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Morphometric and molecular identification, domestication, and potentials of stingless bees (Apidae: Meliponini) in Mount Halimun Salak National Park, West Java, Indonesia

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Abstract. *Pratama MN, Agus A, Umami N, Agussalim, Purwanto H. 2023. Morphometric and molecular identification, domestication, and potentials of stingless bees (Apidae: Meliponini) in Mount Halimun Salak National Park, West Java, Indonesia. Biodiversitas 24: 6107-6118.* The purpose of this study is to identify the stingless bee species found in Mount Halimun Salak National Park, Sukabumi, and investigate the potential for its domestication. Four resorts within the Park, Cimantaja, Mount Koneng, Mount Bodas, and Kawah Ratu, were chosen as the sampling sites. The random sampling method was used to collect samples of stingless bees by working with locals. The collected bees were subsequently identified using their morphological and morphometrics features. Two species of stingless bees, *Tetragonula laeviceps* and *Heterotrigona itama* were found in the Park. The results of the 16S rRNA mtDNA gene sequencing confirmed the identity of the specimen of the stingless bees. Moreover, 37 colonies of *T. laeviceps* and 7 of *H. itama* were collected. The *T. laeviceps* were found in all four resorts, but the *H. itama* were only successfully collected from Kawah Ratu resort. The possibility for domestication and meliponiculture was investigated through observation and interviews with locals who shared their knowledge and the meliponiculture practices surrounding the Park. The study showed that *T. laeviceps* has more potential to be utilized in meliponiculture in the surrounding area of the Park.

Keywords: Domestication, exploration, *Heterotrigona itama,* identification, Mount Halimun Salak National Park, *Tetragonula laeviceps*

INTRODUCTION

Stingless bees (Hymenoptera: Apidae: Meliponini) are widely distributed throughout the tropics and subtropics (Salim et al. 2012). The stingless bee is known as *klanceng* in Javanese. Stingless bees are recorded to reach more than 500 species worldwide (Michener 2013). Stingless bees play a vital ecological role as pollinators of many wild plant species and crops (Kiatoko et al. 2014). Stingless bees live in colonies, which consist of one queen (queen) with several worker bees (worker) and male bees (drone). The bees utilize nectar and resin from several plant species as food sources, nesting materials, chemical defenses, and used to produces honey and propolis (Agussalim and Agus 2022; Agus et al. 2019, 2021; Agussalim et al. 2020, 2022; Erwan et al. 2020, 2021, 2023; Sabir et al. 2021). Indonesia is a tropical country that provides a habitat for many species of stingless bees. There are about 46 species from 10 different genera of stingless bees in Indonesia, which have been successfully documented, mainly from Sumatra and Kalimantan (Kahono et al. 2018).

The potential area for developing the bees industry in Indonesia is quite high, which is around 29,359,235 ha, which spreads in North Sumatra, Riau, West Sumatra, Lampung, West Java, Central Java, D.I. Yogyakarta, East Java, Bali, West Nusa Tenggara, East Nusa Tenggara, South Sulawesi, Central Sulawesi, and Southeast Sulawesi. This indicates that the production of honey and its derivatives can be increased (Directorate General of Land Rehabilitation and Social Forestry 2003). A stingless bee is an insect suitable for living in tropical and lowland climates. The survival of stingless bees in lowland forests in tropical climates depends largely on the availability of food resources. The plant types around the nest of the stingless bee influence propolis production (Agussalim and Agus 2022; Agussalim et al. 2020; Erwan et al. 2021); it contains therapeutic compounds with antioxidant, antimicrobial, and anti-inflammatory properties, which are useful in medicine (Abd Jalil et al. 2017; Lavinas et al. 2019). However, stingless bees must find food sources to optimize high propolis production. Stingless bees are the most important group in pollination compared to other insects (Free 1982), so they can increase crop production; stingless bees can pollinate many flowers (Supeno et al. 2021).

Engel et al. (2019) found 46 stingless bee species identified in Indonesia, constituting the largest number in the Asian region; however, still far behind Brazil, which has 244 species (De Menezes Pedro 2014). Stingless bee found in on the Java Island, are namely *Lepidotrigona nitidiventris*, *L. terminata*, *Heterotrigona itama, Tetrigona apicalis, Tetragonula fuscobalteata, T. drescheri, T. laeviceps*, *T. iridipennis*, *T. sapiens*, *T. sarawakensis,* and *T. biroi* (Priawandiputra et al. 2020; Trianto and Purwanto

2020a, 2020b; Purwanto and Trianto 2021; Rachmawati et al. 2022). *Heterotrigona itama* and *T. laeviceps* are common species and widely kept in Indonesia. Meliponiculture using the two species is more attractive to beekeepers because it is considered more productive and easier to breed than other species of stingless bees (Syafrizal et al. 2020).

The Mount Halimun Salak National Park (TNGHS) area is one of the conservation areas whose appointment began with the Decree of the Minister of Forestry No. 282/Kpts-II/1992 on 28 February 1992, with an area of 40,000 ha as Mount Halimun National Park; The TNGHS area has expanded to 113,357 ha in 10th of June 2003. The area is added to the forest area of Mount Salak, Mount Endut, and the surrounding areas, originally the limited production forests and protected forests managed by Perum Perhutani. Since then, it has been transformed into a single TNGHS conservation area. The TNGHS area's expansion includes settlements, agricultural land, and areas where people conduct economic activities (Hartono et al. 2007). Therefore, it is necessary to survey to provide information on the occurrence and diversity of stingless bees in the Mount Halimun Salak National Park, Sukabumi, West Java, and its potency for meliponiculture in the area surrounding the Park.

MATERIALS AND METHODS

Study area

This research was conducted from October to December 2021. The sampling activities were carried out in the Mount Halimun Salak National Park, Sukabumi region, West Java, Indonesia (106°21'-106°38' E and 6°21'-6°53' S) (Figure 1). The morphological and morphometric identification of the stingless bees was carried out at the Entomology Laboratory Faculty of Biology, and the molecular analysis was carried out at the Faculty of Biology and Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Procedures

Sampling point determination

The sampling points were chosen to represent the diversity of habitats in the Mount Halimun Salak National Park, including the core, rehabilitation, jungle, and utilization zones. Resorts used as sampling sites include Cimantaja Resort, Gunung Bodas Resort, Gunung Koneng Resort, and Kawah Ratu Resort. Four sampling points were determined in each habitat, with a distance between sampling points of ±1 km. Each predetermined sampling point is marked on the GPS tracker.

Stingless bee specimen collection and mounting

Stingless bee collection sampling was done by looking for the nest and taking some worker bees from the hive using the conical by knocking on the nest; this method was done because the weather conditions are in the rainy season, so the use of bait with sugar solution cannot be used. This sampling method was carried out by walking through the forest and assisted by local residents; the obtained bees are immediately given 96% alcohol.

Furthermore, 1 species obtained specimen targets on each sample site are at least 6 individuals: 3 for morphological and morphometric identification, 2 for molecular analysis (16S rRNA gene), and 1 for insectarium. However, during field sampling, 12 of *T. laeviceps* colonies were found in the Cimantaja resort, 8 colonies of *T. laeviceps* were found on Gunung Bodas, 14 colonies of *T. laeviceps* were found on Gunung Koneng, and 3 of *T. laeviceps* colonies and 7 of *H. itama* colonies in Kawah Ratu.

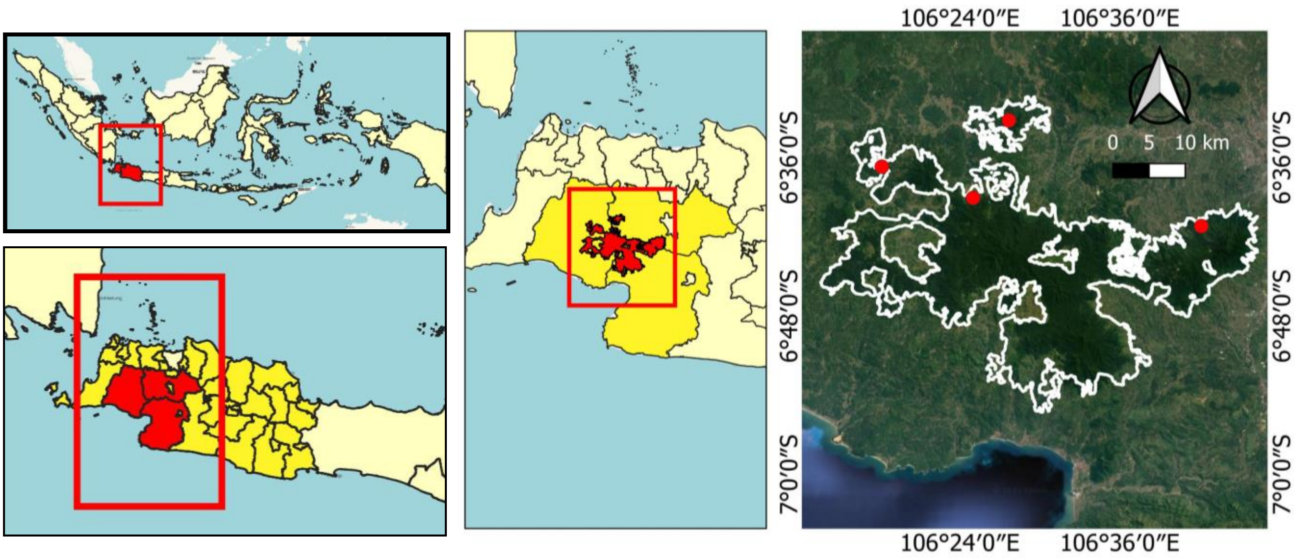

Figure 1. Sampling sites in Mount Halimun Salak National Park, West Java, Indonesia

Collected bee specimens are put into specimen bottle containers containing 90% ethanol and labeled with specimen number, location, and sampling time information. After arriving at the laboratory, the specimen mounting process was carried out so that the important characters on the bee's body are not damaged and are easy to observe during the identification process. The mounting process was carried out by inserting an insect needle into the thorax and tidying up all parts of its body. Next, the bee specimen will be labeled and dried (Salim et al. 2012; Purwanto and Trianto 2021; Trianto and Purwanto 2020a). In addition, stingless bee for PCR analysis was stored at 4ºC (Thummajitsakul et al. 2013).

Morphological identification and morphometric specimens of stingless bee

Specimen identification based on morphological and morphometric characters will be done at the Entomology Laboratory, Faculty of Biology, Universitas Gadjah Mada. The morphological characters were fully described, and the morphometric clans were observed and counted using a stereo microscope. The morphological characters measured included body length (BL), head width (HW), eye length and width (EL and EW), maximum and minimum interorbital distance (JMI and LOD), upper interocular distance (JIA), lower interorbital distance (IOD), ocellocular distance (JO), genealogical width (LG), malar length (PML), IV flagellomere length and width (PF and LF), forewing length from tegula (WL1), length of distance between M-Cu venation (WL2), back tibia length (PTB), back tibia width (LTB), basitarsus width and length (LBB and PBB). In addition, measurements were made for head length (PK), clypeus length (PC), longest and closest interocular distance (JIB and JIA), inter-antennal distance (JI), antennocellar (JA), antennocullar (JO), mandibular length and width (PM, and LM), mesoscutum length and width (PMS and LMS), forewing length and width (PSD and LSD), hind wings length and width (PSB and LSB), number of hamuli (JH), femur length (PJB), width and basitarsus length (LBB and PBB) (Purwanto and Trianto 2021; Trianto and Purwanto 2020a). Measurements were carried out on at least six specimens for both species.

Molecular identification (mtDNA) of stingless bee specimens

The molecular identification of the stingless bees was at three stages: DNA extraction, DNA amplification and sequencing, and bioinformatics analysis. DNA extraction uses all parts of the bee (except the head and wings). Stingless bee DNA was extracted using the CTAB method (Tamura et al. 2021). Polymerase Chain Reaction (PCR) amplification and DNA sequencing were performed using mitochondrial DNA gene primers, namely 16S rRNA (Tamura et al. 2021). Bioinformatics analysis was done by examining and editing DNA sequence data from the sequencing facility using Gene Studio software. Then, the DNA sequences were compared with the GenBank database using Nucleotide BLAST on the NCBI website. Then, the DNA sequences were compared to the GenBank database using the Nucleotide BLAST (Madden 2013). The BLAST results will show the Stingless bee species most closely related to the sample. The phylogenetic reconstruction (phylogenetic tree) was completed using the Neighbor-Joining method with a bootstrap value of 1000 using the Kimura 2-Parameter (K2P) model in the MEGA 11 program (Kumar et al. 2012). The genetic distance for an insect species based on analysis using the 16S rRNA gene was 3.5% (Zemlak et al. 2009), while for analysis using the CO-1 gene, it was 3%. If the genetic distance of two individuals or groups of individuals exceeds this value, they are not in the same species group (different species) (Purwanto and Trianto 2021).

Data analysis

The morphological and morphometric data obtained were then analyzed using Principal Component Analysis (PCA) with PAST4 software. PCA biplot analysis was carried out to determine the character of the grouping to determine its diagnostic character. Then, the scatter plot and loading plot graphs will be observed to determine the specimen pattern based on each character's role in the grouping. The longer the arrow and the higher the graph formed in the loading plot, the higher the character's role in the grouping (Purwanto and Trianto 2021).

RESULTS AND DISCUSSION

This research is important because it aims to collect the native West Java *klanceng* species in the National Park Mount Halimun Salak, Sukabumi, West Java. Therefore, the study aims to identify the native West Java *klanceng* species and understand the opportunities for meliponiculture the *klanceng* species found in Mount Halimun Salak National Park, Sukabumi, West Java. Identification with 16s rRNA gene and morphometric analysis related to domestication all went well. The people domesticated both species by taking colonies from the wild and bringing them home for beekeeping or domestication. The differences in their phylogenetic trees and in the morphological analysis of this research must be carried out to ensure that the species found follow the hypothesis and provide certainty about the species found as specimens. PCA biplot analysis (Figures 4 and 5) must be conducted to determine morphometric identification. The dominant character differences that appear between *T. laeviceps* and *H. itama* are body length (BL), wing length (WL), forewing length (FWL), head width (HW), number of hamuli (HN), and forewing width (FWW). The study highlighted morphometric analysis to find out which stingless bees have the potential to meliponiculture from the Gunung Halimun Salak National Park, West Java. *H. itama* and *T. laeviceps* have been developed and farmed in Indonesia, but this process is carried out by taking colonies from other areas. This can affect the pure strains of stingless bees living in certain areas. Many factors could influence these, including pure strain replacement, intrusion of stingless bees from outside potentially carrying diseases and parasites, and species taken from other areas being invasive.

Stingless bee morphology

Tetragonula laeviceps

The worker bee *T. laeviceps*'s body morphology shows a shiny black color dominates it (Figure 2). Its belly is blackish brown. The head is black and is sparsely covered with fine white hair. The compound eyes are greenish; the ocelli are blackish and large. The clypeus is completely covered with fine white hairs. The antenna has 11 flagellomere, the scape is yellowish brown, socket is gray, pedicel is brown, the first flagellomere is brown, second to tenth flagellomere is brown and slightly black. The mandible has two dental denticles. The mesoscutum is black, the scutellum is black throughout and is covered posteriorly with brownish setae. The tegula is brown, and the forewings are uniformly shiny in color. The hindwings are semi-transparent, and the wing venation is dark brown. The hind tibiae are short, the corbicula are pear-shaped, and there are 5 hamuli on the wings (Trianto and Purwanto 2020a).

Heterotrigona itama

The morphological results show that the bee was without a stinger with the characteristics of the body being covered in dark black, having a dark black stomach or abdomen, the head was black and slightly covered with white hair, and had a mandible that was not jagged, larger in size, and had brownish black eyes (Figure 3). The body is covered with a few hairs of fine white hair; the wings are shiny black, and the ocelli are very clearly visible.

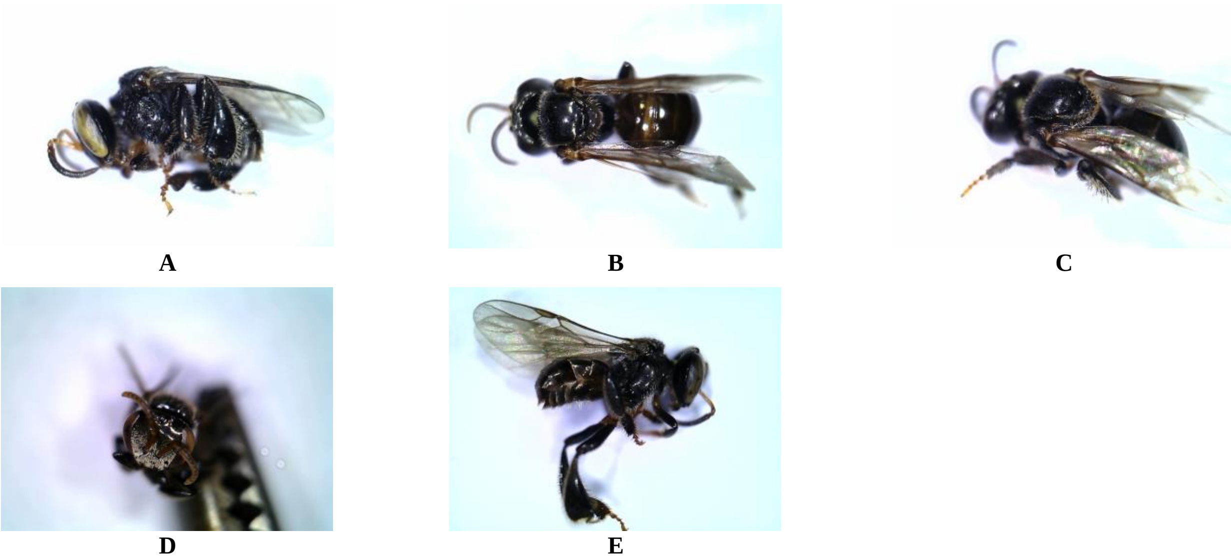

Figure 2. Morphology of *Tetragonula laeviceps* from Mount Halimun Salak National Park, West Java, Indonesia: A. whole body; B. Mesoscutum; C. Tessellation; D. Head width; E. Tegula

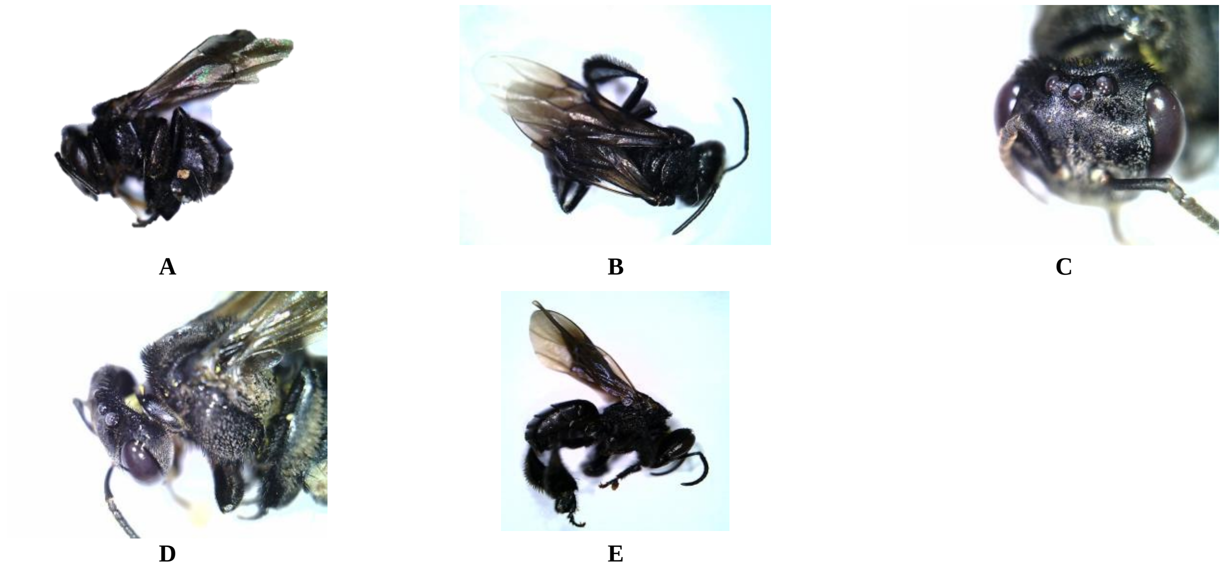

Figure 3. Morphology of *Heterotrigona itama* from Mount Halimun Salak National Park, West Java, Indonesia: A. whole body; B. Mesoscutum C. Ocelli; D. Head width; E. Tegula

The *H. itama* worker bee body is predominantly jet black. Its belly is pitch black. The head is black, the front is completely covered with fine white hair, and a few thick white hairs approach the clypeus. Compound eyes and ocelli are black. Clypeus is black and completely covered with white hair. The antenna consists of 11 flagellomere; the sockets are gray; the scape is black and brown at the base; the pedicel and all flagella are completely black. The mandible has one tooth and is entirely black. The mesoscutum and scutellum are entirely black. Tegulae are dark black. The forewings and hindwings are black, and the wing venation is dark brown and semi-transparent. The hind tibiae and basitarsi are entirely black. The number of hamuli is 7 per hind wing (Trianto and Purwanto 2020a).

Stingless bee morphometric

The morphometry of the two species of bee *klanceng* was found to have the same size difference (Table 1). Next, the morphometric data were analyzed using the main component analysis to investigate the dominant character influencing variations in individual grouping patterns (two species) of bee *klanceng*. Components analysis were performed using PCA from the collected data. The difference between *T. laeviceps* and *H. itama* can be observed by color bodies and features of their body shape. *T. laeviceps* is brownish black, has a narrower malar chamber, hind wing with 5 hamuli, the scutellum protrudes dorsally (hangs down), and a smooth patch on the tibia behind the inside of the basitarsus (Smith 2012; Engel et al. 2018). Temporary, *H. itama* is dark black and black, the mesonotum is covered by a border such as white scales (tessellation), hind wings with 7 hamuli, black scutellum and mesoscutum reaching metanotum, reticulate the propodeal dorsum, and the fore and hind wings are black (Smith 2012; Engel et al. 2018; Sayusti et al. 2021)

Table 1. Stingless bee morphometric characteristics of *Tetragonula laeviceps* and *Heterotrigona itama*

Character	*T. laeviceps* (N=10)				*H. itama* (N=5)			
	Min	Maks	Mean	SD	Min	Maks	Mean	SD
Body Length (BL)	4.25	5.45	4.84	0.46	3.27	8.20	6.94	2.07
Head Length (HL)	1.28	1.88	1.56	0.19	1.83	2.40	2.15	0.23
Head Width (HW)	1.37	2.44	1.87	0.27	2.33	2.98	2.65	0.24
Mandible Length (ML)	0.69	1.02	0.80	0.12	1.25	1.49	1.36	0.12
Mandible Width (MW)	0.11	0.35	0.21	0.08	0.37	0.47	0.41	0.04
Clypeus Length (CL)	0.35	0.69	0.50	0.13	0.74	1.00	0.88	0.10
Lower Interocular Distance (LID)	0.83	1.19	0.99	0.11	1.62	2.00	1.78	0.15
Upper Interocular Distance (UID)	1.10	1.76	1.35	0.18	1.78	2.19	1.99	0.15
Eye Width (EW)	0.33	0.56	0.43	0.08	0.57	0.91	0.71	0.13
Eye Length (EL)	1.04	1.39	1.28	0.12	1.63	2.09	1.76	0.20
Maximum Interorbital Distance (MOD)	1.08	1.88	1.36	0.21	1.92	2.17	2.04	0.11
Lower Interorbital Distance (LOD)	0.93	1.48	1.12	0.17	1.59	1.93	1.73	0.13
Interantennal Distance (IAD)	0.19	0.40	0.27	0.07	0.32	0.39	0.35	0.03
Interocellar Distance (IOD)	0.21	0.56	0.42	0.11	0.67	0.87	0.76	0.08
Ocellocular Distance (OOD)	0.23	0.49	0.37	0.07	0.50	0.89	0.72	0.15
Antennocellar Distance (AD)	0.55	0.96	0.74	0.12	0.84	1.21	1.03	0.14
Antennocullar Distance (AOD)	0.13	0.55	0.40	0.11	0.56	0.81	0.66	0.09
Gena Width (GW)	0.18	0.95	0.49	0.27	0.35	0.78	0.61	0.17
Length of Flagellomere IV (FL)	0.12	0.25	0.17	0.04	0.20	0.28	0.25	0.03
Width of Flagellomere IV (FW)	0.09	1.52	0.30	0.43	0.17	0.22	0.19	0.02
Malar Length (ML)	0.21	0.35	0.28	0.05	0.31	0.42	0.36	0.04
Mesoscutum Length (MCL)	0.96	1.77	1.34	0.30	1.63	2.35	1.94	0.28
Mesoscutum Width (MCW)	0.89	1.61	1.31	0.24	1.85	2.16	1.96	0.13
Length of Forewing Including Tegula (WL1)	3.23	5.79	4.15	0.82	8.07	8.94	8.36	0.34
Distance Between M-Cu Bifurcation (WL2)	0.86	1.30	1.10	0.17	1.28	2.84	1.94	0.70
Fore Wing Length (FWL)	2.77	5.23	3.88	0.76	6.42	7.67	7.10	0.62
Fore Wing Width (FWW)	0.52	1.68	1.23	0.35	2.58	3.00	2.83	0.17
Hind Wing Length (HWL)	2.57	3.83	3.06	0.44	4.66	5.63	5.23	0.36
Hind Wing Width (HWW)	0.51	0.94	0.71	0.13	1.12	1.42	1.29	0.12
Hamuli Number (HN)	5	5	5	0	7	7	7	0
Hind Femur Length (HFL)	1.05	1.62	1.38	0.19	1.50	2.61	1.95	0.48
Hind Tibia Width (HTW)	0.50	1.86	1.24	0.54	0.96	1.35	1.12	0.15
Hind Tibia Length (HTL)	0.46	2.23	1.07	0.75	2.49	3.01	2.77	0.19
Hind Basitarsus Width (HBW)	0.35	0.69	0.50	0.13	0.43	0.80	0.64	0.15
Hind Basitarsus length (HBL)	0.28	0.81	0.52	0.23	0.94	1.12	1.02	0.09

Note: N: Number of samples

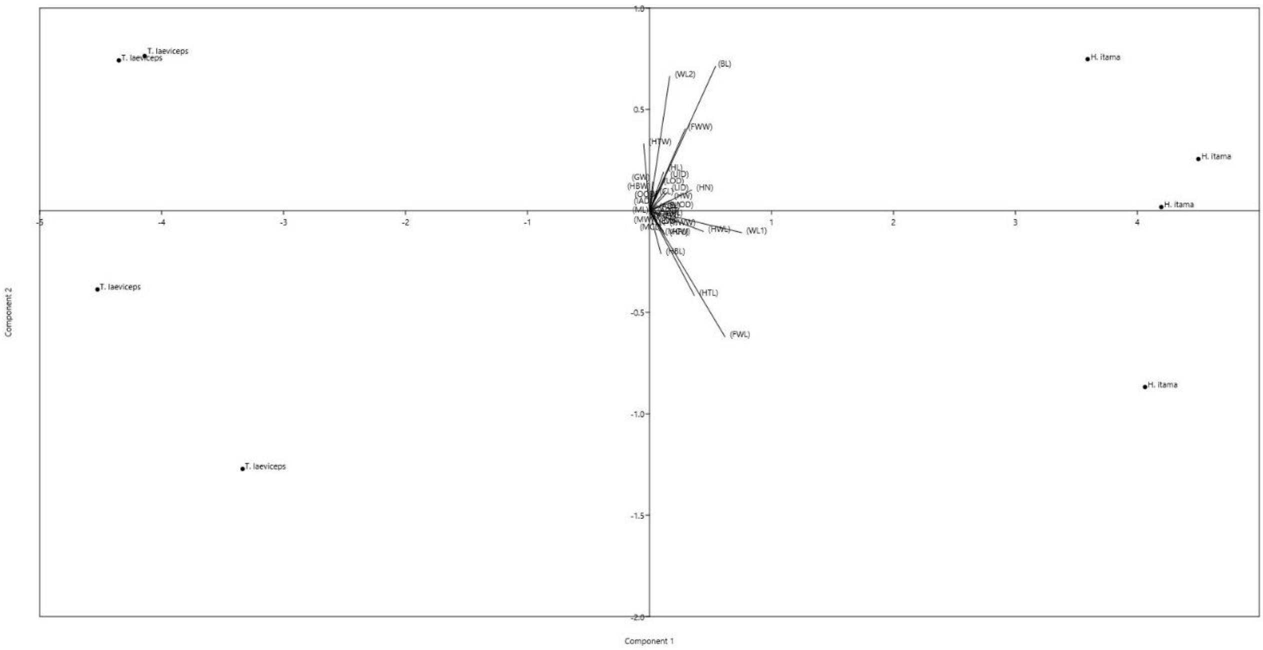

Figure 4. PCA biplot

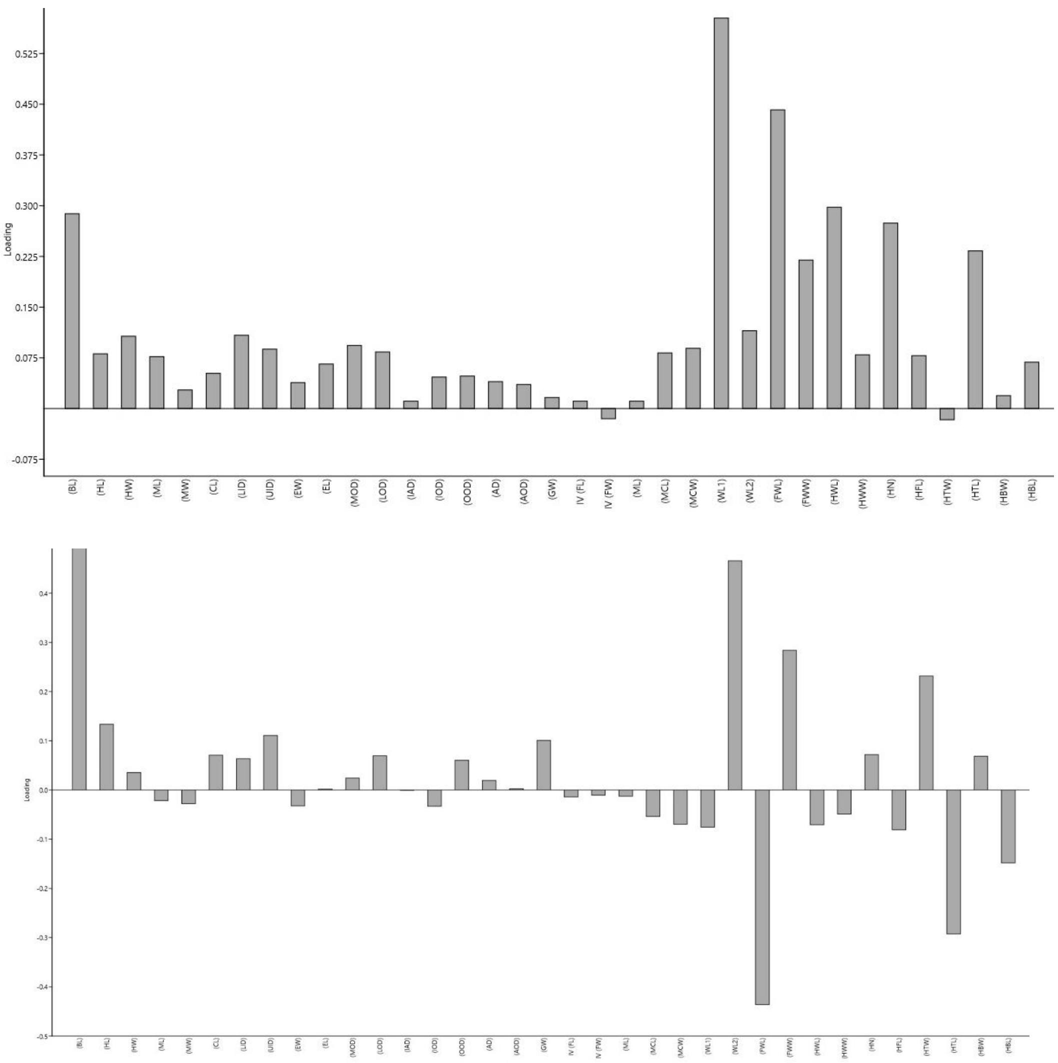

Figure 5. A scatter plot called a biplot graph, combines loading plots and score plots to determine the relationship and know the kinship between the specimens

The morphometric characteristics of the two species based on the dominant PCA differed regarding HTW, WL2, BL, FWW, HN, HWL, WL1, HBL, HTL, and FWL. *T. laeviceps* found in GHSNP had 5 hamuli, while *H. itama* had 7 hamuli; this is the same as the samples found by Purwanto and Trianto (2020) in Yogyakarta, which amounted to 5 hamuli in *T. laeviceps* and 7 hamuli for *H. itama*, the same likewise, the sample found in Kalimantan by Purwanto et al. (2022) showed the number of hamuli in *T. laeviceps* bees was 5 and 7 for *H. itama* hamuli. *T. laeviceps* has 5 hamuli on both hind wings, and *H. itama* has 7 hamuli on its wings (Smith 2012; Engel et al. 2018). Hamuli are hook-like setae on the anterior edge of the hind wings. They allow the two wings to function by interlocking with the curved posterior edge of the forewings. Each species of Hymenoptera has automorphic hamuli (Basibuyuk et al. 2000).

Tetragonula laeviceps obtained from Mount Halimun Salak National Park had a hindwing width (0.50-1.86) close to the body size found by Purwanto et al. (2022) in Yogyakarta, namely (0.46 mm). *T. laeviceps* found in Mount Halimun Salak National Park had WL2 (0.86-1.30 mm), while *the T. laeviceps* specimen found in Yogyakarta by Trianto and Purwanto (2020a) had an average size (1.13 mm). The morphological specimen of *T. laeviceps* obtained from Mount Halimun Salak National Park has a body size (4.25-5.45) close to the body size found by Purwanto et al. (2022) in Kalimantan, namely (4.00 mm) while from the Special Region of Yogyakarta it has a body size BL (3.64-3.68 mm), shorter than the specimen described by Sakagami (1978) using samples from Asia and Sri Lanka (4.0-4.6 mm). The FWW found in *T. laeviceps* had a size (0.52-1.68 mm), while the *T. Laeviceps* specimen found in Yogyakarta by Trianto and Purwanto (2020a) had an average size (1.22 mm). The HN in *T. laeviceps* was found to have as many as 5 pairs located on the hind wings; this is the same as the sample found by Purwanto and Trianto (2020) in Yogyakarta, totaling 5 hamuli.

The HWL found in *T. laeviceps* had a size (2.57- 3.83 mm), while the *T. laeviceps* specimen found in Yogyakarta by Trianto and Purwanto (2020a) had an average size (2.43 mm). WL1 found in *T. laeviceps* had a size (3.23-5.79 mm), while the *T. laeviceps* specimen found in Yogyakarta by Trianto and Purwanto (2020a) had an average size (3.63 mm). The HBL found in *T. laeviceps* had a size (0.28-0.81 mm), while the *T. laeviceps* specimen found in Yogyakarta by Trianto and Purwanto (2020a) had an average size (0.59 mm). The HTL found in *T. laeviceps* had a size (0.46-2.23 mm), while the *T. laeviceps* specimen found in Yogyakarta by Trianto and Purwanto (2020a) had an average size (1.39 mm). The FWL of the *T. laeviceps* specimen found in Mount Halimun Salak National Park is approximately (2.77-5.23 mm); this is smaller than the FWL of the *T. laeviceps* specimen found in Yogyakarta by Trianto and Purwanto (2020a) (3.58mm).

The *H. itama* bees obtained from Mount Halimun Salak National Park have a rear wing width or HTW (0.96-1.35 mm) close to the body size found by Trianto and Purwanto (2022) in Yogyakarta, namely (0.76 mm). *H. itama* bees found in Mount Halimun Salak National Park have WL2 with size (1.28-2.84 mm) while *H. itama* bees found by Trianto and Purwanto (2020a) in Yogyakarta have an average size (1.68 mm). The BL in *H. itama* bees found in Mount Halimun Salak National Park was (3.27-8.20 mm). In contrast, the *H. itama* bees found by Trianto and Purwanto (2020a) in Yogyakarta had an average body size (4.84 mm), so also with the *H. itama* bee specimen found in Kalimantan by Purwanto et al. (2022) having a body size of BL (6.1 mm) which means it shows the similarity of the *H. itama* bee. The FWW found in *H. itama* bees had a size of (2.58-3.00 mm) while the *H. itama* bees found by Trianto and Purwanto (2020a) in Yogyakarta had an average FWW size of (2.83 mm). HN and HWL in *H. itama* bee specimens found in Mount Halimun Salak National Park (7) and HWL numbers (4.66-5.63 mm), while specimens found in Yogyakarta by Trianto and Purwanto (2020a) had HN numbers (7) and has an average HWL size of (4.02), this shows similarities in the comparison of *H. itama* bee specimens found.

Heterotrigona itama bee specimens found in Mount Halimun Salak National Park have WL1 size (8.07-8.94 mm) where WL 1 is counted from the forewing to the tegula, and HBL (0.94-1.12 mm), while the *H. itama* bee specimens found in Yogyakarta by Trianto and Purwanto (2020a) has an average WL1 size (5.67 mm) and an average HBL (0.76 mm). *H. itama* bee specimens found in Mount Halimun Salak National Park have HTL sizes (2.49-3.01 mm), while for FWL *H. itama* bees that were suppressed in Mount Halimun Salak National Park have larger sizes (6.42-7.76 mm) compared to specimens found in Yogyakarta by Trianto and Purwanto (2020a) namely (3.83-5.36) with an average (5.33), but the specimens found in Mount Halimun Salak National Park were similar to the specimens found by Rasmussen and Cameron (2007) in Malaysia (4.78-7.85 mm).

There was variation in measurements of individual stingless bees of *H. itama* and *T. Laeviceps*. The most dominant characters for the formation of 7 clusters that determine species grouping are body length (BL), first wing length (WL 1), forewing length (FWL), head width (HW), number of hamuli (HW), and wingspan front (FWW). Variations in morphology and morphometric characteristics of stingless bees result from adaptations from previous generations. They adapt according to the environment to optimize their strengths and abilities. Regional differences are a factor that causes each bee species to have different sizes and different shapes. Environmental conditions force bees to adapt their morphology to facilitate flight and foraging activities in the environment (Novita and Sutriyono 2013). Each species of stingless bee has variations in body features that can affect its ability to build nests and perform foraging activities.

Molecular identification of stingless bee

Molecular sequence data have become more available to study taxonomy, population genetics, systematics, and bee evolutionary trends (Brito et al. 2013). In addition, genetic distance evaluation and multivariate analysis of morphometric data have been applied to solve taxonomic problems in bees. Molecular identification of the bee *klanceng* sequences of bee specimens used in this study was compared with sequence samples from Yogyakarta (Trianto and Purwanto 2020b) and Kalimantan (Purwanto et al. 2022) and data from the NCBI database. Table 2 provides nucleotide variations between *T. Laeviceps* and *H. itama*, based on molecular analysis using 16S rRNA from West Java.

The DNA sequence data from the sequencing facility was examined and edited using Gene Studio software. Then, the DNA sequences were compared to the GenBank database using Nucleotide BLAST on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cg). The BLAST results will show the bee species most closely related to the sample. Reconstruction of the phylogram (phylogenetic tree) was completed using the Neighbor-Joining method with a bootstrap value of 1000 with the Kimura 2-Parameter (K2P) model in the MEGA XI program (Kumar et al. 2012). The genetic threshold for an insect species based on analysis using the 16S rRNA gene is 3.5% (Zemlak et al. 2009), whereas, for analysis using the CO-1 gene is 3%. Therefore, based on BLAST results, the identity and query cover values for *T. laeviceps* are 95.73% and 97%. In comparison, the identity and query cover values for *H. itama* are 97.53% and 96% if the genetic distance of two individuals or groups of individuals exceeds this value (Table 2). They are not in the same species group (different species) (Purwanto and Trianto 2021).

Using the MEGA 11 program, DNA sequence data from the sequencing facility were reviewed and modified. The DNA sequences were then compared with the GenBank database using the Nucleotide BLAST program on the NCBI website. The results revealed and displayed a stingless bee that most closely resembles the specimen sample. In the MEGA 11 program, a phylogenetic reconstruction (phylogenetic tree) was completed using the Neighbor-Joining method with a bootstrap value of 1000 and the Kimura 2 Parameter (K2P) model (Kumar et al. 2012). Based on the results of the NCBI BLAST for the 16s rRNA mtDNA gene in the stingless bee specimen found in Mount Halimun Salak National Park, the results showed the specimen code H1 (*T. laeviceps*) and the percentage identity in the specimen was 95.73% and the query coverage value was 97%. While the specimen code H2 (*H. itama*). The results per ident are obtained by inputting sequence data into the NCBI (National Center for Biotechnology Information) using BLAST software in the database. The results of the 16s rRNA mtDNA gene sequences in the stingless bee specimen with the specimen code H1 (*T. laeviceps*) found in the Mount Halimun Salak National Park showed a very close relationship with the data in the GenBank, namely 97%, while in the stingless bee specimen code was H2 (*H. itama*) shows a close relationship with the data on GenBank, which is 96%. A value higher than this parameter indicates the more similar the order of the sample bases in the database (Nopriawansyah et al. 2019). It can be concluded that the two specimens found in the Mount Halimun Salak National Park used BLAST with the highest percentage of similarity values in the *Tetragonula* and Genbank samples for morphometric analysis sequences H1 (*T. laeviceps*) and H2 (*H. itama*).

COI was often used before in phylogenetic studies of species in the order Hymenoptera. In addition, the COI gene can be used to distinguish species with the same morphology (Figure 6).

Table 2. Results of nucleotide BLAST NCBI similarity analysis for mtDNA 16S rRNA gene in stingless bees from Mount Halimun Salak National Park, West Java

Specimen code	Per. Ident (%)	Query cover (%)	Species	Accession number
T. laeviceps H1	95.73	97	*T. laeviceps*	KU571748.1
H. itama H2	97.53	96	*H. itama*	KU571761.1

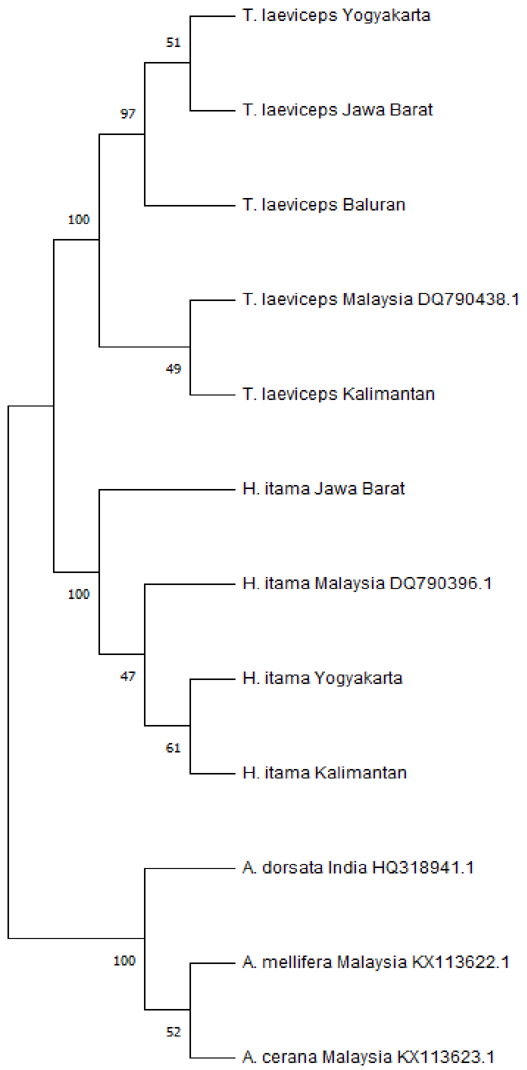

Figure 6. Phylogenetic tree of 4 bee species (2 stingless bees and 2 honey bees) based on 16S rRNA gene sequences generated using MEGA11® software (Tamura et al. 2021).

The paired genetic distance of phylogenetic trees between *T. laeviceps* bees from Mount Halimun Salak National Park and Yogyakarta ranged between 0.00 and 0.02, showing that *T. laeviceps* from Mount Halimun Salak National Park is closer to the Yogyakarta specimen compared to specimens from Kalimantan, and this can be seen in the phylogenetic tree chart (Table 3). Paired genetic distances for *H. itama* bees from Mount Halimun Salak National Park, Yogyakarta, and Kalimantan have a relationship with a difference of 0.01 from 0.02 and 0.03, indicating that the specimens found are significant. The distance between kinships can be seen in the phylogenetic tree graph below. Genetic distance can show how DNA transfers between populations (gene flow), leading to microevolution. The genetic relationships were shown by a phylogenetic tree constructed using the neighbor-joining method with the K2P model. Sequences with NCBI accession numbers published bee sequences retrieved from GenBank. The numbers above the branches indicate the genetic distance, and the numbers below the branches indicate the percentage of bootstrap values of 1000 replicates. Evolutionary analyses were carried out in MEGA11® software. The outer group of the phylogenetic tree uses other members of the family Apidae in a different tribe, namely *Apis cerana* and *Apis mellifera*.

Domestication in Mount Halimun Salak National Park

The results of field sampling by direct observation and interviews with the Kawah Ratu resort area community engaged in Meliponiculture and cultivating bees obtained or domesticated on ceilings, wooden poles, under stones, ceilings of old houses, and weathered trees (Figure 7). Domestication has been envisaged for honey production and has become an incentive for forest conservation (Kiatoko et al. 2014). Based on a study in Brazil, introduced bees are potential hosts for transmissible diseases or parasites to local stingless bees (Nunes-Silva et al. 2016). The introduced bees can also harm the native ecosystem. The sampled bees were the stingless bees *T. laeviceps* and *H. itama*, or the people of the village called *Teuweul* and *Teuweul gagak*. Stingless bees continue to be important in the traditions and cultures of many native communities, such as the Kayapó in Brazil, the Aché in Paraguay (sometimes called the "civilization of honey"), and the Abayanda pygmies in Uganda (Fijn and Baynes-Rock 2018). The community tends to cultivate the stingless bees by taking or domesticating them; they move the colony from the natural nest into the keeping box, which is known as *stup*. The domestication success by transferring colonies from natural nests to *stups* tends to be easy as long as all components are moved, especially the queen. Colonies are collected from the wild and transferred to wooden boxes, logs, or clay pots. Experienced beekeepers can establish new colonies by dividing existing ones into additional ones, a strategy the ancient Mayans already used (Źrałka et al. 2018). The main products of meliponiculture are honey, cerumen or resin, and bee pollen, but many people keep stingless bees simply for pleasure and relaxation (Hrncir et al. 2016). Slessor et al. (2005) reported many aspects of pheromone communication in bees using the queen retinue pheromone (QRP) component as an attractor for worker bees. If the worker does not smell the queen's pheromone, then the worker will try to find another queen. The success of transferring colonies is 94.44% successful if the queen bee is also carried (Putra and Jasmi 2016).

Meliponiculture potential in Mount Halimun Salak National Park

The observations and sampling results in the field showed that the abundance of *T. laeviceps* bee colonies was more than that of *H. itama* bees. The number of colonies of *T. laeviceps* bees found was 37, while the *H. itama* bees had only 7 colonies. The distribution of other stingless bees found in the Park can be seen in Table 4.

Table 3. Estimation of genetic distance based on 16S rRNA gene sequences of stingless bee species generated using Mega11® software (Tamura et al. 2021)

	Tetragonula_laeviceps _TNGHS	*Tetragonula_laeviceps* _Yogyakarta	*Tetragonula_laeviceps* _Kalimantan	*Heterotrigona_itama_* TNGHS	*Heterotrigona_itama_* Yogyakarta	*Heterotrigona_itama_* Kalimantan	*Apis_cerana*_KX11362 3.1
*Tetragonula_laeviceps*_Yogyakarta	0.02						
*Tetragonula_laeviceps*_Kalimantan	0.04	0.06					
*Heterotrigona_itama*_TNGHS	0.12	0.15	0.14				
*Heterotrigona_itama*_Yogyakarta	0.15	0.14	0.16	0.03			
*Heterotrigona_itama*_Kalimantan	0.13	0.15	0.14	0.02	0.03		
*Apis_cerana*_KX113623.1	0.27	0.29	0.28	0.25	0.27	0.25	
*Apis_mellifera*_KX113622.1	0.27	0.29	0.28	0.27	0.29	0.27	0.11

Table 4. Stingless bee species found in Mount Halimun Salak National Park, West Java, Indonesia

Resort	Zone	Species found	Number of colonies	Host plant
Cimantaja	Villages and rehabilitation,	*T. laeviceps*	12	*Bambusa vulgaris* and jengkol
Gunung Bodas	Rehabilitation, utilization, specialty and jungle	*T. laeviceps*	8	*Bambusa vulgaris*
Gunung Koneng	Rehabilitation and utilization	*T. laeviceps*	14	Clove, pine, maniki *Shorea dasyphylla*
Kawahratu	Traditional and utilization	*T. laeviceps*	3	Damar
		H. itama	7	

Figure 7. A. *Tetragonula laeviceps* bee colony in stup box, and B. induction box or *Heterotrigona itama* bee colony graft

Habitat types can influence bee development. A study by Kaluza et al. (2018) shows the group social bees will develop faster in a diverse ecosystem flowering plant, so food sources will always be available. *Tetragonula laeviceps* colonies were spread in all of the studied habitat types. This condition indicated that the *T. laeviceps* colony was well adapted to different environments. Gadhiyah and Pastagia (2019) reported that *T. laeviceps* could visit flowers of many plant species, including vegetables, fruit, oilseeds, nuts, forages, and weeds. Their small bodies allow them to build nests in small spaces in the ground, rock crevices, dry or rotting wood, pithy trunks, plant galls, and tree branches or attach their nests to trunks or rock surfaces (Engel et al. 2020). The entrance to the nest of *T. laeviceps* tends to be sticky because it is covered with propolis, which maintains the nest and temperature stability. Propolis protects the nest from external threats like microbes, predators, and extreme weather (Roubik 2006). *Heterotrigona itama* nest door shape has an entrance in the form of a funnel, and almost everything there is propolis in the vicinity. The entrance of the type *H. itama* has a thin and soft texture, and there is propolis around the entrance, which are used as traps for predators and signals for bee workers flying around (Roubik 2006). The nest entrance properties of stingless bees are related to many factors, such as genetics-bee age, nest age, and the microenvironment, such as rainfall, predation, sunlight, microclimate, parasites, and symbionts. The nest entrance size of stingless bees also varies (Kelly et al. 2014). For *H. itama,* about 10-20% of worker bees have to collect pollen and resin; meanwhile, the rest of the workers gather the nectar from morning to evening (Jaapar et al. 2018)

The sample collection identification obtained from Mount Halimun Salak National Park, West Java, shows two species of stingless bees, namely *T. laeviceps* and *H. itama*. The results of molecular identification show that the bee species *T. laeviceps* and *H. itama* are native species of Mount Halimun Salak National Park, Sukabumi, West Java. The abundance of the *T. laeviceps* colonies was higher than the *H. itama* colonies. *Tetragonula laeviceps* was found in Cimantaja Resort, Bodas Mountain, Koneng Mountain, and Kawah Ratu. In comparison, *H. itama* is only found in the Kawah Ratu Resort. The advantage of the *T. laeviceps* bee is its small body size, which makes it able to reach the deepest part of the flower. This stingless bee easily adapts to new environments and tends not to bite. All these characteristics gave the *T. laeviceps* bees more potential to be developed in meliponiculture in the area of Mount Halimun Salak National Park, West Java.

In conclusion, the sample collection obtained from Gunung National Park Halimun Salak West Java shows low species diversity. The sample collection showed two types, namely *T. laeviceps* and *H. itama*. The morphometric identification showed differences in the dominant characteristic that appears between *T. laeviceps* and *H. itama* are body length (BL), wing length (WL), forewing length (FWL), head width (HW), number of hamuli (HN), and forewing width (FWW). Molecular identification showed that the kinship of *T. laeviceps* bees in Gunung Halimun Salak, Yogyakarta, and Kalimantan at

0.00 and 0.02 means they have a very close kinship. The same is the case between the *H. itama* Jalapeno bee kinship with Mount Halimun Salak National Park, Yogyakarta, and Kalimantan, which are 0.02 and 0.03T. Furthermore, *T. laeviceps* was found at Cimantaja Resort, Mount Bodas, Mount Koneng, and Kawahratu, while *H. itama* is only found in Kawahratu Resort. This shows that the distribution of *T. laeviceps* is broader and more adaptive to various environmental conditions. The abundance of *T. laeviceps* colonies was greater than that of *H. itama*. This makes *T. laeviceps* bees more potent and allows them to be cultivated and developed, but the higher number of colonies will compensate for the lower productivity. There is a cultivation group of *T. laeviceps* and *H. itama* at the Kawahratu Resort, which can be used as a model to develop this stingless bee for other Resorts.

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