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Morphology and Histology of Hypopharyngeal and Mandibular Glands of Five Stingless Bee Genera in Thailand

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Abstract

Stingless bees have an exocrine gland in the head, including hypopharyngeal and mandibular glands, which play essential roles in social insect existence. The research aimed to examine the morphology and histology of five stingless bee genera's hypopharyngeal and mandibular glands (*Homotrigona*, *Lepidotrigona*, *Tetrigona*, *Tetragonilla,* and *Tetragonula*). The morphological properties of the hypopharyngeal gland in all genera are composed of several spherical acini cells connected in a long chain. Each acinus has small tubular secretory cells extending from the acinus to the axial duct. The glandular class III secretory cells found in tissue differ in structure and arrangement among the five genera. There is a remarkable diversity in the mandibular glands of stingless bees. The first type, the ectomandibular gland, features an assembled reservoir at the end of the mandible base for storing the aqueous solution in the tissue found in the glandular epithelial class III secretory cell in all genera. The second type, the intramandibular gland, is found in the secretory cells of the mandible space. A mixture of glandular classes I and III in the secretory cells is present in all genera. Moreover, the morphometrics of the hypopharyngeal gland acini and mandibular gland reservoir indicate that the morphological sizes in each gland are not dependent on the body size of stingless bees, especially the *Lepidotrigona* and *Tetrigona*. The exocrine glands of a stingless bee may demonstrate the relation of their behavior or activity in the colony, which is an important response to their social existence. Furthermore, physiological capabilities may be related to different body sizes and fLowering plant resources in tropical regions such as Thailand.

Introduction

Stingless bees (Hymenoptera, Apidae) are highly eusocial insects in the Meliponini tribe, distributed in the tropical zone of Africa, Asia, and Australia, and the subtropical zone of South America (Heard, 1999; Michener, 2007). In Thailand, stingless bees are important insect pollinators for crop plantations (Chuttong et al., 2015). Hymenopterans are social insects with three exocrine glands in the head: the hypopharyngeal, intramandibular, and salivary (Cruz-Landim & Reginato, 2001). Hymenopterans, such as stingless bees or honey bees, can produce pheromones for communication during foraging or guarding behavior. A pheromone is a chemical communication secreted from the glandular cell (Meer et al., 1998; Wyatt, 2003). The exocrine gland system of the hymenopterans includes the antenna, head, thorax, legs, and abdomen. The head glands in ants, bees, and wasps in the subfamily Apoidea consist of the cephalic salivary, hypopharyngeal, and intramandibular glands (Cruz-Landim, 1967). The head secretory gland of the bee provides natural chemical communication related to the behavioral responses. This signaling system mimics the cleptoparasitic odors in the

genus *Nomada* to its host, genus *Andrena*, demonstrating a close chemical relationship between the solitary bee and cleptoparasite (Galvani & Settembrini, 2013).

The hypopharyngeal glands are secretory structures between the compound eyes and the bee's brain (Snodgrass, 1984). The young adult worker bee performs rearing larvae and queen feeding tasks and has the peak size and secretory capacity for jelly protein production. The hypopharyngeal gland size correlates with the age of the bee and its age-associated tasks and is consequently sensitive to the amount of protein and pollen in the diet of the young adult (Corby-Harris & Snyder, 2018). The evolutionary history of the hypopharyngeal glands in the order Hymenoptera means that the highest levels of these glands developed in the family Apidae are found in the axial duct and pluricellular secretory unit. Focusing on the Apinae and Meliponinae subfamilies, the glandular secretion peak corresponds to the brood care task in worker bees. The glandular types are closely related to the degree of social development. For instance, in eusocial bees, Apinae is type IV and Bombinae type III, while Meliponinae is both types III and IV (Cruz-Landim & Costa, 1998). The secretory unit of the hypopharyngeal gland is classified in the glandular cell type class III (Noirot & Quennedey, 1991). The first type of unicellular acini is constituted by a single cell. In contrast, later, the pluricellular acini are constituted by two or more cells, such as in Meliponinae, *Trigona hypogea*, where the glandular acini are composed of two or three cells (Cruz-Landim & Costa, 1998).

The next head gland of the social bee is the mandibular gland, which comprises two main structures of secretory tissues. The first is the reservoir tissue attached to the mandible, consisting of glandular class III cells (Noirot & Quennedey, 1991). The glandular class III cells in bees differ in number, size, and reservoir characteristics (Cruz-Landim, 1967). The gland development in each bee species and caste (queen, worker, and drone) also differs, such as in the Meliponini bees, *Melipona* sp., and *Scaptotrigona postica* (Gracioli & Silva-de-Moraes, 2002; Gacioli-Vitti et al., 2004). The mandibular gland cells of the queen and workers in *M. quadrifasciata* are different in size, characteristics, and functional state, with the gland being more developed in the nurse worker bee than the forager and queen (Cruz-Landim et al., 2011). The mandibular gland of eusocial hymenopterans such as the formicine ant (*Myrmoteras iriodum*) is very similar among the various ant species: the paired gland is situated at each side of a cluster of class III secretory cells (classification of Noirot & Quennedey, 1974), opening through their accompanying duct cells into a common reservoir. From the reservoir, a main duct connects to the base of the mandible, where secretion is released. The secretory cells are usually round to ovoid with a sinuous end apparatus and lack special intercellular contacts (Billen et al., 2016). The secretory cells in the honey bee, *Apis mellifera*, cover all the reservoirs. At the same time, workers are related to the production of alarm pheromones and, in queens, to sexual pheromones. The mandibular glands of the worker and queen in stingless bees, *M. bicolor*, are formed by a group of secretory cells separated by the reservoir, producing pheromones. In *S. postica*, the gland presents a bifid reservoir, being just one of a branch completely covered by secretory cells and used by the workers for secretion as a trail pheromone (Gracioli-Vitti & Abdalla, 2006). In all *Plebria emerina* workers, the intramandibular glands contain two types of secretory epithelium glandular cells. The mandibular surface of *P. emerina* has some pore clusters at the opening of the conducting canal of the unicellular intramandibular glands. These glands may play a role in propolis manipulation and resin collection because the workers manipulate propolis (Santos et al., 2009).

For social insects, worker body size depends on an adaptation to environmental conditions related to temporal segregation of foraging behavior (Hrncir & Maia-Silva, 2013). The morphological variation in Meliponini of worker body size is 75.5% and has been generally reflected as an adaptation to foraging activity and floral resource exploitation (Pignata & Diniz-Filho, 1996). A strong correlation exists between body size and flight distance in Meliponini, which can be helpful in conserving tropical biodiversity (Araújo et al., 2004). Body size influences the foraging range in many bee species, and some larger stingless bees in the genus *Melipona* are sensitive to disturbance (Vit et al., 2013; Pioker-Hara et al., 2014; Mayes et al., 2019). However, body size strongly determines a worker's sensory organ size, sensitivity, and physiological capabilities. It thus also affects foraging range, flight speed, efficiency by which a certain type of flower can be exploited, and the capability to compete with other species for resources (Hrncir & Maia-Silva, 2013). Examples of the stingless bee, *M. quadrifasciata anthidioides*, indicate more significant evolutionary constraints for the size and shape variation (Nunes et al., 2013). On the other side, the head glands of honey bees and stingless bees are the greatest sizes of the head glands; there was an enlargement in gland alveoli size from newly emerged workers, with the maximum size in forager workers. For example, the head gland may be due to the propolis manipulation performed by foraging honey bee workers and older *P. emerina*. The difference in gland size associated with worker age among *P. emerina* and other stingless bees may be because the bee species do not store viscous clusters of propolis in their nest, as does *P. emerina* (Salles & Cruz-Landim, 1998).

The stingless bee is economically important as an insect pollinator for plant agriculture and meliponiculture in Thailand, especially the genus *Tetragonula* (Chuttong et al., 2014). Stingless bees are different in body size, color, and nesting entrance, and are rarely reported from Thailand. There are more than 35 species of stingless bees in several genera such as *Geniotrigona*, *Heterotrigona*, *Homotrigona*, *Lepidotrigona*, *Lisotrigona*, *Lophotrigona*, *Plattytrigona*, *Tetrigona*, *Tetragonilla*, and *Tetragonula* (Rasmussen, 2008;

Rattanawannee et al., 2015; Attasopa et al., 2020). However, basic knowledge of stingless bee biology is still necessary in Thailand and other countries. This investigation aims to examine the morphology and histology of head glands, such as hypopharyngeal and mandibular glands, in different body sizes of five stingless bee genera: *Homotrigona*, *Lepidotrigona*, *Tetrigona*, *Tetragonilla,* and *Tetragonula*, naturally dispersed in Thailand. Moreover, using light microscopy detection, the research investigates the biological techniques of dissecting stingless bee head glands and their morphological and histological characteristics.

Materials and Methods

Sample collection and fixation

Stingless bee samples of five genera were collected from 17 colonies. Stingless bee samples were collected from 12 colonies in Phayao Province. The first genus had a giant body size: *Homotrigona* in the species *Homotrigona fimbriata* (three colonies), *Lepidotrigona terminata* (three colonies), *Tetrigona apicalis* (three colonies), and *Tetragonilla collina* (three colonies), with an additional *Tetragonilla collina* (one colony) from Chiang Rai Province. Later, bees from the genus with the smallest body size, *Tetragonula pegdini*, were collected from three Chanthaburi colonies and one Kanchanaburi Province. All the bee samples were collected from natural colonies that constructed their nests in a hole in a tree or building. Twenty bee samples were caught in a plastic bag in front of the nest entrance and killed using ethyl acetate. The heads of bees were cut and fixed with Davidson's fixative for 48 hrs, except the giant bee in *Homotrigona,* which was fixed for 72 hrs at room temperature. The fixative solution was discarded and preserved in 70% ethanol until embedded.

Head gland morphology and histology

The morphological and histological procedures were carried out with precision. The morphology of the hypopharyngeal and mandibular glands was sampled for ten stingless bees in each colony. The hypopharyngeal gland was dissected at the head, the opened cuticle, and the facial integument. The hypopharyngeal gland was picked out and placed in a fixative solution with eosin. The mandibular gland was pulled at the mandible using jewelry forceps, and the whole mandibular gland was picked out. The glandular acini of the hypopharyngeal and mandibular glands were captured using OptikaSZP-6 connected with Optikam HDMI – 4083.13H (Optika, Italy). The twelve stingless bee colonies (Table 1) were measured, and the hypopharyngeal glands' radius and circumference were measured by 30 acini per head using the OpikalSview program. The total length of the ectramandibular gland was measured on both the left and right sides in three heads per colony. Also, statistical analysis was performed by Post Hoc Tests ANOVA ($P < 0.05$) using the SPSS IBM 22.0 package.

The stingless bee head in 70% ethanol was dehydrated and embedded in series following Bell & Lightner (1988), a method chosen for its proven effectiveness in preserving tissue structure. The dehydration series comprised 70% ethanol for 1 hr, 90% for 8 hrs, 95% for 12 hrs (twice), n-butanol for 1 hr, and xylene for 1 hr. Then, the paraffin series was started at xylene and paraffin wax at 50 °C for 1 hr (three times), and the wax was kept at 50 °C overnight. The bee head was embedded in paraffin wax and cut into paraffin ribbons with a thickness of 10 µm and a carbon blade 35° angle setting using a Semi-automatic microtome (AMOS Scientific; AMOS, Australia). The head gland tissue was stained using the hematoxylin and eosin series modified by Bell & Lightner (1988), a staining method known for highlighting. The staining slide was started using xylene for 10 mins (twice), absolute ethanol for 1 min (twice), 95% ethanol for 1 min (twice), washed in water for 2 mins, moved to the hematoxylin jar for 2 mins, soaked in water for 2 mins, rinsed with 95% ethanol for 1 min and moved to the eosin jar for 1 min, rinsed with 95% ethanol for 1 min, absolute ethanol for 1 min, and moved to xylene for 4 mins. The slide was permanently mounted using the Permount medium, a choice made for its ability to preserve the stained tissue. Light microscopy was used on both the hypopharyngeal and mandibular glands to examine the glandular cell, duct, and tissue characteristics at 400 and 1,000 magnification and captured images using the compound light microscope Olympus BX43 connected with the Olympus DP73 camera.

Results

Morphology and histology of the hypopharyngeal gland

The hypopharyngeal glands of five stingless bee genera between the compound eyes at the upper brain near the salivary gland were analyzed. The morphological hypopharyngeal gland acini were oval-shaped for all five stingless bee genera. When focusing on the body size of these five genera, *Homotrigona* was found to be the biggest, measuring approximately 7.0 – 10.0 mm, bigger than *Tetrigona* (6.0 – 8.0 mm), *Tetragonilla* (5.0 – 7.0 mm), *Lepidotrigona* (4.0 – 6.0 mm), and *Tetragonula* (2.5 – 4.0 mm), respectively (Fig 1, Table 1). The findings revealed that the average size of the radius and circumference (mm) of acini for the five genera was not dependent on the body size of stingless bees. The biggest body size in the genus *Homotrigona* had an average radius (r) of 0.0301 ± 0.0014 mm while the circumference (c) of 0.1892 ± 0.0142 mm in the genus *Tetrigona* was r = 0.0283 ± 0.0037 mm, c = 0.1706 ± 0.0161 mm; *Tetragonilla,* r = 0.0304 ± 0.0034 mm, c = 0.1883 ± 0.0200 mm; *Lepidotrigona,* r = 0.0306 ± 0.0026 mm, c = 0.1966 ± 0.0188 mm; and *Tetragonula,* r = 0.0267 ± 0.0047 mm, c = 0.1607 ± 0.0116 mm, respectively (Table 1). The results showed a statistical difference in the average size of acini in five genera belonging to three groups of acini. Firstly, the biggest acini in the hypopharyngeal gland were in the *Homotrigona*, *Lepidotrigona,* and *Tetragnilla* group, while

the second was in the *Tetrigona* group, significantly smaller than the first group. The third group, with the smallest body size of *Tetragonula*, also had the smallest acini (Fig 1, Table 1). When focusing on the medium body size, the stingless bees *Lepidotrigona* and *Tetragonilla* had the same hypopharyngeal gland acini size as the giant stingless bees in *Homotrigona*. In contrast, the *Tetrigona* was significantly smaller in acini size than both stingless bees (Table 1).

The histology of the hypopharyngeal gland structure in the acini cells of five stingless bee genera was the same as the secretory cell type in class III, but different in cell size. The secretory cell exhibited good hematoxylin staining at the nucleus, which was round and located at the cell center. The nucleolus was also small and round (Fig 2). Moreover, the secretory cells connected with the axial duct at the end of the hypopharyngeal plate at the hypopharynx were the same as those in the honey bee. The hypopharyngeal gland of the stingless bee was inserted near the salivary gland. Still, the hypopharyngeal gland of the honey bee was in front of the brain, and the salivary gland was behind it. The salivary gland was found to have a secretory cell class III structure, stained with eosin. Inside the salivary secretory cell was a space potentially for storing chemical fluids.

Morphology and histology of mandibular gland

The structure of the mandibular gland in the stingless bee consisted of two main parts. The first part was the ectomandibular gland, a sac-like structure connected to the tip of the molar base. The second part was the intramandibular gland found within the space of the molars. Both parts functioned to secrete substances and store secretions. The anatomy of the mandibular gland consisted of a bifid, sack-like structure. This gland was almost entirely covered with spherical secretory cells. The secretory cells and reservoir were connected to a thin wall by a canal. The paired mandibular gland occurred in the anterior region of the head near the basal margin of the compound eye connected to the mandible. The mandibular glands in stingless and honey bees were the same shape as the thin membrane connecting to the base of the molars. The reservoir or ectomandibular area was of different sizes. The reservoir or ectomandibular gland had a sac-like structure for storing substances (Fig 3). The average length of the reservoir ectomandibular gland was significantly different in the three groups (Table 1). The *Lepidotrigona* had an average length of approximately 0.634 ± 0.008 and was still within the same group as the giant genera *Homotrigona* (0.643 ± 0.003) and *Tetrigona* (0.692 ± 0.001) were significantly bigger than the reservoir ectomandibular gland of the *Homotrigona*. In contrast, the *Tetragonilla* had the same body size, but the average length of the reservoir ectomandibular gland was approximately 0.404 ± 0.006, while the *Tetragonula* was the smallest structure, approximately 0.239 ± 0.006 (Table 1).

Table 1. Comparison of average acini size (radius and circumference) in the hypopharyngeal gland and length of the ectomandibular glands among five genera of stingless bees.

Genera	Range of body size (mm)	Hypopharyngeal glands				Mandibular glands			
		N (H: Acini)	Average size of hypopharyngeal acini (Mean ± SD)			N (H)	Average length of ectomandibular gland (Mean ± SD)		
			Radius (mm)	Circumference (mm)	Area (mm^2)		Left side (mm)	Right side (mm)	Average (mm)
Homotrigona	7.0-10.0	3 (9: 270)	0.030 ± 0.001^{c}	0.189 ± 0.014^{c}	0.0029 ± 0.0003^{c}	3 (9)	0.639 ± 0.029^{c}	0.646 ± 0.042^{c}	0.643 ± 0.003^{c}
Lepidotrigona	4.0-6.0	2 (6:180)	0.031 ± 0.003^{c}	0.197 ± 0.019^{d}	0.0030 ± 0.0006^{c}	2 (6)	0.641 ± 0.040^{c}	$0.625 \pm 0.0.031^{c}$	0.634 ± 0.008^{c}
Tetrigona	6.0-8.0	3 (9: 270)	0.028 ± 0.004^{b}	0.171 ± 0.016^{b}	0.0026 ± 0.0006^{b}	3 (9)	0.690 ± 0.023^{d}	0.694 ± 0.012^{d}	0.692 ± 0.001^{d}
Tetragonilla	5.0-7.0	3 (9: 270)	0.030 ± 0.003^{c}	0.188 ± 0.020^{c}	0.0029 ± 0.0008^{c}	3 (9)	0.397 ± 0.048^{b}	0.411 ± 0.035^{b}	0.404 ± 0.006^{b}
Tetragonula	2.5-4.0	1 (3: 90)	0.027 ± 0.005^{a}	0.161 ± 0.012^{a}	0.0023 ± 0.0007^{a}	1 (3)	0.244 ± 0.025^{a}	0.233 ± 0.030^{a}	0.239 ± 0.00^{6a}

Remarks: N represents the number of bee colonies, H represents the number of bee heads, Acini represents the number of acini in the hypopharyngeal gland, and lowercase letters a, b, c, and d represent statistical significance with Post Hoc Test at $P < 0.05$ among stingless bee genera.

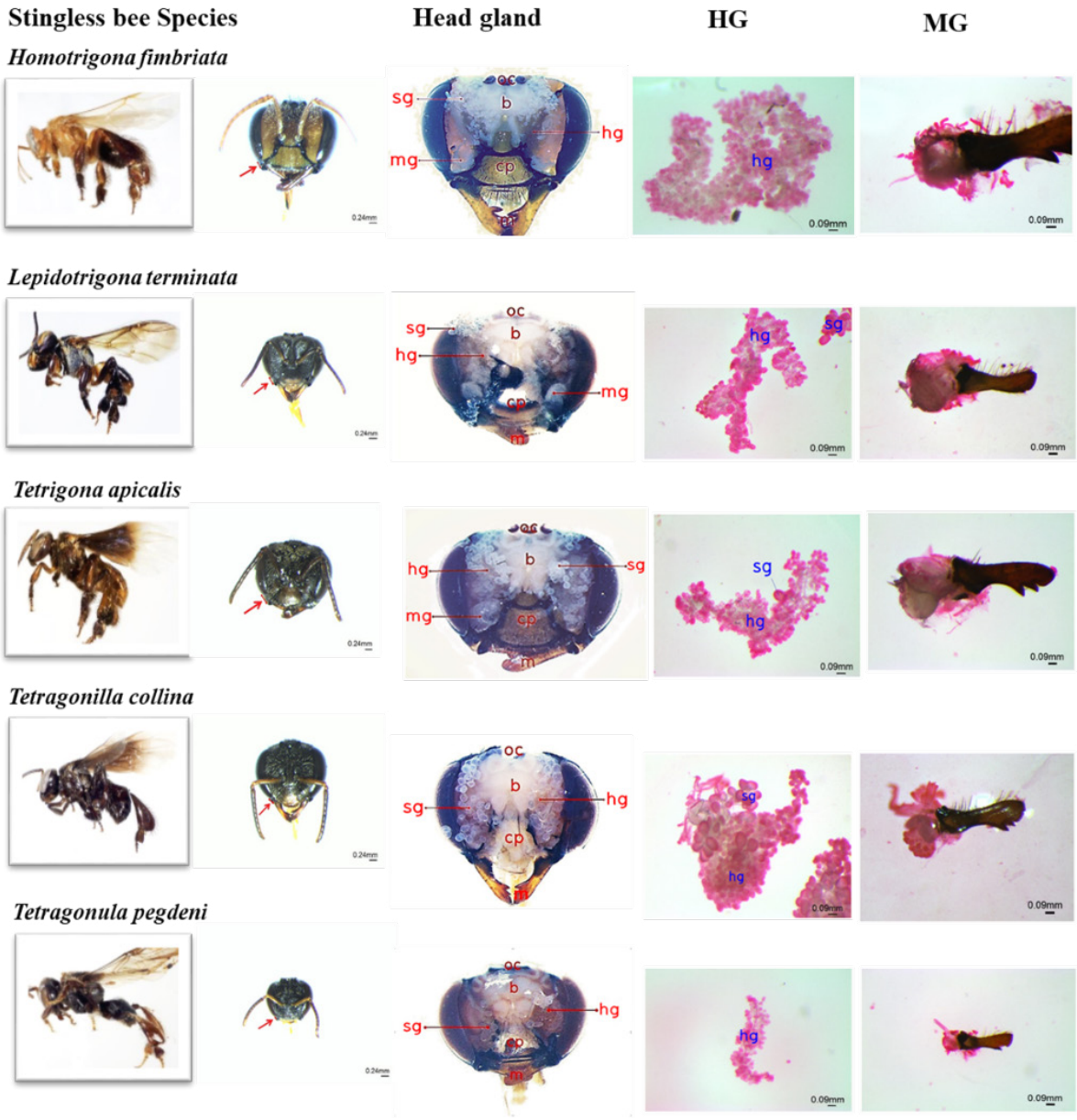

Fig 1. External morphology of the lateral body and head shape (first and second column) in five genera, identified into giant to dwarf stingless bee species as *Homotrigona fimbriata*, *Tetrigona apicalis*, *Tetragonilla collina*, *Lepidotrigona terminata*, and *Tetragonula pegdini*. The head dissection shows the head gland (third column) consisting of the hypopharyngeal gland (hg), salivary gland (sg), and mandibular gland (mg). The stereomicroscopy shows the acini oval-shaped structure of the hypopharyngeal gland (hg) and characteristics of the ectomandible gland (emg) connected to the mandible in the fourth and fifth column, respectively. Photograph of the head dissection hypopharyngeal and mandibular glands of five stingless bee genera, recorded using the same magnification and letter symbols in the head: brain (b), clypeus (cp), mandible (m), and ocelli (oc). The acini gland stained with eosin.

The ectomandibular gland in stingless bees was round or oval-shaped, while the heart-shaped gland was found in the honey bees. This secretion storage sac was round and surrounded by many small granular cells. The ectomandibular was almost entirely covered with spherical secretory cells. In addition, the size of the secretory sacs of each stingless bee genus was different and did not depend on the size of the body, unlike the size of the secretion storage sac in the honey bee, which depends on body size. The histology of the mandibular glands in the area of the ectomandibular gland consisted of a thin, transparent membrane covering the gland. The epithelial glandular cell of the ectomandibular gland was found to have secretory class III cells connected to form a sac-like gland and stained with eosin. Inside the secretory cell, a large nucleus was found in the middle, stained with hematoxylin (Fig 4).

Another mandibular gland, the intramandibular gland, was found within the mandible space. The glandular cell of the intramandibular gland was characterized by class III cells distributed throughout the mandible space. The secretory cell was stained with eosin, and the nucleus within the cell was

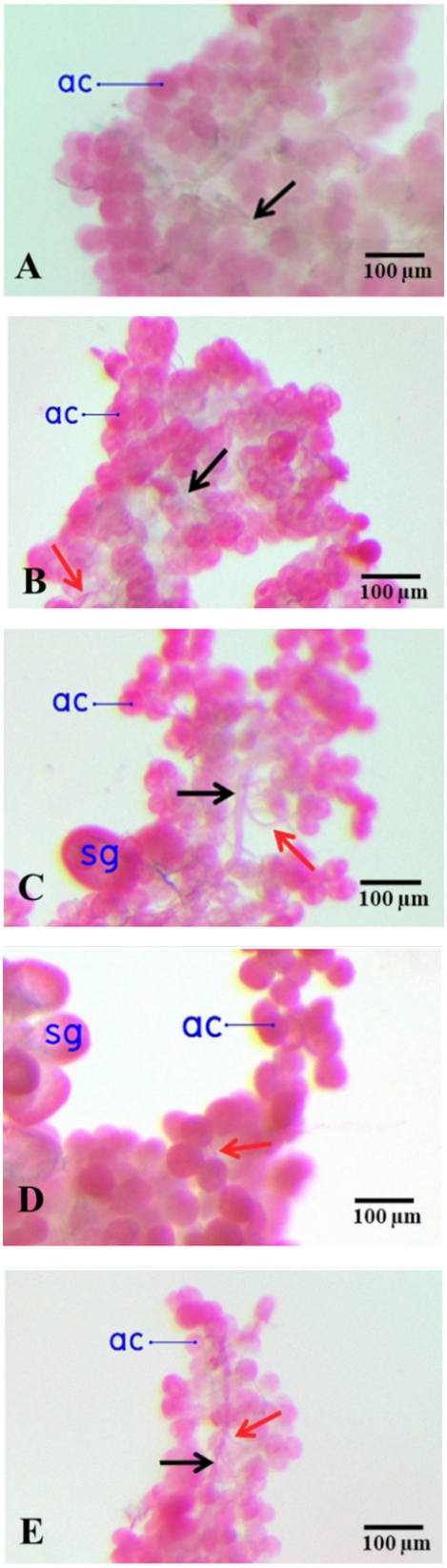

Fig 2. The stereomicroscope focusing on the acini oval-shaped structure and axial duct of the hypopharyngeal gland with eosin staining at 50 magnification. Small letters represented ac = acini of the hypopharyngeal gland and sg = salivary gland. The black arrow represents the intracellular duct and the red arrow the axial duct.

stained with hematoxylin (Fig 5). The histological characteristics of the mandibular glands of the five stingless bee genera revealed that all the mandibular glands had the same glandular structure. However, there were different sizes of secretory cells. In the stingless bee, the secretory cells had characteristics similar to those of the honey bee. Still, the epithelial glandular cells and sacs for storing secretions in the honey bee were more significant than those of the stingless bee.

Additionally, the histological area of the intramandibular gland contained secretory class III cells, which were the general cell type in the stingless bee. Furthermore, secretory class I cells were found in the stingless bee (Fig 5). The intramandibular glands of

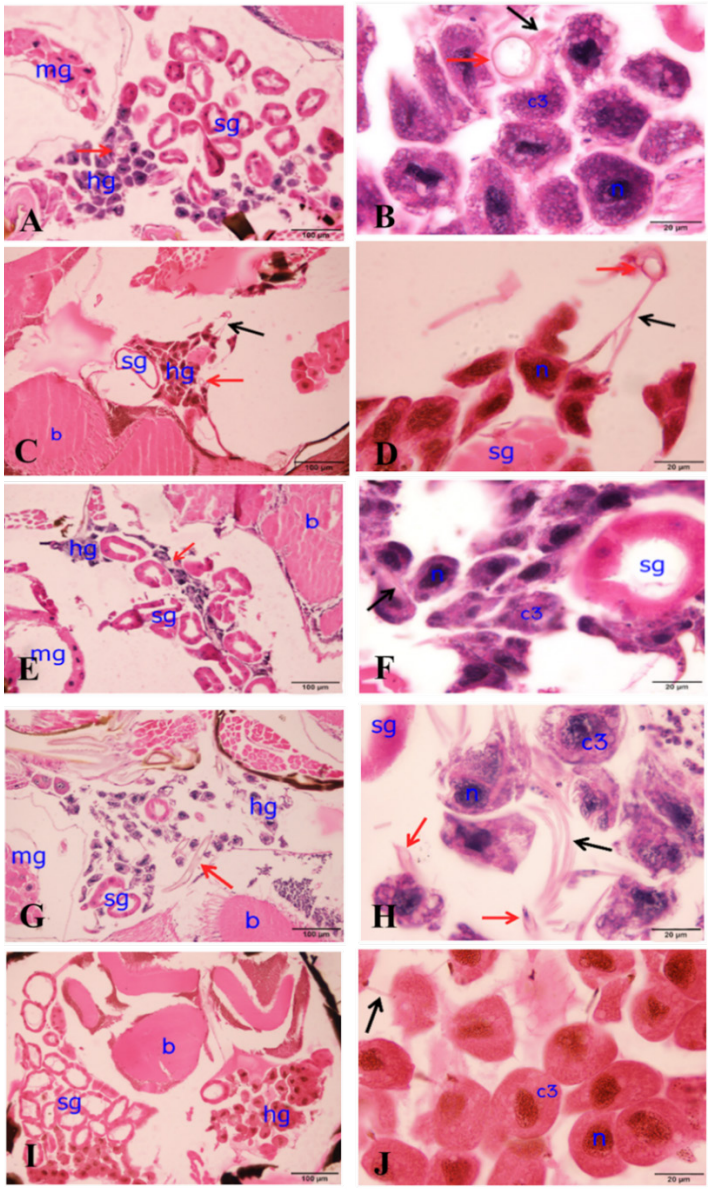

Fig 3. Light microscopy of the hypopharyngeal gland in five stingless bee genera, consisting of *Homotrigona* (A, B), *Lepidotrigona* (C, D), *Tetrigona* (E, F), *Tetrogonilla* (G, H), and *Tetragonula* (I, J). Photographs on the left side are at 400 magnification (A, C, E, G, and I) and 1000 magnification (B, D, F, H, and J), respectively. Small letters refer to hg = hypopharyngeal gland, mg = mandibular gland, sg = salivary gland, b = brain, n = nucleus, and c3 = glandular class III. The red arrow represents the axial duct and the black arrow the intracellular duct.

all five stingless bee genera contained two types of secretory structures. The first constitutes an epithelial glandular class I or epithelial gland; free secretory cells fill the inner space and constitute a class III or unicellular gland. Later, the epithelial gland cells in the epidermis comprised an epithelium of flat cells. The cuticle over the epithelial gland had many pore canals. The mandibular surface of five stingless bee genera had some pore clusters at the opening of the conducting canal of the unicellular intramandibular glands with the cuticle (Fig 5).

Discussion

Many of the exocrine glands in social insects have an epidermal origin. In bees, these glands produce pheromones for communication among colony members. The bees can respond rapidly to changes in both the colony and the environment. Previous studies on the age polymorphism of the exocrine glands of worker bees found that they were associated with worker tasks (Katzav-Gozansky et al., 2001;

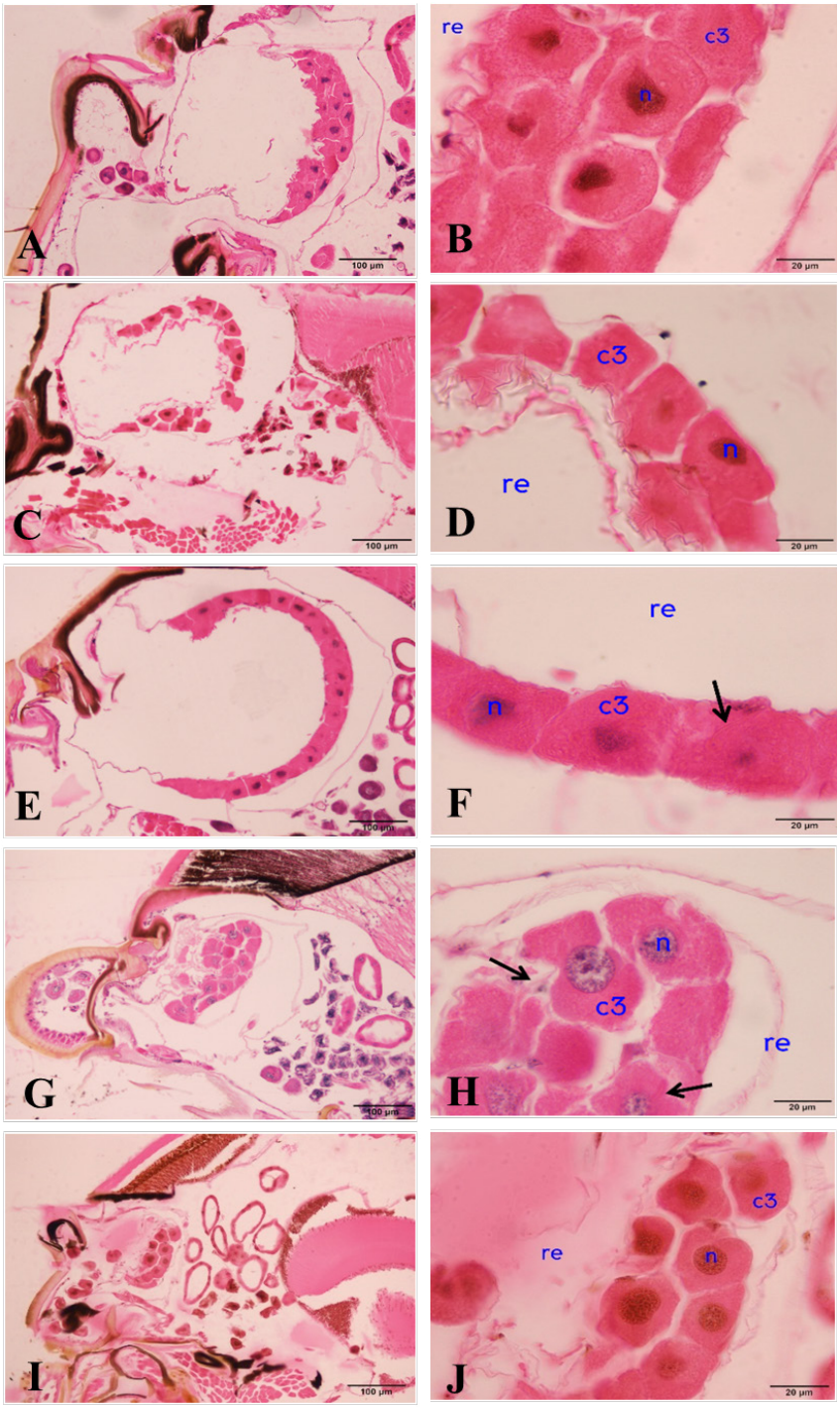

Fig 4. Light microscopy of ectomandibular gland in five stingless bee genera consisting of *Homotrigona* (A, B), *Lepidotrigona* (C, D), *Tetrigona* (E, F), *Tetrogonilla* (G, H), and *Tetragonula* (I, J). The photographs on the left side are at 400 magnification (A, C, E, G, and I) and 1000 magnification (B, D, F, H, and J). Small letters refer to c3 = glandular class III, n = nucleus, and re = reservoir. Black arrows represent the intracellular duct.

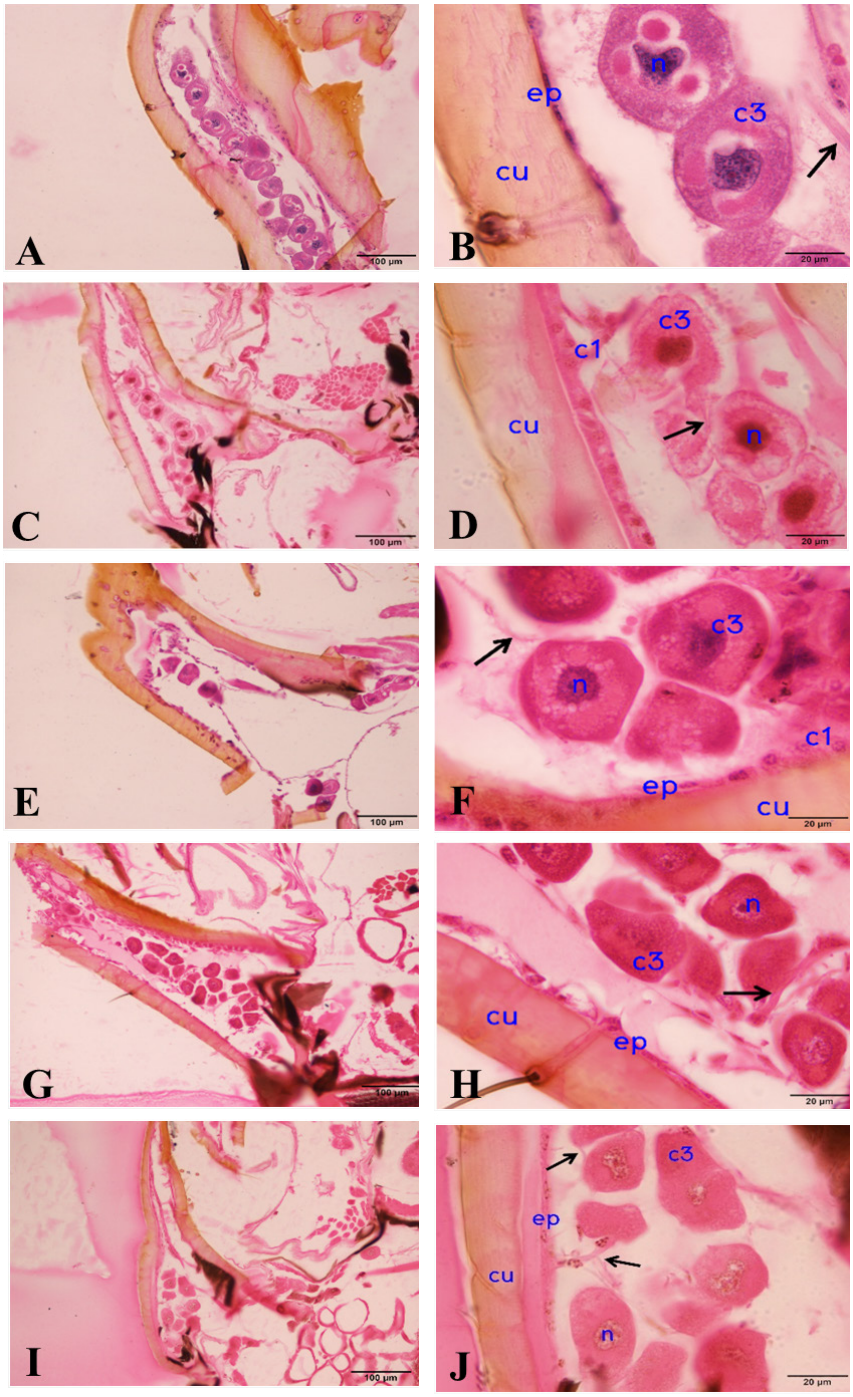

Fig 5. Light microscopy of the intramandibular gland in five stingless bee genera, consisting of *Homotrigona* (A, B), *Lepidotrigona* (C, D), *Tetrigona* (E, F), *Tetrogonilla* (G, H), and *Tetragonula* (I, J). Photographs on the left side are at 400 magnifications (A, C, E, G, and I) and 1000 magnifications, (B, D, F, H, and J). Small letters refer to c1 = exocrine gland class I, c3 = exocrine gland class III, n = nucleus, cu = cuticle, and ep = epithelial cell. Black arrows represent the intracellular duct.

Deseyn & Billen, 2004; 2005). This is the first report to describe the internal structures of the head and glandular structure of exocrine glands, especially the hypopharyngeal and mandibular glands, in the workers of five stingless bee genera in Thailand, following the body size of the genera *Homotrigona*, *Tetrigona*, *Tetragonilla*, *Lepidotrigona,* and *Tetragonula*, respectively. The morphology of body size in giant to dwarf stingless bees varies, but gland size is unrelated to body size, especially *Lepidotrigona*. The acini sac of the hypopharyngeal gland and the reservoir mandibular gland are more significant than the *Tetragonilla*; they are closer to body size. Moreover, the acini sac size of the hypopharyngeal is the biggest, and the length of the reservoir mandibular gland is closely *Homotrigona*. Additionally, the acini sac size of the *Tetrigona* is smaller than *Tetragonilla* but is still bigger *Tetragonula*. On the other hand, the reservoir mandibular

gland is closely *Homotrigona*. Both genera may produce propolis for the nest entrance and involucrum or batumen wall construction. The glandular secretion from the worker glands produces the propolis cluster (Santos et al., 2009). Moreover, body size influences the foraging range in many bee species and is sensitive to disturbance (Vit et al., 2013; Pioker-Hara et al., 2014; Mayes et al., 2019). Also, the worker sensory organ size, sensitivity, and physiological capabilities affect foraging range, flight speed, efficiency by which a certain type of flower can be exploited, and the capability to compete with other species for bee resources (Hrncir & Maia-Silva, 2013). The exocrine glands of bees can be classified into different types according to their secretion-releasing structures. Honey bees and stingless bees have glandular class I glands. In these glands, the columnar epidermis releases its secretion through the body cuticle, using the pore canals, resulting in cuticle deposition. Glandular class III glands are those in which the cellular conducting canals open into pores in the cuticle (Cruz-Landim, 1967; Salles & Cruz-Landim, 1998; Cruz-Landim & Reginato, 1999; Azevedo et al., 2007).

The histological characteristics of the head of these stingless bees include the brain structure, single eye, compound eye, hypopharyngeal glands, mandibular glands, and salivary glands. According to the histology of the epithelial glandular cells in the exocrine glands, the hypopharyngeal glands consist of the same glandular class III secretory cells surrounding the brain structure. In contrast, the mandibular glands comprise glandular class III and class I secretory cells. Likewise, the histological characteristics of the hypopharyngeal gland have been studied in various species of honey bees in the *Apis* genus (Design & Billen, 2005; Suwannapong et al., 2007; 2010; Yousef et al., 2014), stingless bees (Cruz-Landim, 1967; Cruz-Landim & Costa, 1988), and wasps (Cruz-Landim & Costa, 1998; Britto et al., 2004; Galvani & Settembrini, 2013). In honey bees, hypopharyngeal glands are well-developed in worker bees compared to queens and drones, degenerating when the tasks switch from nursing in the hive to foraging in the field (Robinson, 1992; Britto et al., 2004). The morphological characteristics of the hypopharyngeal glands of honey bees and stingless bees comprise a gland structure of spherical acini cells, many cell units grouped, and a bunch of connected ducts. These are connected with a large axial duct that acts as a long canal. The hypopharyngeal gland in the honey bee consists of one pair of core canals, which look like a bunch of grapes, usually requiring the entire brain (Cruz-Landim, 1967; Galvani & Settembrini, 2013). The hypopharyngeal glands of these five stingless bee genera appear to be a single secretory cell, with the nucleus clearly visible in the middle. The glandular class III secretory cells are generally in the hypopharyngeal gland of these five stingless bee genera. The hypopharyngeal glands secrete the royal jelly proteins essential in honey bees' diet and caste differentiation (Kamakura, 2011). Some stingless bees (Meliponini) possess a hypopharyngeal gland in the female castes, while in other species, both the females and males have hypopharyngeal glands (Costa & Cruz-Landim, 1999). In wasps, the hypopharyngeal gland exhibits no anatomical variation among castes and plays a vital role in nest building and food production for larvae (Cruz-Landim & Costa, 1998; Britto et al., 2004). The acini size of the hypopharyngeal gland in these five stingless bee genera is not relative to the body size of the stingless bee worker, which is biggest in *Homotrigona*, *Tetrigona*, *Tetragonilla, Lepidotrigona,* and *Tetragonula,* respectively. The results indicate that stingless bees with medium body size in the genus *Lepidotrigona* have an acini size near the giant stingless bee in the genus *Homotrigona*. The hypopharyngeal gland has been studied in different honey bee species and within the same honey bee workers. The hypopharyngeal glands of *A. florea* and *A. andreniformis* are very similar in histochemical structures, but differences can be observed between pupae, nurses, and foraging bees. The acini sizes (width and length) are larger in the hypopharyngeal gland of *Apis mellifera* and *A. cerana* nurse bees, gradually decreasing in size as the nurses become guards (Suwannapong et al., 2010).

The mandibular glands can be divided into two parts: the part of the gland at the base of the mandible consists of ectomandibular glands of glandular class III secretory cells, and the part within the mandible space consists of intramandibular glands in glandular class III secretory cells and class I secretory cells at the epithelium. The stingless bee's mandibular glands are smaller than the honey bee, although the quantity and density of the secretory cells are higher in the former than in the latter. Like the workers in the stingless bee *Plebeia emerina*, the intramandibular glands consist of secretory epithelium and unicellular glands. The secretory epithelium is in the glandular class I secretory cell, and the unicellular glands are in the glandular class III secretory cell (Santos et al., 2009). However, the difference in gland size associated with worker age among *P. emerina* and other stingless bees may be due to the bee species (Cruz-Landim, 1967; Salles & Cruz-Landim, 1998; Santos et al., 2009). Intramandibular glands, as unicellular glands in the mandible, have been reported in many stingless bee species but are lacking in the honey bee, *A. mellifera*. The unicellular intramandibular glands play a role in mandible lubrication (Cruz-Landim & Reginato, 2001). The intramandibular gland of *P. emerina* workers is well-developed throughout their lifespan. However, the secretory epithelium cells are well-developed at 20-30 days old and are forager workers (Santos et al., 2009; 2015). The results for the intramandibular gland of five stingless bee genera also suggest that this gland may play a role in propolis manipulation and resin collection, since the workers manipulate propolis. In other Meliponini species where mandibular gland secretion is not used to make trails, the workers still use it to perform extranidal tasks (Salles & Cruz-Landim, 1996). In the *Melipona* species, mandibular gland secretion is used to rob other colonies and defend the nest. It has been shown that *M. fasciata* and *M. interrupta triplaridis* respond to their respective mandibular

gland extracts with alarm recruitment and defensive behavior (Smith & Roubik, 1983). Small mandibular glands are present in *M. quadrifasciata,* consisting of a small cluster of gland cells located anteriorly and dorsally to a small, thin-walled reservoir to which the secretion is delivered.

Furthermore, the ultrastructure of the intramandibular gland of *M. bicolor* shows a predominance of SER and lipid-like secretion granules, consistent with the presence of hydrocarbon as a secretion component (Gracioli et al., 2004). The utility of the mandibular secretion in *Melipona* remains unknown. However, *Melipona* species do not employ the mandibular gland to make scent trails or jet air flows when signaling the way from the colony to a food source. The morphological changes in mandibular gland cells over the lifetime of *M. quadrifasciata* workers and queens, taken together with the current knowledge on mandibular gland function in bees (Cruz-Landim et al., 2011).

Stingless bees are different in body size and diversification in Thailand. They indicate more significant evolutionary constraints for size and shape variation in stingless bees. The body and organ size depends on an adaptation to environmental conditions related to bee species and behaviour. This research reveals direct morphology and histology of the hypopharyngeal and mandibular gland in *Homotrigona fimbriata*, *Lepidotrigona terminata*, *Tetrigona apicalis*, *Tetragonilla collina* and *Tetragonula pegdeni*. Another indirect relationship among bee size, the head gland (hypopharyngeal and mandibular glands), and resources will suggest the way of propolis manipulation or chemical production in stingless bees. Further, the stingless bee exocrine head gland of stingless bee species in the same genus, especially the *Lepidotrigona* and *Tetrigona*, may advance our understanding of the relationships among stingless bee's size, resources, and behavior. Moreover, stingless bee sensory organ size, sensitivity, head glands (hypopharyngeal, mandibular, and salivary gland) and physiological capabilities related to different body size and their flowering plant resources in tropical regions, as in Thailand will be further investigated as some stingless bee species have differences in gland size associated with worker age.

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Author's Contribution

All authors participate in all stages of this paper.

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