

Additional Nest Structures and Natural Enemies of Stingless Bees (Hymenoptera: Apidae: Meliponinae)

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Stingless bees (Hymenoptera: Apidae) are widely distributed in tropics and subtropics areas. Now these bees are farmed by the human because they produce honey and propolis. Natural enemies can disturb the colony that affects to nest structure and productivity of these bees. This study aimed to study the common nest structure and additional nest structure of stingless bees which caused by natural enemies. This research was conducted from August to November 2015. Samples were taken from three locations, i.e. Pasuruan (East Java), Tasikmalaya (West Java), and Rangkasbitung (Banten). Descriptive analysis was used to compare the structure and composition of the nest in bamboo and additional nest structures in relation with natural enemies. Generally, the structure and composition of the nest of stingless bees in bamboo consist of nest entrance, storage pots (honey and pollen), and brood cells. We didn't found natural enemies of stingless bees in Pasuruan and Tasikmalaya and these colonies did not create additional nest structures. In contrast, the colonies in Rangkasbitung created additional nest structures i.e. the inner entrance covered by batumen and cerumen and also build a root-like structure. Natural enemies found in the colony at Rangkasbitung were cockroaches (Blattodea: *Blattidae*), histerid beetles, *Platysoma leconti* (Coleoptera: Histeridae), nitidulid beetles, *Carphophilus* sp. (Coleoptera: Nitidulidae), and ants, *Pheidole* sp. (Hymenoptera: Formicidae).

Key words: stingless bees, nest structure, natural enemies, predator, parasite.

INTRODUCTION

Honey bees and stingless bees are classified in the family of Apidae. Their nests located in the cavities. However, the structure and the composition of their nests are different. Honey bee (genus *Apis*) has three types of nesting, i.e., cavity-nesting with multi-combs (*Apis mellifera* and *A. cerana*), single comb nest outside by hanging or attached on the branch (*Apis dorsata*) and single comb nest on tree branches on dwarf honey bees (*A. florea* and *A. andreniformis*). Multi combs of cavity-nesting honeybees are built mainly by wax (Oldroyd and Wongsiri 2006).

Stingless bees have two types of nest i.e., cavity and base nests. Cavity nest is located on a trunk characterized by the outer entrance located on trunk hollows. This type of nests is usually located in a certain height. Base nest is usually located below or at the base of the tree characterized by outer entrance attached to the outer wall of the tree and is usually hidden (Nunes *et al.* 2011). These

bees often built their nest on termite and ants nests (Michener 1974). They generally build their nests in tree holes (Buchwald and Breed 2005) and also in wood, cracks in the walls or under the roof of residence (Kumar *et al.* 2012).

These stingless bees have local name i.e., teuweul (West Java), klanceng (Central Java), lanceng (East Java), galo-galo (Sumatera), and kelulut (Kalimantan). Stingless bees are found in the tropics and subtropics areas (Free 1993). They are also widely distributed in Indonesia i.e., 31 species in Kalimantan, 41 species in Sumatra, and 9 species in Java (Schwarz 1937). Sakagami *et al.* (1990) reported six species of stingless bees found in Java, those are *Tetragonula laeviceps*, *Heterotrigona itama*, *T. drescheri*, *Tetrigona apicalis*, *Geniotrigona thoracica*, and *Lepidotrigona terminata*. Genera of *Homotrigona*, *Lepidotrigona*, and *Heterotrigona* are endemic for the tropics and subtropics areas in Asia (Michener 2000).

The stingless bees can be found in the forests, some types can also be adaptable in open forest areas, grasslands, and there is also in the settlement (Inoue *et al.* 1985). Some of these species also build nests underground (Jalil and Shuib 2014). Nest of

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stingless bees is constructed mainly by resin. The arrangement of the cells in the nest of stingless bees consists of cluster and comb (Michener 1974). Nest is an artificial construction used by bees to lay eggs, spend time, and nurture of young bees. The nest of stingless bees consists of entrance, cerumen, batumen, involucrum, storage pots, and brood cells. Cerumen is a mixture of propolis (resin) and wax to construct storage pots and brood cells. Batumen consists of resin or wax as a layer of cavity nest (Jalil and Shuib 2014). Batumen and cerumen are used to protect the inner part of the hive. Involucrum is a layer around brood cells (Michener 2007).

Stingless bees are social insects that have an important role as plants pollinators (Wille 1983; Inoue *et al.* 1985) and have high economic value related their ability to produce honey and propolis. Honey of stingless bees has a distinctive scent with a lemon fragrance-like taste characterized by the mixture of sweet and sour flavour, compared to the honey produced by honey bees. The honey of stingless bees has darker color, more acidic taste and contains higher phenolic compounds (Garedew *et al.* 2003; Kek *et al.* 2014). Propolis possesses anti-bacterial, anti-microbial, and anti-fungal properties (Sforcin and Bankova 2010). Stingless bees produce high propolis compared to honey bees (Fatoni 2008). However, natural enemies like predators and parasites can reduce the productivity of bees, disturb the colony, as well as affect the structure and composition of the nest or even kill the individual of bees.

Predators are animals that prey on other animals (Estes *et al.* 2001). Some predators attack the colony of stingless bees, i.e. wasps, ants, spiders, and centipedes. Centipedes may damage bee colonies within 2-5 seconds because they have a morphological variation and adaptation to take, catch, and hold prey (Kumar *et al.* 2012). Suicide bite is usually done by the bees as a defense mechanism against predators (Shackleton *et al.* 2015). Parasites are organisms that live on other organisms. The parasites commonly found in honey bees are mites, fleas, moths, and small beetles (Strauss *et al.* 2013). These parasites can be harmful to the health of honey bees (Shen *et al.* 2005). Natural enemies like predators and parasites on the stingless bees have not been widely reported yet. So that these research are really important to conduct. The research aimed to study the natural enemies of stingless bees and additional nest structure related to natural enemies.

MATERIALS AND METHODS

Sample Collections. Collection of stingless bee colonies were taken from August to November

2015. Ten colonies were taken from Pasuruan (East Java), eleven colonies from Tasikmalaya (West Java), and nineteen colonies from Rangkasbitung (Banten province).

Observation of Nest Structure of Stingless Bees. Each colony of stingless bees was observed and classified based on their caste systems. The structure and composition of the nest were observed i.e., the outer entrance, inner entrance, storage pots (honey and pollen), and brood cells. Each colony observed then was moved to wood box, sized 30 x 25 x 15 cm.

Identification of Stingless Bees and Natural Enemies. Description of samples of stingless bees collected from each area were conducted based on morphological study. Identification of stingless bees based on Sakagami (1990) and Michener (2007). Natural enemies studied were collected from the stingless bees in bamboo. Afterward, both samples, stingless bees and their natural enemies were preserved in 70% ethanol. Samples were identified in the Laboratory of Entomology, Indonesian Institute of Sciences (LIPI) at Cibinong, Bogor.

Data Analysis. The species of stingless bees and natural enemies collected from each area were described. Nest structure and additional nest structure arised by natural enemies of stingless bees also were described.

RESULTS

A total of 39 colonies of stingless bees were identified as *Tetragonula* sp. The species is a small genus (about 3.5 mm body length) with a brown-blackish body and transparent wings. A total of 39 colonies found, 64% colonies have the common nest structure and the remaining (36%) with the additional nest structure (Table 1).

Stingless bee colonies of Pasuruan and Tasikmalaya did not create the additional nest structure. Contrary, more colony of Rangkasbitung created additional nest structure (73.68%) and the remaining have common nest-structure (26.32%) (Figure 1). Observation in the fields showed that natural enemies were not found in colonies of Pasuruan and Tasikmalaya. In contrast, more colonies of Rangkasbitung (52.63%) in bamboo attacked by natural enemies (Table 2).

The Common Nests-Structure of Stingless Bees. Commonly, nest structure of stingless bees in bamboo, from outer to inner are nest entrance, cerumen, storage pots, brood cells, and cerumen (Figure 2). Nest entrance is a tunnel for leaving and returning of the colony members. Storage pots consist of honey and pollen pots that are used by colony as nutrient sources. Brood cells have a

Table 1. Common nest-structure and additional nest-structure of stingless bees species from each sampling area

Location of colony	Nest structure	
	Common	Additional
Pasuruan	10	-
Tasikmalaya	10	-
Rangkasbitung	5	9
Total	25	14
Percentage (%)	64	36

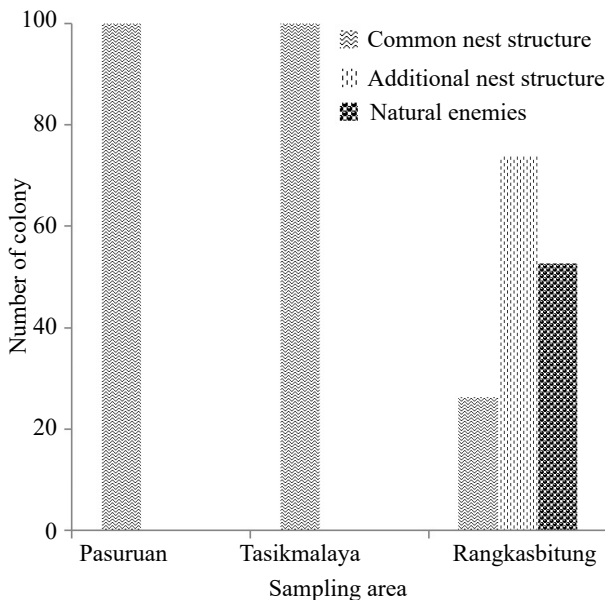


Figure 1. Percentage of common nest structure, additional nest structure, and natural enemies in study sites

smaller size than storage pots that are used by queen to lay eggs.

The Additional Nest Structures of Stingless Bees. Colonies of Rangkasbitung created the additional nest structure, when their nest is damaged or attacked by natural enemies, such as predators and parasites. Results showed that some additional nest structure were inner entrance with much batumen and cerumen and also create the root-like structure (Figure 3).

Natural Enemies of Stingless Bees. Ten colonies in bamboos at Pasuruan and 10 colonies at Tasikmalaya were found without their natural enemies. However, 10 colonies at Rangkasbitung were found with their natural enemies, such as cockroaches (Blattodea: *Blattidae*), *Platysoma leonti* (Coleoptera: Histeridae), *Carpophilus* sp. (Coleoptera: Nitidulidae). We also found several eggs of lizard on the nest and larva of termites at outer layer of bamboo (Table 2, Figure 4).

DISCUSSION

Nests Structure of Stingless Bees. Results showed that the nest structure and composition of stingless bees in bamboo, from the outside to the

Table 2. A number of stingless bee colonies were attacked by natural enemies

Natural enemies	The number of colonies attacked		
	Pasuruan (n=10)	Tasikmalaya (n=10)	Rangkasbitung (n=19)
<i>Platysoma leonti</i>	-	-	6
(Coleoptera: Histeridae)			
<i>Carpophilus</i> sp. (Coleoptera: Nitidulidae)	-	-	2
Cockroaches (Blattodea: Blattidae)	-	-	2
Total	-	-	10
Percentage (%)	-	-	52.63



Figure 2. Structure and composition of the cavity-nesting of stingless bees in bamboo: (a) the nest entrance, (b) storage pots, (c) brood cells

inside, consist of nest entrance, cerumen, storage pots (honey and pollen), brood cells, and storage pots. Generally, the structure and composition of stingless bee nests consist of nest entrance, inner tunnel, brood cells, storage pots (honey and pollen), and batumen layers (Sakagami *et al.* 1983).

The nests are generally built by materials, such as wax, resin, and mud. Wille (1983) stated that bees used olfactory cues to locate the resin and food sources (nectar and pollen). Lack of plant resins causes a pot of food storage and brood cells are transparent (Leonhardt *et al.* 2010). Cerumen is a resin material mixed with wax. The wax of the stingless bee has a higher melting point compared to the wax of honey bees (*A. mellifera*) (Blomquist *et al.* 1985). In a higher concentration of the wax than resin, the texture of the nest becomes harder. The waxy nest can furthermore be hardened by mud to make batumen. While involucrem is cerumen located at around the brood cells to protect the nest from predators and parasites (Michener 2007).

There are two types of brood cells, cluster and comb. Brood cells without eggs have darker in color, whereas those containing eggs are transparent. Chinh *et al.* (2005) stated the new cells were yellow-brownish in color but after the wax eroded to be transparent. Cells are connected with another cell by pillars. Leonhardt *et al.* (2010) showed brood cells associated with one pillar with a small ball

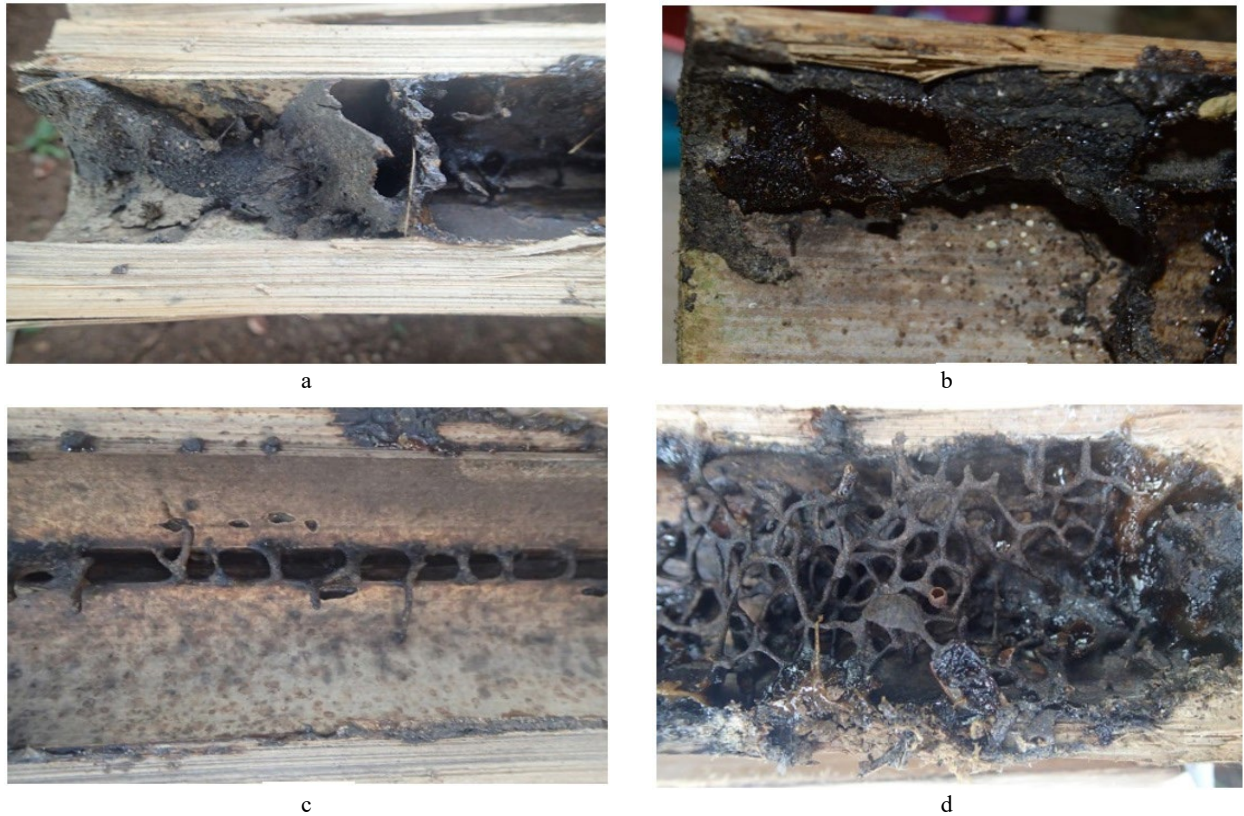


Figure 3. Nest structure of stingless bee: (a) the additional nest structure, the inner entrance with batumen, (b) the inner entrance with cerumen, (c) nest with batumen, (d) and root-like structure



Figure 4. Natural enemies attacked the stingless bee colonies in bamboo: (a) *Platysoma leonti* (mummified), (b) *P. leonti* stuck in sticky cerumen, (c) eggs of lizard on bamboo colony, (d) eggs of lizard on plastic cover of wooden box, (e) and larva of termites at bamboo, (f) and the cockroach

shape. Additional nest structures were found on the bamboo colony in Rangkasbitung, Banten Province. This structure is built in rotted cavities to protect the colony from predators and parasites. Roubik (2006) reported that these bees build the architecture in the inside of the hive to protect from natural enemies. In addition, the colonies use a lot of wax to avoid predators and parasites. Chinh *et al.* (2005) reported that the inner surface of a nest is usually decorated with black cerumen with a thickness of 1.5-2.0 mm.

Natural Enemies of Stingless Bees. Natural enemies were not found in colonies of Tasikmalaya (West Java) and Pasuruan (East Java). Contrary, some predators, such as cockroaches (*Blattidae*), histerid beetles (*Platysoma leconti*), and nitidulid beetles (*Carphophilus* sp.) were found in the colony of Rangkasbitung. Some of the lizard eggs also were found in the bamboo or box colonies. Ants (*Pheidole* sp.) were found in the stingless bee colonies that moved to wood box. In India, Kumar *et al.* (2012) reported some predators attacked the colony of stingless bees, including wasps, ants, spiders, and centipedes. Ants are predator, in high number of individuals, it can interfere the colony of stingless bees.

The resin is used as a defense mechanism against natural enemies (Roubik 2006; Leonhardt *et al.* 2010). Defense mechanism against predators also was showed by workers of the stingless bee by standing and hovering. Standing is the behavior of worker bees which keep the entrance of the hive. While, hovering is the behavior of worker bees which fly near the entrance of the nest. In the evening, some species of stingless bees close the outer entrance by wax with a thickness of <0.05 mm (Gruter *et al.* 2010). If the colonies were disturbed by humans, workers showed defense behavior, like biting and attacking the hair, eyes, nose, and ears (Wille 1983).

Additional Nest Structures of Stingless Bees Arose as a Results of Natural Enemies. The shape of the nest entrance of stingless bees varied. These colonies built nest entrance surrounded by cerumen. Chinh *et al.* (2005) reported the shape of the nest entrance and inner entrance usually are not found in *T. laeviceps*. Nest entrance is usually found at the tree cavity an average height of 90 cm above the ground and or in artificial construction of human (Sakagami *et al.* 1983).

The nests structure of stingless bees has two main parts i.e., storage pots and brood cells. Storage pots are usually distubuted at near of nest entrance and at behind the brood cells, round in form, thin walls, and dark brown in color. The thickness of storage pots is less than 1 mm. Brood cells have the smaller than storage pots (Chinh *et al.* 2005). Brood cells initially

open and closed after the egg is laid. Commonly, brood cells arranged regularly in the horizontal combs and pollen pots are commonly found near the brood cells. Brood cells contain nutrient for the development of eggs and larvae, such as pollen, nectar, and honey (Michener 2000).

Additional nest structures is built to defend against predators. Several predators found in this study were cockroaches (Blattodea: *Blattidae*), histerid beetles *Platysoma leconti* (Coleoptera: Histeridae), nitidulid beetles *Carphophilus* sp. (Coleoptera: Nitidulidae) and ants *Pheidole* sp. (Hymenoptera: Formicidae). The beetle larvae live in colonies of bees to obtain nutrient sources. Whereas, ants in the bee colonies aim to taking a food. Chinh *et al.* (2005) reported that worker bees defend their colony against ants by biting and using a resin to block the outer and inner entrance of the nest.

CONCLUSION

The common nest structure of stingless bees in bamboo are nest entrance, cerumen, storage pots, brood cells, and storage pots. Nest of an underground colony of stingless bees is round-shape and brood cells are covered by storage pots and involucre. The additional nest structure is constructed by batumen, consists of inner entrance lined by batumen or cerumen with a certain thickness, and nest with a roots-like structure to trap the predators and parasites. Predators attacked the colonies of stingless bees in Rangkasbitung were cockroaches (*Blattidae*), histerid beetles (*Platysoma leconti*), nitidulid beetles (*Carphophilus* sp.), and ants (*Pheidole* sp.).

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