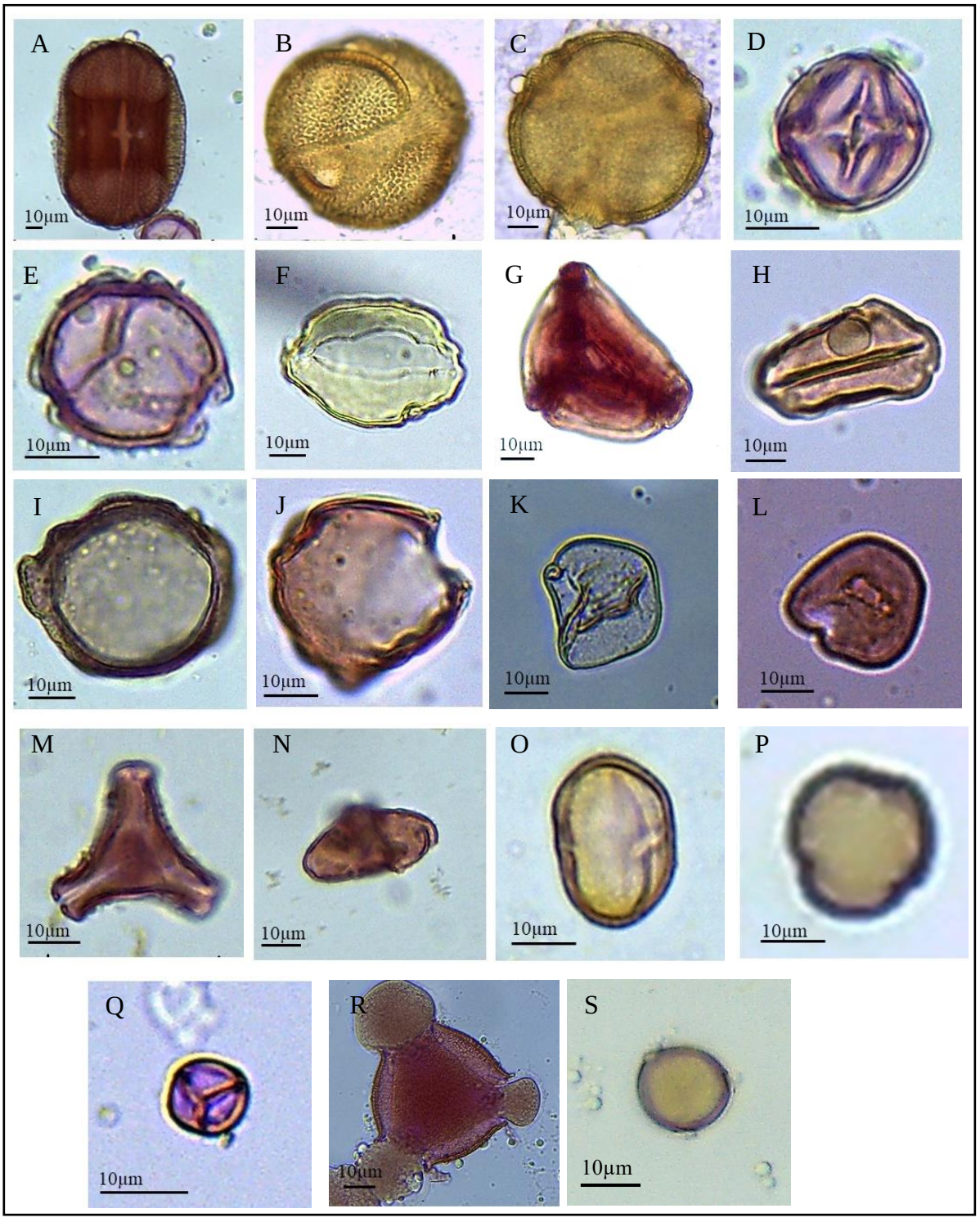

Figure 1. Type of the pollens collected by *Tetragonula* aff. *minor* (E): equatorial; (P): polar). A: *Asystasia gangetica* (E); B: *Caesalpinia pulcherrima* (E), C: *Caesalpinia pulcherrima* (P), D: *Carica papaya* (E), E: *Carica papaya* (P), F-I: *Cocos nucifera* (E), J: *Cocos nucifera* (P), K: Cyperaceae (E), L: Cyperaceae (P), M: *Dendrophthoe pentandra* (E), N: *Dendrophthoe pentandra* (P), O: *Lagerstroemia* sp. (E), P: *Lagerstroemia* sp. (P), Q: *Mimosa* sp. (P), R: *Morinda citrifolia* (E), S: Poaceae (E)

Based on the observations, there is a dominant pollen type that was foraged by each bee individual. Dominant pollens account for more than 90% of the total pollens per sample unit (Table 1, numbers in bold). Besides, based on the calculation of the standard deviation of the average number of pollens for each plant, a value is obtained that illustrates that the pollen of each plant species is not evenly distributed in each individual bee.

The results of relative abundance, relative frequency, and significance value calculation showed that *Cocos nucifera* pollen had the highest value. *Lagerstroemia* sp., Poaceae, *Carica papaya*, *Dendrophthoe pentandra*, Cyperaceae, *Caesalpinia pucherrima*, *Mimosa* sp., *Asystasia gangetica*, and *Morinda* sp. respectively have lower importance value than *C. nucifera* (Table 2). This result suggests that *C. nucifera* is an important plant for *Tetragonula* aff. *minor*. *Tetragonula* aff. *minor*'s preference for *C. nucifera* presumably due to high concentration of sugar its flower contained. Moreover, *C. nucifera* have the ability to produce flower throughout the year, providing food source for T. aff. *minor* (Balderas 2016; Selvaraju et al. 2019).

Table 2. Relative Abundance (RA), Relative Frequency (RF), and Importance Value (IV) of Each Plant Pollen Type Collected by *Tetragonula* aff. *minor*

Species	**RA**	**RF**	**IV**
Cocos nucifera	30.7%	100.0%	65.4%
Lagerstroemia sp.	20.5%	75.0%	47.8%
Poacea	0.4%	87.5%	43.9%
Carica papaya	17.4%	62.5%	39.9%
Dendrophtoe pentandra	29.9%	50.0%	39.9%
Cyperaceae	0.2%	75.0%	37.6%
Caesalpinia pulcherrima	0.9%	50.0%	25.5%
Mimosa sp.	0.0%	25.0%	12.5%
Asystasia gangetica	0.0%	25.0%	12.5%
Morinda citrifolia	0.0%	12.5%	6.3%

The results of Shannon-Wiener index (H') calculation is 1.431 from a maximum value of 2.303 (Lindenmayer et al. 2005). This value indicates that the diversity of bee pollen source plants is moderate. The results of the calculation of the evenness value were 0.621 or 62.1%. This value shows that the distribution of pollen is of moderate value, pollen has not been evenly distributed because some types of pollen are more dominant than others.

Based on the results, it is known that *T.* aff. *minor* collected pollen from 6 types of plant identified to species level, 2 types of plant identified to genus level, and 2 types of plant identified to family level. In general, pollen collected by bees are from 9 different plant families: Acanthaceae, Arecaceae, Caricaceae, Cyperaceae, Fabaceae, Loranthaceae, Lythraceae, Poaceae, and Rubiaceae. This result suggests that *T.* aff. *minor* has a spectrum of polylecty diets. Based on Cane & Sipes (2006), polylecty is a term that describes the broad spectrum of bee's diet that comes from more than 3 families of plant. The polylecty character

of *T.* aff. *minor* related to the other eusocial bee character which mostly also has a broad spectrum of diet. Broad spectrum of diet is an adaptation mechanism of eusocial bees to maintain the existence of the colony. It is because some types of flowers only bloom at one time of year (Cane & Sipes 2006).

Although *T.* aff. *minor* have a broad spectrum of diet; the result indicates that individual worker shows flower consistency in a single foraging trip (Grant 1950). Data shows that in each sample unit there was a dominant pollen type (> 90%) that was collected. Other types of pollen found in the similar sample unit probably are contaminants (Table 1). For example, bee sample number 1 was mostly gathered *C. nucifera* pollen, while it was also taking small number of *A. gangetica, C. papaya, L. gangetica,* Cyperaceae, and Poaceae pollens that considered as contaminants.

This consistent behaviour is also frequently found in other stingless bees, such as *Melipona marginata*, *Melipona quadrifasciata*, *Melipona scuttelaris*, *Nannotrigona testaceicornis*, *Plebeia droyana*, *Plebeia remota*, *Scaptotrigona bipunctata*, *Tetragonisca angustula*, and *Trigona spinipes* (Ramalho et al. 1989). The consistent behavior in flower selection is beneficial for the plant because it is can increases the possibility of flower pollination (Layek & Karmakar 2018). According to Barth et al. (2018) stingless bees generally show high consistency behaviour when large amounts of food source are available. When food sources are not abundant, stingless bees can collect pollen from various flowers in one foraging trip.

CONCLUSION

Tetragonula aff. *minor* collected pollens from 10 taxa of plants and indicate moderate diversity and evenness value. It is also known that *T.* aff. *minor* has polylecty diet spectrum and shows individual floral constancy during a foraging trip. There are at least four important pollen sources for *T.* aff. *minor*: C. *nucifera*, *Lagerstroemia* sp., C. *papaya*, and D. *pentandra*. This finding can be taken into consideration in decision regarding campus land use, so that bee's important pollen sources can be conserved to ensure *T.* aff. *minor*'s sustainability.

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