22 Nutritional Composition of Pot-Pollen from Four Species of Stingless Bees (Meliponini) in Southeast Asia

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22.1 Introduction

Stingless bees are distributed throughout the tropical regions of the world. There are more than 500 described species in 32 genera. The greatest species diversity is found in Central and South America (Michener 2013). For Thailand 32 species in 10 genera have been recorded (Rasmussen 2008). Not all species of stingless bees found in Thailand are adaptable for meliponiculture primarily due to the constraints of their mature tree cavity-nesting preferences. Several species are cultured in human-made hives; of these the most common and widely distributed species are in the *Tetragonula laeviceps* species complex. Additional managed species in Thailand include *Tetragonula fuscobalteata*, *T. testaceitarsis*, *Lepidotrigona doipaensis*, *L. flavibasis*, *L. terminata*, and *Lisotrigona furva*. Chuttong et al. (2014) highlighted that meliponiculture in Thailand is in an incipient stage, but expanding. The utilization of stingless bees, as an economic activity, is largely confined to the southeastern region of Thailand, in the provinces of Chanthaburi and Trat. The estimated number of meliponiculturists in Thailand is more than 700 with approximately 5000 colonies under management (DOAE, Department of Agricultural Extension, Thai Ministry of Agriculture and Cooperatives, Region 3, Chanthaburi, 2014, personal communication).The keeping of stingless bees in this area was initiated by the use of stingless bees as supplemental pollinators for several tropical fruit species. A crucial step in the management of stingless bees in Thailand was the development of methodologies for the division of colonies allowing for colony increase. Presently the primary uses of managed meliponine colonies in Thailand are for pollination and the sales of hive products, principally honey and cerumen. The extraction and sale of stingless bee colony pollen stores is very limited in SE Asia. In Central and South America, honey, pollen, and cerumen of numerous stingless bee species are utilized for traditional purposes such as medicine and food resources (Ayala et al. 2013; Obiols and Vásquez 2013). In Mexico and Guatemala, *Melipona beecheii* pollen is used in traditional therapies (Vit et al. 2004). Menezes

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Fig. 22.1 Pot-pollen of *Tetragonula laeviceps* complex species (Photo: B. Chuttong)

et al. (2013) discussed the use of pollen from *Scaptotrigona* spp. in human diets as an ingredient in beverages and the use of fresh, frozen, and dehydrated pollen. The amount of pollen produced from *Scaptotrigona* spp. colonies is much more than their honey. In Malaysia Omar et al. (2016) evaluated the antioxidant activity of *Lepidotrigona terminata* pollen and the use of its methanol extract to inhibit growth of breast cancer cells. The results showed that stingless bee pollen extract can be useful as a supplementary treatment for chemotherapy drugs. There are no regulations in Thailand regarding the medicinal use of pollen or extracts.

22.2 Shape and Volume of Stingless Bee Pollen Pots

Stingless bees store pollen within pots (made from cerumen) which differs from honey bees where pollen is stored in wax comb cells (Menezes et al. 2013). Stingless bees collect nectar and pollen from plants and place them into separate storage pots within the nest matrix. There is often a pronounced separation of honey pots from pollen pots within the food storage area, but such is not the case for all stingless bee species. When a pollen pot is full, the pot's narrowed entrance is sealed with a cerumen plug, which is unlike pollen storage cells of *A. mellifera.*

The characteristics of pollen pot shape and size vary between bee species, but food storage pots are always much larger than brood cells. The stored pollen varies in flavor, color, and texture dependent on floral species and microbiotic changes which occur during storage (Camargo et al. 1992). From ongoing research in Thailand with the *T. laeviceps* species complex, pollen pots and honey pots are very similar in shape and volume with an average pollen pot volume of 27.7 ± 6.2 mm^3 (Fig. 22.1) and an average honey pot volume of 27.9 ± 6.7 mm^3. Field observations of *T. testaceitarsis*, *L. terminata*, and *L. flavibasis* pollen pots have shown similarities in honey and pollen pot volumes albeit they are larger storage pots than those seen for the *T. laeviceps* species complex. These four stingless bee species have soft and sticky storage pots, but the storage pots of the *T. laeviceps* species complex and *T. testaceitarsis* are shown to be thicker than those for *L. terminata* and *L. flavibasis*. For the *T. laeviceps* species complex (Fig. 22.2) and *T. testaceitarsis*, their pollen pot and honey pot clusters are separated, while for *L. terminata* and *L. flavibasis*, their honey and pollen pots are intermixed (Fig. 22.3). Table 22.1 summarizes pollen pot volume of the four species we examined.

Fig. 22.2 Pot honey of *Tetragonula laeviceps* complex species (Photo: B. Chuttong)

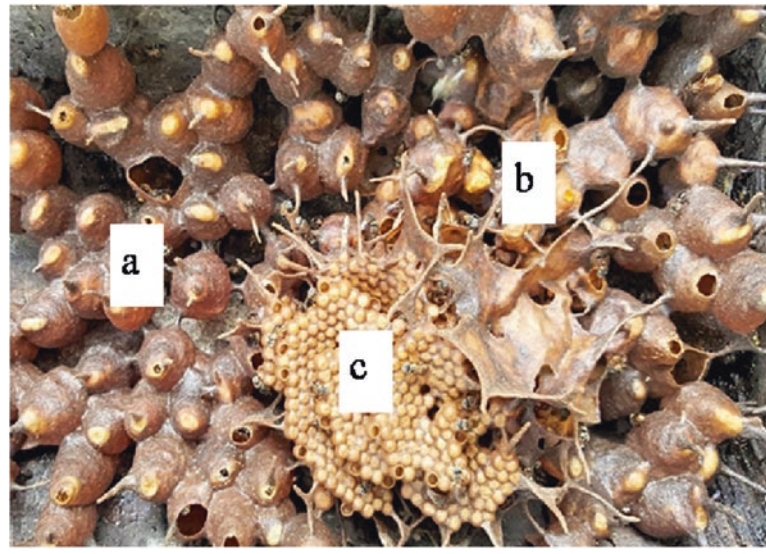

Fig. 22.3 Nest of *Lepidotrigona terminata* (*a*) honey pots, (*b*) pollen pots, (*c*) brood cells (Photo: C. Inkham)

Table 22.1 *T. laeviceps* complex species, *T. testaceitarsis*, *L. terminata*, and *L. flavibasis* pollen pot shape and volume

Stingless bee species	Location	Pollen pot shape	Pollen pot size width × height (mm)
Tetragonula laeviceps complex species	Chanthaburi and Chiang Mai	Ovoid-egg	6 × 7
Tetragonula testaceitarsis	Chanthaburi	Ovoid-egg	8 × 13
Lepidotrigona terminata	Chanthaburi	Ovoid-elongate	14 × 26
Lepidotrigona flavibasis	Chiang Mai	Ovoid-elongate	14 × 20

One closely examined colony of *T. laeviceps* species complex contained 1326 pollen pots and 1578 honey pots, with a brood nest containing 4929 capped cells. There were 2275 adult worker bees.

22.3 Nutritional Composition

Pollen is the male gametophyte formed in the anthers of flowering plants. Pollen is the major source of protein, lipids, vitamin, and minerals for bees (Almeida-Muradian et al. 2005; Campos et al. 2008; Krell 1996; Michener 1974). The chemical composition of pollen differs according to the plant species, nutrient condition of the plant when pollen is developed and storage within the nest (Herbert and Shimanuki 1978).

Research concerning the nutritional value of bee-collected pollen has been dominated by investigations with the Western honey bee (*Apis mellifera*) pollen (Bogdanov 2004; Campos et al. 2008; Herbert and Shimanuki 1978; Human and Nicolson 2006; Serra-Bonvehi and Escolà-Jordà 1997). The nutritional composition of bee-collected pollen shows a high concentration of reducing sugars, essential amino acids, unsaturated and saturated fatty acids, and the presence of minerals which would make bee pollen a valuable addition to the human diet (Almeida-Muradian et al. 2005; Campos et al. 2008; Serra-Bonvehi and Escolà-Jordà 1997). A recommended daily pollen intake as a dietary supplement is 5–10 g/day (M.T. Sancho, personal communication). Bee pollen has been marketed as a health food with an expansive range of putative nutritional and therapeutic properties (Campos et al. 1997, 2008; Wang et al. 1993). There are only a few investigations regarding the chemical makeup of stingless bee-collected pollen (Silva et al. 2006, 2009, 2014; Vit et al. 2016).

22.3.1 Macronutrients of Pot-Pollen

Stored pollen of four species of stingless bee, *T. laeviceps* species complex (n = 3), *T. testaceitarsis*(n = 3), *L. terminata* (n = 3), and *L. flavibasis* (n = 3), was taken from storage pots of living colonies located in the Chanthaburi and Chiang Mai provinces, Thailand. The macronutrient analyses, including moisture, ash, crude fat, and crude protein, followed standard methods of the Association of Official Analytical Chemists (AOAC 2005). The results are expressed as grams per 100 g dry weight.

Moisture content percentage was calculated by drying samples in an oven at 100 °C for 2 h. The dry samples were put into desiccators, allowed to cool, and then reweighed.

Ash percentage was calculated by placing crucibles in a 100 °C oven for 1 h. After cooling the crucible was weighed. Then, weighed samples were placed into a crucible and incinerated in a 500 °C muffle furnace for 2 h prior to weighing.

Crude protein was determined by the Kjeldahl method, and total protein content was calculated as the amount of total N multiplied by a nitrogen to protein conversion factor of 6.25.

Crude fat percentage was calculated by drying fats after extraction in a Soxhlet using diethyl ether.

Total carbohydrate and total energy calculations were performed according to Compendium of Methods for Food Analysis (2003). The total carbohydrate content was calculated by the following formula: Carbohydrate = 100 – percentage of (protein + fat + moisture + ash).

Total energy was calculated by the following equation: Total energy (kcal/100 g) = (% protein × 4) + (% carbohydrates × 4) + (% fat × 9).

The results of the macronutrient analysis (moisture, ash, protein, fat, and carbohydrate) of the four stingless bee species are shown in Fig. 22.4. These results are compared with two studies which looked at corbicular pollen as collected and stored by *A. mellifera* (Herbert and Shimanuki 1978; Human and Nicolson 2006).

The variation in macronutrients between pollen from the four Thai stingless bee species is small, with the exception of the moisture content of *T. laeviceps* complex species that we studied (16.1 ± 1.1 g/100 g), which may represent pollen that had undergone a longer storage period within the colony. Our results in moisture content of stored pollen of *T. testaceitarsis* (31.7 ± 1.2 g/100 g) were higher than the results of moisture content of *A. mellifera* stored pollen from Herbert and Shimanuki (1978) (23.8 g/100 g) and Human and Nicolson (2006) (21.0 g/100 g). But our results in moisture content of stored pollen of *L. terminata* (25.3 ± 0.3 g/100 g) and *L. flavibasis* (22.8 ± 0.5 g/100 g) are within the

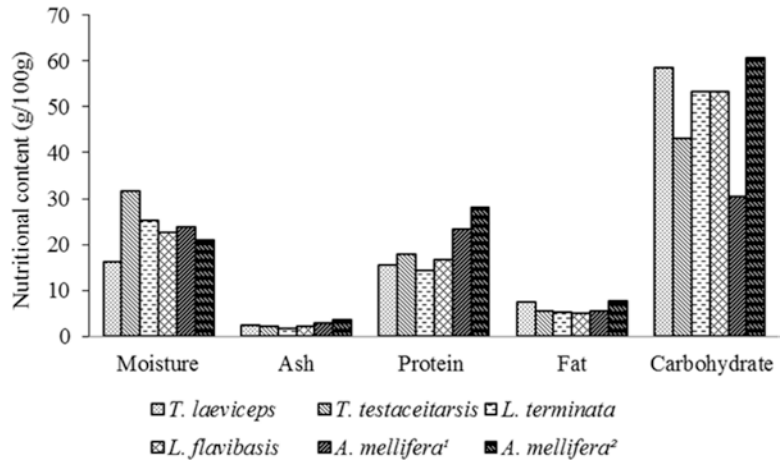

Fig. 22.4 Macro-nutritional analysis of pot-pollen from four stingless bee species compared to corbicular pollen from *A. mellifera*. [1]Herbert and Shimanuki (1978); [2]Human and Nicolson (2006)

ranges as found in *A. mellifera* stored pollen. Our results of moisture content of stored pollen from four species were remarkably lower when compared to the report from Vit et al. (2016) who report the moisture content of *Melipona* sp. aff. *eburnea* (48.5 g/100 g) and *Scaptotrigona* sp. cf. *ochrotricha* (43.5 g/100 g) stored pollen.

The ash content in stingless bee stored pollen of the four species we analyzed was 2.3 ± 0.4 g/100 g (*T. laeviceps* species), 2.2 ± 0.1 g/100 g (*T. testaceitarsis*), 1.8 ± 0.2 g/100 g (*L. terminata*), and 2.2 ± 0.2 g/100 g (*L. flavibasis*); these results fall in the same range with the report of ash content in stored pollen of *Melipona* sp. *aff. eburnea* (2.3 g/100 g) and *Scaptotrigona* sp. cf. *ochrotricha* (1.9 g/100 g) (Vit et al. 2016), but are lower than the reports for *A. mellifera* stored pollen from Herbert and Shimanuki (1978) (2.8 g/100 g) and Human and Nicolson (2006) (3.6 g/100 g).

The stingless bee stored pollen protein content of the four species we examined was 15.5 ± 2.6 g/100 g (*T. laeviceps* species), 17.9 ± 1.9 g/100 g (*T. testaceitarsis*), 14.3 ± 0.6 g/100 g (*L. terminata*), and 16.7 ± 0.4 g/100 g (*L. flavibasis*). This compares to 23.3 g/100 g for *A. mellifera* stored pollen (Herbert and Shimanuki 1978) and 28.1 g/100 g from an *A. mellifera* monofloral stored pollen (Human and Nicolson 2006). The protein content from stingless bee stored pollen from Venezuela (Vit et al. 2016) gave protein contents of 18.3 g/100 g (*Melipona* sp. aff. *eburnea*) and 16.8 g/100 g (*Scaptotrigona* sp. cf. *ochrotricha*) which are within the boundaries of our results.

Our results of fat content in stored pollen showed small variation in four species we examined, 7.4 ± 0.3 g/100 g (*T. laeviceps*), 5.4 ± 0.6 g/100 g (*T. testaceitarsis*), 5.3 ± 0.1 g/100 g (*L. terminata*), and 4.9 ± 0.04 g/100 g (*L. flavibasis*), which are related to the reports of *A. mellifera* fat content in stored pollen from Herbert and Shimanuki (1978) (5.4 g/100 g) and Human and Nicolson (2006) (7.6 g/100 g). Vit et al. (2016) report the fat content of two species of stingless bee stored pollen: 3.2 g/100 g (*Melipona* sp. aff. *eburnea*) which is lower than our results and *Scaptotrigona* sp. cf. *ochrotricha* stored pollen which is in the same boundaries as our study.

Our results in carbohydrate content of stored pollen of *T. laeviceps* species complex (58.7 ± 3.5 g/100 g), *L. terminata* (53.4 ± 1.0 g/100 g), and *L. flavibasis* (53.3 ± 0.8 g/100 g) were related to Human and Nicolson (2006) who report *A. mellifera* stored pollen carbohydrate (60.7 g/100 g). Except for our result of carbohydrate in *T. testaceitarsis* (43.1 ± 2.8 g/100 g), stored pollen was lower. Our results in carbohydrate content of stored pollen were higher when compared to the report of Herbert and Shimanuki (1978) for *A. mellifera* stored pollen (30.4 g/100 g) and the report of Vit et al. (2016) for two species of stingless bee from Venezuela: *Melipona* sp. aff. *eburnea* (27.7 g/100 g) and *Scaptotrigona* sp. cf. *ochrotricha* (31.0 g/100 g).

22.3.2 Mineral Analysis

Mineral content was analyzed by an inductively coupled plasma optical emission spectrophotometer following the standard methods of AOAC (2005). The dried samples were digested with nitric acid and hydrochloric acid (1:3) at 200 °C for 30 min. Each sample was filtered using filter paper (0.45 micron) and stored in washed glass vials before analysis. The samples were diluted to provide concentrations in the proper absorption range.

The results of mineral analysis of the four stingless bee species are shown in Table 22.2. These results are compared to a recent Brazilian study analyzing pollen mineral content including a mineral analysis from the stored pollen of a Brazilian stingless bee species (Morgano et al. 2012). See Table 22.2.

The levels of each mineral examined (K, Ca, Mg, Na) were not significantly different in the stored pollen of the Thai stingless bee species studied here. Potassium is reported as the highest mineral concentration in pollen of *A. mellifera* (Stanley and Linskens 1974; Herbert and Shimanuki 1978; Serra-Bonvehi and Escolà-Jordà 1997) which is similar to that reported for the stingless bee *Melipona subnitida* (Silva et al. 2014) and our results. From our investigation the Na content appears in the same range as the report of Na in Brazilian pollen, but it was not detected in pollen of *Melipona subnitida*. In comparing the Paleotropical pollens of SE Asia to the neotropical pollens examined by Morgano et al. (2012), Ca appears to be the only mineral which is significantly higher than those plant pollens examined from Brazil.

22.3.3 Fatty Acid Analysis

Pollen contains lipids and fatty acids, and their composition is variable depending on the plant species. Fatty acids are essential to honey bees as a source of energy, development, nutrition, and reproduction (Manning 2001). Fatty acids from numerous species of plants and pollen collect by *A. mellifera* have been reported by researchers (Serra-Bonvehi and Escolà-Jordà 1997; Herbert and Shimanuki 1978; Human and Nicolson 2006; Loper et al. 1980: Manning and Harvey 2002; Yang et al. 2013; Weiner et al. 2010). Fatty acids are also necessary for human nutrition. Although humans can synthesize saturated fatty acids and some monounsaturated fatty acids, some essential fatty acids including linoleic acid, alpha-linolenic acid, and omega-3 and omega-6 fatty acids cannot be synthesized and must be obtained from food (Mann and Truswell 2012; Robert and Maurice 1980).

Table 22.2 Comparison of mineral content of Thai pot-pollen and Brazilian references

	Mineral content (mg/kg)			
Bee species	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Sodium (Na)
Tetragonula laeviceps complex species	5656.0 ± 1274.9	2566.0 ± 489.3	1150.0 ± 222.6	89.9 ± 20.0
Tetragonula testaceitarsis	4594.7 ± 521.0	2904.0 ± 546.2	1318.0 ± 95.3	133.5 ± 48.6
Lepidotrigona terminata	4606.3 ± 75.6	2507.3 ± 4.2	1176.0 ± 4.6	77.2 ± 5.9
Lepidotrigona flavibasis	5125.7 ± 30.0	2719.7 ± 94.6	1315.3 ± 2.5	81.7 ± 6.4
Brazilian *Melipona subnitida*[a]	5918.5 ± 98.8	1846.1 ± 19.6	975.4 ± 25.4	ND
Brazilian *Apis mellifera* bee pollen[b]	5089.0 ± 1981.0	2215.0 ± 984.0	1179.0 ± 455.0	85.0 ± 177.0

ND not detected
[a]Silva et al. (2014)
[b]Morgano et al. (2012)

Our detailed fatty acid analysis was confined to the pollen stores from a single stingless bee species (*T. laeviceps* complex sp.). Our findings are shown in Table 22.3. Stingless bee pollen samples were collected from five individual colonies. The fatty acids were analyzed by gas chromatography-flame ionization detector following the standard methods of AOAC (2005).

Our analysis shows the amounts of unsaturated fatty acids to be higher than saturated fatty acids. The ratio of polyunsaturated to saturated fatty acids was 1.59. When the ratio of polyunsaturated and saturated fatty acid in human diet is greater than 1, HDL cholesterol can be slightly reduced by very high intakes of polyunsaturated fatty acids (Mann and Truswell 2012). Our results correspond to Serra-Bonvehi and Escolà-Jordà (1997) who indicated the ratio of unsaturated to saturated fatty acids was 1.96.

Table 22.3 Fatty acid spectra of *Tetragonula laeviceps* species stored pollen

Fatty acids	Content of fatty acids (g/100 g)
Myristic acid (C14:0)	0.02 ± 0.01
Pentadecanoic acid (C15:0)	0.04 ± 0.04
Palmitic acid (C16:0)	1.65 ± 0.49
Stearic acid (C18:0)	0.20 ± 0.05
Arachidic acid (C20:0)	0.07 ± 0.06
Heneicosanoic acid (C21:0)	0.02 ± 0.01
Behenic acid (C22:0)	0.11 ± 0.03
Lignoceric acid (C24:0)	0.12 ± 0.06
Total saturated	***2.30 ± 0.59***
Palmitoleic acid (C16:1n7)	0.04 ± 0.03
cis-9-Oleic acid (C18:1n9c)	0.30 ± 0.07
cis-11-Eicosenoic acid (C20:1n11)	0.11 ± 0.10
Erucic acid (C22:1n9)	0.11 ± 0.12
Total monounsaturated	***0.56 ± 0.25***
trans-Linolelaidic acid (C18:2n6t)	0.13 ± 0.05
cis-9,12-Linoleic acid (C18:2n6)	1.52 ± 0.27
Gamma-linolenic acid (C18:3n6)	0.01 ± 0.01
Alpha-linolenic acid (C18:3n3)	1.40 ± 0.62
cis-11,14-Eicosadienoic acid (C20:2)	0.09 ± 0.12
Arachidonic acid (C20:4n6)	0.01 ± 0.01
Total polyunsaturated	***3.10 ± 0.82***
Total unsaturated fat	***3.66 ± 0.18***
Trans fat	***ND – 0.17***
Omega-3	***1.48 ± 0.73***
Omega-6	***1.54 ± 0.27***
Omega-9	***0.40 ± 0.18***

ND not detected

From our analysis of saturated fatty acids, palmitic acid is shown to be the most prevalent. This result corresponds to the report of Human and Nicolson (2006) who studied saturated fatty acids on *A. mellifera* stored pollen from *Aloe greatheadii* var. *davyana.* Serra-Bonvehi and Escola-Jorda (1997) examined *A. mellifera* collected pollen but did not specify the plant species. Their results showed palmitic acid was the prevalent fatty acid. Yang et al. (2013) investigated fatty acids of *A. mellifera* collected pollen from 12 different locations in China. Their results showed palmitic acid to be the predominant saturated fatty acid in all geographic locations except for one location where palmitic acid was slightly lower than stearic acid.

22.3.4 Amino Acid Analysis

Amino acids are simple organic compounds found in living organisms. About 20 amino acids are common in humans which are necessary for protein assembly (Hardy 1985). The dietary protein requirement for humans should contain sufficient and digestible amounts of nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). Amino acids that can become essential under specific physiological or pathological conditions include cysteine, tyrosine, glycine, arginine, glutamic, and proline. Adequate total amino acid nitrogen can be derived from any of the above amino acids and from nonessential amino acids such as aspartic acid, asparagine, glutamic acid, alanine, and serine or other sources of nonessential nitrogen (WHO/FAO/UNU 2007).

The high protein content and variety of amino acids found in bee-collected pollen have been reported by many researchers (Herbert and Shimanuki 1978; Loper and Cohen 1987;

Szczesna 2006; Weiner et al. 2010), most of which examined pollen collected by *A. mellifera*. Silva et al. (2014) investigated the amino acid content of pollen from the stingless bee species *Melipona subnitida* from Brazil.

We report on the amino acid spectra for only one species of stingless bee (*T. laeviceps* species complex sp.), which is the most commonly encountered species in Thai meliponiculture. The results of our analysis are shown in Table 22.4. Five samples of stingless bee stored pollen were collected from five *T. laeviceps* species colonies. The amino acids were analyzed by mass spectrometry-gas chromatography following the standard methods of AOAC (2005).

Twenty amino acids are identified in our polyfloral pollen samples taken from five *T. laeviceps* colonies. For the essential amino acids, lysine, phenylalanine, and leucine were the dominant amino acids in our samples. Szczesna (2006) who examined amino acids of *A. mellifera* bee-collected pollen from Poland, Korea, and China found lysine and leucine to be the predominant amino acids. Human and Nicolson (2006) also reported leucine and lysine to be the predominant essential amino acids from *A. mellifera* stored pollen. Our results of nonessential amino acids show glutamic acid and tyrosine to be the dominant amino acids, while Human and Nicolson (2006) reported glutamic and aspartic acids at the highest concentration. Our results show lower overall amino acid levels compared to their report with the caveat that their research was based on monofloral pollen from *Aloe greatheadii* var. *davyana*. Silva et al. (2014) examined stingless bee pollen from *Melipona subnitida* in Brazil. This study found proline to be the predominant amino acids (0.11 mg/100 g) which is lower than our result of proline content. Overall Silva et al. (2014) found markedly lower levels of amino acids than our observations of our bee of the *T. laeviceps* complex in SE Asia.

Table 22.4 Amino acids in *Tetragonula laeviceps* complex species pot-pollen

Amino acids	Amino acid content (g/100 g)
Essential amino acids	
Histidine	0.96 ± 0.07
Isoleucine	0.88 ± 0.06
Leucine	1.62 ± 0.12
Lysine	2.46 ± 0.14
Methionine	0.12 ± 0.03
Phenylalanine	1.63 ± 0.26
Threonine	0.17 ± 0.01
Tryptophan	0.13 ± 0.14
Valine	0.65 ± 0.29
Nonessential amino acids	
Alanine	0.40 ± 0.02
Arginine	<0.02
Aspartic acid	0.70 ± 0.06
Cysteine	0.15 ± 0.04
Glutamic acid	1.12 ± 0.01
Glycine	0.26 ± 0.02
Hydroxylysine	<0.02
Hydroxyproline	<0.02
Proline	0.60 ± 0.02
Serine	0.22 ± 0.02
Tyrosine	1.00 ± 0.08

The amino acid composition of plant pollen is recognized as being highly dependent on plant species; however, in a study of 142 plant-specific pollens, Weiner et al. (2010) comment that while amino acid composition varies between plant species, all examined pollen species contained a complete set of essential amino acids. Our examination of the stored pollen from *T. laeviceps* species illustrates that the entire essential as well as the nonessential amino acids are present in the colonial pollen stores of this species.

22.4 Botanical Origin

Pollen samples were homogenized using ethanol, centrifuged and the sediment was resuspended in equal volumes of water and glycerin. One drop of this well-homogenized mixture was put on a microscope slide, covered with a cover glass, and sealed with nail polish. The durability of these preparations is about 10 days (Barth et al. 2010). More than 500 pollen grains per sample were counted at 400 × magnification. Evaluation follows the rule of monofloral patches when more than 90% of the pollen grains belong to a unique plant species or are of 60% when no accessory pollen type (15–45%) was present.

Table 22.5 Palynology analysis of stingless bee (Meliponini) pot-pollen

	Main pollen types				
	Most frequent	Frequent	Less frequent	Rare	
Stingless bee species	++++	+++	++	+	Diagnosis
Tetragonula laeviceps complex species[a]	*Trema micrantha*	*Trema micrantha*, (d), (l)	(j), (k)	*Cocos nucifera*, (a), (d), (j), (k), (l), et al.	Heterofloral
Tetragonula testaceitarsis[a]	(−)	*Cocos nucifera*, (a), (b), (d), (e)	*Cocos nucifera*, (a), (b), (f), (g), (i)	*Cocos nucifera, Acacia*, (a), (h), (g), et al.	Heterofloral
Lepidotrigona terminata[b]	*Trema micrantha*	*Acacia,* (a)	(a), (b), (c)	*Cocos nucifera*, (b), (d), et al.	Heterofloral
Lepidotrigona flavibasis[b]	*Tapirira* sp. (a)	*Tapirira* sp.	(a)	Several	Bifloral

[a]Aggregate of a five-colony sample
[b]Aggregate of a three-colony sample
Unknown pollen types:
(a) = 1-colpate, medium size, reticulate (Monocotiledonea)
(b) = 1-colpate, small size, reticulate (Monocotiledonea)
(c) = 2-colpate, small size, psilate
(d) = 3-colporate, small size, psilate
(e) = 3-colporate, small size, microreticulate (Fabaceae-Faboideae type)
(f) = 1-colpate, large size, psilate (Arecaceae type)
(g) = 2-colpate, small size, spiculate
(h) = 2-porate, small size, psilate (Urticaceae type)
(i) = 4-colporate, small size, strongly reticulate
(j) = 3-colporate, small-medium size, strongly reticulate
(k) = 1-colpate, medium size, microreticulate (Monocotiledonea)
(l) = 3-colporate, very small size, psilate
(−) = absent

Table 22.5 shows the most frequent to the rarest pollen types. The right column gives the interpretation of the data obtained. Many pollen types remain taxonomically unknown, which is not unexpected as pollen keys for SE Asia are very limited, as exemplified by a study of pollen resources for the SE Asian night-flying carpenter bee *Xylocopa* (*Nyctomelitta*) *tranquebarica* (Fabricius, 1804) (Burgett et al. 2005). Pollen type assemblages can group some samples. Monofloral, bifloral and heterofloral pollen batches occurred. The monofloral samples proceed from a unique plant species and the bifloral are a mixture of pollen grains from two plant taxa. The heterofloral samples showed several pollen types that signified no predominant taxon.

Pollen composition was similar for individual bee colonies at specific geographic locations; samples from three colonies of *L. terminata*, as well as five samples from five *T. testaceitarsis* colonies, five colonial samples of *T. laeviceps* complex sp. and three colonial samples from *L. flavibasis* comprised mainly heterofloral pollen batches. Results expressed in Table 22.5 represent species conglomerates. The remaining samples are classified as heterofloral also presenting a variable number of pollen types, all of them at low frequencies.

Among the 16 pot-pollen samples, a total of four important plant taxa could be identified (*Acacia* sp., *Cocos nucifera*, *Tapirira* sp., and *Trema micrantha*) (Fig. 22.5), additionally twelve taxa were recognized and not identified, and there were a large number of sporadic species.

The most significant pollen types were *Trema micrantha* (Cannabaceae), a wind-pollinated

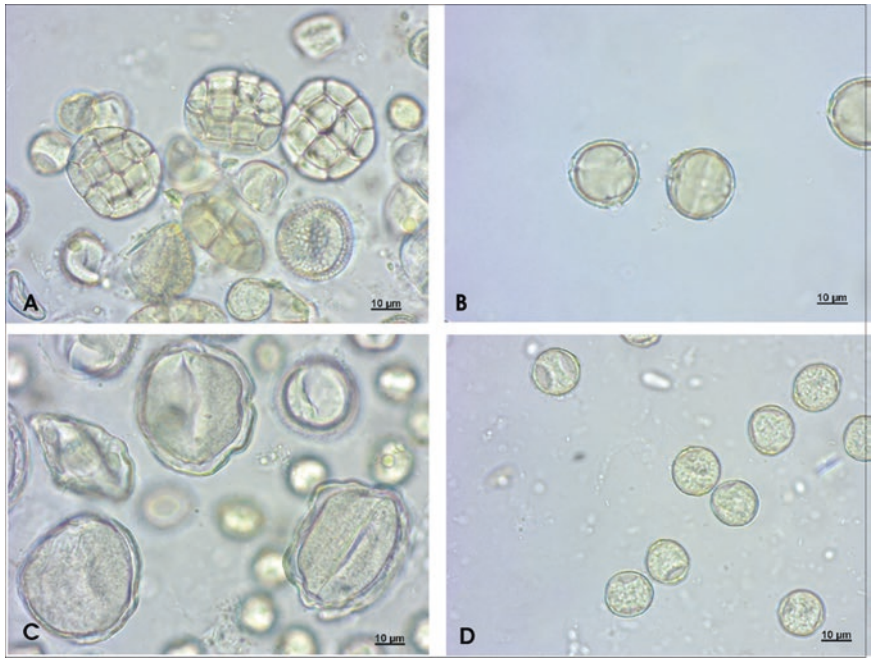

Fig. 22.5 Pollen types found in pot-pollen samples: (**a**) *Acacia* sp., (**b**) *Tapirira* sp., (**c**) *Cocos nucifera*, and (**d**) *Trema micrantha*

invasive, and *Tapirira* sp., a tree of the Anacardiaceae family. The third predominant pollen type belongs to an unknown plant species, possibly a palm tree (pollen type (a) of Table 22.5). Another plant that contributes greatly is *Cocos nucifera*, the coconut palm (Arecaceae), which was present in 11 of the 16 samples. Another important pollen type was *Acacia*, but less common.

As meliponines exhibit polylecty (Eltz et al. 2001; Ramalho et al. 1989, 1990; Roubik and Moreno 2013), the pollen stores found in stingless bee colonies will display great taxonomic variation dependent primarily on geographical location and seasonality. It should be stressed that the pollen identification from our samples represents a geographical and seasonal "snapshot."

22.5 Conclusions, Suggestion, and Future Research

Analyses of pot-pollen nutritional composition are consistent with the numerous researchers on honey beebread (*A. mellifera*) pollen. The macronutrients and minerals are shown to be similar to those reported for beebread pollen. For mineral content, potassium is found to be the major component which correlates to the reports from several studies in honey bee and stingless bee pollen. The results of fatty acids and amino acids content from our stored pollen are compatible with previous findings. The composition of stored pollen from any bee species is primarily dependent on plant species, season, and the geographical location where bees collect pollen. The difference in plant species showed variability in their pot-pollen composition. Our study of the botanical origins shows a relatively restricted view of plants visited intensively by four species of stingless bee. Our study is the first report of the nutritional composition of pollen from four species of stingless bee in SE Asia: a species of the *T. laeviceps* complex, *T. testaceitarsis*, *L. terminata*, and *L. flavibasis*. There are many stingless bee species which have yet to be examined. It will be necessary to carry out further research on stingless bee pot-pollen from SE Asia to better understand a broader scope of nutritional qualities and host plant breadth.

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