ULTRASTRUCTURAL OBSERVATION OF POLLEN MITES ON *TETRAGONULA FUSCOBALTEATA*

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Abstract. Mites are common inhabitants in the nests of eusocial and solitary bees, including stingless bees. In this study, mites associated with *Tetragonula fuscobalteata* were examined using Scanning Electron Microscopy. Stingless bee's specimens were collected in April for pollens studies. The sizes of these mites are in the range of 370-400 µm length and 200-250 µm width. They have four pairs of segmented hairy legs with a claw on each leg. The dorsal body has a unique lining pattern. The mouth part has structure known as pedipalp. They were observed on several areas of the stingless bee's exoskeleton which are on the anterior dorsolateral abdomen, intertagmal region between thorax and abdomen; and dorsal trochanter of the femur. It is believed that these locations are the place where the mites can easily use their piercing mouthparts to penetrate the exoskeleton of their host and where mites are less likely to be removed by the bee's grooming activity. On pollen observation, the mites were predominantly found together with citrus, *Citrofortunella microcarpa*'s pollens. This can be postulated that those mites were closely associated with pollen and transferred from pollen to the nest when the bees foraged the flowers.

Keywords: Mites, *Tetragonula fuscobalteata*, thorax, abdomen

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Introduction

Stingless bees play an important role as pollinators of plants. The occurrence of tiny living things and microorganisms such as mites, yeasts, moulds, and bacteria associated with bee and bee colonies has been reported previously [1-4]. There is no interchange of mites between *Api*s and *Trigona* hosts [5]. There are 29 families and 90 genera of mites reported to be associated with bees in general, where 17 genera are recorded in stingless bees [6]. Laelapidae (Acari: Mesostigmat: Laelapidae) is a family of mites associated with stingless bees which has a genus of mites, *Hypoaspis, Canestrini*, *Bisternalis* Hunter, *Neohypoaspis*, and *Eumellitiphis* Turk [7]. Mite genera always occur in stingless bee nests [1,8,9]. Mites from the genus of *Neotydeolus, Macrocheles, Tyrophagus, Trigonholaspis and Hemileius* help bees to clean the nest, control other mite populations, or even eliminate pathogenic fungi [8]. Some mites such as *Pyemotes, Lasioseius, Glycyphagus, Neocypholaelaps* and *Tyroglyphus* feed on pollen [10,11]. The predaceous laelapid mites such as *Neohypoaspis ampliseta* were responsible for the death of the colony [9,12].

In Malaysia, mite *Neocypholaelaps phooni* Baker was reported found in nests of stingless bees [13]. Other new species of mite *Neocypholaelaps malayensis* Pilsbry was reported found in the nests of *Heterotrigona itama* Cockerell and *Tetragonula iridipennis* in Malaysia [5]. Mites are spread by normal bee behaviour of absconding, swarming, drifting and robbing [12,14]. Bee can also pick up mites while gathering pollen on flowers that have been in contact with other mason bees [15].

The purpose of this paper is to report on the incident observation of mites on the body of Indo-Malaya stingless bees observed using a field emission scanning electron microscope (FESEM). This report will provide more insight into the relationship between stingless bees and mites in their natural environment.

Materials and Methods

Study Area and Sample Collection

Stingless bee specimens for this ultrastructural observation were collected from The Indo-Malaya Stingless Bee Repository Sekayu, Terengganu, Malaysia. This centre is located within a 93 hectares secondary forest which includes fruit orchards [16,17]. The collection of samples was carried out once a week in April between 08.00-11.00 am when foraging stingless bees returned to their nest carrying pollen loads on their corbiculae. Samples were collected by carefully and gently placing the container near the hive entrance of the stingless bees, the bees always end up entering the container which is a small clean bottle or in clean vial [16]. Ten samples of *Tetragonula fuscobalteata* workers (female) were captured for each batch and each bee was kept in separate vials to avoid any cross contamination. They were taken directly to the laboratory for preparation [16,17] and ultrastructural observation under natural observation in low-vacuum mode.

Preparation for Microscopy

The uncoated samples were mounted on stub which was layered with carbon conductive adhesive tape [16,17]. The pollens from references flower were also prepared with similar methods. Analysis of the samples was performed using controlled low vacuum

mode FESEM Quanta FEG 650 at University of Malaya at various applicable magnification. SEM stubs were placed on the SEM stage inside the FESEM chamber. They were viewed under controlled low-vacuum mode FESEM. The accelerating voltage was 1.0 – 5 KV and the spot-size was 2.5 – 5.00 nm with working distance of the specimen was 10 nm [17]. The images were recorded to study the presence and location of mites on stingless bees and measurements for size of mites and pollens. The localisation and measurement were done directly using the supplication in FESEM.

Results and Discussion

Four batches of *T. fuscobalteata* which 10 bees per batch were collected. Mites were observed on some samples of arriving foragers of *T. fuscobalteata* with pollen loads. Figure 1 is electron micrograph of the lateral view of *T. fuscobalteata* at lower magnification and the location of mites found on the body of bees (labelled with alphabet a, b and c). There were only three samples from one batch (Batch No 3) of *T. fuscobalteata* were found to have microscopic organisms on them as on Table 1. Ten mites were observed on one of *T. fuscobalteata* sample No 1, twelve mites on *T. fuscobalteata* sample No 2 and ten mites on *T. fuscobalteata* sample No 3. The rest of seven bee from this batch has no mites detected. The other three batch, mites were found detached from the bee's body which were occurred in only one vial of Batch No 1 and two vials each from Batch No 2 and No 4 (Table 2).

The morphology of the mites which were observed is shown in Figure 2(a) and (b). The size of those mites' ranges from 370-400 μm length and 200-250 μm width. They have four pairs of segmented hairy legs with a claw on each leg. The mouth part region has structures of pedipalp. The dorsal body is oval in shape and showed a unique lining pattern on the edge with many short setae. The mites were found located on a such areas of bee bodies as shown in Table 3 and Figures 3(a), (b) and (c).

Those mites were found together with the collected pollens. The pollens (Figure 4) were identified as *Citrofortunella microcarpa* or other name *Citrus macrocarpa* (family Rutaceae) [17]. This plant is commonly known as calamansi or local name is limau kasturi. It is an ornamental plant with sweet fragrance white flowers [17]. There was a large number of *C. microcarpa* planted in Sekayu near to *T. fuscobalteata*'s hives.

Table 1: The number of mites observed on the body of *Tetragonula fuscobalteata.* (Batch No 3).

Stingless Bees (Batch No 3)	**Number of mites**
Tetragonula fuscobalteata No 1	10
Tetragonula fuscobalteata No 2	12
Tetragonula fuscobalteata No 3	10
Tetragonula fuscobalteata No 4-10	0

Table 2: The number of mites observed in the vials of the samples of *Tetragonula fuscobalteata* on other 3 batches (Batch No 1, 2 and 4).

Stingless Bees	Number of mites (detached)
Batch No 1	
Tetragonula fuscobalteata No 1	3
Tetragonula fuscobalteata No 2 -10	0
Batch No 2	
Tetragonula fuscobalteata No 1	2
Tetragonula fuscobalteata No 2	3
Tetragonula fuscobalteata No 3-10	0
Batch No 4	
Tetragonula fuscobalteata No 1	4
Tetragonula fuscobalteata No 2	3
Tetragonula fuscobalteata No 3-10	0

Table 3: The locations of mites observed on the samples of *Tetragonula fuscobalteata*

Location of mites	The label on Figure
Anterior dorsolateral abdomen, the 1st segment	a
Intertagmal region between thorax and abdomen	b
Dorsal trochanter of femur	c

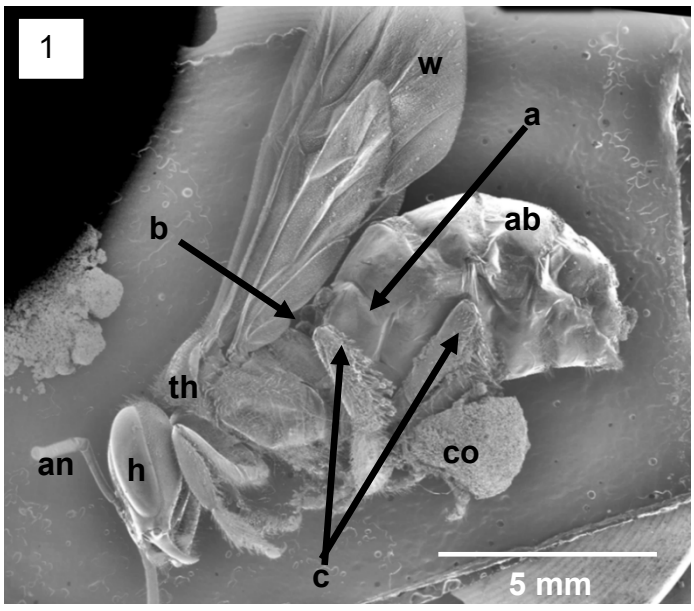
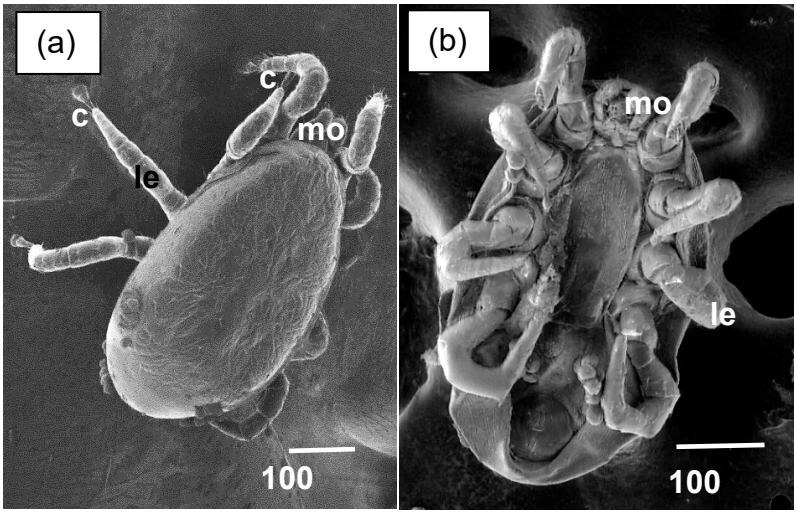
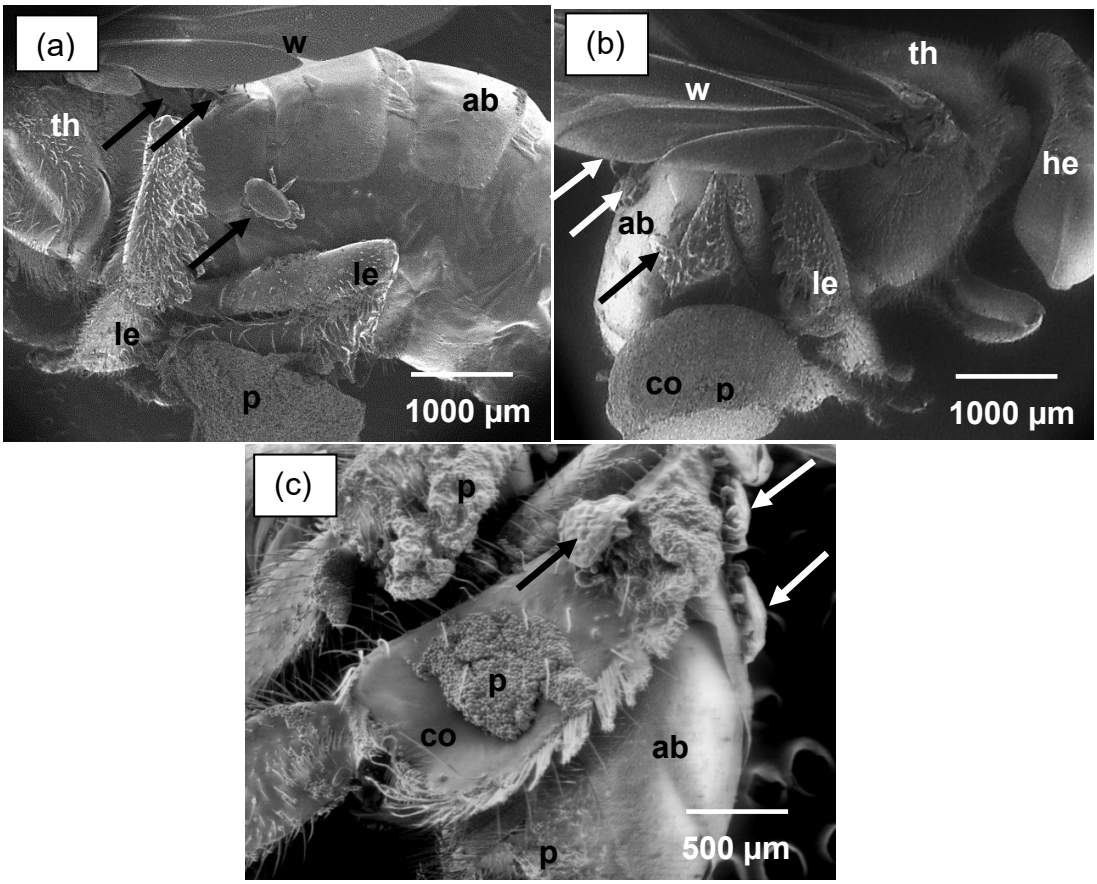


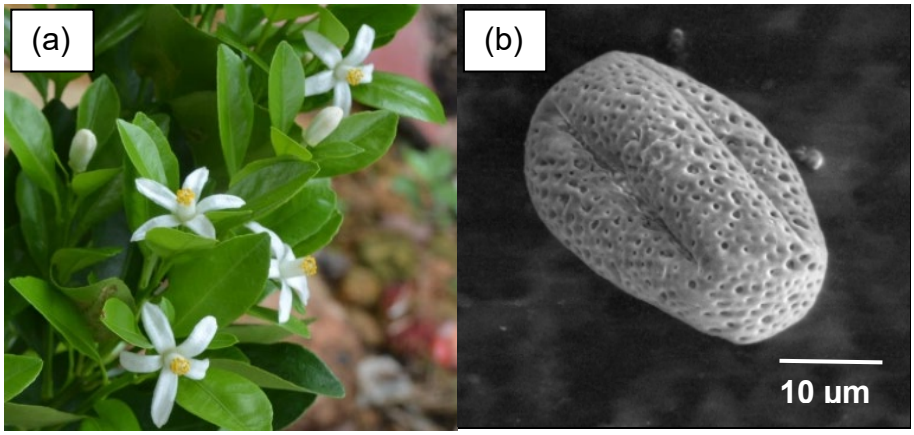
Figure 1: Scanning electron micrographs (at lower magnification mode) of *Tetragonula fuscobalteata* showing the lateral view of stingless bee's morphology. The locations on stingless bee where mites were found are on **a**, Anterior dorsolateral abdomen, the 1st segment, **b**, Intertagmal region between thorax and abdomen, and **c,** Dorsal trochanter of femur. ab, abdomen; an, antenna; co, corbiculae; h, head; th, thorax; w, wing



Figures 2: Higher magnification scanning electron micrographs of mites (a) dorsal view and (b) ventral view showing the morphology of the mites. The mite has four pairs of short and segmented hairy legs with claws. The mouth part region has structures of pedipalp. c, claw; le, leg; mo, mouthparts



Figures 3: Scanning electron micrographs of mites on *Tetragonula fuscobalteata*. (a) sample No 1, (b) sample No 2 and (c) sample No 3 from Batch No 3 show the localizations of mites (arrows) and the mites on the stingless bee. Note of pollens (p) on the surface of corbiculae, abdomen and leg. abdomen; he, head; le, leg; p, pollen; th, thorax; w, wing



Figures 4: (a) The photograph of the reference flower (*Citrofortunella microcarpa or Citrus macrocarpa*) and (b) the electron micrograph of the morphology of pollen. The size of the pollen (27 x 19 µm)

The mites (Acari: Laelapidae) associated with the stingless bee nests have been described by many researchers [1,18]. Those mites are very tiny in sizes, which generally less than 1 mm in length, so they are hardly to be noticed under the naked eyes. The citrus red mite, have an oval-bodied, plump with 4 pairs leg, which the adult female with the length of 0.5 mm [1,19]. The sizes of *Varroa destructor* (Acari: Varroidae), mites on honey bees *Apis mellifera* colony are 0.75 to 0.98 mm long and 0.70 to 0.88 mm wide (males) and 1.00 to 1.77 mm long and 1.50 to 1.99 mm wide (female) [18]. The mites *H. hoffmannae* of stingless bee *T. irridipennis* in Malaysia was reported having the sizes of 444 µm length and 333 µm wide (male) [7]. The sizes of mites observed in this study were in range 370-400 µm length and 200-250µm width (Figures 2(a) and (b)). This mite has four pairs' legs with claws and short fine hairs on the distal parts of the legs. The mouth part region has structures of pedipalp. The sizes and morphology of the mites is a resemblance with the species described by other researchers with setae on the dorsal surface and legs [6,7].

There are not much (are scarce) literatures available on the relationship and localization of mites on the body of stingless bees. Most of the studies were on the mites in the nest [5-7, 21]. Furthermore, information of mites-stingless bees is available only for a few species of stingless bee [4,5]. Interestingly, in this experiment, tiny microscopic mites were observed on returning foragers of stingless bees that carried pollens. Mites have been observed on three of ten samples of *T. fuscobalteata* (Table 1) from one batch and some detached mites inside sample's vials (Table 2). They were found among the collected pollens of *C. microcarpa* (Figures 4(a) and (b)) from the family Rutaceae, either on the pollen basket or on another area of the stingless bees' bodies (Table 3 and Figures 3(a), (b) and (c)). *C. microcarpa* was found grown in large numbers near the *T. fuscobalteata'*s hives. Faegri and Van der Pijl (1979) had mentioned that generally stingless bees collect pollens from plants nearer to their hive to optimise their energy expenditure [20]. Mites from the genus of *Eumellitiphis* Turk, *E. inouei* was reported isolated from the hairy body surface *Geniotrigona thoracica* and *Lophotrigona canifrons* [21].

Peck *et al*, (2016) investigated on the transfer of *V. destructor*, parasite mites that infested honey bees, from flowers to bees [15]. They demonstrated that the mites are able to rapidly infest honey bees' forager at a feeder or flowers by quickly mounting on honey bees engaged in foraging and that despite efforts by the bees to groom off the mites, they always

succeed in leaving the foraging site still attached to a bee. The mites were localized themselves on the specific area on the bees which were dorsal trochanter or femur, intertegmal region between thorax and abdomen, intertegmal region between head and thorax, anterior dorsolateral abdomen, central dorsal thorax; besides the small number of mites on the ventral abdomen, and ventral thorax. Moreover, Vijayakumar et al, (2013) also reported the adherence between 1-3 predatory *Pyemotes* sp. mites on the intersegments, dorsolateral abdomen of queen stingless bee *T. iridipennis* [9]. The mites *Pyemotes tritici* was also reported parasitizing at inter-segmental areas of the abdomen of an adult worker stingless bee *Tetragonisca angustula* [12]. Mites *Varroa* sp are always found mainly on the top of the bee's thorax at the point where the wings attach, between the head and the thorax, between the thorax and the abdomen, or between overlapping segments of the abdomen [3,23].

These locations of mites on bees reported by other workers [9,12,15] are in line with the SEM observations in the present study (Figure 1), which showed the mites located on the dorsal trochanter or femur, intertegmal region between thorax and abdomen, and at the anterior dorsolateral abdomen. These are places where the mites can easily use their piercing mouthparts to penetrate the exoskeleton of their host and gain access to the bee's hemolymph (blood) and are also places where mites are less likely to be removed by the bee's grooming [23].

Since the stingless bees were captured from foraging and carrying pollens, those mites were most likely to be associated with pollens (Figures 1, 3(a), (b), (c) and 4). It is believed that the mites were originated from flowers. There is a report that mites can move from one bee to a neutral location like a flower, and from there to bee from another colony [24]. Therefore, this finding is relevant to the situations where the plausibility of mite transfers between bee colonies via flowers [15,25]. One of the mites associated with pollen is *Carpoglyphus lactis* [9,26], which is generally found in pollen stores and old honeycombs [9,27]. *C. lactis* (L.) was reported infesting the stingless bee *T. iridipennis* in India [9]. Mites are common in citrus plants. They establish their populations because they have food sources (pollen, leaf sap, nectar, and honeydew) [19,28]. Mites may cause damage to citrus crops and may affect the meliponini industry. Phytophagous mites from citrus plants, belonging to the families Eriophyidae, Tenuipalpidae and Tetranychidae in was reported Manaus region, of the Brazilian Amazonia [28,29].

Conclusions

It is suggesting there is a close relationship between mites and stingless bees. The mites were attached to the specific area on the surface of the stingless body. They may play a role in mite transmission from contaminated flowers to a bee colony or to other flowers such as citrus flowers. These findings provide the information on relation of the stingless bee, mites and natural resources. It emphasizes the importance of awareness of the occurrence and transmission of pests with the management of the meliponini industry.

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Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of conflict of interest

The authors report no conflicts of interest in this work.

Compliance with ethical standards

For this type of study formal consent is not required.

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