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Isolation and identification of mites associated with raw and commercial farm edible bird nests

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Abstract. The global demand for edible bird nests (EBNs) is high, especially from Hong Kong and Peoples Republic of China. Recently, this industry was greatly affected when China banned the import of all the EBNs from Malaysia (except for canned version) due to detection of high levels of nitrites. Several cases of anaphylaxis following ingestion of EBNs were reported. The source(s) of these allergens remain unknown. Mites have been reported to trigger allergic responses. Hence, this study was designed to quantify, isolate and identify the mites that are associated with EBNs. The raw EBNs were purchased from swiftlet farms in five locations in Peninsular Malaysia while the commercial nests were purchased from five different Chinese traditional medicinal shops. The average mite density of all the raw nests was 285 ± 603 mites per gram of EBN while the commercial nests had a much lower mean value of 21 ± 32 mites per gram of EBN ($p = 0.082$). Among the raw EBNs, the nests from Kajang had the highest average mite density (946 ± 1443 mites/g of EBN) whereas the nests from Kuala Sanglang had the lowest (54 ± 34 mites/g of EBN). Among the commercial EBNs, the nests from Company D had the highest average mite density (76 ± 18 mites/g of EBN) whereas the nests from Company A were free of mites. Overall, the average densities of mites in the raw nests obtained from southern regions of Malaysia (Selangor and Johor) were higher than those nests obtained from the northern regions (Kedah and Kelantan). Thirty types of mites were isolated from both the raw and commercial nests. Among these, some are probably feather mites (*Eustathia cultrifer*, *Pteroherpus garrulacis*, *Pterodectes amaurochalinus*, *Laminalloptes* sp., *Berlesella alata* and *Neochauliacia* sp.), house dust and storage mites (*Suidasia* sp., *Austroglycyphagus* sp., and *Aleuroglyphus ovatus*), mesostigmatid mites (*Dermanyssus* sp.), prostigmatid mites (*Cheyletus* sp., tarsonemid and cunaxid mites), astigmatid mites (*Collocalidectes* sp., *Streetacarus* sp. and *Hemisarcoptes* sp.) and oribatid mites. This study provides baseline information on the density and type of mites that are probably associated with EBNs. This study also heightens the importance of mites as a possible source of EBN-associated anaphylaxis.

INTRODUCTION

Edible bird nests (EBNs) are nests made from the regurgitated saliva of *Collocalia* (*Aerodramus*) swiftlets (Marcone, 2005). In the past, raw or unprocessed EBNs were harvested mainly from natural caves. However, in recent years, there has been a rapid growth of swiftlet farming in Southeast Asia, including Malaysia. Eighty percent of the EBNs are exported to China. In 2011, there was a scare over high levels of nitrites in the EBNs exported by Malaysia, causing China to ban the import of EBNs from Malaysia. The ban was lifted in November 2011. In February 2012, EBNs (cubilose; except for canned version) were again listed as banned products by the Ministry of Agriculture and the General Administration of Quality Supervision, Inspection and Quarantine, China (http://english.cntv.cn/20120227/116881.sht ml). Due to this ban, there has been greater concern over the contents and the food safety aspects of EBNs.

EBN can be a potential source of life-threatening food allergy to those who are sensitized to its components or contaminants. A number of food allergy and anaphylaxis associated with the ingestion of EBNs has been reported in Singapore, Hong Kong and Malaysia (Goh *et al*., 1999; 2000; Thong *et al*., 2005; 2007; Hon *et al*., 2006; 2009). There has been no other documentation on EBN-associated allergy in other countries despite high global consumption of EBNs. EBN-related food allergy appears to be under-recognized or under-diagnosed in other parts of the world. Characterisation of EBNs revealed 66 and 100 kDa allergens of similar sequence homology with ovoinhibitor (Goh *et al*., 2000; 2001). Marcone (2005) reported another 77 kDa allergen which shares similar sequence homology with the egg's ovotransferrin in unprocessed nests obtained from East Malaysia and Sumatra. EBNs obtained from Sarawak, Thailand and Indonesia showed allergenic heterogeneity (Goh *et al*., 2001).

However, the source(s) of these allergens remain undetermined. The possible source(s) of allergens found in the EBNs may originate from the saliva or feathers of the swiftlets, the insects ingested by the swiftlets [winged ants, fig wasps and bees (Hymenoptera), flies (Diptera) and small beetles (Coleoptera)] (Lourie & Tompkins, 2000), the environment, the microorganisms associated with the nests (such as fungi, bacteria and protozoa), arthropods (such as mites) which inhabit the swiftlets or their nests, the cleaning processes of the raw nests, the adulterants (karaya gum, red seaweed and tremella fungus) added to the commercial nests and/or the contaminants introduced, and the infestation of arthropods or other organisms during the storage of the nests.

Are mites the possible source of these allergens? At least 2,500 species of mites from 40 families are closely associated with birds and their nests (Proctor & Owen, 2000). Mites have been found to be a major source of allergens (Colloff, 2009a) and are associated with various allergic reactions such as asthma, eczema, rhinitis and conjunctivitis. Nearly 130 mite allergens have been identified (Colloff, 2009a). No report on the association of mites with EBNs has been documented so far. Questions were raised on whether mites associated with EBNs are associated with the reported food-induced allergy. This study aims to determine the density of mites in raw unprocessed and processed commercial EBNs, and to identify the type of mites that are associated with EBNs.

MATERIALS AND METHODS

Collection of Raw and Commercial EBNs from Different Localities in Malaysia

The unprocessed (raw and un-cleaned) EBNs were purchased from house farms in five different localities in Malaysia: Kuala Sanglang (Perlis; 6° 16' 0" North, 100° 12' 0" East), Pantai Remis (Perak; 4° 27' 0" North, 100° 38' 0" East), Kluang (Johor; 02° 012 303 North 103° 192 583 East), Kajang (Selangor; 2° 59' 0" North, 101° 47' 0" East) and Kota Bharu (Kelantan; 6° 8' 0" North, 102° 15' 0" East). The commercial EBNs were purchased from five different Chinese traditional medicine shops in Malaysia (Companies A-E). Three to six nests were purchased from each locality/shop. Each of the nests was sealed in a plastic bag and transported to the laboratory.

Processing of the EBN Samples

Upon arrival at the laboratory, the nest was randomly broken up, pressed or gently crushed into tiny fragments, weighed, and examined carefully under a stereo-microscope (Nikon SMZ800, Japan) for the presence of live and dead mites. The inner rim of the petri dish was applied with Vaseline prior to the transfer of the nest onto the dish to prevent the escape of the live mites. Examination of the empty plastic bag was performed under the stereomicroscope and any remaining live and dead mites in the plastic bag were picked for identification. The total number of (live and dead) mites in the each nest was determined. The density of mites in each sample (number of mites per gram of EBN) was calculated. Each mite was picked using a fine applicator stick, cleared

with 90% lactic acid, and mounted in Hoyer's medium. The mounted mites were dried and identified based on morphology.

RESULTS

The average mite density of all the raw nests was 285 ± 603 mites per gram of EBN, while the commercial nests had a much lower value of 21 ± 32 mites per gram of EBNs (Table 1). There was statistically significant difference between the mite densities of the raw and commercial nests ($p < 0.001$; Mann-Whitney test).

Among the raw EBNs, the mite densities of the nests obtained from different locations ranged from 54 to 946 mites per gram of EBN (Table 1a). The raw nests with the highest average mite density were from Kajang (946 mites/g of EBN), followed by Kluang (471 mites/g of EBN). The average mite densities in these locations were much higher than the other three locations, each of which had less than 100 mites/g of EBN. However, this difference was not statistically significant (ANOVA, $p = 0.257$).

The mite densities among the raw nests from the same location varied based on the number of hatchings of the nests (Table 1a). Overall, the new nests without any hatching had the lowest number of mites. For Kuala Sanglang, the nests with multiple hatchings had the highest mite densities (80-101 mites/g of EBN) whereas the nests with no hatching had the lowest mite densities (18-22 mites/g of EBN). The nests from Pantai Remis showed a similar pattern to those nests obtained from Kuala Sanglang with average mite densities of 106-108 and 54 mites/g EBN for those with and without hatchings respectively. For Kluang, the nest with two hatchings had the highest mite density (639 mites/g of EBN) compared with those with single hatching (307 mites/g of EBN). However, the average density of mites in the nest without hatchings (468 mites/g of EBN) was higher than those with one hatching (307 mites/g of EBN). The same trend was seen among the nests obtained from Kajang. The nest with two hatchings had a much higher mite density (2613 mites/g of EBN) than the nests with no hatchings (119 mites/g of EBN) and one hatching (107 mites/g of EBN). The nests from Kota Bharu were all with no hatching. The average mite density of these nests was 94 ± 12 mites/g of EBN and was higher than the mite densities of the nests with no hatching obtained from Kuala Sanglang and Pantai Remis. Statistically, however, there was no significant difference in the mite densities between nests without hatchings and nests with single or multiple hatchings (ANOVA, $p = 0.256$).

Among the commercial EBNs, the densities of mites differed among the companies (Table 1b and Fig. 1). The highest average mite density was found in the nest obtained from Company D (76 mites/g of EBN), followed by Company B (29 mites/g of EBN). These differences were statistically significant (ANOVA, $p = 0.002$). Surprisingly, the commercial nests obtained from Company D showed a higher average mite density than the raw nests obtained from Kuala Sanglang (54 mites/g of EBN).

Thirty types of mites were isolated from both the raw and commercial EBNs. The morphology for each type of mites is illustrated in Figs. 2-4.

DISCUSSION

EBNs are consumed worldwide but the nutritional and medicinal active ingredients and/or allergenic components of this delicacy remain undetermined. In this study, mites were found to be associated with the EBNs. Almost every bird has its own mite fauna except for the penguin (Proctor & Owen, 2000). The swiftlets belong to the same order (Apodiformes) and family (Apodidae) as the swifts which are not exempted from mite-associates (Colloff, 2009b). High numbers of mites have been isolated from the nests of birds (Stoehr *et al.*, 2000). Consistent with the reported findings, high numbers of mites were found in the raw EBNs in this study. This was probably due to Malaysia's tropical climate which is conducive for mite propagation. Lack of sudden changes in relative humidity and temperature favour the reproduction of mites (Nadchatram, 2005).

Table 1. Mite count and mite density of the (a) raw and (b) commercial EBNs

(a) Raw EBNs

Source	No	Type of nest	Weight of sample (g)	Number of mites			Mite density (number of mites/g)
				Live	Dead	TOTAL	
Kuala Sanglang Kedah	1	Very old and dirty, found on ground	2.76	0	99	99	36
	2	After 1 hatching	2.64	1	174	175	66
	3	No hatching	4.01	7	64	71	18
	4	After multiple hatchings	4.69	1	471	472	101
	5	After multiple hatchings, and fell on ground	7.22	5	571	576	80
	6	No hatching	2.05	3	42	45	22
Average				**3**	**237**	**240**	**54 ± 34**
Pantai Remis Perak	1	No hatching	3.27	38	137	175	54
	2	After 2 hatchings	6.75	1	720	721	106
	3	After 1 hatching	7.26	26	761	787	108
Average				**22**	**539**	**561**	**89 ± 31**
Kluang Johor	1	No hatching	2.92	71	1296	1367	468
	2	After 1 hatching	3.39	75	965	1040	307
	3	After 2 hatchings	2.83	188	1622	1810	639
Average				**111**	**1294**	**1406**	**471 ± 166**
Kajang Selangor	1	No hatching	1.32	5	153	158	119
	2	After 1 hatching	1.57	6	162	168	107
	3	After 2 hatchings	1.30	0	3398	3398	2613
Average				**4**	**1238**	**1241**	**946 ± 1443**
Kota Bharu Kelantan	1	No hatching	1.11	0	103	103	92
	2	No hatching	1.30	0	108	108	83
	3	No hatching	1.25	0	134	134	107
Average				**0**	**115**	**115**	**94 ± 12**
Average mite density of all raw nests							**285 ± 603**

(b) Commercial EBNs

Source	No	Weight of sample (g)	Number of mites			Mite density (number of mites/g)
			Live	Dead	TOTAL	
Company A	1	3.01	0	0	0	0
	2	3.01	0	1	1	0
Average			**0**	**1**	**1**	**0 ± 0**
Company B	1	2.23	0	44	44	20
	2	2.19	0	84	84	38
Average				**64**	**64**	**29 ± 13**
Company C	1	1.45	0	1	1	1
	2	1.47	0	0	0	0
Average			**0**	**1**	**1**	**1 ± 1**
Company D	1	2.21	0	194	194	88
	2	2.21	0	138	138	63
Average			**0**	**166**	**166**	**76 ± 18**
Company E	1	1.57	0	2	2	1
	2	1.89	0	5	5	3
Average			**0**	**4**	**4**	**2 ± 1**
Average mite density of all commercial nests						**21 ± 32**

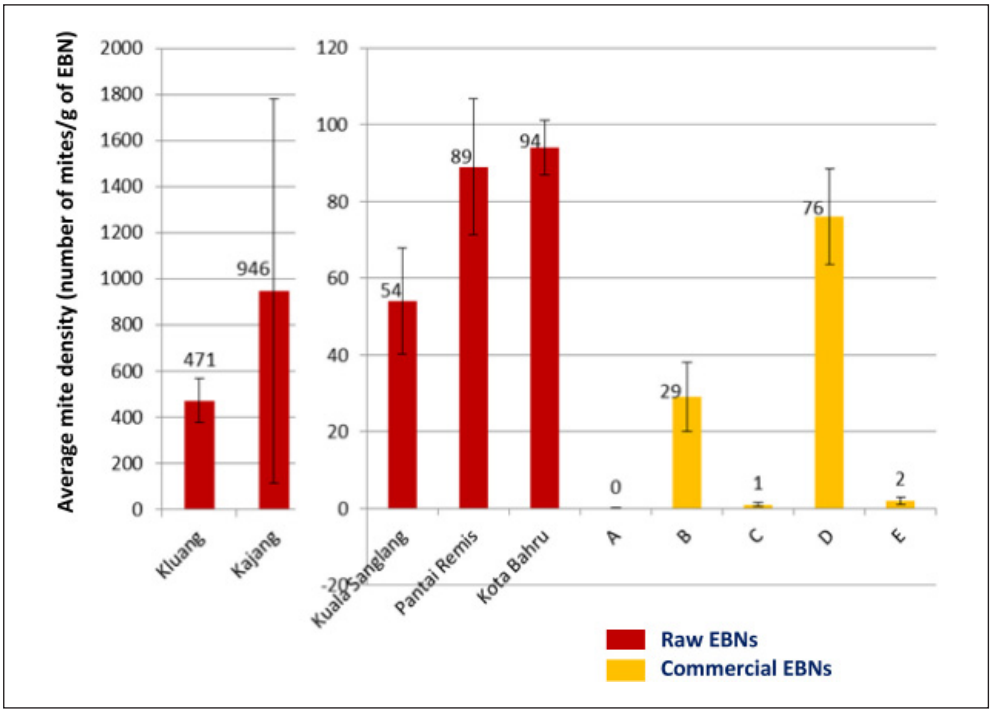

Figure 1. Average mite density of the raw and commercial EBNs.

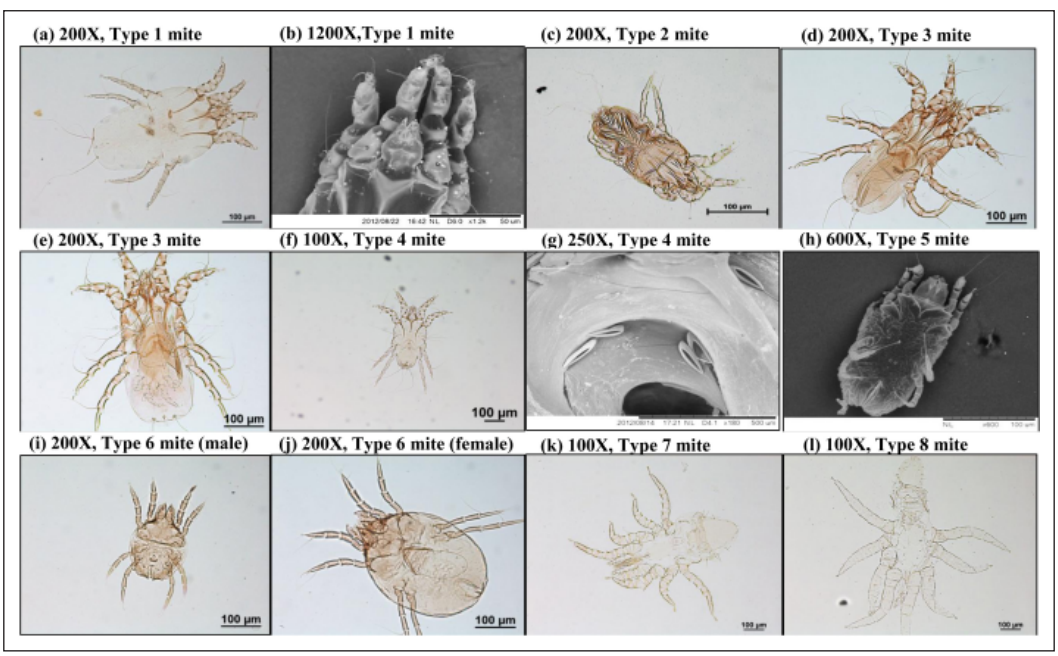

Figure 2. Light (a, c-g, i-l) and scanning electron (b & h) microscopic images of Types 1-8 mites isolated from the EBNs.

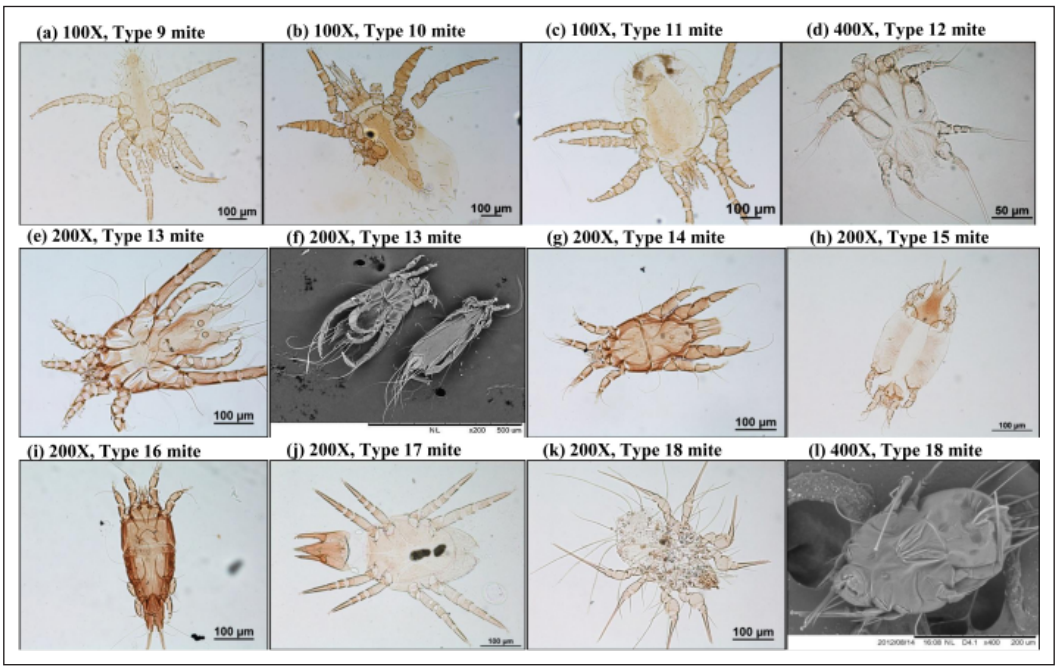

Figure 3. Light (a-e, g-k) and scanning electron (f & l) microscopic images of Types 9-18 mites isolated from the EBNs.

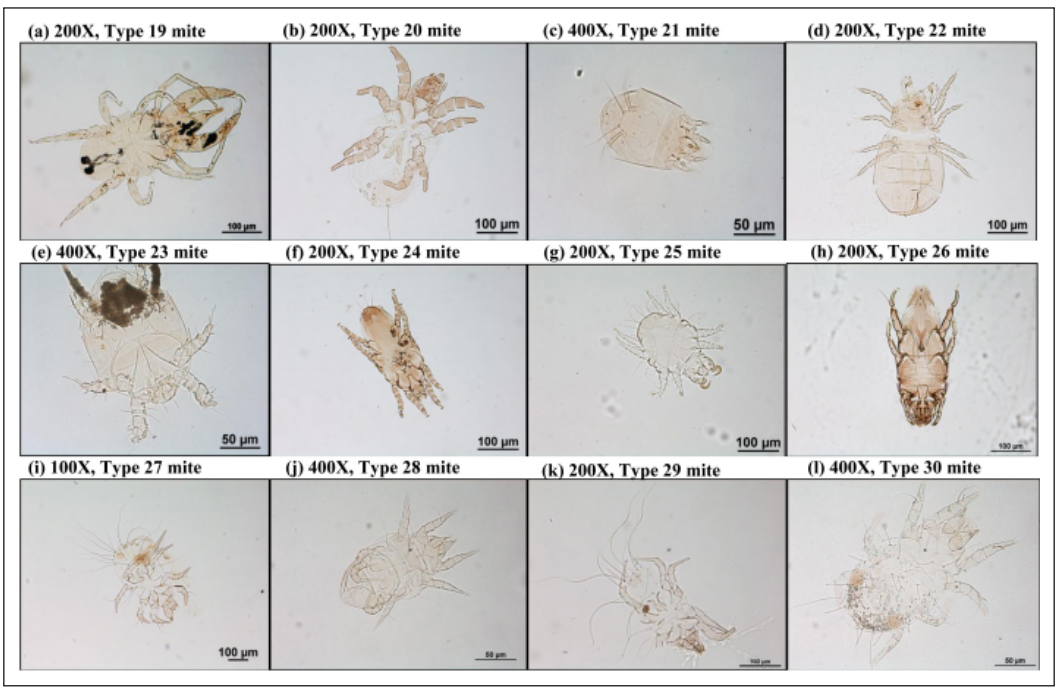

Figure 4. Light microscopic images of Types 19-30 mites isolated from the EBNs.

Overall, greater mite density was found in raw nests with single or multiple hatching(s) compared with the nests with no hatching in this study. This was consistent with the previous findings which reported absence of mites during the un-hatched egg phase but increased through the nestling stage, with the highest mite numbers as the breeding season progressed (Stamp *et al.*, 2002). The probable reasons for this could be due to the absence of chick hosts for blood meals; and the cumulative number of mites since the nest was built. The mites may depend directly on the birds as a food source; or feed on the dermal detritus (Proctor & Owen, 2000); feather lipids and debris, keratin, fungi, algae, bacteria and other arthropods (Stamp *et al.*, 2002). Some parasitic mites seem to be able to survive for some duration without having their hosts during winter, as reported for the genera *Dermanyssus* (Stamp *et al.*, 2002).

Relatively, the average densities of mites in the raw nests obtained from southern regions of Malaysia (Selangor and Johor) were higher than those nests obtained from the northern regions (Kedah and Kelantan). Pantai Remis in Perak was found to have intermediate density of mites. Hence, the location from which the nests are harvested will affect the density of mites. This may probably be attributed to the minute climatic and humidity variation. Urbanization in Selangor and Johor is more intense compared with the other studied regions. Cities with higher population density tend to have higher temperature in general due to heat accumulation, CO_2 emission and decrease green vegetation (Kataokaa *et al.*, 2009). This in fact will affect the growth rates of the mites in urban and rural areas. Mites are very sensitive to their surrounding temperature and relative humidity (Aspaly *et al.*, 2007). Areas with low relative humidity (< 50%) were reported to have lower mite infestations (Arlian *et al.*, 2002). The most optimum temperature for the growth of *Acarus siro*, *A. ovatus* and *Tyrophagus putrecentiae* was at 25ºC. A significant difference in terms of the distribution of mites was also reported between the inland and coastal areas (Macan *et al.*, 2003). In this study, different types of mites were found to infest the nests of different locations as discussed later.

The much lower levels of mites found in commercial nests compared with raw nests seen in this study was likely due to the cleaning process that the commercial nests have been subjected to (Lau & Melville, 1994). Despite the washing and processing of the raw EBNs, mites were still found to be embedded within the strands of the nests, and could only be seen or segregated after breaking the nest into small pieces. The mites on the outer surfaces of the raw EBNs were removed during the soaking of the nests, as mites are light and will float on water. However, those embedded within the strands of the raw EBNs will only be exposed and removed during the crushing, and the subsequent soaking processes. The amount of mites in the commercial nests from various companies differs and this may reflect the stringency and thoroughness of the cleaning processes applied to the raw nests.

In this study, 30 different types of mites were found either in raw, commercial nests or both. Upon ingestion of EBNs, will these mites also cause acariasis? Or, will they trigger a life-threatening allergic reaction? Mites have been known to be a rich source of allergens; nearly 130 mite allergens have been identified (Colloff, 2009a). Several cases of life-threatening anaphylaxis following the ingestion of mite-contaminated food have been reported (Erben *et al.*, 1993; Sanchez-Borges *et al.*, 1997; 2005; Wen *et al.*, 2005; Tay *et al.*, 2008). It has been suggested that mite specific IgE is responsible for mediating oral mite anaphylactic reactions (Sanchez-Borges *et al.*, 2005). Some of the mite allergens are enzymes (e.g. serine proteases and cysteine proteases) which may damage the airway and gastrointestinal mucosa, and subsequently lead to increased permeability and airway hyper-reactivity. Ingestion of mite-contaminated food can trigger allergic reactions even after boiling (Erben *et al.*, 1993; Wen *et al.*, 2005; Tay *et al.*, 2008) as some mite allergens (group 2 allergens) are heat resistant (Sanchez-Borges *et al.*, 2005). If there is a threshold

level for mites and mite allergens in ingested food, above which the consumer will develop allergic symptoms, then the abundance of mites/mite load in EBNs may be of great importance. EBNs with higher mite density will have a greater possibility of triggering allergy and anaphylaxis, compared with those of lower mite densities.

In North America, current standards permit up to 75 "insect fragments" per 50 g of grain (Sanchez-Borges *et al*., 2005). There is no guideline on the permissible level of mites and mite allergens in EBNs neither in Malaysia nor other countries. There are no measures to determine or quantify the amount of mites in these commercial EBNs and no food allergen labelling have been put in place to prompt or alert the public especially those who are allergic to mites. The steps to reduce the mite load in commercial EBNs should be investigated. Prevention of mite contamination can be achieved by storing commercial EBNs in proper storage conditions which are not optimum for the survival and growth of mite colonies. A few steps have been recommended: do not store the food item for more than 6 months, and keep the food in dry and cool places (storage temperature at 0-7°C and humidity of less than 12%) (Sanchez-Borges *et al*., 2005).

Another step to reduce the mite load in EBNs would be to ensure the effectiveness of the cleaning processes and to determine the sources of mites in the raw nests. Mites could be the fauna in the caves or swiftlet farms. Some mites are associated with insects, e.g. *Microlichus* sp. (Philips, 2000). Since swiftlets ingest insects (Lourie & Tompkins, 2000), remnants of insects and possibly mites may be left in their mouths. When the swiftlets produce saliva to build their nests, the mite remnants may be incorporated into the nests.

Another source of mites is from the swiftlets themselves, since many species of mites have been associated with birds. Respiratory mites of the swiftlets may possibly be incorporated into the nests when the swiftlets build their nests with their saliva. Some mites inhabit the feathers and skin of birds (Proctor & Owen, 2000; Philips, 2000), and are likely to get transferred to their nests. Feather mite family Eustathiidae Oudemans is restricted to swifts and crested swifts (Apodiformes: Apodidae and Hemiprocnidae) (Peterson *et al*., 1980). Two monobasic genera of the family Hypoderatidae (*Apodidectes* and *Collocalidectes* species) are reported on glossy swiftlet *Collocalia esculenta* (Mironov & oConnor, 2013).

Some mites are found not predominantly on the birds, but in the nesting material itself. These include mites which feed on the dermal detritus, fungi, algae, bacteria and other arthropods in the nest (Proctor & Owen, 2000; Stamp *et al*., 2002), and blood-feeding mites which only visit their host for short periods of time to feed but remain primarily nest-bound at other times (Proctor & Owen, 2000). One example of such mites is *Dermanyssus gallinae*, also called the red fowl mite or poultry red mite. *D. gallinae* has a wide geographical distribution and low host specificity, having more than 30 avian hosts (Roy & Chauve, 2007). *D. gallinae* has been reported to cause dermatitis (Bellanger *et al*., 2008; Rosen *et al*., 2002). Mesostigmatid mites which are similar in morphology with *D. gallinae* were found in the EBNs in this study; these mites may also cause dermatitis among farmers and workers who are in direct contact with the nests (Hughes, 1976a).

In this study, 30 different types of mites were isolated. Overall, Types 1 and 5 were the two most common mites seen in the raw and commercial EBNs. Type 1 mites were the major mites found in the raw nests obtained from Kuala Sanglang, Pantai Remis and Kota Bharu. Type 5 mites were the major mites picked from the raw nests obtained from Kluang and Kajang. For the commercial nests, 83% (390 out of 469 mites) of the mites counted were embedded between the strands of the nests, and could not be mounted for identification. Therefore, the type of mites found in the commercial nests could not be identified accordingly.

Type 1 mite was found in both the raw and commercial EBNs. Through morphology and taxonomic keys, Type 1 mite resembles the features of the female *Dubininia* or *Eustathia* species. *Dubininia* species is an astigmatid mite in the superfamily

Analgoidea, the family Xolalgidae and the subfamily Ingrassinae (Schmaschkee *et al.*, 2002). *Dubininia* species are associated with budgerigar, cockatiels and many species of terrestrial birds but not with Apodiforms (swiftlets; Albuquerque *et al.*, 2012). Hence, Type 1 mite is more likely to be *Eustathia cultrifer* with Y-shaped epimerites I, narrow epigynum and weakly bilobed terminus which bear two pairs of long terminal setae (Peterson *et al.*, 1980). *Eustathia cultrifer* is one of the reported feather mites of family Eusthathiidae which is associated with *Apus apus* (Apodidae; Peterson *et al.*, 1980).

Type 2 mite was found only in the raw EBNs while Type 3 mite was found in low numbers in both the raw and commercial nests. Through morphology and taxonomic keys, Type 2 and 3 mites were designated into the order Acariformes and suborder Astigmata. Type 2 mite could be nymphal astigmatan which is difficult to be classified into family and requires adult specimen for confirmation and identification. Further verification is needed to confirm the species of these mites.

Type 4 mite was found in moderate amounts in the raw nests, and very low amounts in the commercial nests. According to morphology and taxonomic keys, Type 4 mite was designated to the order Acariformes and the suborder Astigmata. Its features match the features of the female *Pteroherpus garrulacis* (Mironov & Proctor, 2011) which is an astigmatid feather mite in the family Pteronyssidae and superfamily Analgoidea. However, *Pteroherpus garrulacis* has not been reported on Apodiformes (swifts) but was found on Passeriformes.

Type 5 mite was found in both raw and commercial nests. According to its morphological features and taxonomic keys, Type 5 mite was designated to the order Acariformes, the suborder Astigmata and the superfamily Acaroidea. Type 5 mite matches those features of *Suidasia* species (Gisela, 2012). *Suidasia medanensis* is an astigmatid storage mite in the family Suidasiidae.

Type 6 mites are dark brown in colour and were found in both raw and commercial nests. According to morphology and taxonomic keys, Type 6 mite was designated to the order Acariformes and the suborder Oribatida. Most species of oribatid mites inhabit the organic layers of soils and are feed on microbes, detritus and smaller soft-bodied invertebrates like nematodes (Walter *et al.*, 2013).

Types 7-11 mites are the largest mites found in the nests. Types 7 and 8 mites were found in both raw and commercial nests while Types 9-11 mites were found only in the raw nests. Through morphology and taxonomic keys, it was observed that the features listed for Types 7-11 mites match many features of mesostigmatid mites (Hughes, 1976a). Mesostigmatid mites have a wide range of habitats. Many are predators and live in the surface litter of soils where they feed on other arthropods and also fungi (Hughes, 1976a). Some are coprophagous, using dung-eating insects as a vehicle from one source of food to another. Some live in ant nests, either as predators or scavengers, or utilising the food stored by the ants. Many mesostigmated mites are parasites, and live in various degrees of intimacy with their hosts. Types 7-11 mites could be parasitic blood feeders which feed on the blood of the swiftlets. Some of these mounted mites had black substance within their bodies which was very difficult to clear. The black substance most likely is dried blood from a blood meal some time ago (Proctor & Owen, 2000).

The order Mesostigmata is divided into four suborders: Gamasina, Sejina, Antennophorina and Uropodina (Hughes, 1976a). Representatives of the Gamasina and Uropodina are found in house dust and stored food. Some of the families within Gamasina are Parasitidae, Macrochelidae, Digamesellidae, Phytoseiidae, Ascidae, Ameroseiidae and Dermanyssidae (Hughes, 1976a). The family Dermanyssidae contain many species which are nest-dwellers. Within the family Dermanyssidae are a few sub-families; one of them is Dermanyssinae (Hughes, 1976a). Females and nymphs of this sub-family possess chelicerae with minute digits and enormously elongated second segment-like stylets (Hughes, 1976a). This feature can be seen in Type 10 mite. Males have digits which are reduced and their chelicerae are never chelate.

Members of Dermanyssinae are ectoparasites of birds (Hughes, 1976a). *Dermanyssus gallinae* has been the most widely studied species of this subfamily (Proctor & Owen, 2000). *D. gallinae* has a wide geographical distribution, and is an ectoparasite of more than 30 species of wild and domestic birds as it has a low host-specificity (Roy & Chauve, 2007). They remain in the nests of their avian hosts for long periods (Proctor & Owen, 2000), only visiting their hosts to feed (Roy & Chauve, 2007). These mites have very short life cycles: one week under optimum conditions (Proctor & Owen, 2000; Kettle, 1984). A female mite can deposit up to 7 eggs at a time after each blood meal (Hughes, 1976a; Kettle, 1984), laying about 6 batches of eggs in her lifetime (Kettle, 1984). Such features enable *D. gallinae* to build up huge colonies in a very short period of time (Proctor & Owen, 2000; Kettle, 1984). When their avian hosts abandon the nests, *D. gallinae* mites often invade human dwellings, biting humans and causing pruritic dermatitis (Bellanger *et al*., 2008; Rosen *et al*., 2002; Auger *et al*., 1979). For example, in 1977, a few employees and patients in a hospital in Canada presented with papuloerythematous skin lesions and pruritis that lasted for 1-3 weeks (Auger *et al*., 1979). Close examination revealed *D. gallinae* on the skin of two of the patients. Further investigation revealed that these mites were found in the nests of pigeons on the window ledges and air conditioners of the hospital building (Auger *et al*., 1979). *D. gallinae* also transmits diseases such as avian spirocheatosis, fowl cholera and salmonellosis (Roy & Chauve, 2007). It is a concern, then, if these diseases can be transferred to the humans bitten by *D. gallinae*.

Type 12 mites were found in both the raw and commercial nests. Type 12 mites were identified as deutonymph of *Collo-calidectes collocaliae* (astigmatan mites of the family Hypoderatidae), which were reported to be associated with swifts from Java (*Collocalia esculenta* and *C. nidificus* / *Aerodramus fuciphagus*) and occasionally rodents (Mironov & oConnor, 2013). The deutonymph of these mites is morpho-logically heteromorphic with T-shaped anterior and posterior ends of genital sclerite, and lacks mouthparts or mouth opening. However, deutonymph is the main or only feeding stage of these mites (as subcutaneous or visceral tissue parasite). All the other stages of these mites live in the nests of the hosts.

Type 13 mite was found only in raw EBNs. Type 13 mite was identified and designated to the order Acariformes, the suborder Astigmata and the superfamily Analgoidea. Type 13 mite shares a few common characteristics with males of *Dubininia* species which belongs to Analgoidea superfamily (Roy & Chauve, 2007). However, *Dubininia* species has not been reported in Apoiformes, hence, Type 13 mite was more likely to be *Berlesella alata* which has been reported in Apodidae from Borneo (Valim *et al*., 2011).

Type 14 mite was found only in raw nests. Based on morphology and taxonomic keys, Type 14 mite was designated to the order Acariformes, the order Astigmata and the superfamily Analgoidea. It looks quite similar to Type 13 mite. It differs from Type 13 mite in that it has larger and longer legs III and IV, darker brown in colour, has a clear furrow or groove across the middle of its body, and only one pair of long terminal setae arising from its terminal lobe. The similarities it shares with Type 13 mite include its size, small gnathosoma, elongated opisthosoma and a pair of anal sucker above the terminal lobe. However, the opisthosoma of Type 13 mite is bifurcated into two lobes, while the terminal lobes of Type 14 mite are fused together and formed a single lobe projecting out from its opisthosoma. Type 14 mite has a similar morphology to *Laminalloptes* sp., an ectoparasitic feather mite of the avian genera *Phaeton* and *Fregata* (Atyeo & Peterson, 1967) or male *Thysanocercus* sp. which was reported in Apodidae (Barreto *et al*., 2012).

Types 15 and 16 mites were found only in raw nests and were similar in morphology. Based on morphology and taxonomic keys, both of these mites were designated to the order Acariformes, the order Astigmata and the superfamily Analgoidea. Type 16 mite shares some similar morphological features with *Pterodectes amaurochalinus*, a feather

mite belonging to the superfamily Analgoidea, family Proctophyllodidae and subfamily Pterodectinae (Hernandes & Valim 2006). Representatives of the genus *Pterodectes* occur on various Passeriform birds (Hernandes & Valim, 2006).

Type 17 mite was found only in raw EBNs. Type 17 mite was identified and designated to the order Acariformes and the suborder Prostigmata. The possible families within the Prostigamata are the family Bdellidae and Cunaxidae, which have features similar to the features of Type 17 mite (Hughes, 1976b; Skvarla *et al*., 2014). Initially, Cunaxidae was erected as a family separate from Bdellidae by Thor (1902) and kept as a subfamily within Bdellidae by Oudemans (1906).

Type 18 mite was found only in raw nests. Type 18 mite was successfully cultured and identified as *Austroglycyphagus* sp. It has abundant, long, pectinate setae and its morphological features matched the morphology of *Austroglycyphagus* sp. (Colloff, 2009b; Hughes, 1976c). *Austroglycyphagus* sp. has been associated with birds and their nests (Putatunda *et al*., 1989; Hughes, 1976c) e.g. in one study it was found in the feathers of the avian species *Psittacula krameri* (Putatunda *et al*., 1989), and in another report it was mentioned that *A. geniculatus* was found in birds' nests and grain bin residues (Hughes, 1976c). *Austroglycyphagus* sp. is also a known house dust mite (Colloff, 2009b) which may be associated with a variety of allergic diseases, including asthma.

Type 19 mite was found only in raw EBNs. Type 19 mite was identified and designated to the order Acariformes, suborder Prostigmata, family Cheyletidae and genus *Cheyletus*. Species of Cheyletidae are mainly free-living predatory mites (Hughes, 1976b). Cheyletid mites were found in the feathers of the avian species *Lonchura malacca*, *Psittacula alexandri*, *Psittacula cyanocephala*, *Psittacula eupatria*, *Emberiza bruniceps* and *Eudynamys scolopacea* (Putatunda *et al*., 1989).

Type 20 mites were found in both raw and commercial EBNs. Type 20 mite was identified as *Aleuroglyphus* sp., possibly *Aleuroglyphus ovatus*. *A. ovatus* has a life cycle of about 2-3 weeks at 23ºC and a relative humidity of 87%, with wheat germ as their diet (Hughes, 1976c). *A. ovatus* is found in house dust (Colloff, 2009b) and also in bran, wheat, chicken meal, dried fish products, flour, pollards, mice burrows, moles' nests and broiler houses (Hughes, 1976c). Allergens have been isolated from *A. ovatus* (Colloff, 2009a).

Type 21 mite was found in both raw and commercial nests. Type 21 mite was identified and designated in the order Acariformes, the suborder Prostigmata and the family Tarsonemidae (Hughes, 1976b). Type 21 mite seems morphologically similar to *Tarsonemus* sp. females (Padil *et al*., 2009). *T. granarius* is associated with and thrives on fungi, especially species of *Penicillium*, *Aspergillus*, *Chaetomium* and *Hormodendrum* (Hughes, 1976b). One tarsonemid mite, *Tarsonemus confusus* was found in the feathers of the avian species *Cygnus atratus* (Putatunda *et al*., 1989).

Types 22-27 mites were found only in raw nests, but very sporadically and in very small numbers e.g. Type 26 mite was found in only one raw nest, and only one specimen was found. Type 22 mite looks similar to *Eniochthonius crosbyi* (Walter *et al*., 2013). Type 23 mite has quite a round body, and blunt anterior margin and a very small gnathosoma. Its first pair of legs has enlarged tarsi which bear small, recurved claws. Type 23 mite could be deutonymph of *Hemisarcoptes* species which were found beneath the elytra of ladybirds (Coleoptera: Coccinellidae; Fain & Ripka, 1998). Type 24 mite is brown in colour and has an elongated opisthosoma with 2 pairs of long terminal setae. There seems to be some slight variation in the morphology of these mites, some have an opisthosoma with a smooth, round margin whereas others have an opisthosoma with 4 projections where the 4 terminal setae arise. Type 24 mite could be *Neochauliacia minuscula* which have been reported in *Apus apus* (Peterson *et al*., 1980). Type 25 mite has a very round body with fine striations, medium-length idiosomal setae and a thick/fleshy pair of pedipalps which bear large strong claws. Type 25 mite resembles *Cheyletiella* species (*Apodicheles cypsiurus*; Fain, 1979) which had been

reported in Apodidae. Type 26 mite has a dark brown body with distinct tendons on dorsal and ventral sides for movement. Type 26 mite could be *Streetacarus australis* which had been collected from the tail of the quills and *Calyptorhynchus magnificus* (red-tailed black cockatoos; Lukoschus & Lombert, 1979). Type 27 mite has long posterior idiosomal setae especially on its posterior end. Type 27 mite could be *Tyrophagus* species (Fan & Zhang, 2007).

Types 28-30 mites were found exclusively in commercial nests in small quantities, and were not able to be identified to genus due to incomplete mites. Type 28 mite has a nearly transparent, oval-shaped body with clear chelicerae but non-prominent pedipalps, and pointed legs. Type 28 mite could be female of *Apodidectes verrucosus* which was reported in swift nest (Apodiformes: Apodidae) in Philippines (Mironov & oConnor, 2013). Type 29 mite has very long terminal setae, and a gnathosoma with non-prominent pedipalps. Type 30 mite has a round body with setae sticking out all over it, like a pin-cushion. It has short, pointed legs and dentate-chelate chelicerae with non-prominent pedipalps.

In conclusion, the mite densities of the raw nests ranged from 18 to 2,613 mites per gram of EBN. The mite density of the commercial nests ranged from 0 to 88 mites per gram of EBN. The number of mites varied among the place of farming and harvest. If a threshold value for the concentration of mites and mite allergens in ingested food exists, the abundance of mites in EBNs is of great importance. The stringency and effectiveness of the cleaning processes of the raw EBNs should be emphasised, and measures should also be taken to avoid contamination of the commercial EBNs during transportation and storage. Relevant food quality control agencies should perform regular checks on the nest processing cycle, amount of additives added and to determine whether the types and levels of additives added are safe for human consumption. To date, no measures have been taken to determine or quantify the amount of mites in the commercial EBNs. No indicator has been put in place to prompt or alert the public especially those who are allergic to mites. A proposal on food allergen labelling and consumer protection could be introduced in order to reduce the number of mite or food-induced anaphylactic cases. Various steps need to be taken in order to reduce the mite load in commercial EBNs also.

Thirty different types of mites were found in the raw and commercial nests, out of which 17 types were found exclusively in the raw nests, 3 types were only found in the commercial nests, and the remaining 10 types were found in both the raw and commercial nests. It is possible that these mites inhabit swiftlets and their nests, and may cause adverse effects to humans who come into contact with them or ingest the EBNs. Steps to reduce the mite loads in EBNs should be developed to minimize the risk of occupational hazards to farmers and workers who deal with the nests. To ensure the well-being of EBN consumers, an effective cleaning system should also be developed in order to thoroughly clean the EBNs of their contaminants, and optimum storage conditions should be determined to avoid contamination of the nests. Further investigations should also be performed to identify the additives added to the EBNs and to determine if these additives are detrimental to the health of consumers. Future studies should be designed to investigate the potential roles of these mites in triggering allergic responses subsequent to ingestion of mite contaminated EBNs.

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