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THE POLLEN LOAD ON STINGLESS BEES (APIDAE: MELIPONINAE) FORAGED IN URBAN AREA

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Abstract

Stingless bees (Apidae: Meliponinae) are eusocial insects that distributed in the tropics and subtropics. Stingless bees are pollinators for various plant species. Foraging activities of worker stingless bees collect pollens, nectar, resin or water as nutrients for individuals and colony needed. This study aimed to measures pollen load and pollen composition carried by four species of stingless bees, i.e., *Tetragonula laeviceps*, *Lepidotrigona terminata*, *Heterotrigona itama*, and *Geniotrigona thoracica* in urban area at Bogor, Indonesia. Acetolysis method was used for pollens preparation and pollens were counted by using hemacytometer under light microscope embedded with camera. The study showed that the highest pollen load occurred in *H. itama* (59181 pollen grains), followed by *L. terminata* (27806 pollen grains), *T. laeviceps* (20816 pollen grains), and *G. thoracica* (11775 pollen grains). The number of pollens collected by *T. laeviceps, L. terminata*, and *H. itama* positively correlated with body size. Thirteen types of pollen were identified on the body of stingless bees. Pollen composition collected by *T. laeviceps* were dominated by Chlorantaceae (50%) and Polygonaceae (20%), *L. terminata* and *H. itama* were dominated by Asteraceae (70.19% and 62.76%) and Arecaceae (22.87% and 29.65%), while in *G. thoracica* was dominated by Apocynaceae (53.53%) and Acanthaceae (34.32%). *Lepidotrigona terminata* and *H. itama* carried of small pollen-size and *G. thoracica* carried moderate pollen-size.

Keywords: foraging activity, *Tetragonula*, *Lepidotrigona*, *Heterotrigona*

INTRODUCTION

Stingless bees (Apidae: Meliponinae) belong to social insect and are distributed in the tropics and subtropics (Hrncir *et al.*, 2016). In Southeast Asia, there were reported 50 species of stingless bees (Inoue *et al.*, 1985) and in Indonesia, 31 species of stingless bees were reported in Kalimantan, 41 species in Sumatra, and 9 species in Java. As eusocial bees, stingless bees shows division of labor, overlap generation, and communication among colony members. The caste consists of workers, queen, and males (Michener, 2000).

Worker bees have an important task to collect foods, i.e., pollen and nectar and other materials, such as resin, water, and nest materials (Eltz *et al.*, 2002; Michener, 2007). By stingless bees, pollen and nectar are used as nutrients for individual and their colonies (Ramalho *et al.*, 1994). During foraging, pollens are deposited in the corbicula located in the hind-tibia. Proboscis, a special structure of mouthpart, is used for feeding of nectar and water (Michener, 2007). Total pollen load on various parts of the body is called total pollen load. While, pollen on the parts which may be indirect contact with the receptive stigma while visiting flower is called functional pollen load. The chances for pollination depend on the functional pollen load (Dafni, 1992).

In one of foraging trip, individual of bees often restricted their visits to one species flower (flower constancy). The phenomenon of flower constancy is crucial for pollination ecology. More than 100 plant species visited by stingless bees, such as belong to family Begoniaceae, Caesalpiniaceae, Malvaceae, Myrtaceae, Rutaceae (Engel and Bakels, 1980), Euphorbiaceae (Ramalho *et al.*, 1994), Rubiaceae, Sapindaceae, and Solanaceae (Slaa *et al*., 2006). Uji (1987) reported *H. itama* carried pollens and as agent pollination of 'rambutan' (*Nepheuum lappaceum*). In strawberry, Harahap (2013) reported pollination by *T. laeviceps* increased fruit formation, fruit size and weight, and reduce abnormal fruits.

In this study, four species of stingless bees with different body size were used, i.e., *Tetragonula laeviceps*, *Lepidotrigona terminata*, *Heterotrigona itama*, and *Geniotrigona thoracica*. *Tetragonula laeviceps* has a small body size (3.44–4.88 mm in length), brown-blackish in color, and transparent wings (Sakagami, 1978). *Lepidotrigona terminata* is characterized by body size ranged 4.0–5.5 mm in length, yellow or white ring of hairs on the thoracic and in outside of mesocutum and mesoscutellum (Schwarz, 1939; Smith, 2012). *Heterotrigona itama* has a larger body size (6.15 mm in length), body is predominantly black, with one tooth on the mandible (Sakagami, 1978). *Geniotrigona thoracica* has a body sized ranged 8.12–8.65 (Sakagami *et al*., 1990). This study aims to measure the pollen load and composition of pollens carried by four species of stingless bees, i.e., *T. laeviceps*, *L. terminata*, *H. itama*, and *G. thoracica* at Bogor, Indonesia.

MATERIALS AND METHODS

Collection and Measuring of Body Size of Stingless Bee

Stingless bee colonies used in this study located in fruit garden in Bogor, Indonesia with an area of about 0.5 ha. The environmental parameters measured during sample collections were an average 29.1 °C of temperature, 78.17% of humidity, and 6016.67 lux of light intensity. Twenty individuals of each species that returning to the hive with pollens in their hind-tibia were collected using insect nets. Ten individuals of each species used for measuring body length were preserved by dry method. For measuring pollen load, each individual of bee was put into 1.5 ml tube containing 0.5 ml of 70% ethanol: glycerol (4 : 1). Ten individuals of each species of stingless bee were photographed by using the Opti-Lab camera embedded on stereo microscope. The body length of each species of bee then was measured using Image Raster 3 software.

Pollen Preparation and Identification

Pollen carried by each species of stingless bee was analyzed using acetolysis method (Erdtman, 1960). Each individual of bee preserved in 70% ethanol: glycerol (4 : 1) solution then was rotated for 24 hours and then individual of bee was removed. The solution was centrifuged in 2000 rpm for 3 minutes and the supernatant was removed and then leaving pollen deposits (Dafni, 1992). Deposit of pollens was added by 1 ml acetolysis solution (acetic anhydrides and acetic acid, 9 : 1) and heated in 80 °C in the water bath for 5 minutes and then supernatant was removed. The remaining deposit was rinsed with distilled water and centrifuged in 2000 rpm for 3 minutes. The supernatant was removed by leaving 0.1 ml of pollen deposit. This procedure was replicated for 10 individuals of each species.

Pollen is identified based on several characters, such as type of aperture and pollen size on polar and equatorial view. Identification of pollen based on Erdtman (1972), Huang (1972), APSA (http://apsa.anu.edu.au/), and Gosling *et al*. (2013). Generally, pollen diameters are 15–60 μm (Kearns and Inouye, 1993). Polar and equatorial view are valuable characteristics in pollen identification (Huang, 1972). Based on the size, pollen is classified into very small (< 10 μm), small (10–25 μm), moderate (25–50 μm), large (50–100 μm), very large (100–200 μm), and giant (> 200 μm) (Erdtman, 1972).

Measurement of Pollen Load and Flower Constancy

The remained solution (0.1 ml of pollen deposit) was dropped into a Neubauer-type of hemocytometer and the number of pollens were counted under the compound microscope. The number of pollen load on an individual of bee was calculated by the formula:

$$N_2 = (N_1 \times V_2)/V_1, \quad (1)$$

where

V_2 ... volume of sample solution (0.1 ml = 100 mm^3),

N_1 ... number of pollens in four quadrants of hemocytometer,

V_1 ... volume of four quadrans of hemocytometer (0.4 mm^3).

Flower constancy was measured by examining the composition of pollen loads expressed as the percentage of individual pure loads (Willmer, 2011). Flower constancy on stingless bees were measured on 10 individuals of each species, i.e., *L. terminata*, *H. itama*, and *G. thoracica* collected from fruit garden in Bogor and *T. laeviceps* collected in the campus area of IPB University, Bogor.

Data Analysis

Pollen load of each species of stingless bees were showed in a bar chart and analyzed by using Kruskal-Wallis and Mann-Whitney. The relationship between pollen load and body size of stingless bees were analyzed by Spearman's rank correlation test using Paleontological Statistics (PAST) 4.02 (Hammer *et al.*, 2001) and displayed in a scatter plot. Flower constancy of stingless bees expressed as percentage were shown in bar chart.

RESULTS

Morphology of Stingless Bees

The worker of *T. laeviceps* (3.89 ± 0.10 mm in body length) has a shiny-black color, narrow malar space,

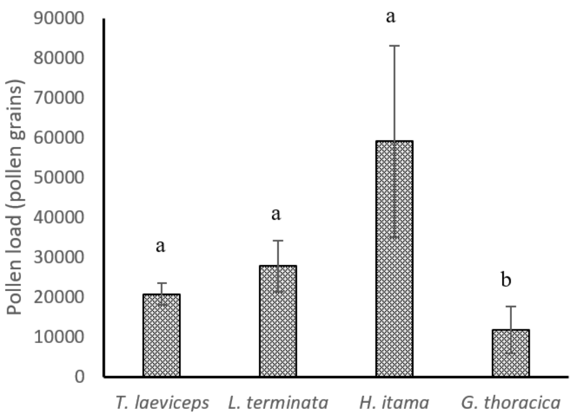
a b c d

1: *Morphology of stingless bees: 1a T. laeviceps, 1b L. terminata, 1c H. itama, and 1d G. thoracica*

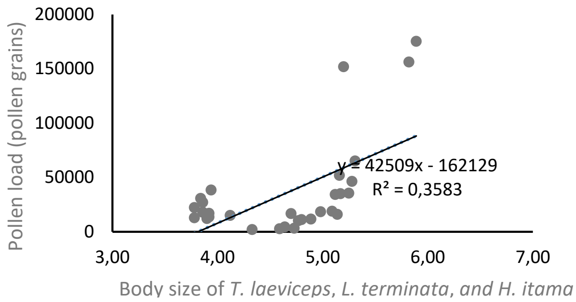
mesoscutum without hairbands, and monotonous and transparent wings. Worker of *L. terminata* (4.9 ± 0.32 mm in body length) has dominantly black and yellow hairs in the thorax and abdomen, black mesoscutum with short and yellow hairs, and wings with six hammuli. Worker of *H. itama* (5.09 ± 0.45 mm in body length) is characterized by blackish color, mesothorax distinctly dullish, and scutellum with dark hairs. While, worker of *G. thoracica* (7.94 ± 0.11 mm in body length) is characterized by rufous thorax, scutellum hairs are bright orange-brown, and scutum is chestnuts (Fig. 1).

Pollen Load and Flower Constancy of Stingless Bees

The effectiveness of stingless bees as pollinating insects can be measured from pollen load. The number of pollens carried by four species of stingless bee varied. The highest pollen load occurred in *H. itama* (59,181 pollen grains), followed by *L. terminata* (27,806 pollen grains), *T. laeviceps* (20,816 pollen grains), and *G. thoracica* (11,775 pollen grains). Based on the Kruskal-Wallis and Mann-Whitney analysis, pollen load on *T. laeviceps*, *L. terminata*, and *H. itama* were not significantly different ($p = 0.3447$, $p = 0.6776$), $p = 0.9698$), but significantly different with *G. thoracica* ($p = 0.005795$, $p = 0.03121$, $p = 0.03764$) (Fig. 2). The number of pollens collected by



2: *Pollen load on four species of stingless bees (n = 10 individuals of each species). The same letter showed no significant difference (Kruskal-Wallis and Mann-Whitney tests)*

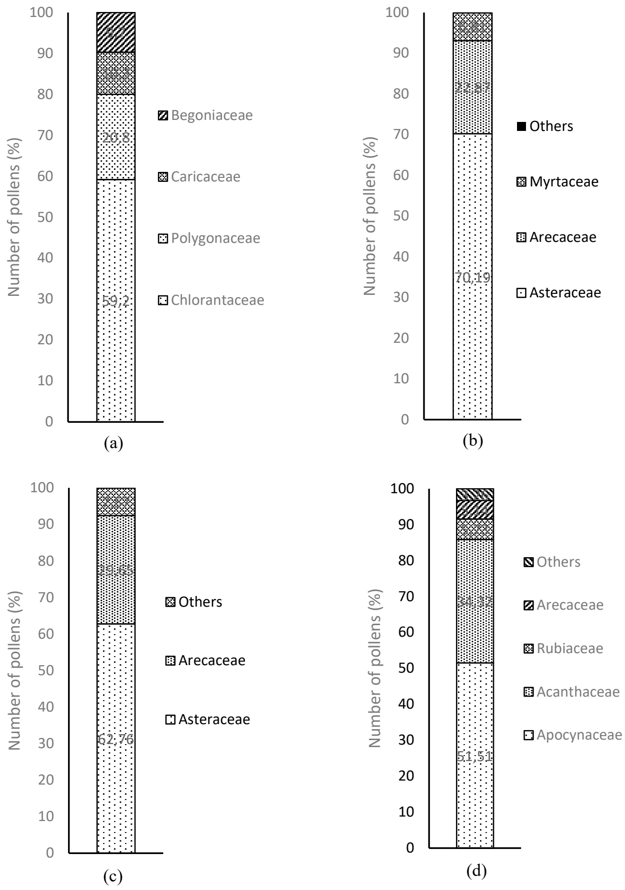


3: *Scatter plot showed the relationship between body size of stingless bees and pollen load*

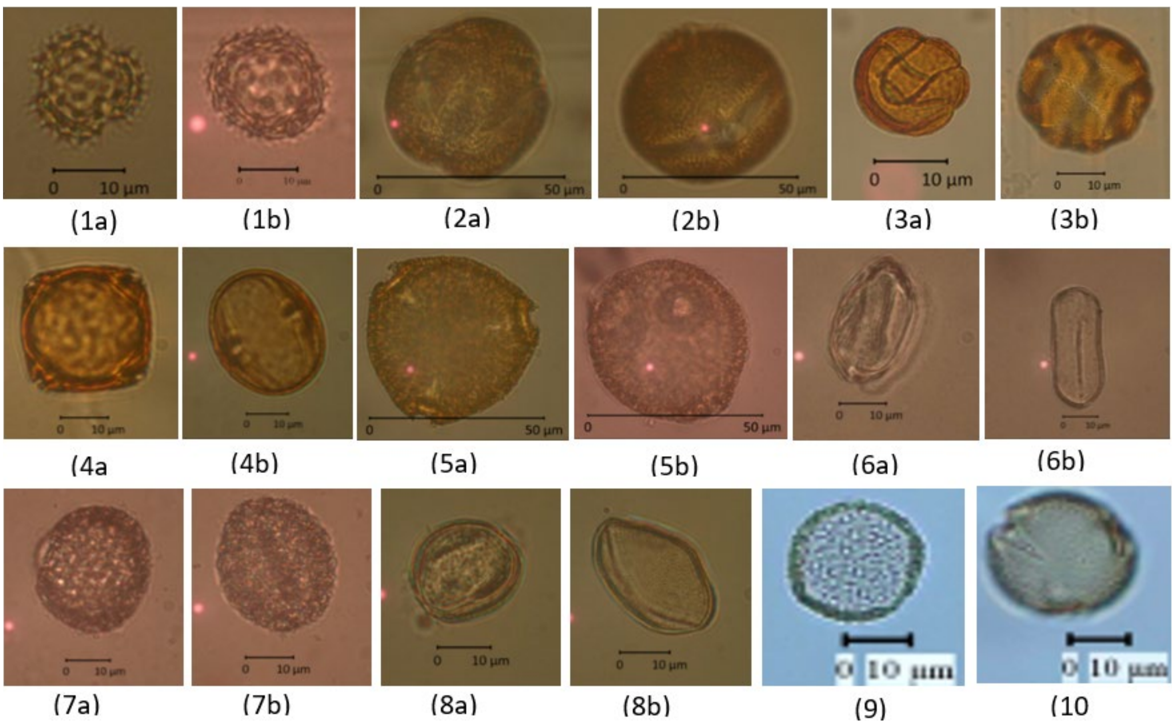
T. laeviceps, *L. terminata*, and *H. itama* was significantly correlated with body size ($rs = 0.519$, $p = 0.003$) (Fig. 3). In *G. thoracica* which has the largest body size (7.94 mm in body length) carried low number of pollens (11,775 pollen grains)

During foraging, bees showed flower constancy. Flower constancy plays an important role in cross-pollination. The plant species visited by bees in one area are differ with other areas depends on the availability of flowering plants. Pollens collected by stingless bees in fruit garden were identified from thirteen plant families, i.e., Caricaceae, Begoniaceae, Polygonaceae, Chlorantaceae, Myrtaceae, Arecaceae, Asteraceae, Rubiaceae, Acanthaceae, Apocynaceae, Capparaceae, Flagellariaceae, and Sapindace (Fig. 4 and 5). In the IPB university campus area, the dominant pollen composition carried by *T. laeviceps* was Chlorantaceae (50%). This species also visited flowers of Polygonaceae (20.8%), Caricaceae (10.3%), and Begoniaceae (9.7%). In fruit garden in Bogor, the dominant pollens collected by *L. terminata* and *H. itama* was Asteraceae (70.19% and 62.76%) and Arecaceae (22.87% and 29.65%). Whereas in *G. thoracica* was dominanted by Apocynaceae (53.53%) and Acanthaceae (34.32%) (Fig. 4).

The pollen characteristics carried by stingless bees varied in shape and size. Thirteen types of pollens were identified from stingless bees (Tab. I). Those pollens are monocolpate, colporate, and tricolporate. Based on the polar and equatorial view, those pollens are circular, triangular, oblate, trilobate, and subprolate. The diameter of polar view ranged 7.28–53.70 um. The pollen found on



4: *Composition of pollen carried by stingless bees: 4a T. laeviceps, 4b L. terminata, 4c H. itama, and 4d G. thoracica*



5: *Morphology of pollen carried by stingless bees: 5-1 Acanthaceae, 5-2 Apocynaceae, 5-3 Arecaceae), 5-4 Asteraceae, 5-5 Myrtaceae, 5-6 Polygonaceae, 5-7 Rubiaceae, 5-8 Sapindaceae, 5-9 Chlorantaceae, 5-10 Caricaceae. a. polar view, b. equatorial view*

I: *Characteristics of pollen collected by stingless bees*

Plant family (species)	Aperture type	Polar view	Euatorial view	Pollen size (μm)		
				Polar view	Equatorial view	
				Diameter	Length	Width
Acanthaceae-1	Tricolporate	Triangular	Circular	12.45	22.15	15.20
Acanthaceae-2	Dicolporate	Circular	Circular	41.87	61.49	42.67
Apocynaceae	Dicolporate	Circular	Circular	33.02	35.04	28.54
Arecaceae (*Areca macrocalyx*)	Monocolpate	Circular	Subprolate	15.32	33.54	15.67
Arecaceae (*Chypokentia* sp.)	Monocolpate	Circular	Subprolate	20.22	32.43	21.24
Arecaceae	Tricolporate	Circular	Subprolate	17.53	35.54	12.42
Asteraceae	Tricolporate	Triangular	Subprolate	17.78	19.43	16.48
Capparaceae	Tricolporate	Trilobate	Subprolate	22.21	30.52	18.34
Flagellariaceae	Monocolpate	Circular	Circular	7.28	8.58	7.24
Myrtaceae	Tricolporate	Triangular	Oblate	18.34	18.81	12.00
Polygonaceae (*Antigonon leptopus*)	Tricolporate	Triangular	Subprolate	53.70	56.36	45.15
Rubiaceae	Monocolpate	Circular	Subprolate	53.13	58.48	49.25
Sapindaceae (*Lepisanthes* sp.)	Tricolporate	Triangular	Oblate	23.49	27.30	22.32

stingless bees varied from very small (diameter < 10 μm) belong to Flagellariaceae, small (10–25 μm diameter) belong to Acanthaceae-1, Asteraceae, and Myrtaceae, moderate (25–50 μm) belong to Apocynaceae, Arecaceae (*Areca macrocalyx*, *Chypokentia* sp.), Capparaceae, and Sapindaceae (*Lepisanthes* sp.), and large (50–100 um) belong to Acanthaceae-2, Rubiaceae, and Polygonaceae (*Antigonon leptopus*) (Tab. I). *Lepidotrigona terminata* and *H. itama* dominantly carried pollens of Asteraceae and Arecaceae which are generally small (10–25 um). *Lepidotrigona terminata* also carried small number pollens of *Antigonon leptopus*, *Areca macrocalyx*, Acanthaceae, and Myrtaceae, whereas *H. itama* carried small number of pollens of *Antigonon leptopus*, *Lepisanthes* sp., Acanthaceae, and Apocynaceae.

DISCUSSION

Stingless bees are widely used as pollinators for cultivated plants. Compared to honey bees, stingless bees have more resistance to pests, parasites, and climate change (Heard, 1999). Foraging activities of stingless bees are started in the morning until afternoon. Their returning activities to nest carried water, nectar, pollen, or resin, whereas leaving activities carried or without garbage. The foraging activity of stingless bee is affected by environmental parameters, such as in *T. laeviceps* occurred at temperatures 26–34 °C (Amano *et al.*, 2000) and at humidity 48–98% (Ferreira *et al.*, 2010). Foraging activity of *T. laeviceps* positively correlated with temperature and light intensity, and negatively correlated with humidity (Wati, 2013). Foraging activity for pollens and nectar were high in the morning. Tangmitcharoen and Owens (1997) reported the high visits of pollinator in the morning related to the number of pollens and nectar secretion. In stingless bees, pollens and resin are carried in the corbicula located in the hind-legs, while nectar is carried in the honey crop (Michener, 1974).

Previous studies reported the number of pollen load on stingless bees were positively correlated with body size. Current study showed pollen load on *T. laeviceps*, *L. terminata*, and *H. itama* positively correlated with body size ($rs = 0.519$, $p = 0.003$) (Fig. 3). These results supported Pangestika *et al.* (2018) that the highest pollen load occurred in *H. itama* (31,392 pollen grains), followed by *L. terminata* (23,017 pollen grains) and *T. laeviceps* (8,015 pollen grains). Results of this study were different with Atmowidi *et al.* (2018) that reported the highest pollen load (270,950 pollen grains) on *L. terminata*, followed by *H. itama* (69,802 pollen grains), and *T. laeviceps* (40,802 pollen grains). In this study, *G. thoracica* that has the largest body size, but the number of pollens collected was lowest compared to other species. Our data showed that *G. thoracica* collected medium-sized pollen (25–50 μm), such as pollen of Apocynaceae and Acanthaceae that caused the number of pollens collected was low.

Flower constancy is behavior of flowers-visiting insects in one plant species in a foraging time (Kobayashi-Kidokoro and Higashi, 2010). At Ecology Park of the Indonesian Institute of Sciences, Bogor, Indonesia, Pangestika *et al.* (2017) reported flower constancy of *T. laeviceps* in Poaceae (76.49%), *L. terminata* in Euphorbiaceae (80.46%), and *H. itama* in Solanaceae (83.33%). Atmowidi *et al.*

(2018) also reported *H. itama* preferred to visit Acanthaceae. *Geniotrigona thoracica* predominantly carried pollens of Apocynaceae and Acanthaceae and small number pollens of *Lepisanthes* sp., *Areca macrocalyx*, *Chypokentia* sp., Capparaceae, Asteraceae, and Rubiaceae. Generally, this species collected medium-sized pollens (25–50 um). Zaki and Razak (2018) reported in rubber smallholding environment at Tepoh, Terengganu, Malaysia, *H. itama* collected 37 types of pollens and 29 pollen types were successfully identified. The dominant pollen collected was pollen of *Ixora coccinea.* Barth *et al.* (2020) also reported in the Parque Nacional da Tijuca urban forest at Rio de Janeiro, two species of stingless bees (*Melipona rufiventris* and *Melipona quadrifasciata anthidioides*) dominantly collected pollens of Alchornea, Eucalyptus, *Mimosa caesalpiniifolia*, *M. scabrella*, Melastomataceae, Myrcia, and Solanum. The two species of bees showed the different preference among plant species. In açaí palm (*Euterpe oleracea*), two species stingless bees (*Trigona branneri* and *T. pallens*) collected 128.8 and 58.4 pollen grains (median pollen load), respectively (Bezerra *et al*., 2020). Stingless bees are generalist visitor and play important role in plant pollination, therefore conservation and management of pollinators are needed.

CONCLUSION

The foraging activity of worker stingless bees includes activity of collect pollen, nectar, water, and resin and the activity of removing garbage. In *T. laeviceps*, *L. terminata*, and *H. itama*, pollen load was positively correlated with body size. Flower constancy of stingless bees in an area depends on the availability of flowering plants. In this study *T. laeviceps* showed flower constancy in Chlorantaceae (59.2%), *L. terminata* and *H. itama* in Asteraceae (70.19% and 62.76%), and *G. thoracica* in Apocynaceae (51.51%). *Lepidotrigona terminata* and *H. itama* tend to carry small-sized pollens, while *G. thoracica* carried medium-sized pollen.

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