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Type and size of pollen collected by *Tetragonula laeviceps* at various altitudes

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Abstract. *Abbas M, Sukarsa. 2022. Type and size of pollen collected by* Tetragonula laeviceps *at various altitudes. Biodiversitas 23: 1567-1575.* Pollen's type and size are affected by altitude. No study about pollen types and sizes collected *Tetragonula laeviceps* at various altitudes in Banyumas District, Central Java, Indonesia. This study was aimed to analyze pollen types and sizes collected at various altitudes in Banyumas District. The pollen samples were collected using a random sampling technique at each altitude, namely 46.7 m asl, 317 m asl, and 912 m asl. The pollen samples were collected from flowers around and inside the nest, then prepared using acetolysis. The parameters include pollen morphology, pollen's polar (P) diameter, and the pollen's equatorial (E) diameter. The morphological characteristics and pollen diameter were analyzed statistically using analysis of variance. Pollens' type was not affected by altitudes. However, pollen's diameter was significantly different among altitudes, with the largest pollen size was observed at the highest altitude and the smallest were observed at the lowest altitude. The data are vital for meliponid culture at various altitudes.

Keywords: Diameter, flower, morphology, ornamentation, stingless bee

INTRODUCTION

Tetragonula laeviceps belongs to Tribe Meliponini, Family Apidae. Previously was placed in the Genus *Trigona* (Dollin et al. 2015). Contrary to other members of Apidae, the *Trigona* bee, including *T. laeviceps*, belongs to the stingless apid bee group (Kwapong et al. 2010; Trianto and Purwanto 2020). This species is a significant insect that produces honeybee and propolis (do Nascimento et al. 2015; Utispan et al. 2017; Bisui et al. 2019; Georgieva et al. 2019; Agussalim et al. 2020). Furthermore, stingless bees play an essential role in the process of pollination together with other bee species (Corlett 2011; Putra et al. 2014; Das et al. 2015; Agussalim et al. 2017; Efin et al. 2019; Sayusti et al. 2021). Therefore, they have high prospects of being developed as plant pollinators because of their small size (Das et al. 2015), no sting (Kek et al. 2014), high adaptability to environmental stresses, easy handling, high activity, ease to domesticate and produce various products (Slaa et al. 2006; Jalil 2014; Ya'akob et al. 2018).

Like other pollinators, *T. laeviceps* lives closely with the plant community. Their nest is commonly found in dead trees (Kumar et al. 2012) and inside the tree's hollow (Suriawanto et al. 2017; Absy et al. 2018). The previous study proves that the maximum foraging distance of *Trigona* bees was 500 m apart from their hives (Nugroho and Soesilohadi 2014; Widhiono and Sudiana 2016). The primary purpose of *Trigona* visiting the flower plants is to collect nectar and pollen as their main foods (Suwannapong et al. 2012). Foraging visits of *Trigona* bees to the flowering plant is dependent on the size and shape, even though they originated from the same flower (Nugroho and Soesilohadi 2014). Therefore, *Trigona*'s life depends on the flowering plant availability (Aleixo et al. 2018). Previous studies reported that pollen diversity inside beehives could be used to estimate flowering plant diversity living around the nest (Sajwani et al. 2014; Chauhan et al. 2017; Bareke and Adamassu 2019; Abbas and Sucianto 2020).

Environmental factors such as precipitation, temperature, humidity, and light intensity are also essential factors for the survival of stingless bees (Aleixo et al. 2018). Such ecological factors are also affected by altitude. A previous study proved a positive correlation between altitudes and vegetation density, where denser vegetation was observed in high altitudes (Pratama et al. 2018). As the carrom effect, flowering plant diversity as a source of pollen for bees is influenced by altitude. The presence of flowering plants determines the honey and propolis produced by stingless bees (Nugroho and Soesilohadi 2014). Therefore, differences in altitude produce pollen of varying amounts and sizes (Suwannapong et al. 2012). Jayuli et al. (2018) stated that altitude affects the pollen diameter of honey bees, such as *Apis cerana*, and supported by Pratama et al. (2018), who noted that the pollen collected by honey bees is varied at different altitudes. Previous studies reported pollen diversity collected by *Trigona* bees. The studies were conducted outside Indonesia (Azmi et al. 2015; Bisui et al. 2019). Studies on pollen diversity collected by honey bees at different altitudes have been done outside Banyumas District, Central Java, Indonesia (Jayuli et al. 2018; Pratama et al. 2018; Hugg et al. 2020). There was no study about pollen diversity collected by *T. laeviceps* from different altitudes in the Banyumas District, especially on pollen type and size.

Determining the potential and cultivation efforts of *T. laeviceps* bees is necessary to investigate the types of pollen collected by stingless bees at various altitudes (Hugg et al. 2020). It is also essential to measure its diameter to determine the source of feed that supports productivity (Abbas and Sucianto 2020). Therefore, we could discover an ideal meliponiculture location based on pollen's diameter and altitude. This study aimed to analyze pollen type and size collected at various altitudes in the Banyumas District, Central Java, Indonesia.

MATERIALS AND METHODS

Sampling sites and times

The sampling was performed at three different locations, namely Kaliwangi Village (L1: 46.7 m asl, -7.470328279172163, 109.1023079032395) Purwojati Sub-district, Kalikesur Village (L2: 317 m asl, -7.353448890192117, 109.20835783347272) Kedung-banteng Sub-district; and Glempang Village (L3: 912 m asl, -7.316998931791332, 109.11145437652368), Pekuncen Sub-district, Banyumas District, Central Java, Indonesia. This leads to results in differences in environmental conditions between locations. Samples examination was conducted at the Laboratory of Plant Structure and Development, Faculty of Biology, Jenderal Sudirman University, Banyumas, Indonesia from May to September 2021.

Sampling

The study has used a survey method using a random sampling technique. The pollens were collected from flowering plants about 200 m around and inside the nest, then prepared by acetolysis (Purnobasuki et al. 2014). The pollens were observed under a scanning electron microscope (SEM) and photographed.

Variables and parameters

The variable was pollen diversity collected by *T. laeviceps* bees at various altitudes. However, the main parameters were the pollen's morphological character, i.e., unit, polar (P) diameter, equatorial (E) diameter, type, size, aperture, and ornament. At the same time, air temperature and humidity were the supporting parameters.

Plant identification

We were photographed the flowering plants to identify and observe their morphological characteristics. Furthermore, plant identification was performed using the identification keys available in the PlanNet Identification Application (https://identify.plantnet.org) and Steenis et al. (2013). Flowering plants identification was conducted to know the pollen sources utilized by stingless bees by comparing pollen obtained from the identified flowering plants from around the nest and pollen observed inside the nest.

Pollen preparation and measurement

Pollen was prepared using a method developed by Purnobasuki et al. (2014) called the acetolysis method. The procedure was started by placing the pollen into a falcon bottle containing glacial acetic acid. This fixation step was conducted for 24 h. Fixed pollen was then precipitated by centrifugation with rotator speed at 1000 rpm for ten minutes. Glacial acetic acid was replaced by a mixture of 9 parts of new glacial acetic acid and 1 part of concentrated sulfuric acid. These mixtures were heated at 60ºC inside a water bath for 5 min and centrifuged at 1000 rpm for 10 min. The pellets were then washed using distilled water and were centrifuged at 1000 rpm for 10 min. The centrifugation step was repeated twice to rinse all fixative solutions. The last step was staining the pollen using safranin in glycerin jelly.

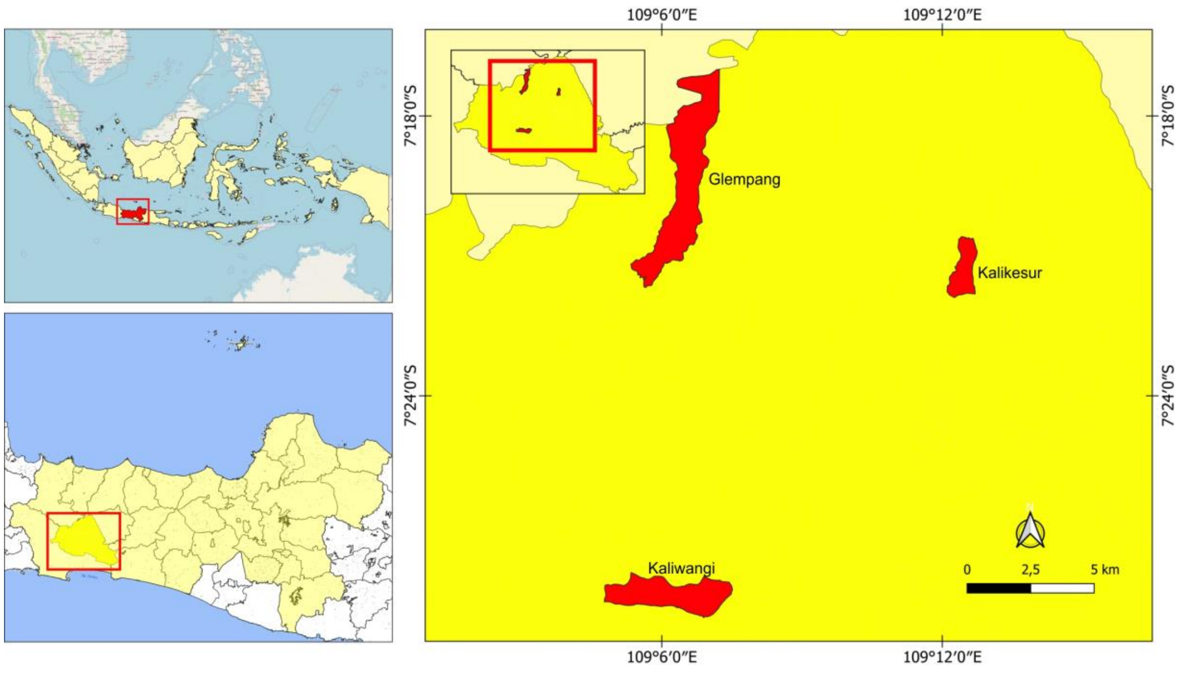

Figure 1. Study sites in Banyumas District, Central Java, Indonesia. 1: Kaliwangi Village, 2: Kalikesur Village, 3: Glempang Village

The stained pollens were observed under a scanning electron microscope (SEM) at 400 times. First, the pollen samples from the stingless bee hive were measured using a micrometer and then identified the pollen type. Second, pollen samples collected from the flowers around the nest were treated the same way. Pollen identification was conducted according to the identification book (Hesse et al. 2009; Halbritter et al. 2018); and Palynological Database (www.paldat.org). The measurements were conducted for polar (P) diameter and equatorial (E) diameter (Rajbhandary et al. 2012). Classification of the pollen types followed the formula from Halbritter et al. (2018), which considered the ratio between polar axis length and equatorial diameter. Pollen types were classified as follow, Preoblate (ratio <50), oblate (50-70), suboblate (75-88), oblate-spheroidal (88-99) spheroidal (100), prolate-spheroidal (101-104), Sub-prolate (114-133), Prolate (133-200) and Per-prolate (>200) (Halbritter et al. 2018). The pollen was classified as very small, small, medium, large, very large, and giant. Tiny pollen has a size of fewer than ten micrometers, while small pollen has a range size between 10 and 25 micrometers. Medium pollen has a size from 25 to 50 micrometers. Large and very large pollen have range sizes from 50 to 100 micrometers and from 100 to 200 micrometers, respectively. The giant pollen has a size larger than 200 micrometers (Halbritter et al. 2018).

Data analysis

The data on pollen types were analyzed descriptively according to literature (Hesse et al. 2009; Halbritter et al. 2018) to determine the pollen collected by stingless bees. Comparison on pollen diameter among sampling sites was analyzed statistically using analysis of variance (ANOVA), followed by a t-test with a confidence level of 5% in SPSS 21 (IBM SPSS Statistics 21).

RESULTS AND DISCUSSION

Pollen type obtained from plants around *Tetragonula laeviceps* nest

Based on the results of identification, the type of flower pollen around the hive, which is a source of food for stingless bees, varies depending on the difference in altitude from three locations in Banyumas District. We found 22 flowering plant species in the first location (L1), Kaliwangi Village. In the second location (L2), Kalikesur Village, 17 flowering plants species were identified during the study, while in the third sampling site (L3, Glempang Village), we observed 15 flowering plants species. The different flowering plant diversity could be caused by the different environmental conditions, such as temperature and humidity (Table 1). Kurniawan and Parikesit (2018) stated that altitude, soil moisture, and light intensity are environmental factors that influence the distribution of plant species. According to Table 1, sampling sites have significantly different altitudes. This difference implied different temperatures and humidity, which will select which plant species could cope with each ecological condition in each location. The selection result was expressed in different flowering plant diversity.

The pollen identification that collected around the beehive from the three locations at different altitudes show three different pollen types, i.e., spheroidal prolate, subprolate, and prolate. Four different sizes of pollen were observed, i.e., very small, small, medium, and large. We found two pollen units from three sites: monad and tetrahedral pollen. The pollens' apertures collected from three locations were tricolpate, monoporate, pantoporate, inaperture, hexacolpate, tetracolpate, tetra aperture and heterocolpate. Furthermore, the pollen ornamentation at the three locations was varied. The ornaments were psilate, echinate, verrucate, reticulate, scrabate, bireticulate, and gemmate. The pollen types were prolate, subprolate, and prolate spheroidal, while pollen sizes were tiny, small, medium, and large. Although only three types and four sizes of pollen were observed, they have various ornamentations and apertures (Table 2).

Other information that we could be retrieved from Table 2 is that each flowering plant species has specific pollen morphology. Therefore, pollen collected by *T. laeviceps* bees could be utilized for estimating flowering plants diversity as a food source for *T. laeviceps* bees. The utilization of pollen diversity as a plant identification tool had been reported by previous studies elsewhere (Sajwani et al. 2014; Chauhan et al. 2017; Bareke and Adamassu 2019; Abbas and Sucianto 2020). According to Salamah et al. (2019), pollen has specific morphology characters that could be used for plant identification.

Pollen observed inside *Trigona's* nest

Pollen identification was conducted by matching pollen of the flowering plants that live around and pollen inside the *T. laeviceps* nest. At Kaliwangi Village (L1), 13 plant species were identified based on the pollen found inside the nest. We could identify ten samples into species level because this flowering plant species live around the *T. laeviceps* nest (Table 2 and Figure 1). However, we could not identify the three remaining samples to the species level because we did not find these three flowering plants living around the nest. At L2, 11 plant species were identified based on the observed pollen inside the nest, in which ten species were matched with the flowering plant living around the *T. laeviceps* nest, and one species was not matched. While at L3, 14 plant species were discovered as a pollen source for *T. laeviceps* bee, of which 11 flowering plant species matched with the species listed in Table 2, and three species did not match and therefore could not be identified (Table 3 and Figure 2). The data indicated that *T. laeviceps* were not utilized all the flowering plants lives closely to their nest as a food source. Food selection by *T. laeviceps* depends more on pollen characteristics than on their distance to the nest. The phenomena could be caused by the pollen's aroma, size, and shape. It has been reported that pollen could attract the *Trigona* because of their type, dimension, and aroma (Nugroho and Soesilohadi 2014).

Based on the pollen found inside the nest, the unidentified flowering plant species could be because the plant lives outside the sampling radius 200 m from the nest. This sampling radius was less than the maximum foraging radius of *Trigona*, which is 500 m (Nugroho and Soesilohadi 2014; Widhiono and Sudiana 2016). In this case, we have not identified flowering plants that live over a radius of 200 m from the *T. laeviceps* nest. Therefore, we could not identify some plant species based on the observed pollen inside the nest without comparing them with the pollen collected from the flowering plants.

Furthermore, the pollen collected by *T. laeviceps* bees had varied morphological characteristics. At L1, the pollens mainly were in small size, with the dimension ranging from 14.7µm-24.6µm. At L2, the size was small and medium, ranging from 14.9µm-45.7µm. At L3, the size was small to medium and large, ranging from 15.3µm-68.9µm. The largest size collected by the stingless bee was discovered at L3 with 68.9um. The types of pollen at the three locations were spheroidal prolate, subprolate, and prolate. These data indicated that *T. laeviceps* bees collected highly diverse pollen types. It has been reported that such phenomena were also observed in other regions in Indonesia (Jayuli et al. 2018; Pratama et al. 2018) and outside Indonesia (Azmi et al. 2015; Bisui et al. 2019).

Meanwhile, the Aperture were tricolporate, tricolpate, monoporate, pantoporate, tetra aperture, and inaperture. The pollen ornamentation in the three areas was varied. The ornaments were echinate, psilate, reticulate, scrabate, bireticulate, and verrucate (Table 3). The characteristics of pollen are used to identify plants by observing their morphology. Additionally, the exine layer has a distinctive structure and ornamentation. Previous studies reported that the morphological structure of exin is specific to plant groups to be used in the identification process. Therefore, the producing plant is discovered by identifying and classifying a pollen grain (Chatterjee et al. 2014; Theodorou et al. 2017; Puspitasari et al. 2018).

Furthermore, the adaptability of *Ageratum conyzoides* plants at different altitudes flowers throughout the year is a good food source for stingless bees. The pollen from the Ageratum conyzoides flower was discovered at three locations: Kaliwangi, Kalikesur, and Glempang, where the plant grows. Pollen of *Zinnia elegans* was found in two locations which include L1 and L3. *Physalis angulata* and *Kyllinga brevifolia* were found in L1 and L2, and *Peperomia pellucida* was found in L1 and L3. The *Trigona* bees visited flowers of various sizes, specifically small and brightly colored flowers and open flowers or specific flower shapes that make it easier for bees to take pollen. Previous studies have reported that bee is so small that it takes pollen from small flowers. In addition, stingless bees prefer flowers with bilateral symmetry because it is the basis for bee landing (Garbuzov and Ratnieks 2014; Salisbury et al. 2015).

It was reported that Trigona bees collected pollen far from their nest (Abbas and Sucianto 2020). The pollen collected by the stingless bee was not compatible with the flowers around the nest, which resulted in difficulty identifying the producing plants (Figure 2). This condition was possible because the location of flowers is outside the observation radius, which was 200 m. According to Pratama et al. (2018) and Nugroho and Susilohadi (2014), stingless bees have a flight distance of a 500m radius from the nest but usually tend to seek food from flowers close to the nest.

Pollen's type collected by stingless bees at various altitudes

Pollen's type collected by stingless bees at three sampling sites with different altitudes is summarized in Table 3. Table 3 showed that similar pollens types were collected by *Trigona* bee across sampling sites (i.e., prolate, subprolate, and prolate spheroidal). The phenomena were also reported in other regions (Azmi et al. 2015; Jayuli et al. 2018; Pratama et al. 2018; Bisui et al. 2019). Another interesting finding summarized in Table 3 was that altitude did not significantly affect the pollen types collected by a stingless bee because prolate spheroidal was the dominant type in all altitudes. It has been previously reported that *Trigona* visits flowering plants because of the pollen's aroma, size, and form (Nugroho and Soesilohadi 2014).

Diameter of pollen collected by stingless bees across sampling sites

Two types of diameters were measured, i.e., polar and equatorial diameters. ANOVA analysis indicated that polar and equatorial diameters were significantly different among altitudes ($p<0.005$). The average polar and equatorial diameters of pollen at each altitude are presented in Table 4. However, careful observation proved that a significant difference was only observed between lowland (L1) and mountain hill (L3). In contrast, L2 was not different either from L1 or L3.

Trigona bee collected larger pollen at a higher altitude (Table 4). Our results were similar to previously reported data from other regions in Indonesia (Jayuli et al. 2018; Pratama et al. 2018) that altitude significantly impacted pollen type collected by *Trigona* bee. However, whether the phenomenon indicates a direct effect of altitude on pollen size or plant species was not clear. The most reliable argument would be that altitude directly impacts plant distribution because different altitudes have different ecological characteristics. Therefore, different altitudes would have different plant diversity, with some species could distribute across altitudes while others could not (Neri et al. 2017; Mahmoudi et al. 2018; Admassu et al. 2020. For example, the largest pollen diameter collected by *Trigona* was 68.9 m, which is *Portulaca grandiflora* pollen. This plant species was not found in L1 and L2.

Table 1. Environmental conditions at three different sampling sites

Ecological Parameter	Sampling sites		
	Kaliwangi Village	Kalikesur Village	Glempang Village
Altitude (m asl.)	46.7	317	912
Topography	Lowland hill	Plateau	Mountain plateau
Temperature (°C)	30.67 ± 0.58^a	27.67 ± 1.15^b	23.33 ± 0.58^c
Humidity (%)	72.33 ± 1.15^a	80.67 ± 0.58^b	84.67 ± 0.58^c

Note: values followed by different words indicates significant different

Table 2. Morphological characteristics of pollen found around stingless bee nest in three locations

Plant species	Pollen morphology						
	Unit	Polar axis length (P) µm	Equatorial diameter (E) µm	Type	Size	Aperture	Ornament
Kaliwangi Village							
Ageratum conyzoides	Monad	14.7 ± 16.5	14.0 ± 16.0	Prolate spheroidal	Small	Tricolporate	Echinate
Zinnia elegans	Monad	20.2 ± 22.4	19.9 ± 22.7	Prolate spheroidal	Small	Tricolpate	Echinate
Tithonia rotundifolia	Monad	22.8 ± 32.6	21.3 ± 32.3	Subprolate	Small	Tricolpate	Verrucate
Melampodium divaricatum	Tetrahedral	14.2 ± 19.3	13.5 ± 19.2	Prolate spheroidal	Small	Tricolpate	Echinate
Cyanthillium cinereum	Monad	26.4 ± 27.5	23.8 ± 27.3	Prolate spheroidal	Small	Tricolpate	Psilate
Panicum trichoides	Monad	16.7 ± 25.0	15.0 ± 22.7	Prolate spheroidal	Small	Monoporate	Psilate
Leersia virginica	Monad	29.4 ± 37.4	26.5 ± 30.6	Subprolate	Medium	Tricolpate	Psilate
Amaranthus spinosus	Monad	18.6 ± 23.1	17.5 ± 22.8	Prolate spheroidal	Small	Pantoporate	Psilate
Gomphrena globosa	Monad	14.6 ± 18.3	14.0 ± 18.2	Prolate spheroidal	Small	Pantoporate	Echinate
Mimosa pudica	Tetrahedral	7.1 ± 7.8	6.8 ± 7.7	Prolate spheroidal	Very small	Tricolpate	Psilate
Sesbania grandiflora	Monad	32.2 ± 35.4	27.9 ± 33.1	Prolate spheroidal	Medium	Triporate	Reticulate
Ocimum basilicum	Monad	42.1 ± 49.7	41.9 ± 48.7	Prolate spheroidal	Medium	Hexacolpate	Bireticulate
Turnera subulata	Monad	37.1 ± 50.0	36.8 ± 45.3	Prolate spheroidal	Medium	Tricolpate	Reticulate
Impatiens balsamina	Monad	28.4 ± 34.1	18.8 ± 23.5	Prolate	Medium	Tetracolpate	Reticulate
Physalis angulata	Monad	18.9 ± 20.0	18.6 ± 19.9	Prolate spheroidal	Small	Tricolpate	Psilate
Dimocarpus longan	Monad	16.7 ± 19.2	15.8 ± 18.3	Prolate spheroidal	Small	Tricolpate	Reticulate
Kyllinga brevifolia	Monad	13.5 ± 18.6	12.1 ± 18.0	Prolate spheroidal	Small	Tetra aperture	Psilate
Ruellia simplex	Monad	51.2 ± 60.1	45.8 ± 54.4	Prolate spheroidal	Large	Triporate	Gemmate
Ixora acuminata	Monad	18.0 ± 21.2	15.0 ± 15.8	Subprolate	Small	Tricolporate	Reticulate
Antigonon leptopus	Monad	48.1 ± 50.0	46.5 ± 48.6	Prolate spheroidal	Medium	Tricolpate	Reticulate
Carica papaya	Monad	36.8 ± 40.9	25.1 ± 34.2	Subprolate	Medium	Tricolporate	Reticulate
Jatropha integerrima	Monad	59.8 ± 68.2	58.2 ± 60.3	Subprolate	Large	In aperture	Reticulate
Kalikesur Village							
Ageratum conyzoides	Monad	16.6 ± 20.6	16.2 ± 19.4	Prolate spheroidal	Small	Tricolporate	Echinate
Cosmos sulphureus	Monad	27.2 ± 32.2	25.4 ± 31.8	Prolate spheroidal	Medium	Tricolporate	Echinate
Sphagneticola trilobata	Monad	26.5 ± 27.6	20.9 ± 26.7	Prolate spheroidal	Medium	Tricolporate	Echinate
Emilia sonchifolia	Monad	27.7 ± 41.4	27.6 ± 32.9	Prolate spheroidal	Medium	Tricolporate	Echinate
Physalis angulata	Monad	25.3 ± 31.0	22.6 ± 25.1	Prolate spheroidal	Medium	Tricolporate	Reticulate
Kyllinga brevifolia	Monad	11.5 ± 16.7	10.1 ± 14.0	Subprolate	Small	Tetra aperture	Psilate
Acalypha indica	Monad	28.0 ± 36.2	27.4 ± 34.9	Prolate spheroidal	Medium	Tricolporate	Psilate
Peperomia pellucida	Monad	37.4 ± 47.7	26.4 ± 32.1	Prolate	Medium	In aperture	Verrucate
Capsicum annuum	Monad	26.5 ± 38.3	22.1 ± 25.9	Prolate spheroidal	Medium	Tricolporate	Reticulate
Hippobroma longiflora	Monad	50.3 ± 51.2	46.4 ± 47.4	Prolate spheroidal	Large	Tricolporate	Echinate
Cuphea hyssopifolia	Monad	16.5 ± 17.9	15.6 ± 17.7	Prolate spheroidal	Small	Tricolporate	Psilate
Episcia cupreata	Monad	26.0 ± 33.0	25.7 ± 33.0	Prolate	Medium	Tricolporate	Reticulate
Dicliptera chinensis	Monad	24.9 ± 26.0	15.9 ± 17.9	Prolate	Small	Heterocolpate	Reticulate
Rosa chinensis	Monad	28.8 ± 35.3	25.2 ± 33.9	Prolate spheroidal	Medium	Tricolporate	Psilate
Trimezia martinicensis	Monad	27.2 ± 33.6	24.3 ± 31.3	Prolate spheroidal	Medium	Monocolpate	Psilate
Mirabilis jalapa	Monad	26.7 ± 39.2	25.6 ± 38.8	Prolate	Medium	Pantoporate	Psilate
Bougainvillea spectabilis	Monad	25.9 ± 28.6	24.7 ± 28.3	Prolate spheroidal	Medium	Tricolpate	Scrabate
Glempang Village							
Ageratum conyzoides	Monad	17.1 ± 22.0	15.2 ± 15.8	Prolate spheroidal	Small	Tricolporate	Echinate
Melampodium divaricatum	Monad	15.5 ± 18.4	15.0 ± 16.4	Prolate spheroidal	Small	Tricolpate	Echinate
Symphyotrichum lanceolatum	Monad	16.6 ± 23.7	15.9 ± 20.1	Prolate spheroidal	Small	Tricolpate	Echinate
Zinnia elegans	Monad	20.7 ± 24.6	20.3 ± 24.4	Prolate spheroidal	Small	Tricolpate	Echinate
Punica granatum	Monad	16.9 ± 21.3	16.1 ± 17.5	Prolate spheroidal	Small	Tricolporate	Psilate
Portulaca grandiflora	Monad	52.5 ± 73.1	50.3 ± 68.8	Prolate spheroidal	Large	Pantocolpate	Echinate
Impatiens balsamina	Monad	26.7 ± 29.6	17.6 ± 19.5	Prolate	Medium	Tetracolpate	Reticulate
Antigonon leptopus	Monad	51.2 ± 57.0	49.5 ± 54.9	Prolate spheroidal	Large	Tricolporate	Verrucate
Peperomia pellucida	Monad	34.4 ± 45.2	25.1 ± 38.6	Prolate	Medium	Inaperture	Psilate
Rosa chinensis	Monad	30.4 ± 35.1	24.5 ± 35.0	Prolate spheroidal	Medium	Tricolporate	Reticulate
Perilla frutescens	Monad	23.6 ± 31.8	19.3 ± 28.6	Prolate spheroidal	Medium	Tricolpate	Bireticulate
Jatropha integerrima	Monad	31.2 ± 44.5	16.5 ± 25.1	Subprolate	Medium	Inaperture	Reticulate
Begonia hirtella	Monad	10.8 ± 12.1	7.9 ± 9.2	Prolate	Small	Tricolporate	Psilate
Calathea warscewiczii	Monad	42.2 ± 43.8	28.8 ± 38.7	Prolate	Medium	Tricolpate	Psilate
Cynodon dactylon	Monad	25.0 ± 30.4	23.5 ± 30.4	Prolate spheroidal	Medium	Monoporate	Psilate

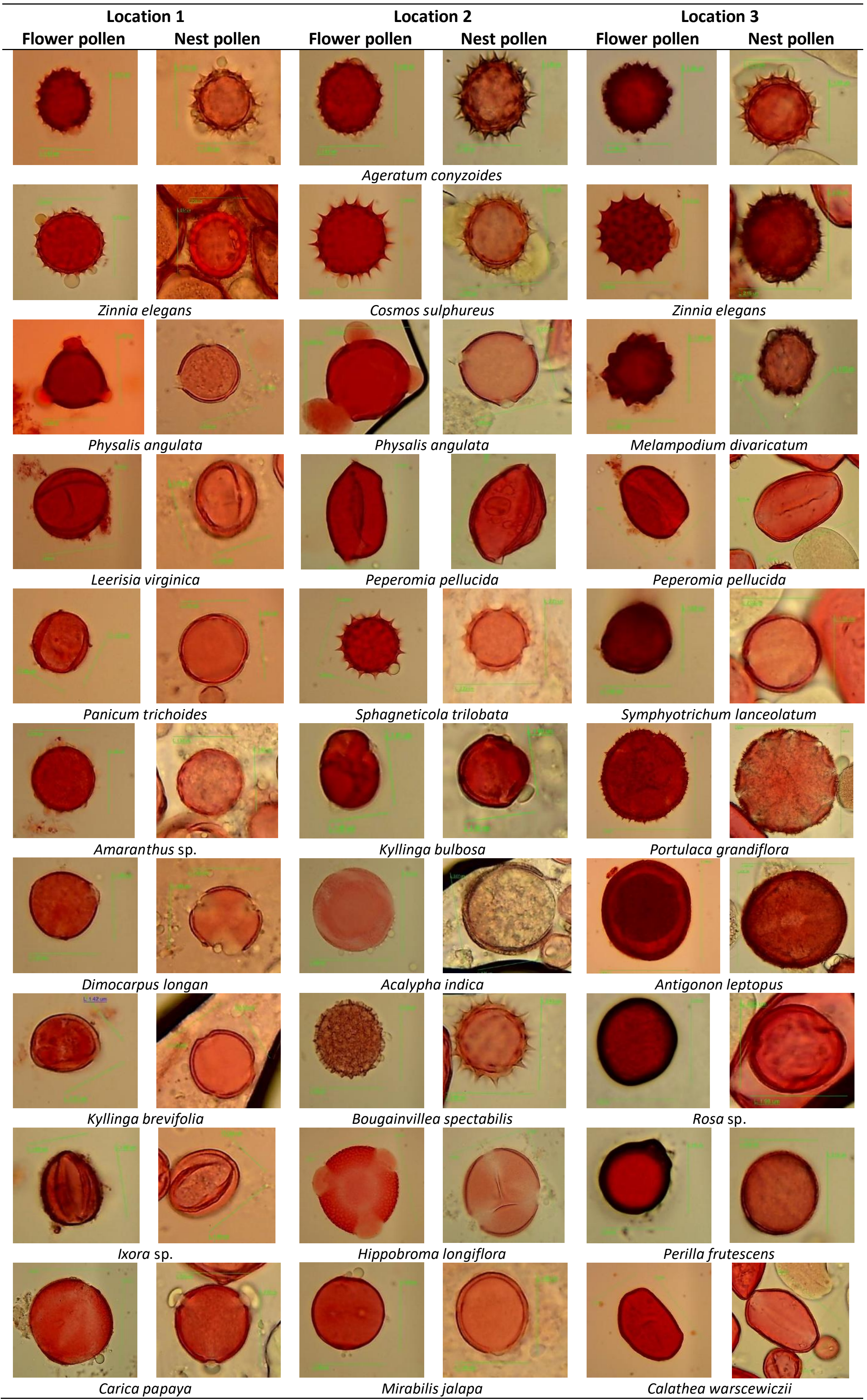

Figure 1. The pollen from the flowering plants lives around and from inside the hive at various altitudes

Table 3. Morphological characteristics of pollen contained in stingless bee nest at the three locations

Location	No.	Plant species	Unit	Polar axis length (P) µm	Equatorial diameter (E) µm	Type	Size	Aperture	Ornament
Kaliwangi	1	*Ageratum conyzoides*	Monad	14.7 ± 17.3	14.4 ± 15.4	Prolate spheroidal	Small	Tricolporate	Echinate
	2	*Zinnia elegans*	Monad	21.1 ± 23.8	19.4 ± 23.6	Prolate spheroidal	Small	Tricolpate	Echinate
	3	*Panicum trichoides*	Monad	17.1 ± 19.3	16.1 ± 17.1	Prolate spheroidal	Small	Monoporate	Psilate
	4	*Leersia virginica*	Monad	17.0 ± 20.6	16.3 ± 19.2	Prolate spheroidal	Small	Tricolpate	Psilate
	5	*Amaranthus spinosum*	Monad	19.1 ± 19.3	17.8 ± 18.8	Prolate spheroidal	Small	Pantoporate	Psilate
	6	*Physalis angulata*	Monad	18.9 ± 20.7	17.0 ± 19.9	Prolate spheroidal	Small	Tricolpate	Psilate
	7	*Dimocarpus longan*	Monad	19.3 ± 19.6	18.8 ± 19.3	Prolate spheroidal	Small	Tricolpate	Reticulate
	8	*Kyllinga brevifolia*	Monad	17.2 ± 18.5	16.2 ± 17.4	Prolate spheroidal	Small	Tetra aperture	Psilate
	9	*Ixora acuminata*	Monad	18.4 ± 19.8	12.9 ± 16.4	Subprolate	Small	Tricolporate	Reticulate
	10	*Carica papaya*	Monad	23.6 ± 24.6	23.1 ± 23.2	Prolate spheroidal	Small	Tricolporate	Reticulate
Kalikesur	1	*Ageratum conyzoides*	Monad	17.1 ± 20.2	16.8 ± 19.3	Prolate spheroidal	Small	Tricolporate	Echinate
	2	*Cosmos sulphureus*	Monad	24.2 ± 24.6	23.9 ± 24.3	Prolate spheroidal	Small	Tricolporate	Echinate
	3	*Sphagneticola trilobata*	Monad	21.5 ± 24.7	20.2 ± 25.2	Prolate spheroidal	Small	Tricolporate	Echinate
	4	*Physalis angulata*	Monad	21.2 ± 22.9	20.7 ± 22.1	Prolate spheroidal	Small	Tricolporate	Reticulate
	5	*Kyllinga brevifolia*	Monad	14.9 ± 16.8	13.4 ± 15.2	Subprolate	Small	Tetra aperture	Echinate
	6	*Acalypha indica*	Monad	25.4 ± 26.7	22.2 ± 22.4	Subprolate	Medium	Tricolporate	Psilate
	7	*Mirabilis jalapa*	Monad	36.0 ± 38.2	22.8 ± 29.9	Prolate	Medium	Pantoporate	Psilate
	8	*Bougainvillea spectabilis*	Monad	25.1 ± 26.8	22.5 ± 25.6	Prolate spheroidal	Medium	Tricolpate	Scrabate
	9	*Hippobroma longiflora*	Monad	34.1 ± 43.6	33.2 ± 41.2	Prolate spheroidal	Medium	Tricolporate	Echinate
	10	*Peperomia pellucida*	Monad	41.0 ± 45.7	26.1 ± 26.4	Prolate	Medium	Inaperture	Verrucate
Glempang	1	*Ageratum conyzoides*	Monad	17.1 ± 22.0	16.5 ± 17.5	Prolate spheroidal	Small	Tricolporate	Echinate
	2	*Melampodium divaricatum*	Monad	15.3 ± 16.2	15.0 ± 15.9	Prolate spheroidal	Small	Tricolpate	Echinate
	3	*Symphyotrichum lanceolatum*	Monad	18.9 ± 23.3	16.2 ± 23.3	Prolate spheroidal	Small	Tricolpate	Echinate
	4	*Zinnia elegans*	Monad	22.0 ± 23.3	20.3 ± 21.9	Prolate spheroidal	Small	Tricolpate	Echinate
	5	*Portulaca grandiflora*	Monad	65.3 ± 68.9	62.9 ± 67.5	Prolate spheroidal	Large	Pantocolpate	Echinate
	6	*Antigonon leptopus*	Monad	46.3 ± 49.9	42.0 ± 46.6	Prolate spheroidal	Medium	Tricolporate	Verrucate
	7	*Peperomia pellucida*	Monad	35.6 ± 42.5	23.0 ± 23.4	Prolate	Medium	Inaperture	Verrucate
	8	*Rosa chinensis*	Monad	26.3 ± 35.1	26.0± 35.0	Prolate spheroidal	Medium	Tricolporate	Reticulate
	9	*Perilla frutescens*	Monad	20.6 ± 23.1	19.7 ± 21.9	Prolate spheroidal	Medium	Tricolpate	Bireculate
	10	*Calathea warscewiczii*	Monad	38.7 ± 42.1	25.0 ± 25.8	Prolate	Medium	Tricolpate	Psilate
	11	*Cynodon dactylon*	Monad	25.1 ± 26.0	24.5 ± 25.3	Prolate spheroidal	Medium	Monoporate	Psilate

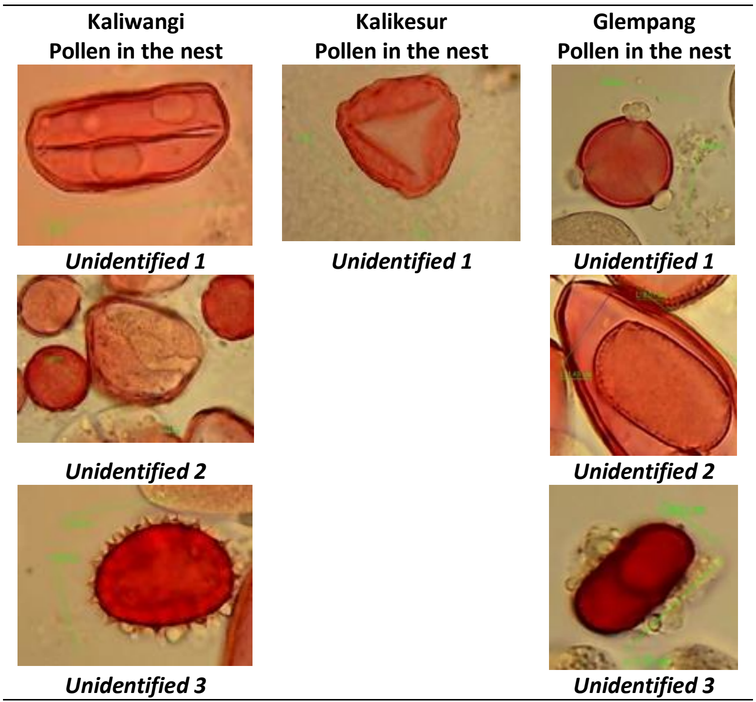

Figure 2. The unidentified pollen collected inside *Tetragonula laeviceps* bee nest from different altitudes

Table 4. The average polar and equatorial diameters of pollen at each altitude

Altitude (m asl)	Polar diameter (um)	Equatorial diameter (um)	Significant*
L1 (0-300)	19.7 ± 3.256203	18.4625 ± 3.647284	a
L2 (300-600)	29.05714 ± 11.63799	23.9875 ± 7.527936	ab
L3 (600-100)	35.5 ± 16.5743	30.5375 ± 17.43756	b

Note: *) Numbers followed by the same letter indicate different mean values

In conclusion, based on our results it could be concluded that *Tetragonula laeviceps* collected similar pollen types but different pollen sizes at various altitudes. These data are essential for meliponid culture development, especially in the Banyumas District, Central Java Province, Indonesia.

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