International Journal of Agricultural Technology 2025 Vol. 21(2):421-432
DOI: https://doi.org/10.63369/ijat.2025.21.2.421-432
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Stingless bees collecting pollen in durian orchards, Khao Khitchakut District, Chanthaburi Province

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Anutrakunchai, S., Thongkamngam, T., Puramongkon, P. and Charoensuk, K. (2025). Stingless bees collecting pollen in durian orchards, Khao Khitchakut District, Chanthaburi Province. International Journal of Agricultural Technology 21(2):421-432.

Abstract Stingless bees are small social insects that collectsnectar and pollens from plants for food and play a crucial role in pollination. The results are identified into two species: *Tetragonula pagdeni* and *Lisotrigona furva*. *T. pagdeni* primarily collected the dominant pollen from nectar-rich flowering plants which were *Asystasia gangetica* (83%), *Tridax procumbens* (65%), and *Wedelia trilobata* (53%). Interestingly, *T. pagdeni* collected the occasional pollen from *Durio zibethinus* (L.) which was "Mon-Thong (1%) and Kradum Thong (1%). Unlike *T. pagdeni*, the smaller *L. furva* focused on various plants and weedy plants with smaller flowers, which, collecting pollen from *A. gangetica* (12-20%), *T. procumbens* (14-18%), *Bougainvillea glabra* (15%), *Gomphrena globosa* (8%), *Eleutheranthera ruderalis* (11%), and *Eleusine indica* (13%), and however, no pollen of *D. zibethinus* was observed. Overall, the study shows that both species adapt their foraging behavior in response to environmental changes, such as El Niño and La Niña, highlighting their resilience and adaptability to survive in varying climatic conditions.

Keywords: Stingless Bees, Pollen, Durian orchards, *Tetragonula pagdeni*, *Lisotrigona furva*

Introduction

Stingless bees are small social insects that gather nectar from flowers and pollen as food, similar to honeybees. They belong to the family *Apidae* but differ from honeybees in that stingless bees lack a stinger and cannot defend themselves by stinging. Instead, they have strong mandibles that they use to bite enemies in order to protect their nests. Stingless bees are distributed in tropical and subtropical regions (Toledo *et al.,* 2018; Engel *et al.,* 2023).

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Currently, over 400 species have been discovered worldwide across 50 genera. In Thailand, 39 species have been reported, each with different distribution patterns depending on the abundance of food sources, environmental conditions, temperature, and altitude above sea level in their respective habitats. Stingless bees can inhabit cracks and crevices in rock layers, trees, wood cavities, wooden posts, cement or brick walls, gaps in house walls, as well as in old termite mounds or ant nests (Neto *et al.,* 2017; Bareke, 2019). These are typically undisturbed areas. The habitats of stingless bees can be clearly divided into two types: 1) Natural cavities: These are often found in nature, such as in forests, community areas, temples, schools, and agricultural land. 2) Artificial cavities: These are man-made structures or various types of beekeeping boxes. Stingless bees frequently inhabit cracks or gaps in buildings, such as walls and posts, whether made of cement, wood, or metal, as well as in pipes, steel tubes, and various boxes (Canche *et al.,* 2020; Ng *et al.,* 2024).

Currently, Chanthaburi Province in eastern Thailand is renowned for its fruit cultivation, largely due to its favorable geographic conditions, such as fertile soil and a tropical climate with high rainfall. The province is especially famous for producing high-quality durians, which are exported globally. Other fruits like longan, rambutan, salak, and mangosteen also thrive in the region, contributing to its reputation as a major agricultural hub. This strong fruit production supports both the local economy and international trade, making Chanthaburi a key player in Thailand's fruit export industry. Consequently, farmers are raising stingless bees to assist with pollination. However, there are limitations concerning the food sources for these bees, as each fruit species has different flowering seasons, which can lead to food shortages (Cardona *et al.,* 2019). Additionally, heavy rainfall during certain periods can hinder plant growth, resulting in a decline in stingless bee populations, prompting them to abandon and relocate their foraging areas. In Thailand, three species of stingless bees can be cultivated: *T. pegdeni*, *Trigona laeviceps* and *Lepidotrigona terminata* (Jongjitvimol and Wattanachaiyingcharoen, 2007; Thakodee *et al.,* 2018). Moreover, *L. furva* is a species of stingless bee in the family Apidae, belonging to the tribe Meliponini. Like other stingless bees, *L. furva* is relatively small compared to other bee species, and this size allows it to forage in a variety of floral environments. As with most stingless bees, *L. furva* plays an important role in pollination, visiting various plants to collect nectar and pollen (Michener, 2007). However, stingless bees, as their name suggests, are unable to sting, making them important and safe pollinators in tropical regions. Furthermore, stingless bee farming can be adapted to thrive in economic crop areas. Beyond their role in pollination, stingless bees also produce honey, providing farmers with an income through honey sales (Engel

et al., 2023; Ng *et al.,* 2024). Therefore, the research aimed to study stingless bees collecting pollen from food plants in durian orchards in Khao Khitchakut District, Chanthaburi Province, Thailand.

Materials and methods

Sampling of Stingless bee in durian orchard

Ten samples of stingless bees were collected from the durian orchard of the Department of Plant Production Technology and Landscape, Rajamangala University of Technology Tawan-ok, Chanthaburi Campus during the summer of 2024. Adult stingless bees were collected from the hive using an aspirator pump and stored in a plastic tube. All samples were kept at -20°C in a zip lock bag until further analysis. Subsequently, the morphological characteristics of the stingless bees were studied by observing the features of the nest, the population within the nest (adults, larvae, and pupae), pollen pots, and honey pots, along with capturing photographic.

Diversity of stingless bees collecting pollen in food plant

Surveying the food plants of stingless bees in the durian orchard of the Department of Plant Production Technology and Landscape, Rajamangala University of Technology Tawan-ok, Chanthaburi Campus, Thailand. The survey began by establishing points within the durian orchard along the line transect sampling method, covering a distance of 2,500 meters. Observations were conducted from 6:00 to 10:00 a.m., which corresponds to the active foraging period of stingless bees. The stingless bees foraging behavior was observed based on their flower selection (Figure 1). Food plant samples were collected, focusing specifically on floral parts, colors, and shapes. The methodology adapted from Momose *et al.* (1998) categorized flower shapes into the following: bilabiate, brush-like, caesalpinaceous, campanulate, funnelform, papilionaceous, tubular, salverform, and other floral forms. Pollen samples were then transferred onto slides to study the characteristics of the food plant pollen under a microscope.

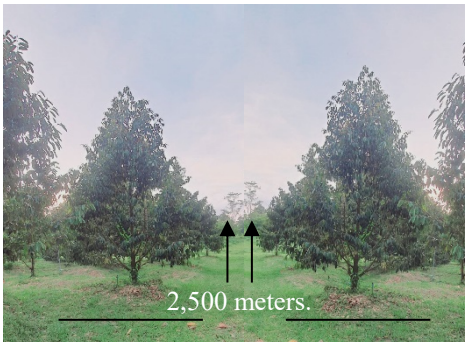

Figure 1. Investigating the foraging plants of stingless bees in the durian orchard

Palynological analysis

The palynological preparation of microscope slides, 2 g. of each pollen sample from stingless bees were taken and homogenized separately in 10 mL of distilled water, followed by centrifugation for 10 minutes at 4,500 rpm, after which the supernatant was discarded. Another 20 mL of distilled water was added, and the mixture was centrifuged again for 5 minutes. The supernatant was discarded, and the remaining pellet was subjected to acetolysis (Thakodee *et al.,* 2018). Approximately 500 pollen grains per sample were identified to classify the pollen types by percentage: dominant pollen (D > 45%), accessory pollen (A = 15-45%), isolate pollen (I = 3-15%), and occasional pollen (O < 3%) (Barth, 1989). Samples were collected at random during February, April, and June of 2024.

Results

Morphological characteristics of stingless bee in durian orchard

From the collection of stingless bee samples from the durian orchard of the Department of Plant Production Technology and Landscape, Rajamangala University of Technology Tawan-ok, Chanthaburi Campus during the summer of 2024, a total of 10 samples were collected. Two species of stingless bees were found inhabiting the durian orchard. Subsequently, the study of the Morphological characteristics and Stylized representation of the stingless bee nests revealed that the nest structure comprised adults, larvae, pupae, pollen pots, and honey pots. The stingless bees were identified as *T. pagdeni* is a small bee, approximately 3-4 mm in length. Its body is black or dark brown with

some lighter or yellowish markings. The wings are translucent with a light brown tint. It has a broad, rounded head with short, segmented antennae, and its large eyes help with navigation and foraging. The thorax is strong, with sparse hairs that aid in collecting pollen, and it has two pairs of wings, with the forewings being longer than the hindwings. The slender legs are well-suited for pollen collection, and the hind legs have a pollen basket (corbicula). The wings have a simple venation pattern typical of stingless bees, and the mandibles are used for handling wax and resin to build the nest (Figure 2) and *L. furva* is a very small stingless bee, with a body length of about 2-3 mm, smaller than *Tetragonula* and other stingless bees. It has a dark brown or black, glossy body. The head is small and round with large eyes, short antennae, and multiple segments. The thorax is sturdy with minimal hair. The wings are thin, translucent, and clear or light brown. The legs are slender and long, and the hind legs lack a pollen basket since it feeds on nectar instead. The abdomen is slender and segmented, and it has small mandibles used for building nests from wax or resin (Figure 3).

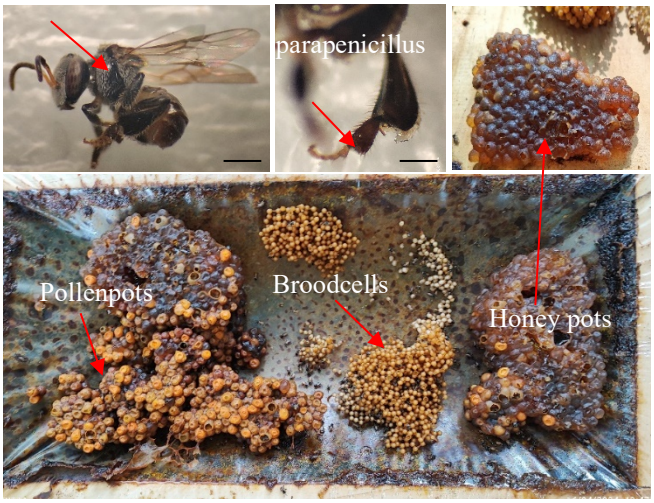

Figure 2. Morphological characteristics of *T. pagdeni* and stylized representation of *T. pagdeni* nest structure

Diversity of stingless bees collecting pollen in food plant

The survey began by establishing points within the durian orchard using the line transect sampling method, covering 2,500 meters. Observations were conducted from 6:00 to 10:00 a.m., which corresponds to the active foraging period of stingless bees. The foraging behavior of stingless bees was observed

based on their flower selection. It was found that the food plants of interest to the stingless bees included a total of 13 species: *A. agangetica* (L.), *T. procumbens* (L.), *Wedelia trilobata* (L.), *Bougainvillea glabra* (L.), *Eleutheranthera ruderalis* (Sw.), *Vernonia cinerea* (L.), *Gomphrena globosa* (L.), *Eleusine indica* (L.), *Mimosa pudica* (L.), *Lindernia crustacea* (L.), *Durio zibethinus*; Mon-Thong, Chanthaburi 3 and Kradum Thong (Figure 4). Afterward, when examined under a microscope, the pollen displayed various forms, such as capsular, pendulous, spherical, and oval shapes, among others (Figure 5).

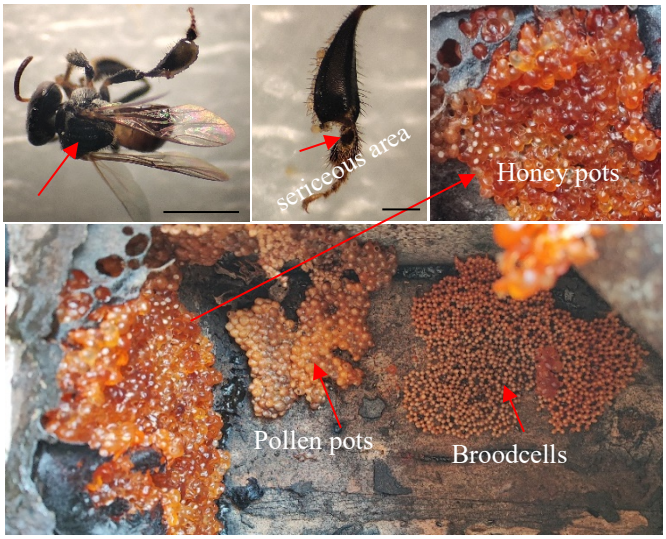

Figure 3. Morphological characteristics of *L. furva*is and stylized representation of *L. furva* nest structure

Palynological analysis

The collection of pollen pots in the nests of *T. pagdeni* and *L. furva*, the study examined the characteristics of the food plants from which the stingless bees gathered pollen. It was found that both species collected pollen from food plants, but there were significant differences between them. The *T. pagdeni* demonstrated a greater capacity for pollen collection, which could be categorized into four groups: Dominant pollen (D >45%): The frequencies of the pollen types were *A. agangetica* (L.) (83%), *T. procumbens* (L.) (65%), and *W. trilobata* (L.) (53%). Accessory pollen (A = 15–45%): Found were *V. cinerea* (L.) (16%) and *M. pudica* (L.) (18%). Isolate pollen (I = 3–15%): Included *M. pudica* (L.) (5%) and *L. crustacea* (L.) (3%). Occasional pollen (O <3%): Only 1% was recorded, specifically *D. zibethinus* (L.); Mon-Thong and

Kradum Thong (Table 1; Figure 5). In contrast, *L. furva*, which is smaller than *T. pagdeni*, primarily collects pollen from weedy plants with small flowers. The frequencies of the pollen types collected included *B. glabra* (L.) (15%), *G. globosa* (L.) (8%), *E. ruderalis* (Sw.) (11%), and *E. indica* (L.) (13%). Overall, *L. furva* does not focus on collecting pollen from flowering plants that provide nectar for sustenance in the nest more than other stingless bee species (Table 1; Figure 6).

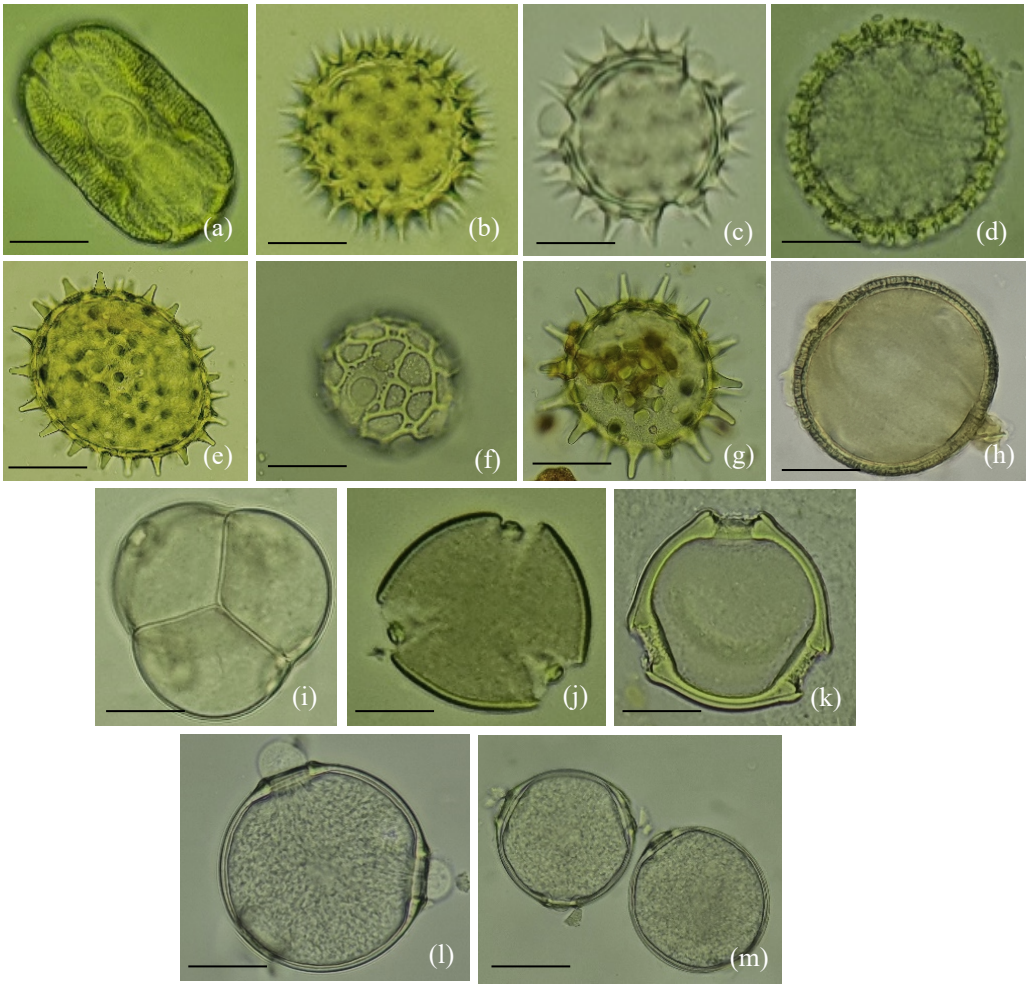

Figure 4. Stingless bees collecting pollen on food plant in durian orchad: (a)*A. gangetica* (L.), (b)*T. procumbens* (L.), (c)*W. trilobata* (L.), (d) *B. glabra* (L.), (e)*E. ruderalis* (Sw.), (f) *V. cinerea* (L.), (g) *G. globosa* (L.), (h)*E. indica* (L.), (i)*M. pudica* (L.), (j)*L. crustacean* (L.), (k)*D. zibethinus* (L.) "MonThong", (l)Chanthaburi 3 and (m)Kradum Thong

Figure 5. The botanical sources of pollen collected of stingless bees in this study (photo by Titi Thongkamngam)

Table 1. Main pollen types identified in pollen pots of Stingless bees *T. pagdeni* and *L. furva*, collected in February 2024 (SP-1), April 2024 (SP-2) and June 2024 (SP-3)

Percentage class of the pollen type	Frequencies (%) of the pollen types					
	T. pagdeni			*L. furva*		
	SP-1	SP-2	SP-3	SP-1	SP-2	SP-3
Dominant pollen (D > 45%)						
A. gangetica (L.)	83	-	-	20	15	12
T. procumbens (L.)	-	65	-	18	13	14
W. trilobata (L.)	-	-	53	-	-	-
Accessory pollen (A = 15–45%)						
V. cinerea (L.)	-	16	-	-	-	-
M. pudica (L.)	-	-	18	-	-	-
Isolate pollen (I = 3–15%)						
M. pudica (L.)	3	5	2	-	-	-
B. glabra (L.)	-	-	-	15	12	14
G. globosa (L.)	-	-	-	8	9	11
E. ruderalis (Sw.)	-	-	-	11	15	17
E. indica (L.)	-	-	-	13	13	8
L. crustacea (L.)	3	2	3	-	-	-
Occasional pollen (O < 3%)						
D. zibethinus						
- Mon-Thong	1	-	-	-	-	-
- Chanthaburi 3	-	-	-	-	-	-
- Kradum Thong	1	-	-	-	-	-

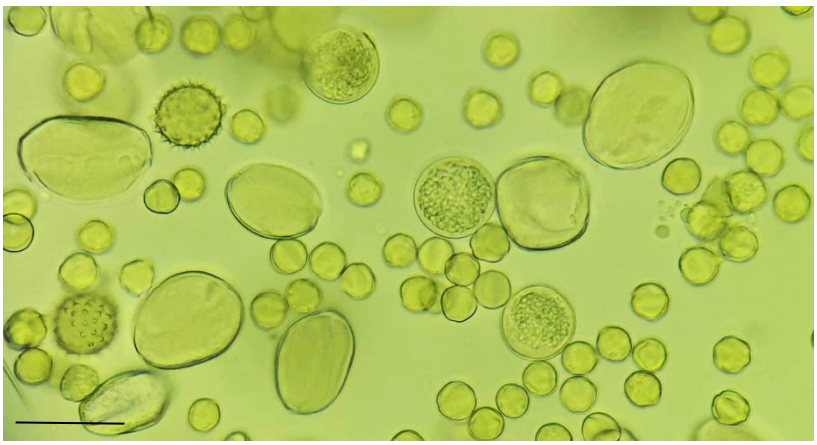

Figure 5. Pollen grain types from *T. pagdeni* and *L. furva* in pollen pots observed under a light microscope

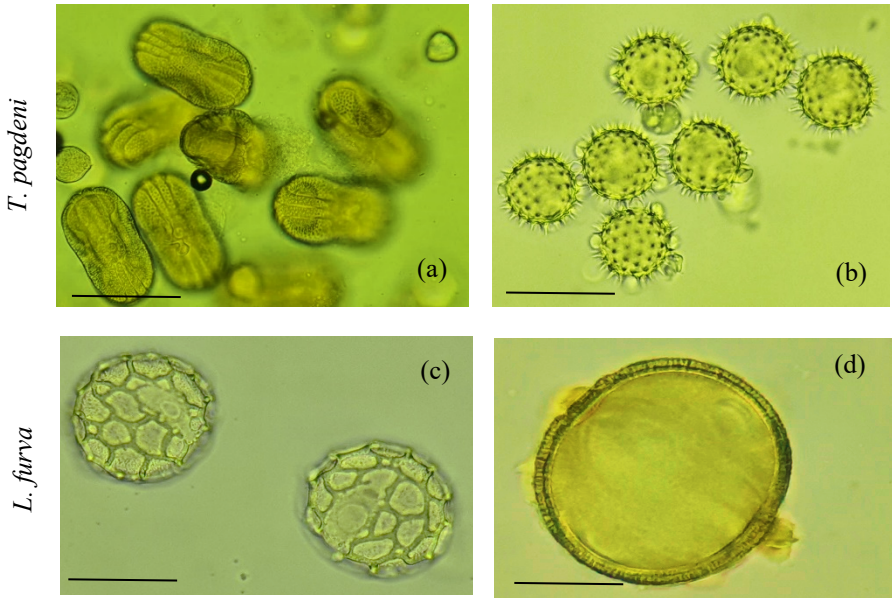

Figure 6. Verification of pollen types identified in samples collected from stingless bees of *T. pagdeni* and *L. furva*on foraging plants: (a) *A. agangetica* (L.) (b) *T. procumbens* (L.), (c) *B. glabra* and (d) *E. indica* (L.)

Discussion

During the summer of 2024, stingless bee samples were collected from the durian orchard of the Department of Plant Production Technology and Landscape, Rajamangala University of Technology Tawan-ok, Chanthaburi Campus. The foraging behavior of the bees was observed based on their flower selection. It was found that the stingless bees were interested in 13 food plant species: *A. agangetica* (L.), *T. procumbens* (L.), *W. trilobata* (L.), *B. glabra* (L.), *E. ruderalis* (Sw.), *V. cinerea* (L.), *G. globosa* (L.), *E. indica* (L.), *M. pudica* (L.), *L. crustacea* (L.), *D. Zibethinus*; Mon-Thong, Chanthaburi 3, and Kradum Thong. In line with the experiment by Chelog (2021) studying the food plants of stingless bees, it was found that the pollen pots of *H. itama* included pollen from Wedelia, Acacia, Cocos, and Elaeis (Song *et al.,* 2012; Toledo *et al.,* 2018). Most pollen types came from the families Mimosaceae, Graminae, and Asteraceae.A total of 10 samples were collected, including two species: *T. pagdeni* and *L. Furva* (Boontop *et al.,* 2008; Cardona *et al.,* 2019; Karabagias *et al.,* 2020; Engel *et al.,*2023; Ng *et al.,* 2024).

The study involved collecting pollen pots from the nests of *T. pagdeni* and *L. Furva* to examine the characteristics of the food plants from which the

stingless bees gathered pollen. The pollen types identified in the samples were categorized into four groups: Dominant pollen (D > 45%): The frequencies of the pollen types included *A. agangetica* (L.) (83%), *T. procumbens* (L.) (65%), and *W. trilobata* (L.) (53%). Accessory pollen (A = 15-45%): This group included *V. cinerea* (L.) (16%) and *M. pudica* (L.) (18%). Isolate pollen (I = 3-15%): This category comprised *M. pudica* (L.) (5%) and *L. crustacea* (L.) (3%). Occasional pollen (0 < 3%): Only 1% was recorded, specifically from Mon-Thong and Kradum Thong. Similarly, in the experiment by Syafrizal *et al.* (2020), which surveyed 12 species of stingless bees, only two species were found *Heterotrigona itama* and *Tetragonula laeviceps*that collected the most pollen in Indonesia. *L. furva* does not focus on collecting pollen from flowering plants that provide nectar for sustenance in the nest more than other stingless bee species. The frequencies of the pollen types collected included *Bougainvillea glabra* (L.) (15%), *G. globosa* (L.) (8%), *E. ruderalis* (Sw.) (11%), and *E. indica* (L.) (13%) (Cuttong *et al.,* 2016).

In summary, the study of pollen collection by the two species, *T. pagdeni* and *L. furva*, demonstrates that food stability and environmental conditions, such as El Niño and La Niña events, significantly impact their foraging behavior. These environmental fluctuations compel the stingless bees to adapt their living habits to survive in changing conditions. As a result, the bees modify their flower selection and foraging strategies to ensure access to suitable food sources, reflecting their resilience and ability to cope with the variability in their environment. This adaptability is crucial for their survival and overall success in fluctuating climatic conditions.

Acknowledgements

The authors would like to thank the Rajamangala University of Technology Tawan-ok, Chanthaburi Campus, Thailand, the Department of Plant Production Technology and Landscape and the Faculty of Agro-Industrial Technology for providing all the facilities both in entomology and plant pathology laboratory.

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(Received: 10 October 2024, Revised: 8 January 2025, Accepted: 13 January 2025)