SPECIES DESCRIPTION, MORPHOMETRIC MEASUREMENT AND MOLECULAR IDENTIFICATION OF STINGLESS BEES (HYMENOPTERA: APIDAE: MELIPONINI) IN MELIPONICULTURE INDUSTRY IN WEST JAVA PROVINCE, INDONESIA

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ABSTRACT

This study was aimed to identify stingless bees commonly used in meliponiculture in Ciamis Regency, West Java Province, Indonesia based on morphological, morphometric, and molecular characters. Six species from three genera, namely *Tetragonula*, *Heterotrigona,* and *Lepidotrigona*, were obtained. There were *Tetragonula laeviceps* with morphological characteristics of a shiny black body and a body length of 3.42±0.02 mm (mean±SD); *T. sarawakensis* with morphological characteristics of a predominantly black body and a body length of 4.63±0.01 mm (mean±SD); *T.* cf. *biroi* with morphological characteristics of a body dominated by black and a body length of 3.94±0.02 mm (mean±SD); *T. drescheri* with morphological characteristics of a body dominated by a brownish-black color and body length of 4.64±0.01 mm (mean±SD); *Heterotrigona itama* with morphological characteristics of a dark black dominant body color and a body length of 4.84±0.02 mm (mean±SD); and *Lepidotrigona terminata* with morphological characteristics of a black dominant body color and body length of 4.63±0.01 mm (mean±SD). The sequences of stingless bees from Ciamis Regency, West Java Province have similarities (89-97%) with those of similar species of stingless bee in the GenBank database. The results of this study provide preliminary data contributing to the conservation and management as one the Indonesia's important biodiversity resources.

Keywords: Beekeepers, Ciamis Regency, conservation, characteristics.

ABSTRAK

Kajian ini bertujuan untuk mengecamkan spesies lebah kelulut yang digunakan dalam peternakan lebah di Kabupaten Ciamis, Provinsi Jawa Barat, Indonesia berdasarkan ciri morfologi, morfometrik dan molekul. Enam spesies dari tiga genus iaitu *Tetragonula*, *Heterotrigona* dan *Lepidotrigona* telah dikenal pasti. *Tetragonula laeviceps* dengan ciri morfologi badan berwarna hitam berkilat dan panjang tubuh 3.42±0.02 mm (purata±SD); *T. sarawakensis* dengan ciri morfologi tubuh didominasi warna hitam dan panjang tubuh 4.63±0.01 mm (purata±SD); *T.* cf. *biroi* dengan ciri morfologi tubuh didominasi warna hitam dan panjang tubuh 3.94±0.02 mm (purata±SD); *T. drescheri* dengan ciri morfologi tubuh yang didominasi warna hitam kecoklatan dan panjang tubuh 4.64±0.01 mm (purata±SD); *Heterotrigona itama* dengan ciri morfologi warna tubuh dominan hitam pekat dan panjang tubuh 4.84±0.02 mm (purata±SD) dan *Lepidotrigona terminata* dengan ciri morfologi warna tubuh dominan hitam dan panjang tubuh 4.63±0.01 mm (purata±SD). Jujukan DNA spesies lebah kelulut dari Kabupaten Ciamis, Provinsi Jawa Barat menunjukkan persamaan (89-97%) dengan spesies lebah kelulut di pangkalan data GenBank. Hasil kajian ini memberikan data awalan bagi menyumbang kepada pengurusan dan pemuliharaan salah satu sumber biologi yang penting di Indonesia.

Kata Kunci: Penternak lebah, Kabupaten Ciamis, pemuliharaan, ciri.

INTRODUCTION

Stingless bees (Hymenoptera: Apidae) are belong to a group of eusocial insects that live together in a hive (Michener 2007). In nature, stingless bees play an essential role in the process of plant pollination (Norowi et al. 2010; Kelly et al. 2014). The bees also produce significant amounts of honey and propolis (Francoy 2009; Kumar et al. 2012; Lourino et al. 2006; Mohamad et al. 2020). Indonesia has many species of stingless bee that are widespread throughout the island (Rasmussen 2008). Java Island is one of the islands with a high level of organism endemicity, as it was formed of a long ecological and geological process, which created a unique island landscape with a diversity of flora and fauna (Whitmore 1975).

Several *Apis* species namely *A. cerana*, *A. dorsata* and *A. mellifera* (Hadisoesilo 2001), and *A. koschevnikovi* have been discovered in Java (Mathew & Mathew 1988; Tingek et al. 1988). Meanwhile, species of stingless bee found in Java including *Trigona (Tetragonula) iridipennis, Trigona (Tetragonula) laeviceps, Trigona (Tetrigona) apicalis, Trigona (Lepidotrigona) nitidiventris, Trigona (Lepidotrigona) ventralis, Trigona (Lepidotrigona) terminata, Trigona (Tetragonula) fuscobalteata,* and *Trigona (Heterotrigona) itama* (Erniwati 2013; Sakagami et al. 1990). Thus, through an exploration and identification of stingless bees, it is likely that new species or new records will be found on the island of Java.

This study was aimed to identify stingless bees species commonly used in meliponiculture in Ciamis Regency, West Java Province, Indonesia based on morphological, morphometric, and molecular characters. There are several beekeepers focusing on the stingless bee culture in Ciamis. The bee stock for their culture was obtained from the surrounding area. It is expected that in the future this area can be used as a centre for stingless bee conservation and culture in West Java Province. However, lack information on the bees species from the West Java Province, therefore this research is urgently needed.

MATERIALS AND METHODS

Sampling Location

Sampling of the stingless bee was conducted from October to December 2019 from beekeepers in Ciamis Regency, West Java Province (7^{o}19’44.14” S 108^{o}21’19.59” E), Indonesia.

Morphological Identification and Morphometric Measurement

The identification of stingless bees based on morphological characters of the head, antennae, thorax, wings, legs, and abdomen structure and body coloration (Figure 1). Furthermore, as many as 35 morphological characters of stingless bee were selected as parameters for morphometric measurement (Table 1) following Dollin et al. (1997), Samsudin et al. (2018), Sakagami (1978), Sakagami & Inoue (1987), Sakagami et al. (1990), Smith (2012), Trianto & Marisa (2020), Trianto & Purwanto (2020a) and Suprianto et al. (2020).

Table 1. List of parameters used for morphometric measurement

No.	Body characters
1	Body Length (BL)
2	Head Length (HL)
3	Head Width (HW)
4	Mandible Length (ML)
5	Mandible Widht (MW)
6	Clypeus Length (CL)
7	Lower Interocular Distance (LID)
8	Upper Interocular Distance (UID)
9	Eye Width (EW)
10	Eye Length (EL)
11	Maximum Interorbital Distance (MOD)
12	Lower Interorbital Distance (LOD)
13	Interantennal Distance (IAD)
14	Interocellar Distance (IOD)
15	Ocellocular Distance (OOD)
16	Antennocellar Distance (AD)
17	Antennocullar Distance (AOD)
18	Gena Width (GW)
19	Length of Flagellomere IV (FL)
20	Width of Flagellomere IV (FW)
21	Malar Length (ML)
22	Mesoscutum Length (MCL)
23	Mesoscutum Width (MCW)
24	Length of Forewing Including Tegula (WL1)
25	Distance Between M-Cu Bifurcation (WL2)
26	Fore Wing Length (FWL)
27	Fore Wing Width (FWW)
28	Hind Wing Length (HWL)
29	Hind Wing Width (HWW)
30	Hamuli Number (HN)
31	Hind Femur Length (HFL)
32	Hind Tibia Width (HTW)
33	Hind Tibia Length (HTL)
34	Hind Basitarsus Width (HBW)
35	Hind Basitarsus Length (HBL)

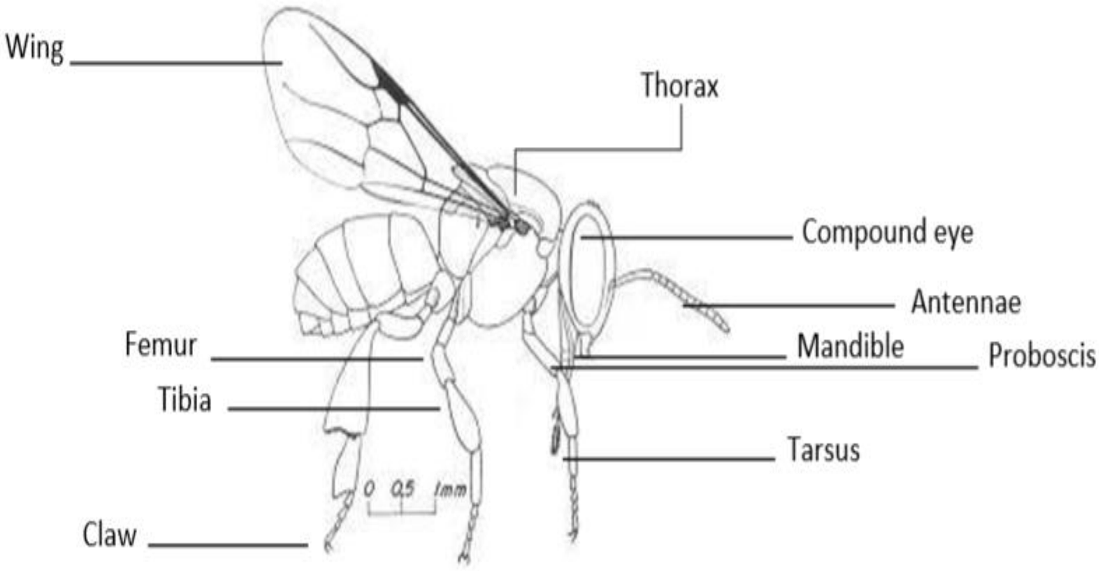

Figure 1 Schematic dof stingless bees showing principal body parts for measurement purposes (Samsudin et al. 2018)

Morphological and morphometric analyses were carried out at the Entomology Laboratory, Faculty of Biology, Universitas Gadjah Mada. Ten individual specimens from each species were taken for morphological measurement and observation, especially the color of each body part. Images of the body parts such as the head, thorax, wings, and legs (Table 1) for each species were taken and included in the description of each species (Smith 2012). Morphological and morphometric analyses were performed using a binocular XSZ-107 BN Microscope with an Optilab viewer and Image Raster software.

Furthermore, morphometric data were analyzed using the Principal Component Analysis (PCA) to observe the pattern of grouping five species of stingless bees based on the 35 morphometric characters analyzed in this study. Correlation analysis of between-group results in eigenvalue and percentage variance are shown in Table 5, whereas the plot distribution can be seen in Figure 8.

Table 5 Eigenvalue and % Variance

PC	Eigenvalue	% Variance
1	2.09942	88.470
2	0.14281	6.0183
3	0.07752	3.2667
4	0.04604	1.9405
5	0.00318	0.1342

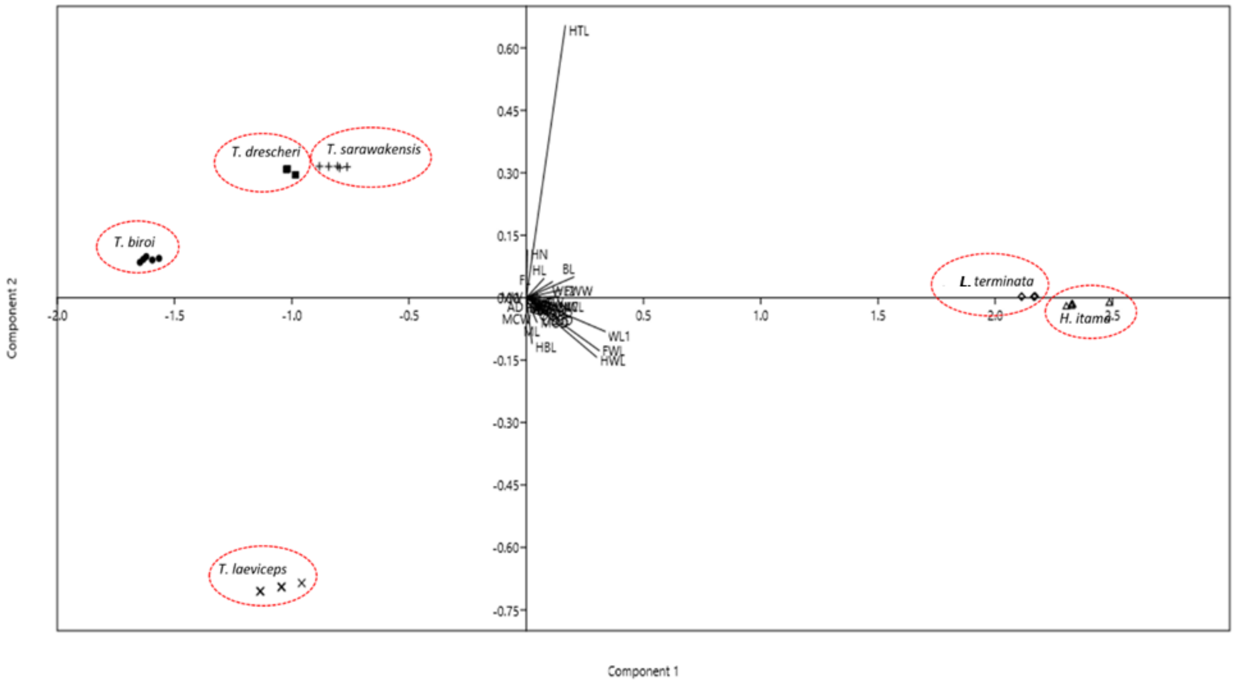

Figure 8 PCA results for stingless bees. The dominant characters in the formation of the six groups are HTL, followed by HWL, FWL, WL1, and BL. This can be seen from the arrows. The longer the arrow, the higher the character's role in group formation

Molecular Identification

After the morphological and morphometric identification processes had been completed, a molecular identification process was carried out. Molecular analysis was carried out at the Biotechnology and FALITMA Laboratory, Faculty of Biology, Universitas Gadjah Mada. The analysis process consists of three stages, namely DNA extraction, amplification of Polymerase Chain Reaction (PCR), and DNA sequencing analysis. For DNA extraction, all parts of the bee (except the head and wings) were used for the DNA extraction process. DNA extraction was performed using the CTAB method described by Thummajitsakul et al. (2011) and Trianto & Purwanto (2020b). One individual of stingless bee/species was ground using an ice-cold pestle with 500 ul CTAB buffer and then transferred to a 1.5 mL microtube. The sample was incubated at 55^{o}C-65^{o}C for 30 mins, then 500 µl chloroform was added, the DNA preparations were shaken at 120 rpm for 30 mins. After shaking, the preparations were centrifuged at 5.000 rpm for 5 min. The supernatants were transferred to new microtube. Then, an equal volume of isopropanol (1:1, v/v) was added to the tube. The DNA preparations were then incubated at room temperature for 10 min. The samples were centrifuged at 5.000 rpm for 5 min; subsequently, the supernatant was removed. DNA pellets were washed using 70% ethanol, then centrifuged at 5.000 rpm for 5 min. Finally, the DNA pellets were dried for 15 min and resuspended by using 50 µl TE buffer.

Polymerase Chain Reaction (PCR) amplification and DNA sequencing were conducted using the mitochondrial 16S rRNA gene primers following Thummajitsakul et al. (2013). PCR was completed in 35 cycles in a 30 µl reaction volume, based on Na-Nokorn et al. (2006) and Mahendran et al. (2006). PCR reactions were performed using GoTaq Green master mix (Promega) under the following conditions: Pre-denaturation 95°C for 2 min; 35 cycles of denaturation at 95°C for 30 s; annealing at 50°C for 30 s; extension at 72°C for 30 s; and a final hold at 4°C. Amplified DNA (amplicon) was resolved on 1% agarose gel and visualized under UV transillumination. The amplicons were sent to the 1st Base DNA sequencing facility.

The DNA sequence data from the sequencing facility were checked and edited using the Gene Studio software. Then, the DNA sequences were compared to the Genbank database using a Nucleotide BLAST (Madden 2013) search on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cg). The results showed the stingless bees most closely related stingless bees to the samples (Table 2). The phylogram reconstruction (phylogenetic tree) is completed using the Neighbour-Joining method with 1000 bootstrap value with the Kimura 2-Parameter (K2P) model in the MEGA X program (Kumar et al. 2012).

Table 2 Result of the 16S rRNA mitochondrial gene BLAST analysis

No.	**Sample**	**Sequence in Genebank**	**Accesion Number**	**Identity**
1.	*T. laeviceps* Jawa Barat	*T. laeviceps*	DQ790420.1	99%
2.	*T.* cf. *biroi* Jawa Barat	*T. pagdeni*	DQ790413.1	74%
3.	*T. sarawakensis* Jawa Barat	*T. sarawakensis*	DQ790435.1	97%
4.	*T. drescheri* Jawa Barat	*T. drescheri*	MH453963.1	98%
5.	*H. itama* Jawa Barat	*H. itama*	DQ790396.1	99%
6.	*L. terminata* Jawa Barat	*L. terminata*	DQ790398.1	98%

RESULTS AND DISCUSSION

Six species under three stingless bee genera were identified (Table 3). Furthermore, the morphological, morphometric, and molecular characters of each species of stingless bee are displayed and described and are accompanied by photographs of the parts observed (Figure 2-7). There are six species of stingless bee found in West Java belonging to the genera *Tetragonula* (4 species), *Heterotrigona* (1 species), and *Lepidotrigona* (1 species). In this study, we found several types of morphological characters in the genus *Tetragonula,* namely a mandible with two small teeth, a malar area shorter than the diameter of the antennal flagellum, a rounded gena, shorter than compound eye, a propodeum of moderate size, a smooth, hairless and shiny basal area, a hind basitarsus narrower than the tibia, an abdomen slightly narrower than the thorax, and a total of five hamuli per wing. The morphological characters observed in the genus *Heterotrigona*, were a mandible with one denticle, a long malar area, a rounded gena as broad as the eye, a short scutellum, a rather short, propodeum, a smooth and hairless basal area, a hind basitarsus as broad as half its length, an abdomen narrower than the thorax, and a total of 7 hamuli per wing. Finally, the morphological characters of the genus *Lepidotrigona* were a mandible with two small teeth, a compound eye narrower than the gena, a short scutellum, a hind basitarsi broader than half its length, an abdomen narrower than the thorax, and a total of 6 hamuli per wing.

Table 3 Species checklist of stingless bees

No.	Species
1.	*Tetragonula laeviceps* (Smith 1857)
2.	*Tetragonula* cf. *biroi* (Fries 1898)
3.	*Tetragonula drescheri* (Schwarz 1939)
4.	*Tetragonula sarawakensis* (Schwarz 1939)
5.	*Heterotrigona itama* (Cockerell 1918)
6.	*Lepidotrigona terminata* (Smith 1987)

Morphology of Stingless Bees

Tetragonula laeviceps (Smith 1857)

Figure 2 *Tetragonula laeviceps* (Smith 1857); a. Habitus, Lateral view; b. Frons; c. Mesoscutum; d. Forewing; e. Hind tibia & basitarsus. Scale: 1.0 mm.

Head: Small, width almost double the length and covered with short setae. Clypeus and frons separated by episomal sulcus and covered by fine white hair. Compound eyes large and reddish in colour, ocelli blackish and large. Antennae geniculate, 10 flagellomeres, uniformly covered with short, erect setae, long scape, pedicel, and entire flagella double the scape. The antennal socket grey, pedicel, and scape are yellowish-brown; the first flagellomere is brown; and the second to tenth flagellomeres are brown and slightly black. Mandible with two teeth, brown and slightly black at the basal.

Thorax: Mesoscutum finely punctuated, small, black, and fully covered with yellowish setae, scutellum entirely black and covered with yellowish setae at the posterior end. Tegulae rounded, large, and highly sclerotized. Wing venation weak, length of forewing almost three times the width, tegulae black, forewing coloration uniform and semitransparent, wing venation dark brown. Total of five hamuli on hindwings. Hind tibiae short, sparsely covered with long setae at the apex but short at the base, pear-shaped corbicula, short hind basitarsi, and tibia three times the length of the basitarsus.

Abdomen: First to third gastral tergite smooth, fourth to sixth tergite fully covered with short setae. Sternite covered with short setae.

***Tetragonula* cf. *biroi* (Friese 1898)**

Figure 3 *Tetragonula* cf. *biroi* (Friese 1898); a. Habitus, Lateral view; b. Frons; c. Mesoscutum; d. Forewing; e. Hind tibia & basitarsus. Scale: 1.0 mm.

Head: Small, almost twice the length of the head, and covered with short setae. Clypeus and frons are separated by episomal sulcus and covered by fine brown hair. Compound eyes and ocelli brown. Geniculate antennae, 11 flagFellomeres, scape yellowish-brown, socket grey, pedicel brown, first flagellomere brown, second to tenth flagellomeres brown and slightly black. Mandible with two teeth, brown and slightly black at base.

Thorax: Mesoscutum small, black, and fully covered with black hair, scutellum black, and covered with brown setae at the posterior end. There are two pairs of transparent wings, wing venation dark brown, tegula rounded, and dark. Total of five hamuli on hindwings. Hind tibiae entirely blackish, corbicula pear shape, and basitarsi wholly black.

Abdomen: Blackish. Sternite covered with short setae.

***Tetragonula drescheri* (Schwarz 1939)**

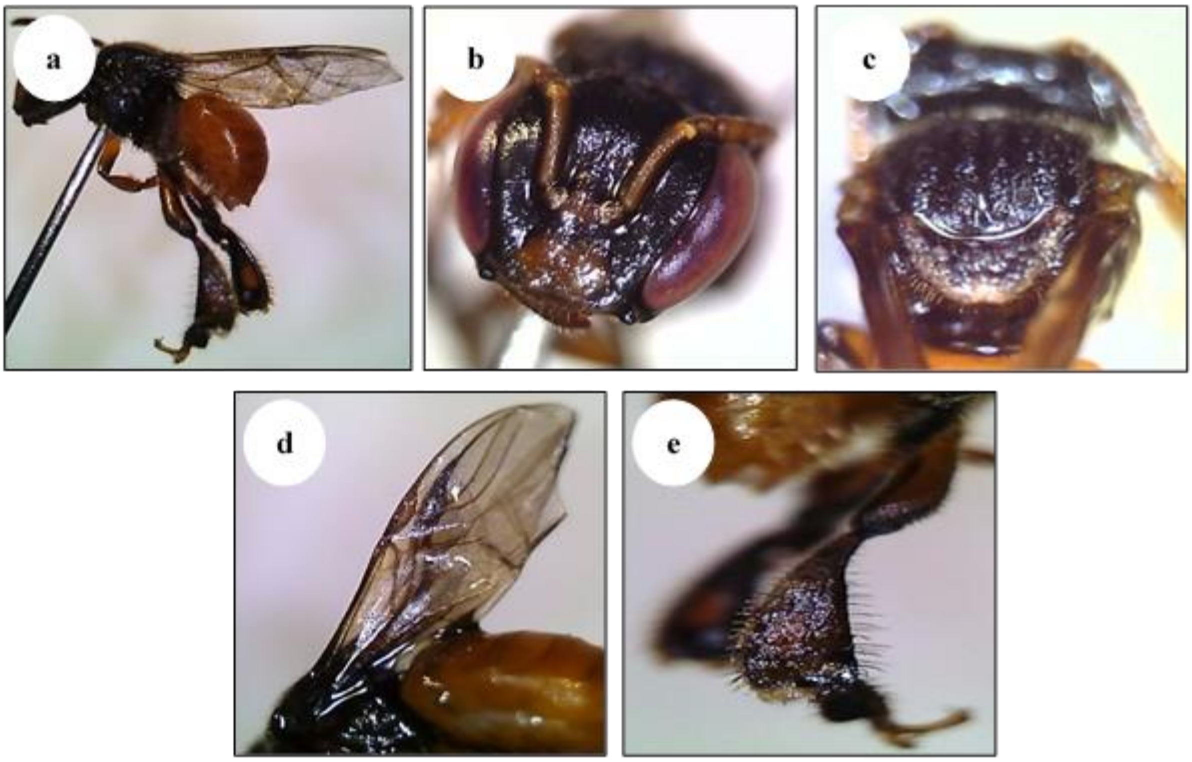

Figure 4 *Tetragonula drescheri* (Schwarz 1939); a. Habitus, Lateral view; b. Frons; c. Mesoscutum; d. Forewing; e. Hind tibia & basitarsus. Scale: 1.0 mm.

Head: Width almost twice the length and covered with short setae at apex. Clypeus and frons separated by episomal sulcus and covered with fine brown hair. Compound eyes and ocelli are large and blackish. Antennae geniculate, 10 segmented flagella, uniformly covered with erecting short setae, scape long, length of the pedicel, and entire flagella twice the length of the scape. Mandibles broader on their basal half, narrower on their apex, brown, slightly black at the base, blackish-brown at the apex, and possessing two teeth.

Thorax: Mesoscutum black and covered with brownish-black hair, scutellum small, brownish-black, and covered with brownish setae, and unclear hairband pattern. Tegulae rounded, highly sclerotized, and obvious. Wing venation weak, length of forewing almost twice the width, covered with fine and short setae. Total of five hamuli on hindwings. Hind tibiae long, pear shaped corbicula, and sparsely covered with short setae. Hind basitarsi short, length of tibia three times that of the basitarsus, and covered with short setae. Hind tibiae and basitarsi are entirely black.

Abdomen: First to fourth gastral tergite smooth; fifth, and sixth tergite slightly coarser, and covered with fine setae. Sternite covered with fine setae.

***Tetragonula sarawakensis* (Schwarz 1939)**

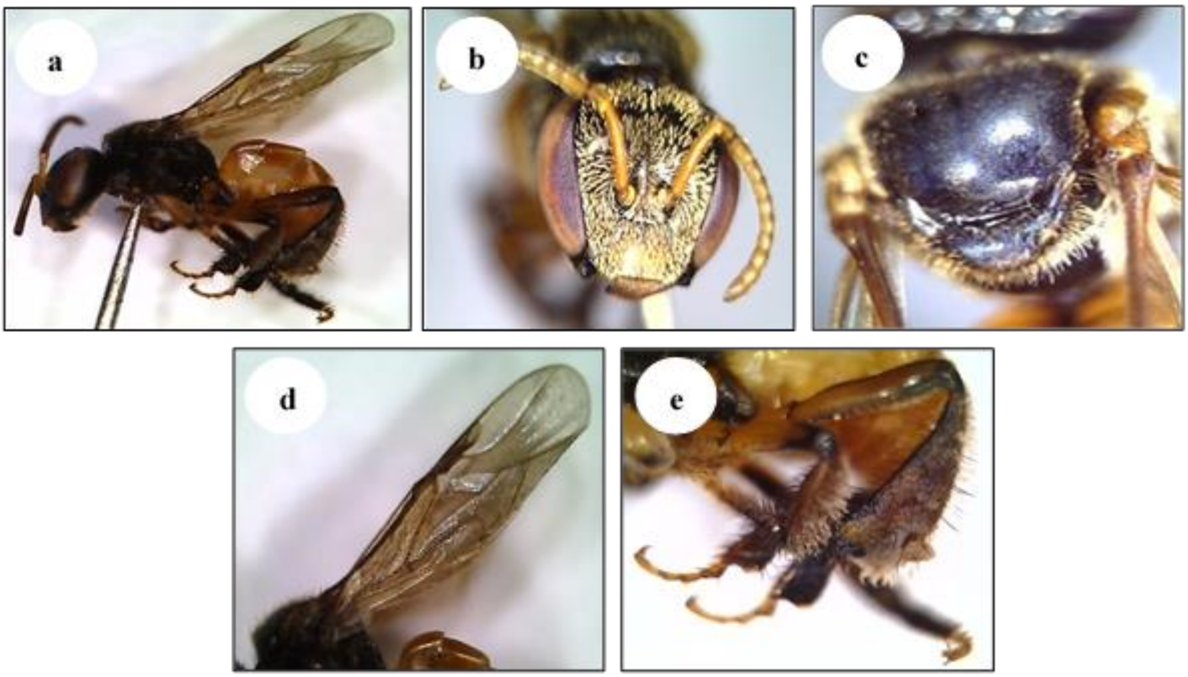

Figure 5 *Tetragonula sarawakensis* (Schwarz 1939); a. Habitus, Lateral view; b. Frons; c. Mesoscutum; d. Forewing; e. Hind tibia & basitarsus. Scale: 1.0mm.

Head: Width almost twice the length, and covered with short and smooth setae. Clypeus and frons are separated by episomal sulcus and covered with fine brown hair. Compound eyes brownish and ocelli brown. Antennae brownish and geniculate, 11 segmented flagella, uniformly covered with short erecting setae, scape long, length of pedicel, and entire flagella twice that of the scape. Mandible with two teeth, brown and slightly black at the base.

Thorax: Mesoscutum blackish-brown and covered with brownish to black hair, scutellum small and rarely covered by brownish setae, and unclear hairband pattern. There are two pairs of transparent wings, tegula black, and wing venation dark brown. Total of five hamuli on hindwings. Hind tibiae testaceous, pear shaped corbicula sparsely covered with short setae, and basitarsi wholly testaceous.

Abdomen: First to fourth gastral tergite smooth, fifth, and sixth tergite slightly coarser, and covered with fine setae. Strenite covered with short setae.

***Heterotrigona itama* (Cockerell 1918)**

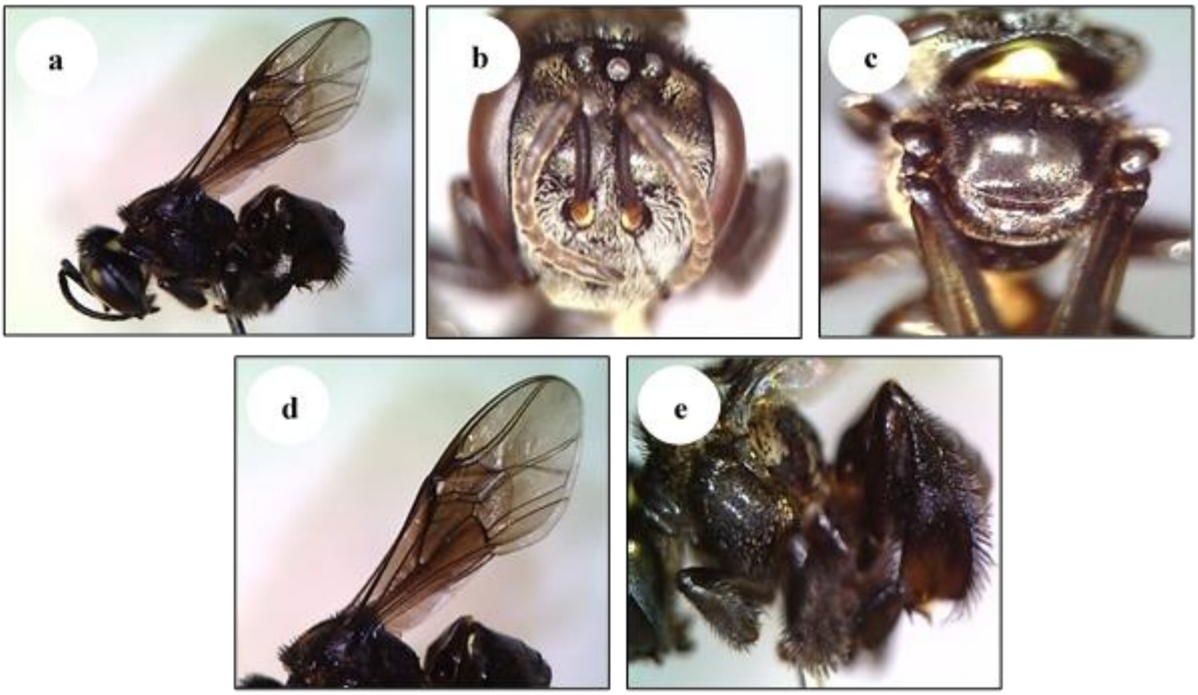

Figure 6 *Heterotrigona itama* (Cockerell 1918); a. Habitus, Lateral view; b. Frons; c. Mesoscutum; d. Forewing; e. Hind tibia & basitarsus. Scale: 1.0 mm.

Head: Width almost twice the length and covered with short setae. Clypeus and frons separated by episomal sulcus. Compound eyes large and blackish, and ocelli blackish. Antennae geniculate, 11 segmented flagella, uniformly covered with short erecting setae, scape long, length of the pedicel, and entire flagella twice that of the scape. Mandibles broader on their basal half, narrower on their apex, and possessing one tooth.

Thorax: Mesoscutum black and coarser, covered with long setae at anterior, anterior edge broader than the posterior. Tegulae black, rounded, highly sclerotized, and obvious. Wing venation weak, and wings covered with fine and short setae. Total of seven hamuli on hindwings. Hind tibiae long, corbicula pear shape and obvious, sparsely covered with long setae at the apex and short setae at the base. Hind basitarsi long, length of the tibia almost twice that of the basitarsus and sparsely covered with short setae.

Abdomen: First to third gastral tergite smooth, fourth tergite coarser, fifth to sixth tergite coarser and covered with fine setae. Sternite fully covered with fine setae

Lepidotrigona terminata **(Smith 1987)**

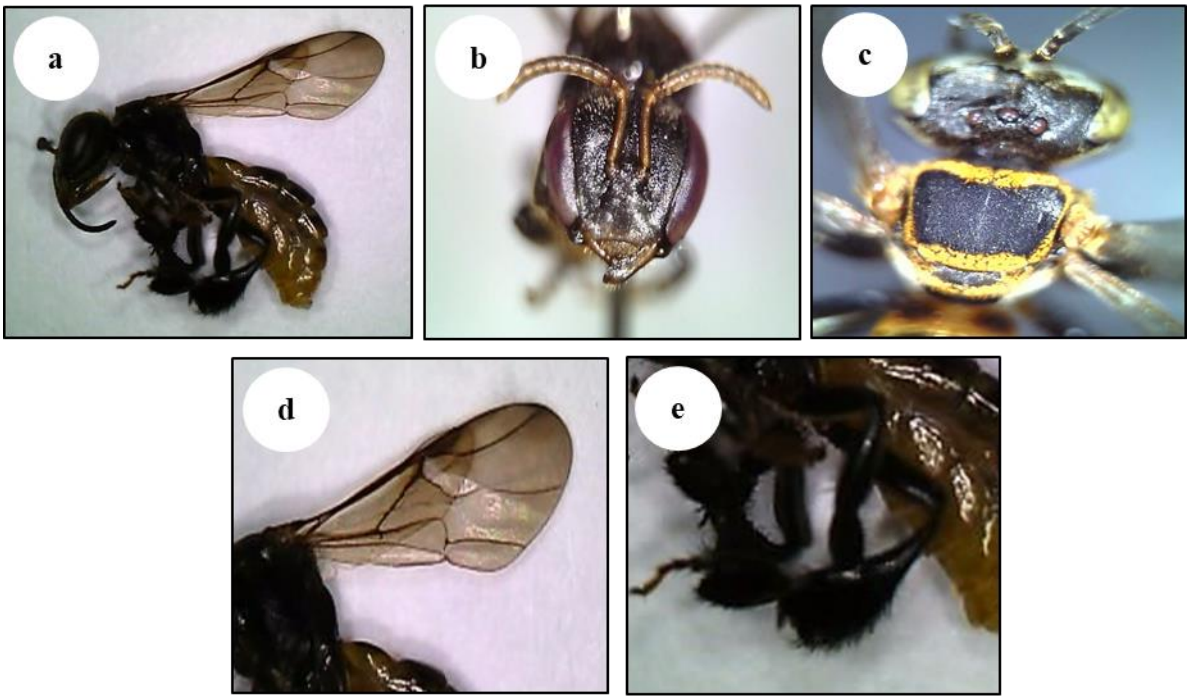

Figure 7 *Lepidotrigona terminata* (Smith 1987); a. Habitus, Lateral view; b. Frons; c. Mesoscutum; d. Forewing; e. Hind tibia & basitarsus. Scale: 1.0 mm.

Head: Width almost twice the length and covered with short setae. Clypeus and frons separated by episomal sulcus. Compound eyes and blackish ocelli. Antennae geniculate, 11 segmented flagella, uniformly covered with short erect setae, scape long, length of the pedicel, and entire flagella twice the length of the scape. Mandibles broader on their basal half, narrower on their apex, and possessing two teeth.

Thorax: Mesoscutum black, frame with a thick, golden-yellow, scale-like and covered with brown setae at the anterior margin, anterior edge broader than the posterior one. Tegulae brownish-yellow, rounded, highly sclerotized, and obvious. Wing venation weak, and wings covered with fine, short setae. Total of eight hamuli on hindwings. Hind tibiae long, corbicula pear shaped and obvious, sparsely covered with long setae at the apex and short setae at base. Hind basitarsi long, length of tibia almost twice that of the basitarsus, and sparsely covered with short setae.

Abdomen: First to fourth gastral tergite smooth, fifth to sixth tergite coarser and covered with fine setae. Sternite fully covered with fine setae.

Morphometric Measurements of Stingless Bees

The morphometry of the six species of stingless bee obtained comprised a variety of sizes (Table 4). Based on the results of PCA analysis the variation in morphometric characteristics among stingless bee resulted in different sample grouping patterns. Six different groups were formed on the basis of species (Figure 8). Groupings were also formed on the basis of the role of each character in each individual analysed. According to Izhaki et al. (2002), principal component analysis is an analytical technique that is often used in taxonomic research because it can identify the role of each character in each group formed. The results of analysis of the main components are displayed in a PCA diagram (Figure 8). PCA analysis obtained results that support grouping based on cluster analysis. Of the several individual stingless bees analyzed, five groups were formed based on the role of each character, namely the *Tetragonula laeviceps, T. drescheri, T.* cf. *biroi*, *T. sarawakensis*, *Heterotrigona itama* and *Lepidotrigona terminata* groups. The diagram shows the pattern of sample grouping based on the role of each character in the grouping process. The most dominant characters in the formation of the six groups are Hind Tibia Length (HTL), followed by Hind Wing Length (HWL), Fore Wing Length (FWL), Length of Forewing Including Tegula (WL1), and Body Length (BL). This can be seen from the length of the resulting line (Figure 8). In addition, this can also be seen in the image loading component plot 1 as the x-axis (Figure 9) and the loading component 2 plot as the y-axis (Figure 10) generated. The longer the arrows and the higher the graph formed, the higher the character's role in a grouping.

The six stingless bee species successfully analyzed in this study had morphologies were similar to those of specimens of the same type from other regions. For example, the morphological character of *Tetragonula laeviceps* in this study has morphological characteristics following the description of Efin et al. (2019), Karimah (2017), Manarudin (2019), Rasmussen & Michener (2010), Sakagami (1978), Sakagami et al. (1990), Smith (2012), Suriawanto et al. (2017) and Trianto & Purwanto (2020a). *Tetragonula* cf. *biroi* in this study are in accordance with the description of Schwarz (1939), Sakagami (1978), Vijayakumar et al. (2014), Suriawanto et al. (2017), and Trianto & Purwanto (2020a). Furthermore, according to the results of the present study. *Tetragonula sarawakensis* has morphological characters that are in accordance with the description of Moure (1961), Sakagami (1978), Sakagami & Inoue (1987), Sakagami et al. (1990), Schwarz (1939) and Trianto & Purwanto (2020a). The description of *Tetragonula drescheri* in this study agrees with that of Smith (2012) and the morphology of *Heterotrigona itama* described in this study is in accordance with the description of Schwarz (1939), Smith (2012), and Trianto & Purwanto (2020a). *Lepidotrigona terminata* in this study agrees with that of Suprianto et al. (2020) and Trianto & Purwanto (2020a).

The morphometry of the five species of stingless bee has size variation in comparison with the same species reported from other areas. Differences in the sizes of worker bees are a morphological adaptation to different environmental conditions. Novita et al. (2013) concluded that changes in temperature or environmental conditions would cause living things to adapt morphologically as a form of adjustment of their flight and foraging activities to the environment. Also, this variation is supported by the statements of Ruttner (1988) that in honey bees, changes in environmental conditions will cause organisms to adapt morphology as a form of adjustment to the environment.

Table 4 Morphometric characters measurements of stingless bees

No.	Body characters	Morphometry of stingless bees (mm)																	
		T. laeviceps			*T.* cf. *biroi*			*T. drescheri*			*T. sarawakensis*			*H. itama*			*L. terminata*		
		Min - Max	**Mean**	**SD**	**Min - Max**	**Mean**	**SD**	**Min - Max**	**Mean**	**SD**	**Min - Max**	**Mean**	**SD**	**Min - Max**	**Mean**	**SD**	**Min - Max**	**Mean**	**SD**
1	Body Lenght (BL)	3.42 - 3.45	3.42	0.02	4.02 - 4.19	3.94	0.02	4.63 - 4.64	0.64	0.01	4.62 - 4.63	4.63	0.01	4.83 - 4.86	4.84	0.02	4.68 - 4.71	4.63	0.01
2	Head Length (HL)	1.35 - 1.37	1.36	0.01	1.48 - 1.51	1.49	0.02	1.71 - 1.73	1.72	0.01	1.66 - 1.68	1.67	0.02	1.88 - 2.01	1.97	0.02	1.74 - 1.75	1.72	0.01
3	Head Width (HW)	1.62 - 1.64	1.63	0.01	1.84 - 1.87	1.85	0.02	2.23 - 2.26	2.25	0.02	2.02 - 2.12	2.10	0.02	2.20 - 2.49	2.43	0.02	2.25 - 2.26	2.24	0.01
4	Mandible Length (ML)	0.63 - 0.65	0.64	0.01	0.58 - 0.64	0.61	0.02	0.75 - 0.77	0.76	0.01	0.72 - 0.73	0.73	0.01	0.90 - 0.93	0.91	0.01	0.76 - 0.77	0.76	0.01
5	Mandible Widht (MW)	0.21 - 0.24	0.23	0.02	0.25 - 0.28	0.27	0.02	0.24 - 0.26	0.25	0.01	0.26 - 0.27	0.27	0.01	0.24 - 0.26	0.25	0.01	0.25 - 0.26	0.25	0.01
6	Clypeus Length (CL)	0.42 - 0.47	0.45	0.03	0.50 - 0.52	0.51	0.01	0.58 - 0.60	0.59	0.01	0.55 - 0.56	0.56	0.01	0.61 - 0.65	0.63	0.04	0.59 - 0.60	0.59	0.02
7	Lower Interocular Distance (LID)	0.89 - 0.91	0.90	0.01	0.94 - 0.97	0.95	0.02	1.27 - 1.30	1.29	0.02	1.28 - 1.29	1.28	0.01	0.94 - 1.32	1.30	0.01	1.29 - 1.30	1.28	0.02
8	Upper Interocular Distance (UID)	1.01 - 1.03	1.01	0.02	1.07 - 1.11	1.09	0.02	1.57 - 1.59	1.59	0.01	1.58 - 1.59	1.59	0.01	1.57 - 1.67	1.64	0.02	1.58 - 1.59	1.58	0.01
9	Eye Width (EW)	0.40 - 0.43	0.42	0.02	0.43 - 0.55	0.51	0.02	0.49 - 0.51	0.50	0.01	0.52 - 0.53	0.53	0.01	0.40 - 0.41	0.40	0.01	0.50 - 0.51	0.50	0.03
10	Eye Length (EL)	0.99 - 1.05	1.01	0.02	1.23 - 1.33	1.29	0.02	1.38 - 1.40	1.39	0.01	1.39 - 1.40	1.40	0.01	1.40 - 1.44	1.42	0.03	1.39 - 1.40	1.39	0.01
11	Maximum Interorbital Distance (MOD)	1.09 - 1.16	1.12	0.02	1.25 - 1.26	1.25	0.01	1.66 - 1.68	1.67	0.01	1.63 - 1.65	1.64	0.01	1.63 - 1.74	1.73	0.02	1.67 - 1.68	1.67	0.04
12	Lower Interorbital Distance (LOD)	0.89 - 0.93	0.90	0.02	0.94 - 0.98	0.96	0.02	1.40 - 1.42	1.41	0.01	1.35 - 1.36	1.35	0.01	0.94 - 1.44	1.42	0.02	1.41 - 1.42	1.41	0.01
13	Interantennal Distance (IAD)	0.19 - 0.21	0.20	0.01	0.19 - 0.21	0.20	0.01	0.29 - 0.31	0.29	0.01	0.30 - 0.31	0.31	0.01	0.28 - 0.32	0.30	0.02	0.30 - 0.31	0.30	0.01
14	Interocellar Distance (IOD)	0.16 - 0.26	0.20	0.02	0.19 - 0.26	0.22	0.02	0.60 - 0.62	0.61	0.01	0.56 - 0.57	0.57	0.01	0.53 - 0.56	0.54	0.02	0.61 - 0.62	0.61	0.01
15	Ocellocular Distance (OOD)	0.24 - 0.25	0.25	0.01	0.24 - 0.28	0.25	0.02	0.48 - 0.50	0.49	0.01	0.44 - 0.45	0.45	0.01	0.44 - 0.55	0.52	0.01	0.49 - 0.50	0.49	0.01
16	Antennocellar Distance (AD)	0.64 - 0.69	0.66	0.02	0.67 - 0.72	0.70	0.02	0.69 - 0.70	0.69	0.01	0.67 - 0.68	0.68	0.01	0.69 - 0.74	0.71	0.01	0.69 - 0.70	0.70	0.01
17	Antennocullar Distance (AOD)	0.29 - 0.32	0.30	0.02	0.29 - 0.33	0.31	0.02	0.41 - 0.42	0.42	0.01	0.35 - 0.36	0.35	0.01	0.40 - 0.47	0.46	0.01	0.42 - 0.42	0.42	0.00
18	Gena Width (GW)	0.22 - 0.27	0.25	0.02	0.23 - 0.28	0.26	0.01	0.38 - 0.39	0.39	0.01	0.39 - 0.40	0.40	0.01	0.36 - 0.43	0.41	0.02	0.39 - 0.39	0.39	0.00
19	Length of Flagellomere IV (FL)	0.13 - 0.14	0.14	0.01	0.14 - 0.16	0.15	0.01	0.16 - 0.17	0.17	0.01	0.18 - 0.19	0.18	0.01	0.16 - 0.20	0.18	0.02	0.16 - 0.17	0.17	0.01
20	Width of Flagellomere IV (FW)	0.15 - 0.15	0.15	0.00	0.15 - 0.17	0.16	0.01	0.13 - 0.14	0.14	0.01	0.16 - 0.17	0.17	0.01	0.11 - 0.14	0.12	0.01	0.14 - 0.14	0.14	0.00
21	Malar Length (ML)	0.08 - 0.10	0.09	0.01	0.05 - 0.07	0.06	0.01	0.11 - 0.12	0.12	0.01	0.13 - 0.14	0.14	0.01	0.04 - 0.10	0.09	0.02	0.11 - 0.12	0.12	0.01
22	Mesoscutum Length (MCL)	0.82 - 0.92	0.86	0.02	0.83 - 0.99	0.90	0.02	1.51 - 1.52	1.52	0.01	1.50 - 1.51	1.51	0.01	0.90 - 1.58	1.55	0.02	1.51 - 1.52	1.52	0.01
23	Mesoscutum Width (MCW)	1.05 - 1.09	1.08	0.02	1.11 - 1.26	1.20	0.02	1.22 - 1.23	1.23	0.01	1.21 - 1.21	1.21	0.00	1.21 - 1.29	1.27	0.02	1.22 - 1.23	1.23	0.01
24	Length of Forewing Including Tegula (WL1)	3.62 - 3.78	3.70	0.02	4.11 - 4.22	4.15	0.02	4.54 - 5.55	5.25	0.01	4.36 - 4.36	4.36	0.00	4.16 - 5.70	5.67	0.02	5.54 - 5.55	5.55	0.01
25	Distance Between M-Cu Bifurcation (WL2)	1.12 - 1.18	1.14	0.02	0.95 - 1.18	1.10	0.02	1.66 - 1.67	1.67	0.01	1.67 - 1.68	1.67	0.01	1.02 - 1.70	1.68	0.02	1.66 - 1.67	1.67	0.01
26	Fore Wing Length (FWL)	3.56 - 3.64	3.61	0.02	3.72 - 3.87	3.78	0.02	5.26 - 5.27	5.27	0.01	4.56 - 4.57	4.57	0.01	4.93 - 5.36	5.28	0.02	5.26 - 5.27	5.27	0.01
27	Fore Wing Width (FWW)	1.06 - 1.33	1.20	0.02	1.09 - 1.33	1.19	0.02	2.20 - 2.21	2.21	0.01	2.02 - 2.12	2.10	0.00	1.61 - 1.85	1.77	0.02	2.00 - 2.01	2.01	0.01
28	Hind Wing Length (HWL)	2.41 - 2.48	2.45	0.02	2.39 - 2.71	2.58	0.02	3.96 - 3.97	3.98	0.01	3.29 - 3.30	3.29	0.01	3.37 - 3.68	3.65	0.02	3.95 - 3.97	3.97	0.01
29	Hind Wing Width (HWW)	0.50 - 0.64	0.55	0.02	0.49 - 0.75	0.61	0.02	0.90 - 0.90	0.90	0.00	0.88 - 0.89	0.88	0.01	0.80 - 0.93	0.92	0.02	0.89 - 0.90	0.90	0.01
30	Hamuli Number (HN)		5			5			5			5			7			8	
31	Hind Femur Length (HFL)	0.98 - 1.02	1.00	0.02	1.12 - 1.19	1.14	0.02	1.22 - 1.22	1.22	0.00	1.28 - 1.29	1.29	0.01	1.36 - 1.53	1.46	0.01	1.22 - 1.22	1.22	0.00
32	Hind Tibia Width (HTW)	0.45 - 0.50	0.47	0.02	0.52 - 0.56	0.54	0.02	0.73 - 0.74	0.74	0.01	0.63 - 0.64	0.64	0.01	0.53 - 0.79	0.67	0.02	0.72 - 0.74	0.74	0.01
33	Hind Tibia Length (HTL)	1.37 - 1.43	1.41	0.02	1.50 - 1.62	1.59	0.02	2.14 - 2.15	2.15	0.01	2.15 - 2.16	2.16	0.01	2.13 - 2.17	2.10	0.02	2.13 - 2.15	2.15	0.01
34	Hind Basitarsus Width (HBW)	0.26 - 0.33	0.30	0.02	0.27 - 0.37	0.32	0.02	0.46 - 0.47	0.47	0.01	0.44 - 0.45	0.44	0.01	0.44 - 0.55	0.47	0.02	0.45 - 0.47	0.47	0.01
35	Hind Basitarsus Length (HBL)	0.59 - 0.63	0.61	0.02	0.53 - 0.62	0.57	0.02	0.70 - 0.70	0.70	0.00	0.69 - 0.70	0.70	0.01	0.58 - 0.79	0.69	0.02	0.69 - 0.70	0.70	0.01

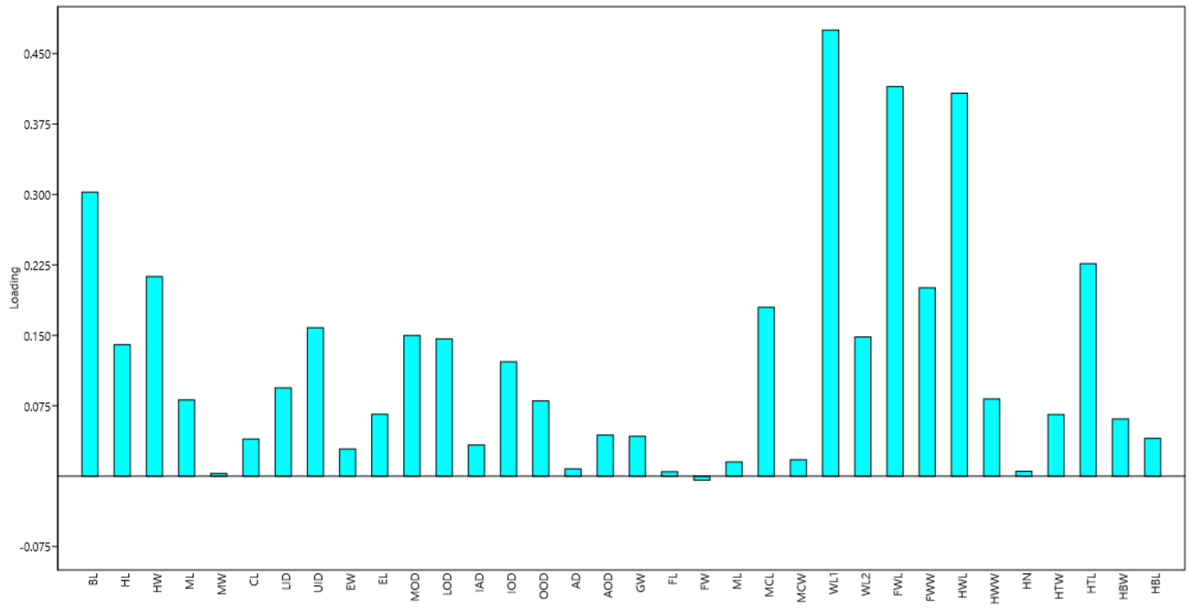

Figure 9 Loading plot of component 1 as the x-axis. The higher the graph formed, the larger the character's role in group formation is also high

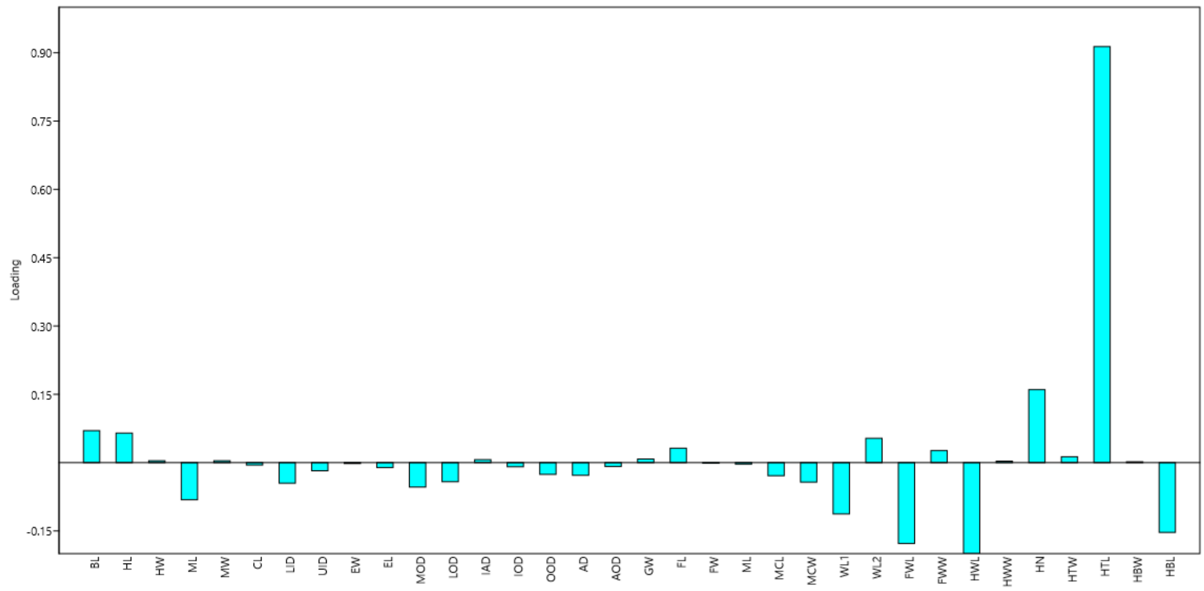

Figure 10 Loading plot of component 2 as the y-axis. The higher the graph formed, the larger the character's role in group formation is also high

Molecular Analysis of Stingless Bees

The phylogram reconstruction (phylogenetic tree) in this study uses the Neighbor-Joining method with a bootstrap value of 1000 times with the Kimura 2- Parameter (K2P) model in the MEGAX program. In this study, *A. cerana* and *A. dorsata* which are also included in the Apidae family members, are used as an outgroup while members of each species from different locations in Rasmussen and Cameron (2007) research are used as the building data in the group.

The results of phylogenetic analysis based on 16S rRNA mitochondrial gene nucleotide sequences (Figure 11) show that several clades formed with each genus and species are in the same clade supported by bootstrap values and genetic distances indicating that the individual is still in the same species. According to Zemlak et al. (2009), the genetic threshold for a species is 3.5% whereas, according to an analysis of BOLD Systems that use the COI gene, the genetic distance threshold for a species is 3%. If the genetic distance of two individuals or groups of individuals exceeds this value, then they are not in the same species group (different species).

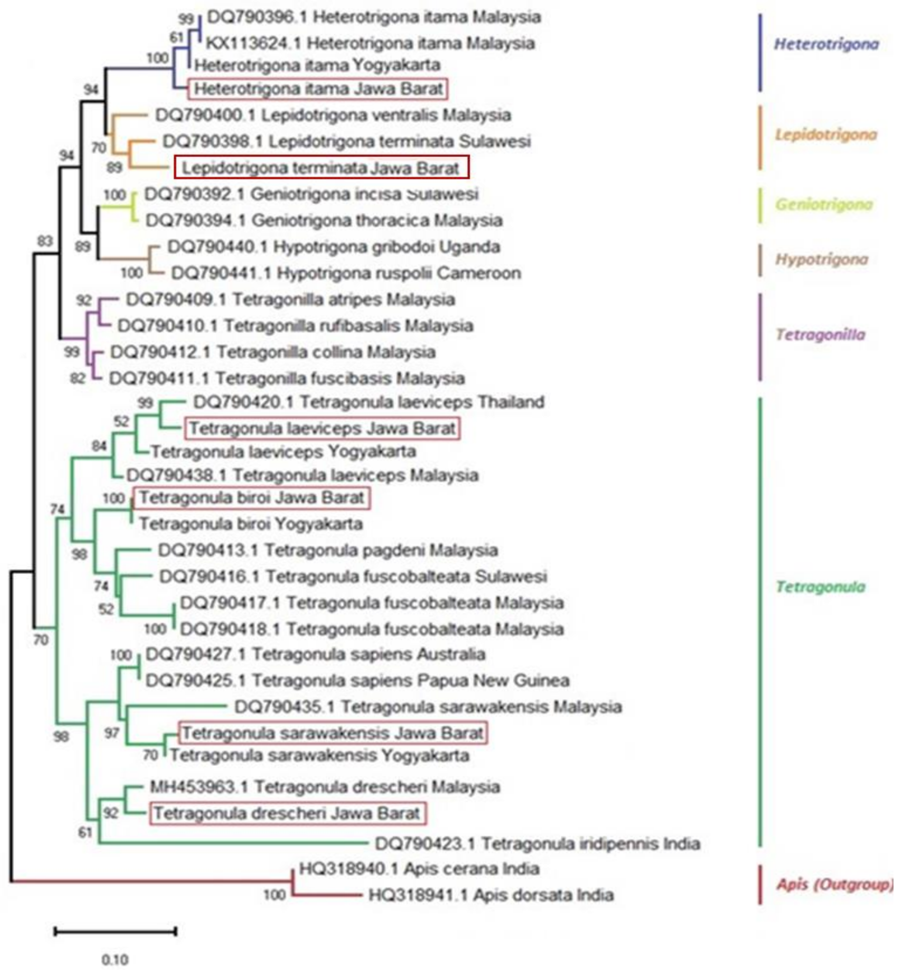

Figure 11 Phylogeny tree of stingless bees used in meliponiculture in West Java Province, Indonesia

Based on phylogenetic tree reconstruction (Figure 12), it appears that the six species of stingless bees from West Java Province analyzed to have the closest genetic relationship with similar species whose data are from GenBank. The *H. itama* West Java sample is in the same clade as the *H. itama* sample from Yogyakarta and GenBank with access numbers DQ790396.1 and KX113624.1 originating from Malaysia with a bootstrap value of 61%-100%. The genetic distance between the three is 3.0%, which indicates that all three are still in the same species. The same phenomenon also occurred in samples of *T. laeviceps* West Java and *T. laeviceps* Yogyakarta, *T. sarawakensis* West Java and *T. sarawakensis* Yogyakarta, *T. drescheri* West Java and *T. drescheri* Malaysia from GenBank with accession numbers MH453963.1, and *T.*

cf. *biroi* West Java and *T. biroi* Yogyakarta. This is supported by a high bootstrap value of 70%-100% and a low genetic distance under 3.5% (Table 6).

Table 6 The genetic distance of stingless bees

	Heterotrigona_itama_Jawa_Barat	Heterotrigona_itama_Yogyakarta	DQ790396.1_Heterotrigona_itama_Malaysia	KX113624.1_Heterotrigona_itama_Malaysia	Tetragonula_laeviceps_Jawa_Barat	Tetragonula_laeviceps_Yogyakarta	DQ790420.1_Tetragonula_laeviceps_Thailand	DQ790438.1_Tetragonula_laeviceps_Malaysia	Tetragonula_sarawakensis_Jawa_Barat	Tetragonula_sarawaekensis_Yogyakarta	DQ790435.1_Tetragonula_sarawakensis_Malaysia	Tetragonula_drescheri_Jawa_Barat	MH453963.1_Tetragonula_drescheri_Malaysia	Tetragonula_biroi_Jawa_Barat	Tetragonula_biroi_Yogyakarta	Lepidotrigona_cf_terminata_Jawa_Barat	DQ790398.1_Lepidotrigona_terminata_Sulawesi	HQ318940.1_Apis_cerana_India	HQ318941.1_Apis_dorsata_India
Heterotrigona_itama_Jawa_Barat																			
Heterotrigona_itama_Yogyakarta	0,03																		
DQ790396.1_Heterotrigona_itama_Malaysia	0,03	0,03																	
KX113624.1_Heterotrigona_itama_Malaysia	0,03	0,03	0,00																
Tetragonula_laeviceps_Jawa_Barat	0,17	0,18	0,15	0,15															
Tetragonula_laeviceps_Yogyakarta	0,21	0,22	0,19	0,19	0,03														
DQ790420.1_Tetragonula_laeviceps_Thailand	0,16	0,17	0,15	0,15	0,05	0,09													
DQ790438.1_Tetragonula_laeviceps_Malaysia	0,17	0,18	0,17	0,17	0,04	0,08	0,06												
Tetragonula_sarawakensis_Jawa_Barat	0,19	0,21	0,20	0,20	0,15	0,18	0,15	0,13											
Tetragonula_sarawaekensis_Yogyakarta	0,20	0,21	0,20	0,20	0,15	0,18	0,15	0,14	0,02										
DQ790435.1_Tetragonula_sarawakensis_Malaysia	0,27	0,26	0,26	0,26	0,21	0,25	0,21	0,16	0,08	0,07									
Tetragonula_drescheri_Jawa_Barat	0,17	0,19	0,19	0,19	0,14	0,18	0,16	0,12	0,07	0,09	0,16								
MH453963.1_Tetragonula_drescheri_Malaysia	0,16	0,18	0,18	0,18	0,15	0,19	0,16	0,11	0,07	0,09	0,15	0,03							
Tetragonula_biroi_Jawa_Barat	0,15	0,15	0,14	0,14	0,10	0,14	0,10	0,10	0,15	0,14	0,21	0,15	0,14						
Tetragonula_biroi_Yogyakarta	0,15	0,15	0,14	0,14	0,10	0,14	0,10	0,10	0,15	0,14	0,21	0,15	0,14	0,00					
Lepidotrigona_cf_terminata_Jawa_Barat	0.19	0.18	0.18	0.19	0,20	0,20	0,15	0,18	0.16	0.19	0.18	0.19	0.18	0.18	0.18				
DQ790398.1_Lepidotrigona_terminata_Sulawesi	0.21	0.20	0.21	0.20	0,20	0,20	0,15	0,18	0.18	0.20	0.21	0.20	0.20	0.20	0.21	0.07			
HQ318940.1_Apis_cerana_India	0,59	0,58	0,57	0,57	0,51	0,54	0,53	0,59	0,47	0,44	0,58	0,50	0,50	0,47	0,47	0.56	0.53		
HQ318941.1_Apis_dorsata_India	0,63	0,62	0,61	0,61	0,56	0,57	0,58	0,65	0,51	0,48	0,63	0,55	0,55	0,51	0,51	0.61	0.58	0,03	

Unlike the case of the above sample, the sample *T. laeviceps* West Java when compared with DQ790420.1 *T. laeviceps* Thailand and DQ790438.1 *T. laeviceps* Malaysia, and *T. sarawakensis* West Java and DQ790435.1 *T. sarawakensis* Malaysian, and *L. terminata* and DQ790398.1 *L. terminata* Sulawesi have quite a high genetic distance, namely above 3.5%. It is suspected that the samples of *T. laeviceps*, *T. sarawakensis*, and *L. terminata* from West Java are different types or at least come from separate populations. Schwarz (1939) and Sakagami et al. (1990) once reported that this species was found on the island of Java

CONCLUSION

Based on the morphological, morphometric, and molecular identification results, there are six species of stingless bee from Ciamis Regency, West Java Province, namely *Tetragonula laeviceps, T.* cf. *biroi*, *T. drescheri*, *T. sarawakensis*, *Heterotrigona itama,* and *Lepidotrigona terminata*.

ACKNOWLEDGMENTS

We would like to thanks Mr. Asep Sulaeman and his colleagues that provides stingless bee samples from their farm in Ciamis Regency, West Java Province, and also staffs from the Laboratory of Entomology, Biotechnology and FALITMA Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia for providing facilities during the research.

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