Unraveling the floral preference: bee pollen identification and characterization of *Tetragonula laeviceps*

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Abstract. The stingless bee (*Tetragonula laeviceps*) is capable of yielding products such as honey, propolis, and bee pollen, which hold promise as potential sources of nutrition or alternatives in the field of medicine. The physical and chemical attributes of these products are influenced by intrinsic factors, including dietary sources and bee species, as well as extraneous variables such as management techniques, seasonal variations, and environmental parameters. The Campus of the Biology Faculty UGM encompasses a rich repository of flowering plant species that present a viable resource for the sustenance of stingless bees. Consequently, this research endeavor seeks to delineate and characterize the plant species that serve as the sustenance of stingless bees by elucidating the pollen types discovered within their nest. Pollen specimens were directly procured from the nests and subjected to be analyzed with light microscopy and scanning electron microscopy (SEM). At least four distinct varieties of pollen have been identified, originating from divergent plant species, namely those belonging to the Pinaceae, Fabaceae, Myrtaceae, and Brassicaceae families. The dietary preferences of stingless bees, as discerned through the identification of pollen types, exhibit congruence with the plant species in the vicinity of their nesting sites.

1 Introduction

Stingless bees (Apidae: Meliponini) represent the largest group of eusocial bees, comprising over 500 described species [1]. In Indonesia, a minimum of 46 species of stingless bees are distributed across Java, Sumatra, and Kalimantan [2].

Tetragonula laeviceps, also locally known as "klanceng," is a common species of stingless bee found in tropical and subtropical regions, including both Indonesian forests and residential areas [3]. *T. laeviceps* typically construct nests within bamboo, wall crevices, and even underground [4, 5]. The nests of stingless bees take an oval shape and are compartmentalized for the separation of eggs and food storage [6].

Stingless bees exhibit preferences in foraging behavior around their nests. These preferences are influenced by factors such as the location of food sources and the selection

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of plants that can provide both nectar and pollen [7]. Stingless bees utilize pollen and nectar as sources of protein, fats, vitamins, and minerals for the development of their colonies. The pollen preferences of *T. laeviceps* are influenced by the type, aroma, and dimensions of the pollen [8]. Additionally, the distance between plants also affects the pollen selection of *T. laeviceps*, as these bees can forage for food up to a distance of 200 meters [9].

Forager bees collect flower pollen, which is then mixed with salivary gland secretions. The resulting secretion is stored in corbiculae, which are located on the hind legs, allowing it to be transported back to the nest. *T. laeviceps* has the ability to segregate food sources between pollen and honey, storing both products in propolis pots [10, 11].

Bee-collected pollen, known as bee pollen, holds significant potential in the field of pharmacy due to its high nutritional value [12]. On the other hand, the collected pollen also has a direct impact on bee products, notably honey. The quality of honey can be analyzed based on the presence of hydroxymethylfurfural (HMF) compounds. These compounds are highly sensitive to heat, making them useful indicators for distinguishing between natural honey and artificial or mixed honey products. In this study, the aim is to identify and characterize the types of pollen found in *T. laeviceps* nests. Additionally, the quality of natural honey from the nests will be analyzed for its HMF content.

2 Materials and Methods

Pollen and honey samples were obtained from the nests of *T. laeviceps* located in the Faculty of Biology at Gadjah Mada University. The materials employed in the preparation of the pollen included pollen samples, aquades, object glass, and cover glass. The reagents utilized encompassed a 25% solution of HCl, 25% solution of KOH, 1% safranin solution in aquades,and glycerin.

2.1 Collection of pollen and honey from *T. laeviceps* nests

Pollen and honey samples were collected from the nests of *T. laeviceps* at the Faculty of Biology, UGM. The collection process involved carefully opening the nests. Typically, these nests contain dark-colored propolis, as well as honey and pollen sacs. The pollen was gathered without causing any harm to the honey sacs or the stingless bee colony's eggs. Meanwhile, honey samples are pressed and filtered before being placed into sealed containers.

2.2 Pollen preparation with acetolysis

Sample preparation of pollen was carried out at the Plant Development Structure Laboratory, Faculty of Biology, UGM. The samples were prepared using a modified acetolysis method based on [13]. This method enabled the purification of pollen from propolis residue and other impurities [13].

Five grams of pollen samples were placed into a conical tube, and then 10 ml of aquades was added. Subsequently, the sample was heated in a water bath for a period, vortexed, and centrifuged at 1000 rpm for 5 minutes. The supernatant was carefully removed, and the pellet was then treated with additional aquades until the volume reached the 10 ml limit of the conical tube. This mixture was once again heated in a water bath, vortexed, and centrifuged at 1000 rpm for 5 minutes. This process was repeated until the supernatant became clear. The supernatant was removed, and the pellet was then treated with 25% HCl up to the 10 ml limit of the conical tube. After vortexing, the tube was heated in a water bath at 100 ºC for 30 minutes and then centrifuged at 1000 rpm for 5 minutes. The

supernatant removed again, and the pellet was treated with 25% KOH up to the 10 ml limit of the conical tube. After centrifugation at 1000 rpm for 5 minutes, the supernatant was removed. The pellet was thoroughly washed with aquades and centrifuged until no organic material remained.

2.3 Pollen identification and characterization

The purified pollen was subsequently subjected to an initial morphological analysis using a light microscope. A 1% safranin solution was employed as a stain to enhance contrast in the prepared samples. Further clarification of the identification results was achieved through ultrastructure observations conducted with a scanning electron microscope. Ultrastructural observations did not involve staining with 1% safranin. Instead, the prepared samples were dried. Pollen identification is conducted based on the Illustrated Pollen Terminology and the PalDat - Palynological Database (https://www.paldat.org) [14, 15].

2.4 HMF analysis of honey with spectrophotometry

Fresh honey is stored in sealed containers without the presence of air bubbles. Honey samples undergo analysis for reduced sugar content levels using hydroxymethylfurfural (HMF) analysis. The analysis of HMF is conducted via spectrophotometry based on AOAC officialmethod 980.23.

3 Results and Discussion

Based on initial observations using a light microscope, at least five types of pollen grains originating from different plant species were discerned, as showed in the following image.

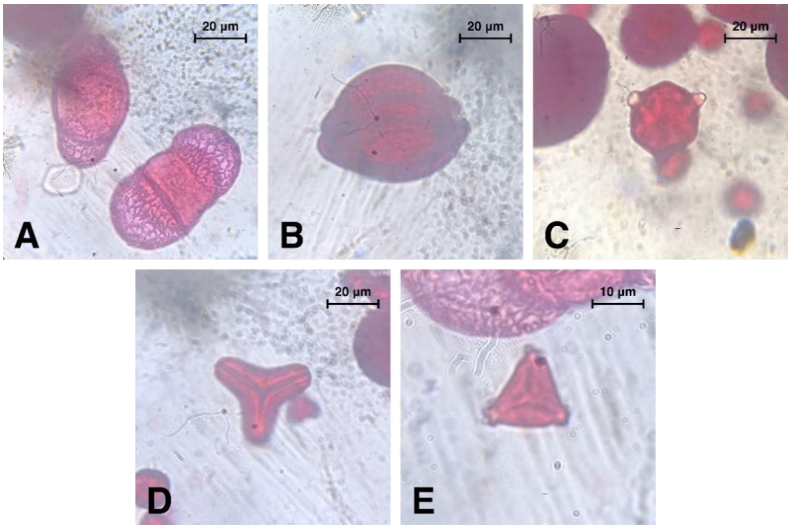

Fig. 1. Pollen morphology with 1% safranin found within the *T. laeviceps* nest. A. *Pinus* sp.; B. Arecaceae; C. Fabaceae; D. Loranthaceae; E. Myrtaceae

The pollen discovered within the nests of *T. laeviceps* is predominantly derived from *Pinus* sp., a group of coniferous trees. The distinctive morphology of *Pinus* sp. pollen is characterized by the presence of two wings, referred to as sacci, with a smaller central body known as the corpus [16]. These sacci are specialized structures that enable the efficient wind-mediated dispersal of pine pollen over considerable distances. The presence of such winged pollen is indicative of anemophily, a pollination mechanism where pollen is dispersed by the wind.

The prevalence of *Pinus* sp. pollen in the nests of *T. laeviceps* may also be linked to the foraging behavior of these stingless bees for resin. Resin from *Pinus* sp. is the preferred primary source for stingless bees in the production of propolis [17]. Propolis is a sticky substance that bees use to seal cracks and protect their nests. This preference for *Pinus* sp. resin might explain why stingless bees frequently visit *Pinus* sp. plants, which offer both resin and pollen as their food source.

Additionally, the nests of *T. laeviceps* also contain various other types of pollen, including those from the Arecaceae, Fabaceae, Loranthaceae, and Myrtaceae. These diverse pollen sources reflect the foraging habits of *T. laeviceps* and provide insights into their dietary preferences and ecological interactions within their habitat.

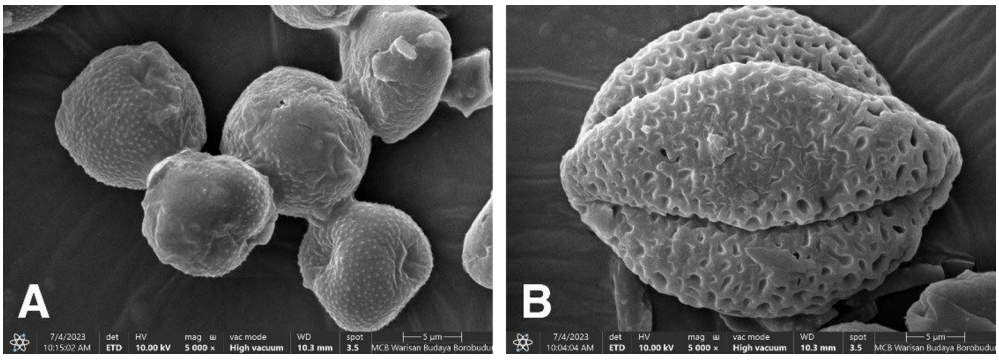

Fig. 2. Pollen ultrastructure found whitin *T. laeviceps* nest using Scanning ElectronMicrosope. A. Malvaceae; B. *Citrus* sp.

Ultrastructural observations reveal the presence of additional pollen from the Malvaceae family and the *Citrus* sp. species. Malvaceae pollen typically exhibits a spheroid or globular shape, with colporate or porate apertures featuring echinate sculpturing [18]. On the other side, *Citrus* sp. pollen is characterized by a prolate grain shape with tetracolporate apertures [19]. With the diversity of identified pollen, the value of HMF was determined through spectrophotometric analysis in the honey samples, and it was found to be 3,74 mg/kg.

4 Conclusion

T. laeviceps exhibits a dynamic range of pollen preferences for their food source, and these preferences appear to be influenced by both the type and size of the pollen. Initial observations conducted with a light microscope have revealed that the predominant pollen within their nests is derived from *Pinus* sp., which also functions as a resin source for *T. laeviceps*.

Moreover, ultrastructural examinations have yielded additional insights by uncovering the presence of *Citrus* sp. and Malvaceae pollen in their nests. This demonstrates the species' capacity to forage and collect a diverse array of pollen types, reflecting their adaptability and

foraging behavior. The specific types and dimensions of pollen consumed can play a pivotal role in the nutritional intake and ecological interactions of *T. laeviceps*. With the diversity ofpollen identified, the HMF value in the honey sample obtained was 3.74 mg/kg.

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