ภาคผนวก 1

Palynological Analysis of Stingless Bee (*Trigona collina* Smith) Pollen Loads for Botanical Origin Determination in Relation to Floristic Composition in Northern Thailand

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ABSTRACT – Stingless bee (*Trigona* sp.) need pollen as food source for growth of colony. The number of colony depends on available supply of melliferous species. We studied morphology of pollen from hive-returning stingless bee pollen load and analyzed the plant community in the deciduous forest of Queen Sirikit Botanic Garden, Chiang Mai provinec, Thailand. The *Trigona collina* Smith. visited 18 from 70 flowering species around the year. These pollen florae were Litchi (*Litchi chinensis* Sonn.), Tin tukkae (*Tridax procumbens* L.), Plao yai (*Croton roxburghii* N.P. Balakr.), Rok faa (*Terminalia alata* Heyne ex Roth), Phang rae yai (*Trema orientalis* (L.) Blume), Chaa paen (*Callicarpa arborea* Roxb.), Kamlung suea khrong (*Betula alnoides* Buch.-Ham. ex G. Don), Phrik (*Capsicum frutescens* L.), Arang (*Peltophorum dasyrachis* (Miq.) Kurz), Thalo (*Schima wallichii* (DC.) Korth.), Maiyarap ton (*Mimosa pigra* L.), Po lom pom (*Thespesia lampas* (Cav.) Dalz. & Gibs.), Khaa hot (*Engelhardtia serrata* Blume), Ya yung (*Capillipedium parviflorum* (R.Br.) Stapf), Sieo dok khaao (*Bauhinia variegata* L.), Kaafaak (*Scurrula sp.*), Graminae and Verbenaceae. The flora species visited by the stingless bees was not related with the importance value index (IVI) and density of the given species. The result suggests that there is the preference behavior of pollen collection for the stingless bee. This information is useful for monitoring and conservation management of this bee species.

Key words : Stingless bee, *Trigona collina*, melliferous plants, palynology, pollen load, importance value index

1. Introduction

Stingless bee is important for crop pollinations in Thailand, beside other bee pollinators such as asian bee (*Apis cerana F.*), giant bee (*Apis dorsata*) and european bee (*Apis mellifera*). Twenty four *Trigona* species are recorded in Thailand (Saiboon, 1996), including *Trigona collina* Smith. In the past, the stingless bees were widely distributed around the country. However, due to an increase of human being population and modernization, the reduction of forest fragments largely spreads throughout the country, the number of the bee colony rapidly undergoes an extinction process (Pechhacker *et. al*, 1991, Pechhacker and Juntawong, 1994) and the beekeeper preferred to keep the

domesticated european honey bee more than the asian honey bee (Wongsiri and Chen (1995).

Nowadays, stingless bee species in Thailand can be apparently found in protected national park such as Queen Sirikit Botanic Garden (QSBG), Chiangmai province because of the tremendous availability of pollen and nectar sources. The QSBG located in Doi Pui-Suthep national park between 19-20 °N latitude and 91-92 °N longitude and covered with common species of Dipterocarpaceae. However, the melliferous plant species for the stingless bee have never been surveyed in the QSBG area.

Vorwohl (1954) used the pollen-honey analysis to identify the botanical and geographical origin of nectar plants. Juntawong and Pechhacker (1991) analyzed the pollen in honey sac of european bee (*Apis mellifera*) to determine the nectar plant source whereas the pollen from hair body of the bee was observed by Cotmee (1999). Bastos *et al.* (2004) determined 14 honey bee (Apis sp.) pollen loads in the states of Sao Paulo and Minas Gerais, Brazil and several pollen types were identified. Eucalyptus (Myrtaceae) and Eupatorium (Asteraceae) pollen types were the most common among those sampled. The nondestructive analysis of honey by Raman spectroscopy is alternatively used to identify the floral origin of honey (Good *et al.*, 2002). Nevertheless, this highly specialized mellisopalynological technique costs expensively. Sriputachat (1972) reared *Apis cerana* Far. at Sakaerat forest, Nakorn Ratchasima Province, Thailand and found that the majority of the pollen collected came from *Pterocarpus macrocarpus* Kurz (Family Leguminoceae).

In this paper we analyzed pollen loads from hive-returning stingless bee to survey and identify plants which the bee used pollen as food source. It also aims to identify the relationship among the available plant species and its relative importance. This study focused on the assessment of pollen plants in Queen Sirikit Botanic Garden in northern Thailand as a case study. The inventory directory of melliferous plant species is illustrated. This information will be useful for the future improvement of cultural practice and management of stingless bees in Thailand.

2. Materials and Methods

Study area : The decidous forest at Queen Sirikit Botanic Garden (QSBG), Chiangmai was selected as a model site for the study. The forest is still virgin, located between 18 54 57 °N, 98 51 11 °S and 18 55 43 °N, 98 48 47 °S and the elevation between 750-1,030 m from mean sea level (MSL) (Figure 1), never fired.

Stingless bee species : The *Trigona collina* Smith stingless bee colony was selected for the study. The bee hive located at 18 54 55 °N and 98 51 13 °S and at the elevation 1,000 m from mean sea level.

Pollen load study : Ten pollen loads from the stingless bees were collected monthly during 9-11.00h around the year by trapping the hive-returning bees in front of the hive with small plastic bags. The pollen loads were then transferred to a small glass vial and kept in refrigerator for further analysis. In the laboratory, the pollen were acetolyzed according to the method of Erdtmann (1961). The triplicates of permanent slides were prepared by the paraffin method (Johannsen, 1942) for Olympus light microscopic observation. The sculpturing structures of the pollen were studied with JACSCO scanning electron microscope.

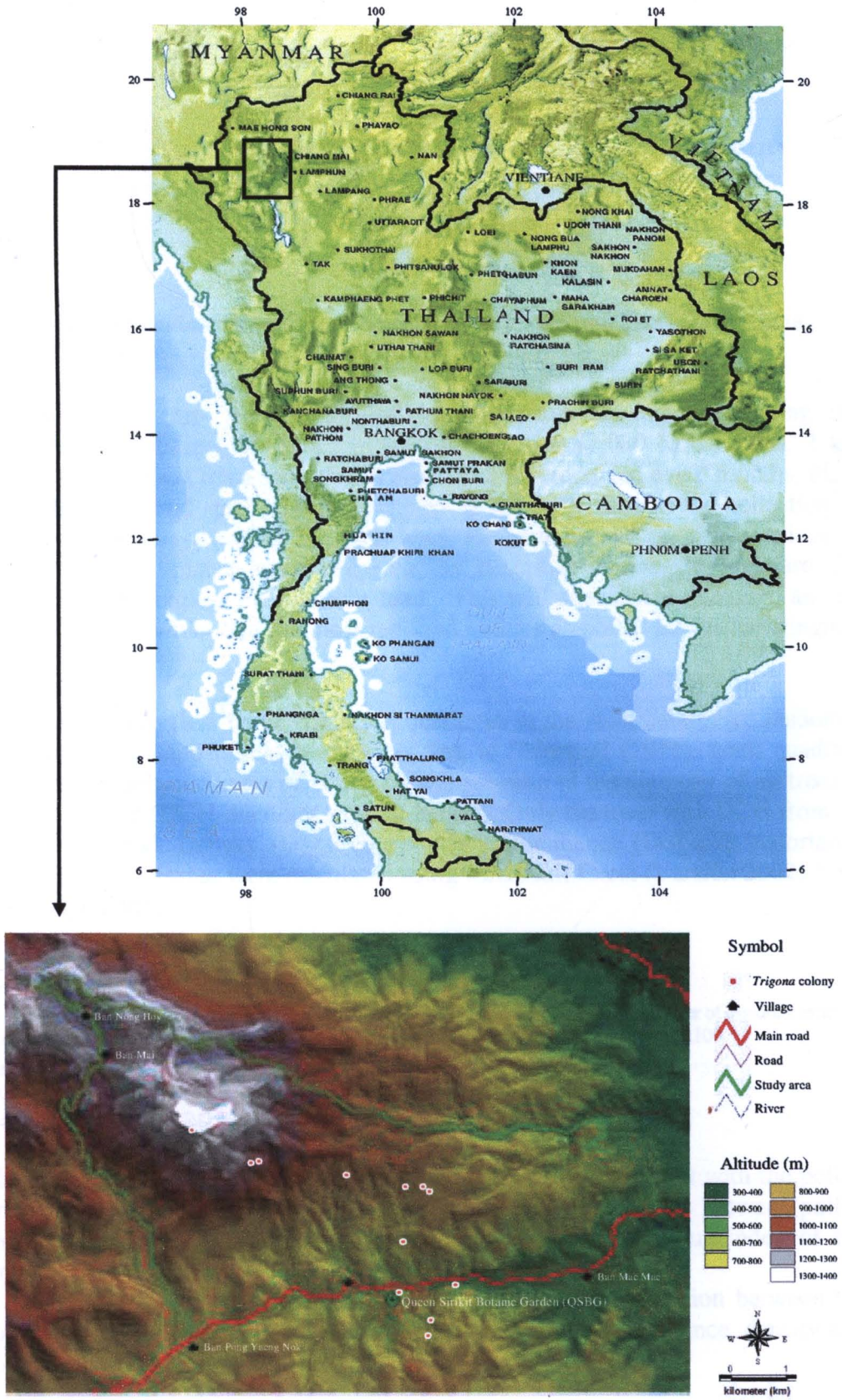

Figure 1. Study area in deciduous forest, Queen Sirikit Botanic Garden (QSBG), Chiangmai province between 18 54 57-18 55 43 °N and 98 48 47-98 51 11 °S, altitude at 750-1,030 m from mean see level.

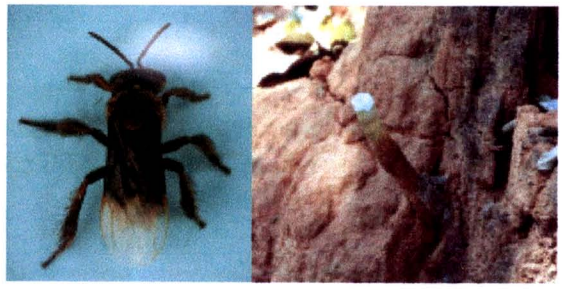

***Figure 2. Trigona collina* Smtih stingless bee hive under termite hive in study area**

Flowering plant collection : An inventory of blooming plant was carried out along the walking way from the base (750 MSL) to the hill direction (1,030 MSL) at ca. 2 km distance, 50 m widely apart from each sides. Blooming was recorded every months. Plant specimens with flower and pollen were collected as the same time as the collection of pollen load from the hive-returning stingless bees. Herbariums and permanent slides of the pollen from these plants were prepared, as described above, to compare the morphology with the stingless bee pollen's load. This will answer the question, among the inventoried species which are the most reported species as pollen source for stingless bee.

Plant community analysis : Three 20x50 m^2 quadrates in the study area were randomly selected for floristic compositional study according to Shimwell (1971). Each quadrate was divided into ten sub-quadrates of 10x10 m, were measured the diameter of all trees at 1.3 m from the ground (DCH-diameter at chest height). Only the trees with DCH from 15 cm up were considered. Density (D), Frequency (F), Dominance (Do) and Importance Value Index (IVI) of the trees were calculated using Microsoft Excel (Version 2002) from the following equations :

1) Density, D = Total number of individuals/area sampled
   Relative Density, RD = (Density of a given species/sum of densities of all species)x100
2) Frequency of a species, F = Total number of quadrats in which a species occurs/total number of quadrats examined
   Relative Frequency, RF = (Frequency of a given species/sum of frequency of all species)x100
3) Dominance of a species, Do = Total percent cover of a species/total area sampled
   Relative Dominance, RDo = Dominance of a species/sum of the dominance of all species
4) Importance Value Index, IVI = RD + RF + RDo

The structural parameters of the plant community were correlated with the pollen plants for stingless bee to obtain answers whether there is a relationship between pollen plants and the dominance, density and frequency and importance value index of the species or not.

A linear regression analysis was applied to identify the correlation between the number of pollen plants and number of flowering species and its dominance, density and frequency and importance value index.

3. Results

Flowering plant collection

A total amount of 70 accessions of flowering plants were inventoried around the year in QSBG and classified into 33 families, 57 genus and 70 species (Table I).

Pollen load study

Of the 70 inventoried flowering species, by comparing to the morphology of pollen, 18 species were found from the pollen load (Table II and Figure 3-21). Among the known species, the Thalo (*Schima wallichii* (DC.) Korth.) and Arang (*Peltophorum dasyrachis* (Miq.) Kurz) were frequently observed in the samples from March, April and June. Interestingly, no pollen was collected between July-September. In October, 80.5% of pollen came from Maiyarap ton (*Mimosa pigra* L). In November 2003, the Gramineae pollen was predominantly found in pollen load. Khaa hot (*Engelhardtia spicata* Blume) was a dominant species in December. The number of pollen species in the pollen load increased during December-January. The most important pollen source in February came from Litchi (*Litchi chinensis* Sonn.). The number of pollen species (y) did not correlate with the number of flowering species (x) ($y = 0.0181x + 1.9063$, $R^2 = 0.006$, $p > 0.05$) (Table II).

Plant community analysis

Thirty six tree species with DCH > 15 cm were recorded in 20x50 m^2 quadrates area (Table III). Only four species of pollen plants for stingless bee, Rok faa (*Terminalia alata* Heyne ex Roth), Chaa paen (*Callicarpa arborea* Roxb.), Thalo (*Schima wallichii* (DC.) Korth.) and Khaa hot (*Engelhardtia serrata* Blume), were observed in the quadrates area with the density of 0.3, 0.1, 0.07 and 0.03 plants/m^2, respectively. Families that presented higher value for density, frequency, dominance and importance value index were not the same one found in pollen load. The importance value index did not correlate with the percent of pollen of the same species in pollen load ($p > 0.05$) (Figure 22).

4. Discussion

In the study area the blooming species were found around the year (Table I, II) even in July, August and September. However, during July-September, there was not much flights activity and no pollen load was found on the hive-returning stingless bee. This is because it is in rainy season in Thailand. As reported, high humidity and lower temperature outside the hive obstructs foraging activity of the bees. No correlation between the number of pollen species found in pollen load and the number of blooming species (Table II). This indicates that the preferential behavior of pollen collection exists in the bee.

Among the inventoried species, Thalo (*Schima wallichii* (DC.) Korth.), Phrik (*Capsicum frutescens* L.), Maiyaraap ton (*Mimosa pigra* L.), Khaa hot (*Engelhardtia spicata* Blume), Rok faa (*Terminalia alata* Heyne ex Roth) and Litchi (*Litchi chinensis* Sonn.) are the most reported species as pollen source for stingless bee.

Although the number of melliferous species is limited (18 species), compared to the total blooming species (70 species), we expect that the number of pollen plants should be more than observed. Three reasons can be implied the result. Firstly, because the pollen morphology is closely similar to each others, this makes the difficulty for the

identification. Second, due to the line transect sampling method, not all flowered species in the area were collected. Some species locates in the deep volley area where we could not go through. Thirdly, because the pollen load was colleted from hive-returning stingless bee between 9.00-11.00h, however, various plant species bloomed in the afternoon.

In the study area the forest type was dipterocarp which consisting mainly of Ko phae (*Quercus kerrii* Craib), Teng (*Shorea obtusa* Miq.), Yaang hiang (*Dipterocarpus obtusifolius* Teijsm. ex Miq.), Yaang phluang (*Diptrocarpus tuberculatus* Roxb.), Mueat lot (*Aporusa villosa* (Wall. ex Lindl.) Baill.) and Kwaao (*Haldina cordifolia* (Roxb.) Ridsdale). These predominant species present the greatest number of dominance, density, frequency and importance value index (Table III). However, there is no correlation between these species and the species of pollen collected by the *Trigona collina* Smith stingless bee.

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Table I

Lists plant species which flowered around the year in the deciduous forest, Doi Monrong, Queen Sirikit Botanic Garden. The pollens were collected and analyzed to compare with the pollens in pollen loads of *Trigona collina* Smith stingless

Acc. no.	Family	Genus/species	Local name	Flowering period	Habitat	Reference
191	Anacardiaceae	*Buchanania lanzan* Spreng.	Mamuang hua maeng wan	Dec-Mar	T	BKF 067647
194	Anacardiaceae	*Gluta usitata* (Wall.) Ding Hou	Rak yai	Nov-Mar	T	BKF 049231
200	Anacardiaceae	*Spondias pinnata* (L.f.) Kurz	Ma kok	Dec-May	T	BKF 026355
377	Apocynaceae	*Holarrhena pubescens* Wall. ex G. Don	Mok yai	Mar-May	T	BKF 037046
233	Burseraceae	*Garuga pinnata* Roxb.	Ta khram	Feb-Apr	T	BKF 017689
287	Burseraceae	*Protium serratum* Engl.	Ma faen	Jan-Apr	T	BKF 063802
351	Caesalpiniaceae	*Peltophorum dasyrachis* (Miq.) Kurz	Arang	Mar-Apr	T	BKF 037998
620	Celastraceae	*Lophopetalum duperreanum* Pierre	Song salueng	Jun-Aug	T	BKF 040639
380	Combretaceae	*Quisqualis indica* L.	Lep mue naang	Mar-May	T	BKF 009984
283	Combretaceae	*Terminalia bellirica* (Gaertn.) Roxb.	Samo phi phek	Feb-Apr	T	BKF 008511
436	Combretaceae	*Terminali alata* Heyne ex Roth	Rok faa	Jan-Jun	T	BKF 003696
627	Compositae	*Tridax procumbens* L.	Teen tukkae	Dec-Feb	H	BKF 008520
633	Compositae	*Mikania cordata* (Burm.f.) B.L.Rob.	Khee kai yaan	Oct-May	H	BKF 052790
634	Compositae	*Ageretum conyzoides* L.	Saapraeng saapkaa	Dec-Feb	H	BKF 037342
601	Convolvulaceae	*Argyreia kerrii* Kerrii	Khruea phuu muang	Sep-Nov	C	BKF 112016
193	Dilleniaceae	*Dillenia ovata* Wall. ex Hook.f & Thom.	Saan bai lek	Feb-May	T	BKF 133514
250	Dilleniaceae	*Dillenia pentagyna* Roxb.	Saan naa	Jan-May	T	BKF 063766
211	Dipterocarpaceae	*Shorea roxburghii* G.Don	Phayom	Dec-Mar	T	BKF 003802
251	Dipterocarpaceae	*Shorea siamensis* Miq.	Rang	Jan-Mar	T	BKF 016287
248	Dipterocarpaceae	*Dipterocarpus tuberculatus* Roxb.	Phluang	Dec-Apr	T	BKF 063671
549	Ericaceae	*Craibiodendron stellatum* (Pierre) W.Sm.	Taa-chee-khoei	Aug-Nov	T	BKF 045840
106	Euphorbiaceae	*Croton roxburghii* N.P.Balakr	Plao yai	Nov-Feb	T	BKF 042315
192	Euphorbiaceae	*Aporosa villosa* (Wall. ex Lindl.) Baill.	Mueat lot	Jan-Mar	T	BKF 033217
357	Euphorbiaceae	*Antidesma acidum* Retz.	Mamao	Apr-Jul	S	BKF 045834
364	Euphorbiaceae	*Antidesma bunius* (L.) Spreng.	Mamao dong	Mar-May	S	BKF 009595
478	Euphorbiaceae	*Antidesma sootepense* Craib	Mamao saai	May-Jul	S	BKF 002176
441	Euphorbiaceae	*Flueggea virosa* (Roxb. ex Willd.) Voigt	Kaangplaa khaao	May-Jun	S	BKF 069888
600	Euphorbiaceae	*Bridelia tomentosa* Blume	Seefan krabue	May-Dec	S	BKF 009098
252	Fabaceae	*Dalbergia oliveri* Gamble	Chingchan	Jan-May	T	BKF 010691
378	Fabaceae	*Butea superba* Roxb.	Kwaao khruea	Mar-May	C	BKF 116415
555	Fabaceae	*Codariocalyx motorius* (Houtt.) Ohashi	Choi naang ram	Aug-Sep	H	BKF 067679
612	Fabaceae	*Dunbaria bella* Prain	Khaang khrang	Sep-Jan	C	BKF 055792
190	Fagaceae	*Lithocarpus lindleyanus* (Wall.) A.Camus	Ko daang	Apr-May	T	BKF 004488
210	Fagaceae	*Castanopsis indica* L.	Ko lim	Jan-Mar	T	BKF 011684
284	Fagaceae	*Castanopsis diversifolia* (Kurz) King	Ko paen	Jan-May	T	BKF 005860
404	Fagaceae	*Castanopsis hystrix* A.DC.	Ko daeng	Jan-May	T	BKF 093266
411	Fagaceae	*Castanopsis ferox* (Roxb.) Spach	Ko lame	Jan-May	T	BKF 104865
244	Fagaceae	*Quercus ramsbottomii* A.Camus	Ko talab	Mar-Apr	T	BKF 010740
425	Fagaceae	*Quercus vestita* Rehder & Wills.	Ko aep	Dec-Feb	T	BKF 102667
550	Fagaceae	*Quercus kingiana* Craib	Ko daeng	Nov-Jan	T	BKF 002428
613	Gentianaceae	*Exacum tetragonum* Roxb.	Yaa liam	Nov-Dec	H	BKF 005045
199	Gramineae	*Dendrocalamus strictus* (Roxb.) Nees	Phai saang	Feb-Mar	H	BKF 019206
621	Gramineae	*Capillipedium parviflorum* (R.Br.) Stapf	Yaa yung	Nov-Dec	H	BKF 034131
383	Guttiferae	*Cratoxylum formosum* (Jack) Dyer	Tiu khon	Jan-May	T	BKF 004357
206	Juglandaceae	*Engelhardtia spicata* Blume	Khaa hot	Dec-Jan	T	BKF 092498
526	Labiatae	*Tectona grandis* L.f.	Sak	Jun-Oct	T	BKF 005997
350	Labiatae	*Callicarpa arborea* Roxb.	Chaa paen	Jan-Jul	S	BKF 002014
247	Malpighiaceae	*Hiptage benghalensis* (L.) Kurz	Kamlang chaang phueak	Jan-Feb	S	BFK 029889
438	Malvaceae	*Abelmoschus moschatus* Medic.	Som chabaa	Mar-Apr	H	BKF 046795
22	Mimosaceae	*Mimosa pundica* L.	Maiyaraap	Jan-Dec	H	BKF 070326
51	Mimosaceae	*Mimosa pigra* L.	Maiyaraap ton	Nov-Jun	S	BKF 059406
217	Mimosaceae	*Xylia xylocarpa* (Roxb.) Taub.	Daeng	Feb-Apr	T	BKF 016289
196	Myrtaceae	*Syzygium glaucum* Chantar & J.Parn.	Daeng	Feb-Apr	T	BKF 068467
445	Myrtaceae	*Syzygium fruticosum* DC.	Waa khee kwaang	Feb-Apr	T	BKF 001118
602	Orobanchaceae	*Aeginetia indica* Roxb.	Dok din daeng	Aug-Oct	H	BKF 122305
209	Palmae	*Phoenix acaulis* Roxb.	Peng bok	Feb-Mar	T	BKF 071998
434	Proteaceae	*Helicia nilagirica* Bedd.	Mueat khon tua phuu	Jan-Feb	S	BKF 048140
215	Rhamnaceae	*Ziziphus rugosa* Lam.	Ma khwat	Feb-Apr	S	BKF 017573
373	Rhamnaceae	*Gardenia sootepensis* Hutch.	Kham mok luang	Mar-Jul	T	BKF 017513
440	Rhamnaceae	*Pavetta petiolaris* Wall. ex Craib	Khem phae	May-Jun	S	BKF 024758
282	Sapindaceae	*Schleichera oleosa* (Lour.) Oken	Ta khro	Jan-Apr	T	BKF 007671
222	Sapindaceae	*Litchi chinensis* Sonn.	Linchee	Feb-Apr	T	BKF 080768
557	Stemonaceae	*Stemona collinsae* Craib	Non taai yaak	Aug-Sep	C	BKF 077197

245	Sterculiaceae	*Firmiana colorata* (Roxb.) R.Br.	Po huu chaang	Feb-Apr	T	BKF 017242
376	Strychnaceae	*Strychnos nux-blanda* A.W. Hill	Salaeng chai	Feb-Mar	S	BKF 001750
249	Symplocaceae	*Symplocos racemosa* Roxb.	Mueat hom	Nov-Feb	T	BKF 024912
348	Theaceae	*Schima wallichii* (DC.) Korth.	Tha lo	Jan-Apr	T	BKF 000267
198	Theaceae	*Anneslea fragrans* Wall.	Saaraphee paa	Nov-Feb	T	BKF 047588
435	Tiliaceae	*Grewia lacei* Drumm. ex Craib	Naat nok	May-Jun	S	BKF 003869
360	Tiliaceae	*Grewia eriocarpa* Juss.	Po yaap	Jul-Sep	T	BKF 005912

Abbreviations : C = climber, S = shrub, T = tree, H = herb, BKF = Bangkok Royal Forestry Department Herbariums

Table II

Number of plant species which supplied pollen as food source for *Trigona collina* Smith and percent of pollen of each species in pollen loads between April 2003-March 2004

Month	Year	Flowering species*	No. species in pollen load	Plant species	Pollen (%)
April	2003	30	2	Thalo (*Schima wallichii* (DC.) Korth. : TC10 **)	89.9
				Arang (*Peltophorum dasyrachis* (Miq.) Kurz : TC9)	10.1
May	2003	21	1	Phrik (*Capsicum frutescens* L. : TC8)	100.0
June	2003	14	2	Thalo (*Schima wallichii* (DC.) Korth. : TC10)	90.8
				Arang (*Peltophorum dasyrachis* (Miq.) Kurz : TC9)	9.2
July	2003	13	-	No pollen load	-
August	2003	18	-	No pollen load	-
September	2003	5	-	No pollen load	-
October	2003	8	3	Maiyaraap ton (*Mimosa pigra* L. : TC11)	80.5
				Po lom pom (*Thespesia lampas* (Cav.) Dalz. & Gibs. : TC12)	12.8
				Verbenaceae (TC18)	6.7
November	2003	14	2	Gramineae (TC17)	97.8
				Po lom pom (*Thespesia lampas* (Cav.) Dalz. & Gibs. : TC12)	2.2
December	2003	12	4	Khaa hot (*Engelhardtia spicata* Blume : TC13)	77.8
				Ya Yung (*Capillipedium parviflorum* (R.Br.) Stapf : TC14)	12.0
				Kaafaak (*Scurrula sp.* : TC16)	8.9
				Sieo dok khaao-like (*Bauhinia variegata* L. : TC15)	1.3
January	2004	20	9	Rok faa (*Terminalia alata* Heyne ex Roth : TC4)	23.1
				Gramineae (TC17)	15.7
				Cha paen (*Callicarpa arborea* Roxb. : TC6)	15.4
				Tin Tukkae (*Tridax procumbens* L. : TC2)	12.9
				Kamlung suea Khrong-like (*Betula alnoides* Buch.-Ham. ex G.Don : TC7)	10.7
				Phang rae yai (*Trema orientalis* (L.) Blume : TC5)	10.0
				Phrik (*Capsicum frutescens* L. : TC8)	8.1
				Litchi (*Litchi chinensis* Sonn. : TC1)	2.2
				Plao yai (*Croton roxburghii* N.P.Balakr. : TC3)	1.9
February	2004	40	2	Litchi (*Litchi chinensis* Sonn. : TC10)	99.9
				Plao yai (*Croton roxburghii* N.P.Balakr. : TC3)	0.1
March	2004	33	2	Thalo (*Schima wallichii* (DC.) Korth. : TC10)	85.9
				Arang (*Peltophorum dasyrachis* (Miq.) Kurz : TC9)	14.1
		228	27		

* Due to the overlapping of flowering period, some flowering species were repeatedly counted to give the total summary of 228 instead of 70 actual flowering species (Table I) and 27 instead of 18 actual species in pollen load.

** TC10 means pollen from *Trigona collina* Smith, code no. 10

Table III

Plant species with DCH > 15 cm in the deciduous forest at Doi Mon Rong, Queen Sirikit Botanic Garden, Chiang Mai province.

Plant species	Local name	D	F	Do	RD	RF	RDo	IVI
Quercus kerrii Craib	Ko phae	1.83	0.73	0.000524	15.49	11.06	20.25	46.80
Shorea obtusa Miq.	Teng	1.97	0.70	0.000391	16.62	10.55	15.11	42.28
Dipterocarpus obtusifolius Teijsm. ex Miq.	Yaang hiang	0.97	0.50	0.000428	8.17	7.54	16.53	32.24
Diptrocarpus tuberculatus Roxb.	Yaang phluang	0.93	0.40	0.000328	7.89	6.03	12.66	26.58
Aporusa villosa (Wall. ex Lindl.) Baill.	Mueat lot	1.27	0.70	0.000081	10.70	10.55	3.13	24.38
Haldina cordifolia (Roxb.) Ridsdale	Kwaao	0.83	0.43	0.000215	7.04	6.53	8.31	21.88
Wendlandia tinctoria (Roxb.) DC.	Khaeng kwaang	0.60	0.40	0.000034	5.07	6.03	1.30	12.40
Terminalia alata Heyne ex Roth	Rok faa	0.30	0.27	0.000104	2.54	4.02	4.02	**10.58***
Anneslea fragrans Wall.	Saaraphee paa	0.37	0.20	0.000063	3.10	3.02	2.44	8.56
Craibiodendron stellatum (Pierre) W.Sm.	Taa-chee-khoei	0.33	0.23	0.000039	2.82	3.52	1.49	7.83
Lithocarpus sootepensis (Wall.) A.Camus	Ko hua muu	0.23	0.23	0.000038	1.97	3.52	1.47	6.96
Shorea siamensis Miq	Rang	0.20	0.17	0.000043	1.69	2.51	1.65	5.85
Xylia xylocarpa Taub.	Daeng	0.17	0.17	0.000043	1.41	2.51	1.66	5.58
Colona flagrocarpa (C.B.Clarke) Craib	Po yaap	0.23	0.17	0.000028	1.97	2.51	1.06	5.54
Gluta usitata (Wall.) Ding Hou	Rak yai	0.17	0.17	0.000033	1.41	2.51	1.27	5.19
Flemingia sootepensis Li	Kaa saam peek	0.13	0.13	0.000010	1.13	2.01	0.40	3.54
Bombax anceps Pierre var. *anceps*	Ngiu paa	0.07	0.07	0.000049	0.56	1.01	1.90	3.47
Diospyros ehretioides Wall. ex G.Don	Ruean kwaang	0.13	0.10	0.000012	1.13	1.51	0.44	3.08
Schima wallichii (DC.) Korth.	Tha lo	0.10	0.07	0.000027	0.85	1.01	1.06	**2.92***
Dalbergia cultrata Grah. ex Benth.	Kraphee khao khwaai	0.10	0.10	0.000012	0.85	1.51	0.46	2.82
Terminalia chebula Retz.	Samo Thai	0.10	0.10	0.000010	0.85	1.51	0.40	2.76
Vaccinium sprengelii (G.Don) Sleumer	Som pae	0.10	0.07	0.000016	0.85	1.01	0.64	2.50
Gardenia sootepensis Hutch.	Kham mok luang	0.10	0.07	0.000006	0.85	1.01	0.23	2.09
Dillenia pentagyna Roxb.	Saan chaang	0.07	0.07	0.000004	0.56	1.01	0.17	1.74
Engelhardtia spicata Blume	Khaa hot	0.07	0.07	0.000004	0.56	1.01	0.16	**1.73***
Dalbergia oliveri Gamble	Chingchan	0.10	0.03	0.000007	0.85	0.50	0.28	1.63
Cratoxylum cochinchinense (Lour.) Blume	Tiu kliang	0.07	0.03	0.000009	0.56	0.50	0.34	1.40
Careya sphaerica Roxb.	Kradon	0.03	0.03	0.000015	0.28	0.50	0.58	1.36
Callicarpa arborea Roxb.	Chaa paen	0.03	0.03	0.000004	0.28	0.50	0.16	**0.94***
Shorea roxburghii G. Don	Phayom	0.03	0.03	0.000003	0.28	0.50	0.13	0.91
Markhamia stipulata Seem.	Khae haang khaang	0.03	0.03	0.000002	0.28	0.50	0.06	0.84
Strychnos nux-blanda A.W. Hill	Tuumkaa khaao	0.03	0.03	0.000002	0.28	0.50	0.06	0.84
Castanopsis hystrix A. DC.	Ko daeng	0.03	0.03	0.000001	0.28	0.50	0.05	0.83
Phyllanthus emblica L.	Makhaam pom	0.03	0.03	0.000001	0.28	0.50	0.03	0.81
Buchanania lanzan Spreng.	Mamuang hua maeng wan	0.03	0.03	0.000001	0.28	0.50	0.02	0.80
Ziziphus rugosa Lamk.	Ma khwat	0.03	0.00	0.000002	0.28	0.00	0.06	0.34
Total	**36**	**11.83**	**6.63**	**0.0026**	**100.00**	**100.00**	**100.00**	**300.00**

Use categories : D = Density, F = Frequency, Do = Dominance, RD = Relative Density, RF = Relative Frequency, RDo = Relative Dominance and IVI = Importance Value Index.

* = Visited by *T. collina* Smith

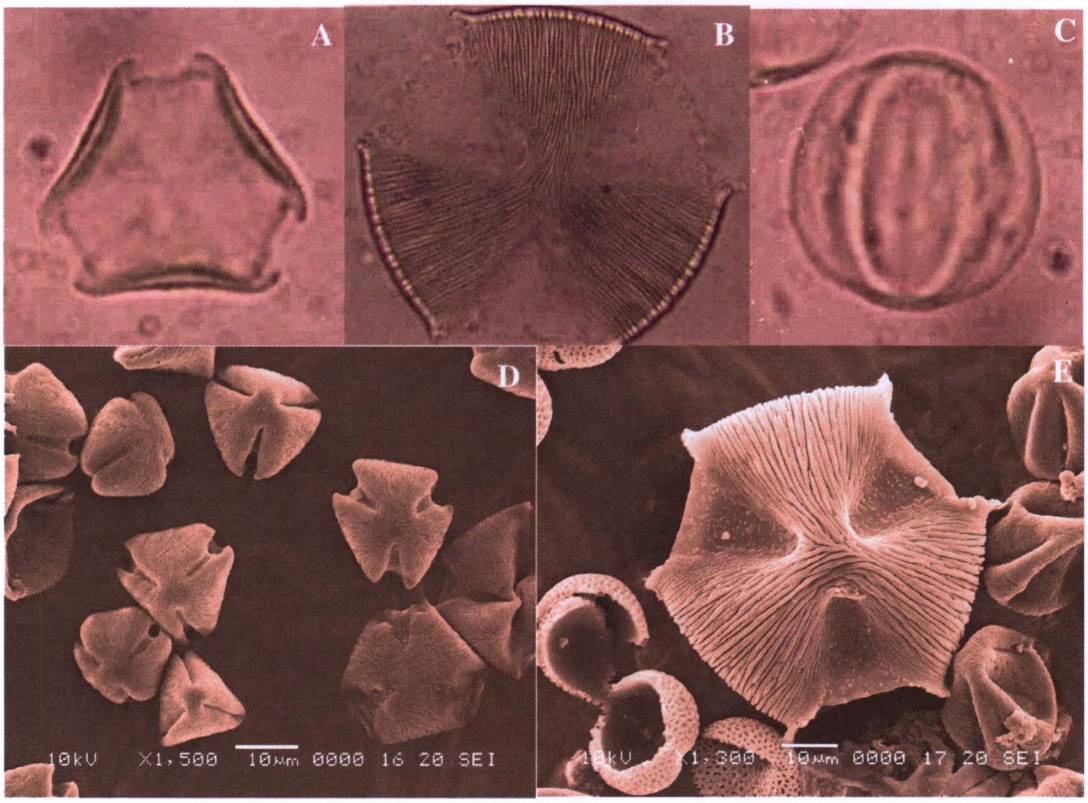

Figure 3. Litchi *(Litchi chinensis* Sonn) pollen (Code TC1), Sapindaceae , LM : A) polar view, 40x, B) polar view, 100x, C) equatorial view, 40x, SEM : D) tricolporate pollen (500x) and E) striate exine sculpturing (1,300x). **Pollen descriptions** : Monad, tricolporate, isopolar, radial symmetry, polar view : peroblate, equatorial view : circular, large grain; equatorial axis 55 – 70 µm, exine thickness 3 µm, sexine 2 µm, nexine 1 µm, striate exine sculpturing

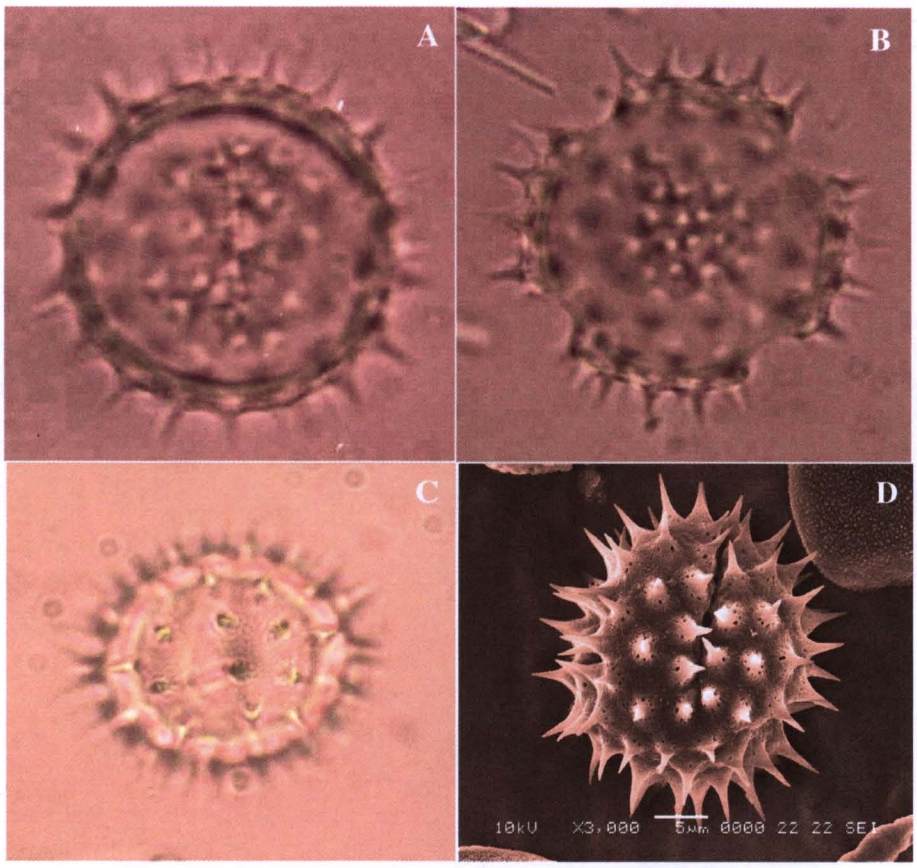

Figure 4. Tin Tukkae (*Tridax procumbens* L.) pollen (Code TC2), Compositae, LM 100x : A) polar view, B) equatorial view, C) tricolporate aperture, SEM 3,000x : D) aperture and echinate sculpturing. **Pollen descriptions** : Monad, stephanocolporate aperture, isopolar, radial symmetry, oblate-spheroidal to suboblate in polar view, circular in equatorial view, medium grain, polar axis 21.88 - 23.67 μm in length, equatorial axis 26.81 – 29.65 μm, exine thickness 2 μm, sexine and nexine 1 μm, scabrate exine sculpturing (<1 μm) and eclinate (2.5 – 3 μm x 4 -5 μm

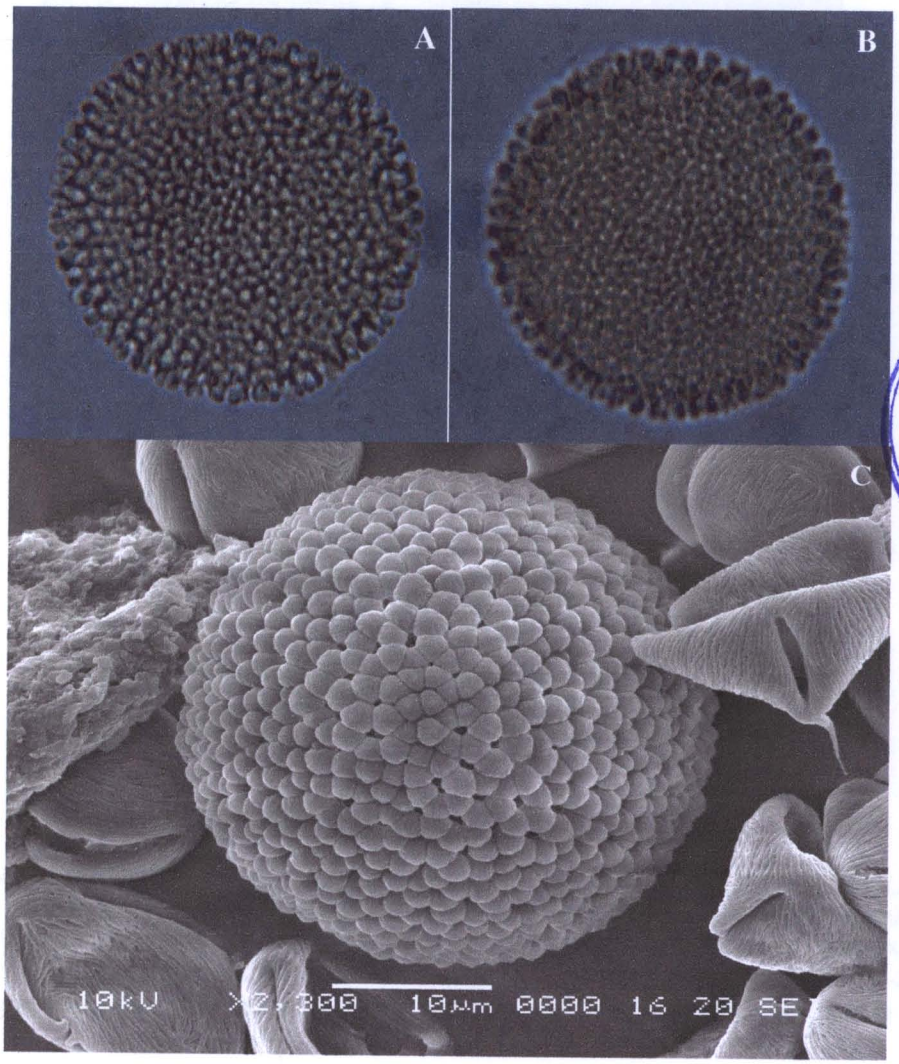

Figure 5. Plao yai (*Croton roxburghii* N.P. Balakr.) pollen (Code TC3), Euphorbiaceae, LM 40x : A, B) spheroidal, SEM 2,300x : C) exine sculturing. **Pollen descriptions** : Monad, inaperturate, apolar, radial symmetry, spheroidal, large grain, diameter 60-70 µm, croton type exine sculpturing

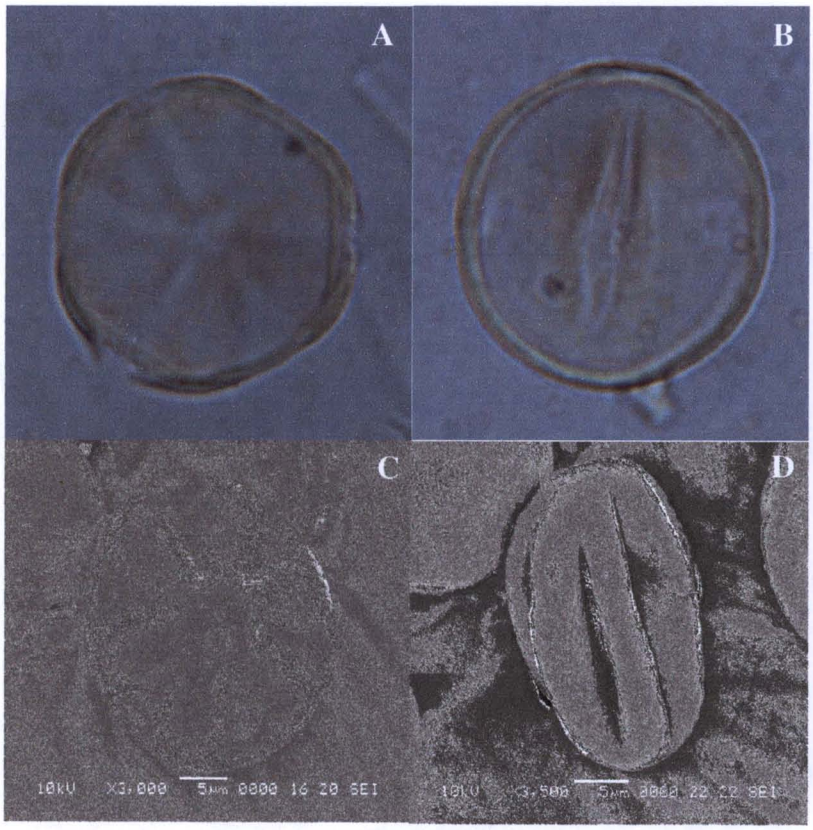

Figure 6. Rok Pha (*Terminalia alata* Heyne ex Roth) pollen (code TC4), Combretaceae, LM 100x : A) equatorial view, B) polar view, SEM : C) polar view (3,000x) and D) equatorial view (3,500x) **Pollen descriptions** : Monad, heterocolpate, isopolar, radial symmetry, polar axis : prolate-spheroidal, equatorial axis : (amb.) semiangular, medium grain, polar axis 26.2-29.3 μm, equatorial axis 24.4-29.2 μm, exine thickness 2 μm, striato-reticulate exine sculpturing

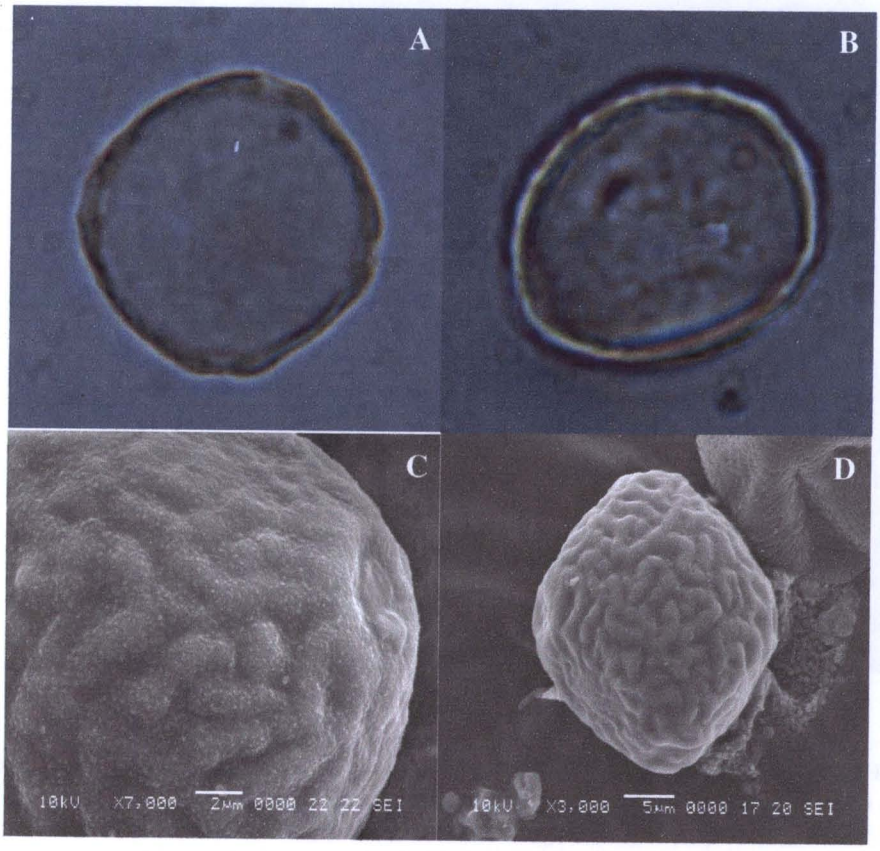

Figure 7. Pang lae yai (*Trema orientalis* (L.) Blume) pollen (code TC5), Ulmaceae, LM 100x : A) equatorial view, B) polar view, SEM : C) polar view (7,000x) and D) equatorial view (3,000x). **Pollen descriptions** : Monad, stephanopolate, isopolar, radial symmetry, polar view : oblate – spheroidal, equatorial view : (amb.) rectangular, small grain, polar axis 22.1 μm in length, equatorial axis 24.0 μm, exine thickness 1 μm, regulate exine sculpturing

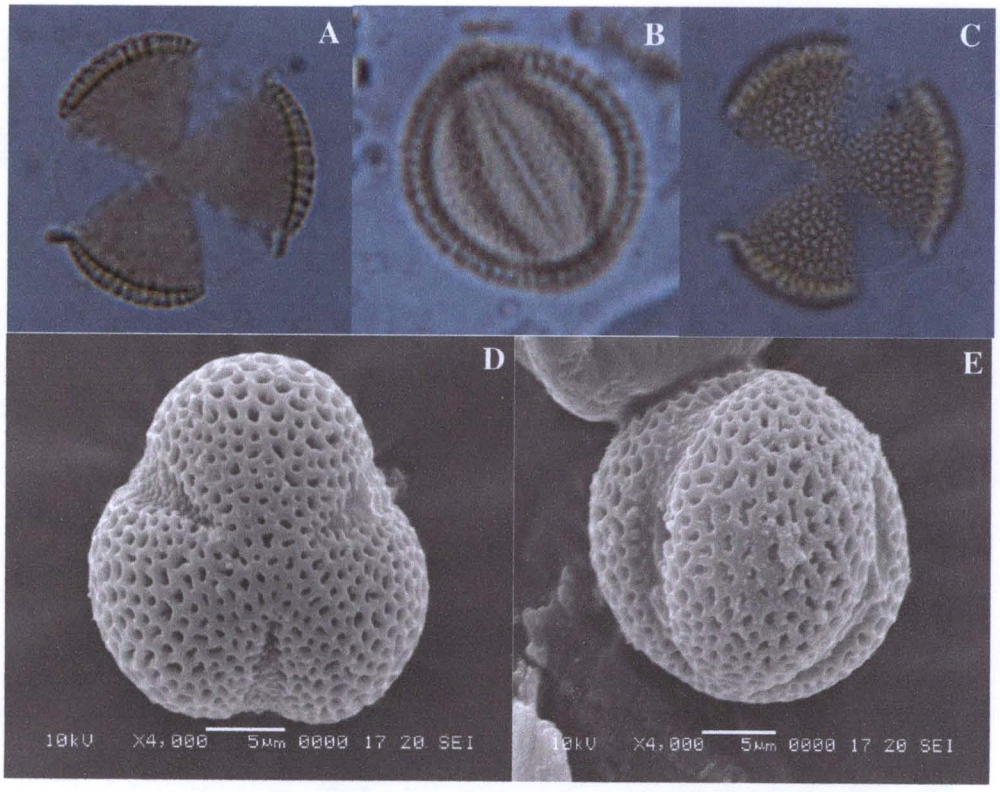

Figure. 8 Cha paen (*Callicarpa arborea* Roxb.) pollen (code TC6), Labiatae, LM 100x : A) equatorial view, B) polar view, C) reticulate sculpture, SEM 4,000x : D) polar view and E) equatorial view.. **Pollen descriptions** : Monad, tricolpate, isopolar, radial symmetry, polar view : spheroidal, equatorial view : (amb.) inter – semiangular, small grain, polar axis length 24.1 µm, equatorial axis 24.5 µm ; exine thickness 2.5 µm, sexine ¾ of exine and nexine ¼ of exine, finely reticulate exine sculpturing

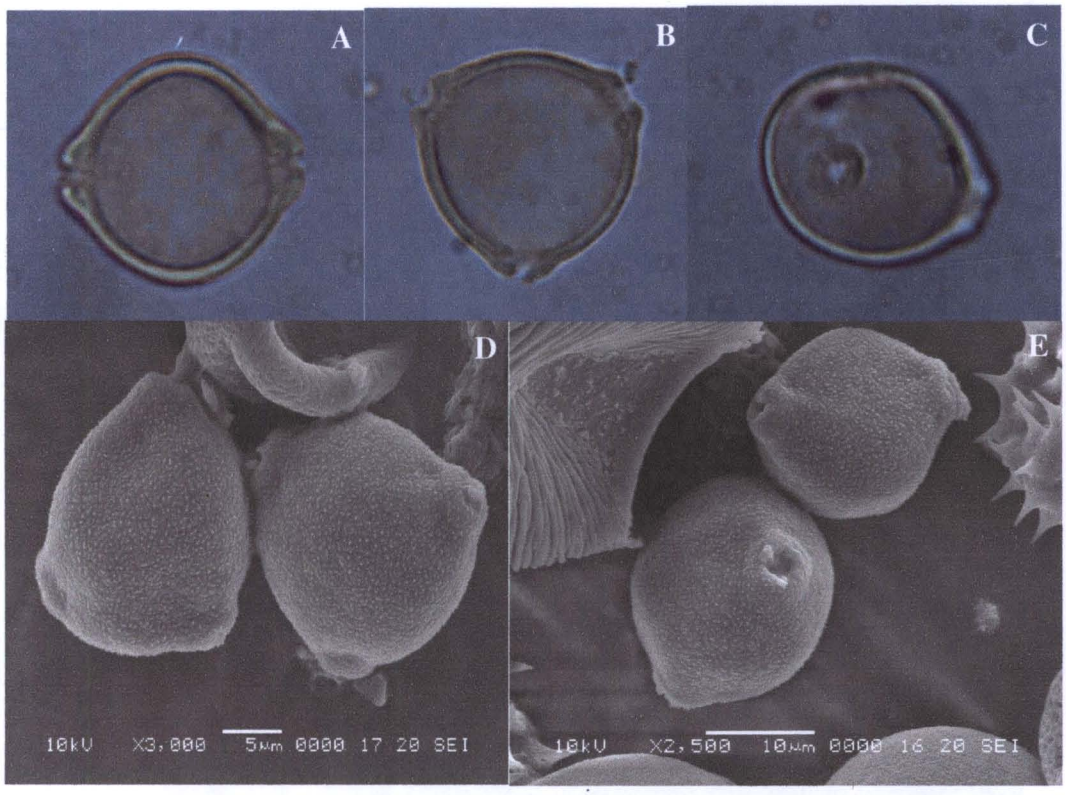

Figure 9. Kamlung suea Khrong (*Betula alnoides* Buch. – Ham. ex G.Don) pollen (code TC7), Betulaceae, LM 100x : A) equatorial view, B) polar view, C) triporate aperture, SEM : D) exine sculpturing (3,000x) and E) aperture (2,500x). **Pollen descriptions** : Monad, triporate, isopolar, radial symmetry, polar view : oblate – spheroidal, equatorial view : [amb.] semiangular, small grain, polar axis length 20.6 µm and equatorial axis 21.1 µm, exine thickness 1 µm.

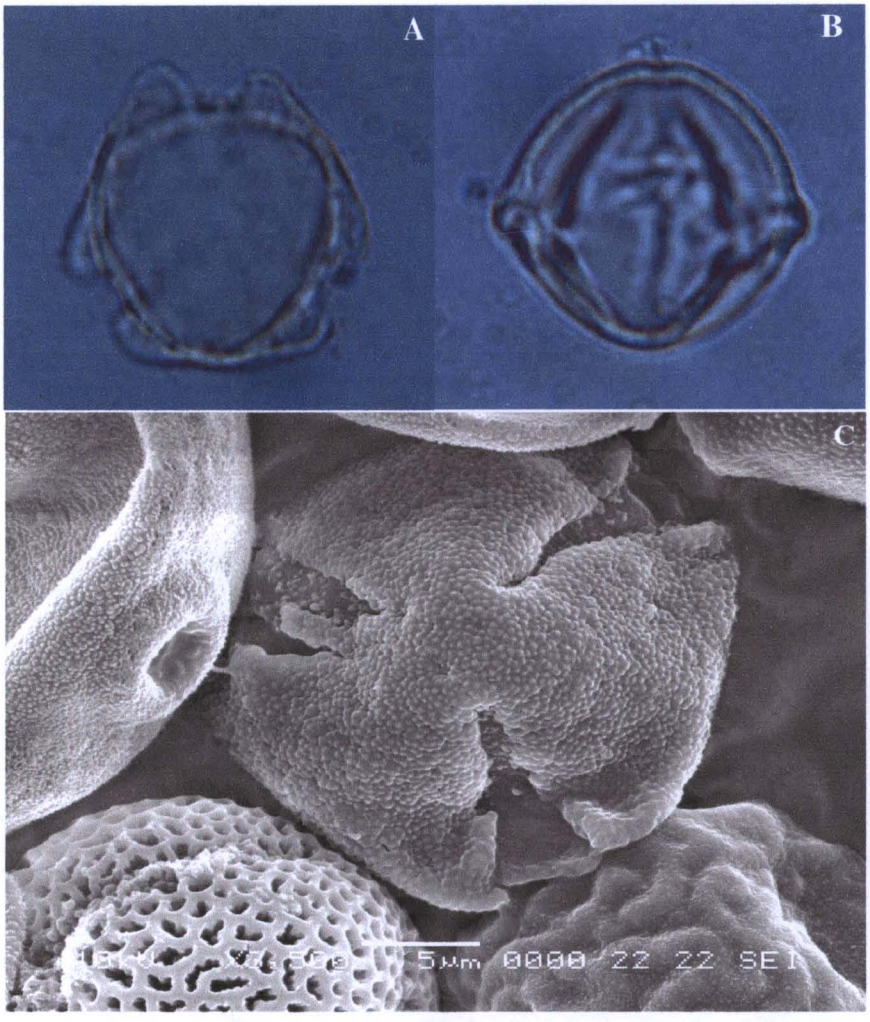

Figure 10. Prik (*Capsicum frutescens* L.) pollen (code TC8), Solanaceae, LM 100x : A) equatorial view, B) polar view, SEM 3,500x : c) tricolporate aperture and exine sculpture. **Pollen descriptions** : Monad, tricolporate; isopolar, radial symmetry, polar view : prolate-spheroidal or spheroidal, equatorial view : (amb.) semiangular, small grain, polar axis 21.2-24.9 µm, equatorial axis 18.9-22.4 µm, exine thickness 1.6 µm

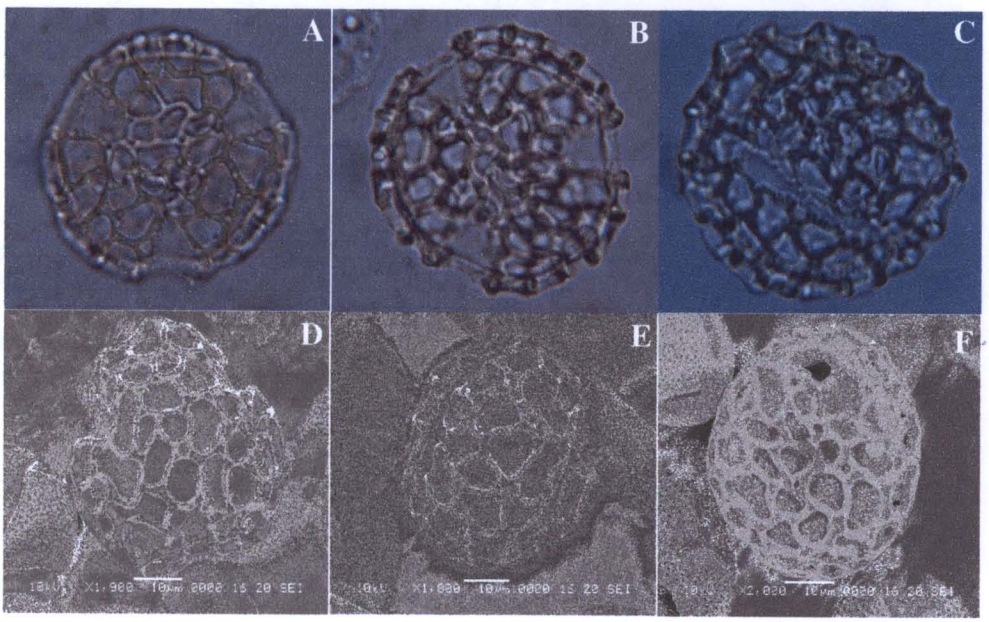

Figure. 12 Arang (*Peltophorum dasyrachis* (Miq.) Kurz) pollen (code TC9), Caesalpiniaceae, LM 100x : A, B) polar view, C) equtorial view, SEM : D) polar view (1,900x), E) equatorial view (1,800x) and F) lopho-reticulate exine sculpture (2,000x). **Pollen descriptions** : Monad, tricolporate, isopolar, radial symmetry, polar view : spheroidal, equatorial view : (amb.) circular, medium grain, polar axis 50.1 μm, equatorial axis 49.9 μm, exine structure not clear, lopho-reticulate exine sculpturing

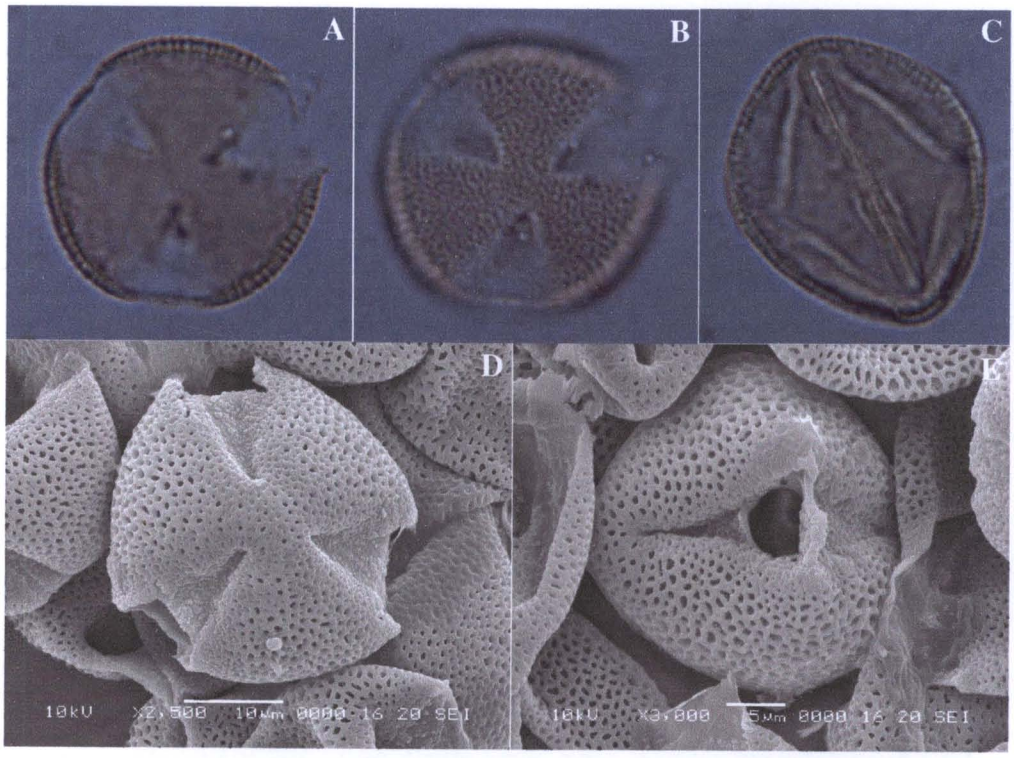

Figure 13. Thalo (*Schima wallichii* (DC.) Korth.) pollen (code TC10), Theaceae, LM 100x : A, B) polar view, C) equtorial view, SEM : D) polar view (2,500x) and E) equatorial view (3,000x) with finely reticulate exine sculpturing. **Pollen descriptions** : Monad, tricolpolate, isopolar, radial symmetry, polar view : spheroidal or oblate-spheroidal, equatorial view : (amb.) circular, medium grain, polar axis 28.1-35.1 µm, equatorial axis 32.1-39.7 µm, exine thickness 2 µm, sexine 1.5 µm, nexine 0.5 µm, finely reticulate exine sculpturing

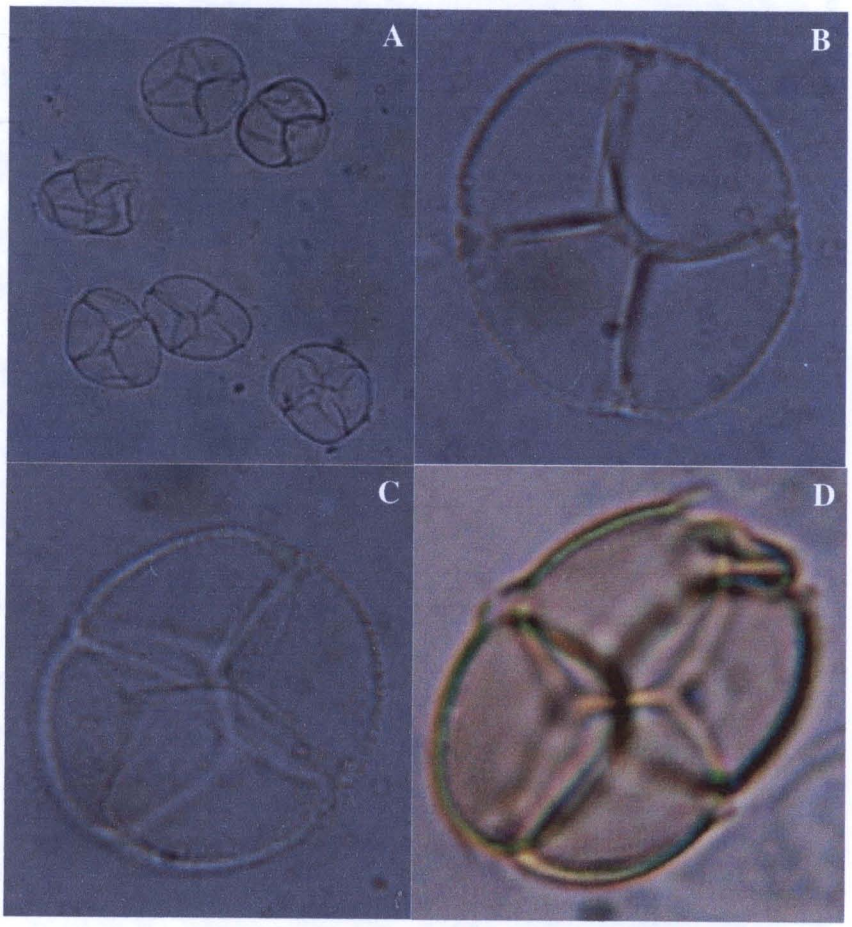

Figure 14. Maiyarap (*Mimosa pigra* L.) pollen (code TC11), Mimosaceae, LM : A) tetrad pollen (40x), B, C) apolar and asymmetry (100x) and D) rhomboidal shape (100x). **Pollen descriptions** : Tetrads, apolar and asymetry, rhomboidal shape, medium grain, 29x37 µm in size, exine thickness 1 µm, scrabate exine sculpturing

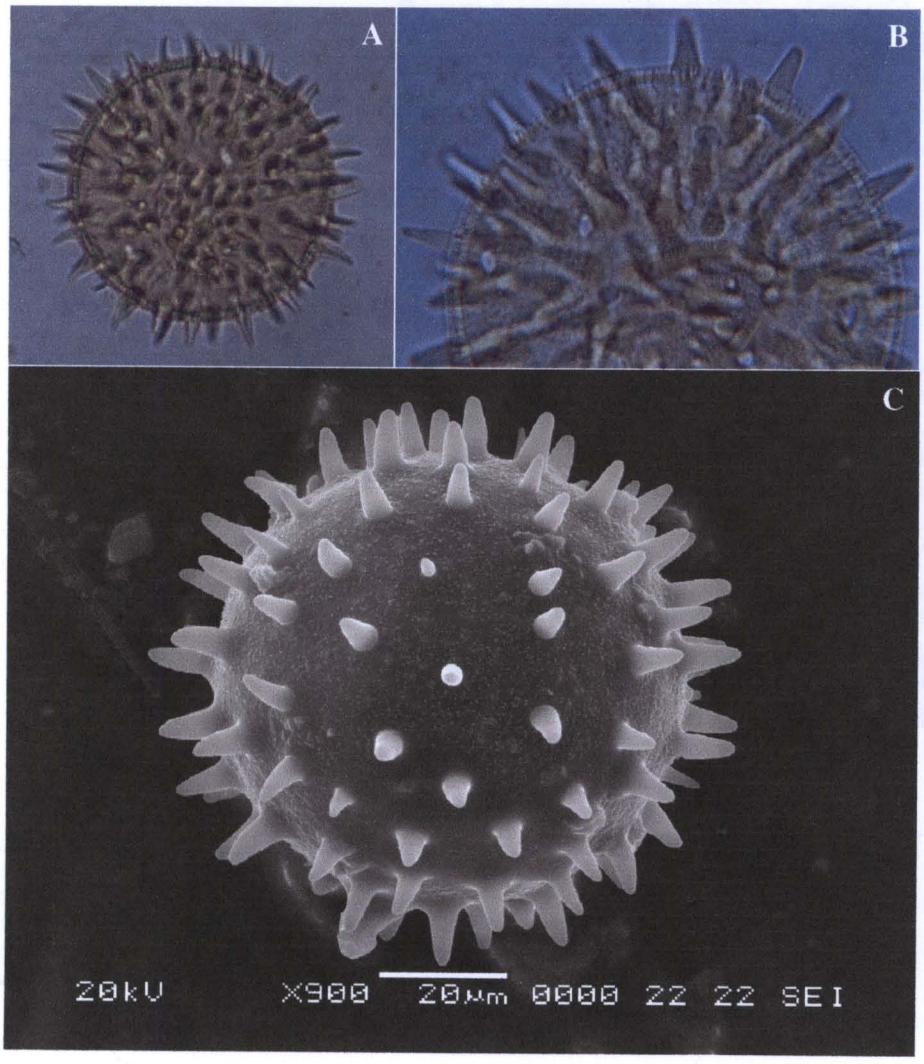

Figure 15. Po lom pom (*Thespesia lampas* (Cav.) Dalz. & Gibs.) pollen (code TC12), Malvaceae, LM : A) spheroidal shape (40x), B) periporate aperture (100x), SEM : C) , scabrate and baculate exine sculpturing (900x). **Pollen descriptions** : Monad, periporate, apolar, radial symmetry, spheroidal and circular, large grain, 80-90 µm diameter, exine thickness 4 µm, scabrate and baculate (4.1x9.2 µm) exine sculpturing

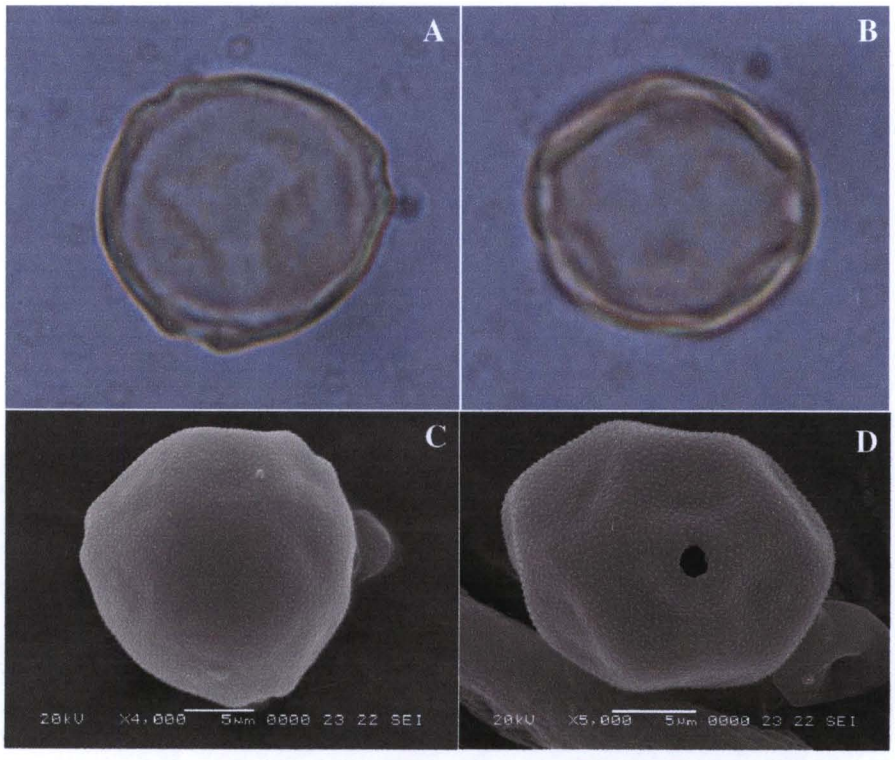

Figure. 16 Kha hot (*Engelhardtia spicata* Blume) pollen (code TC13), Juglandaceae, LM 100x : A) quatorial view, B) polar view, SEM : C) triporate aperture (4,000x) and D) scabrate exine sculpturing (5,000x). **Pollen descriptions** : Monad, triporate, isopolar and radial symmetry, polar view : oblate-spheroidal, equatorial view : (amb.) circular, small grain, polar axis 17.5 μm, equatorial axis 19.6 μm, exine thickness 1.5 μm and scabrate exine sculpturing

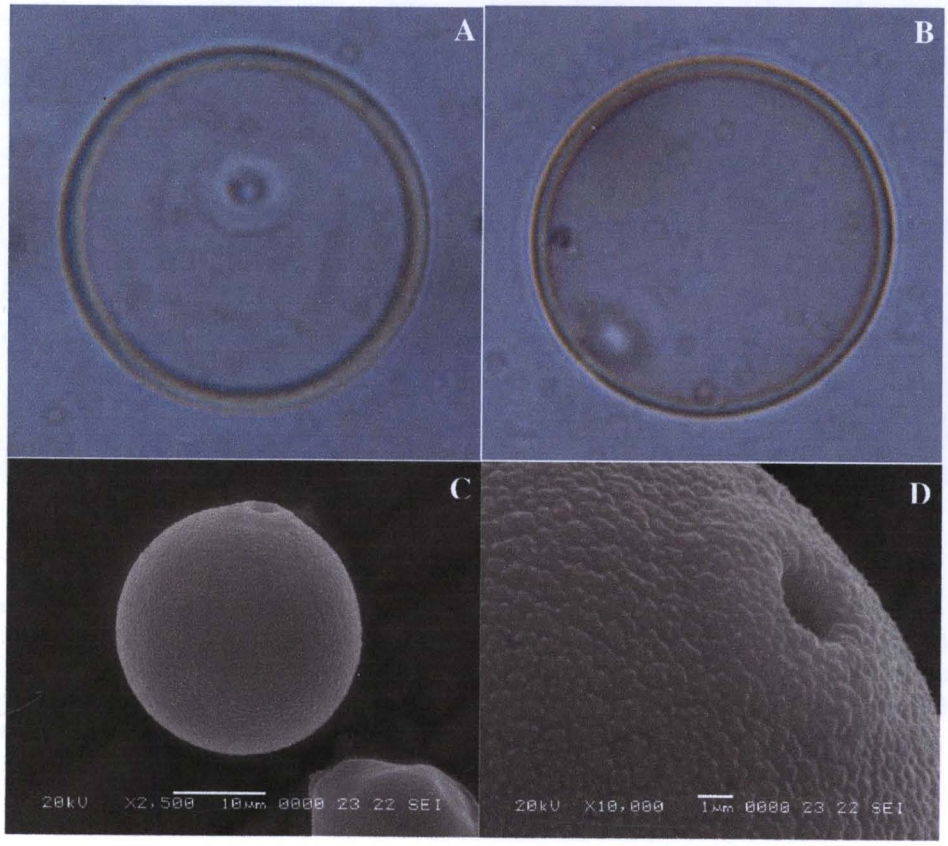

Figure 17. Ya yung (*Capillipedium parviflorum* (R.Br.) Stapf.) pollen (code TC14), Gramineae, LM 100x : A) equatorial view, B) polar view, SEM : C) monoporate aperture (2,500x) and D) scabrate exine sculpturing with annulus (10,000x). **Pollen descriptions** : Monad, monoporate, heteropolar and bilateral symmetry, polar view : spheroidal, equatorial view : (amb.)circular, medium grain, polar axis 26.4-27.9 µm, equatorial axis 26.6-28.6 µm, annulus 3 µm, exine thickness 2µm, scabrate exine sculpturing

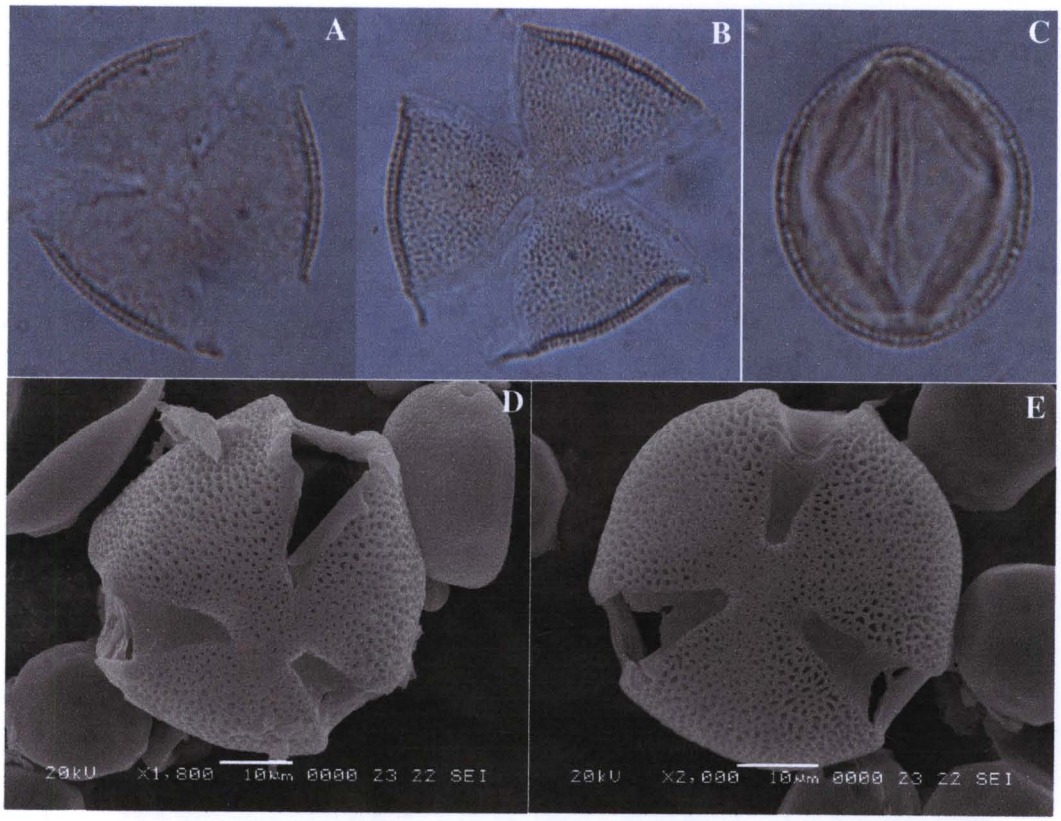

Figure. 19 Sieo dok khaao (*Bauhinia variegata* L.) pollen (code TC15), Caesalpiniaceae, LM 100x : A) polar view, B) tricolpate aperture, C) equatorial view, SEM : D) polar view (1,800x) and E) apocolpium and mesocolpium exine sculpturing (2,000x). **Pollen Description** : Monad, tricolporate ; isopolar and radial symmetry, polar view : spheroidal, equatorial view : (amb.) circular, large grain, polar axis 50x55 µm, equatorial axis 50 – 55 µm, exine thickness 2 µm, exine sculpturing apocolpium and mesocolpium

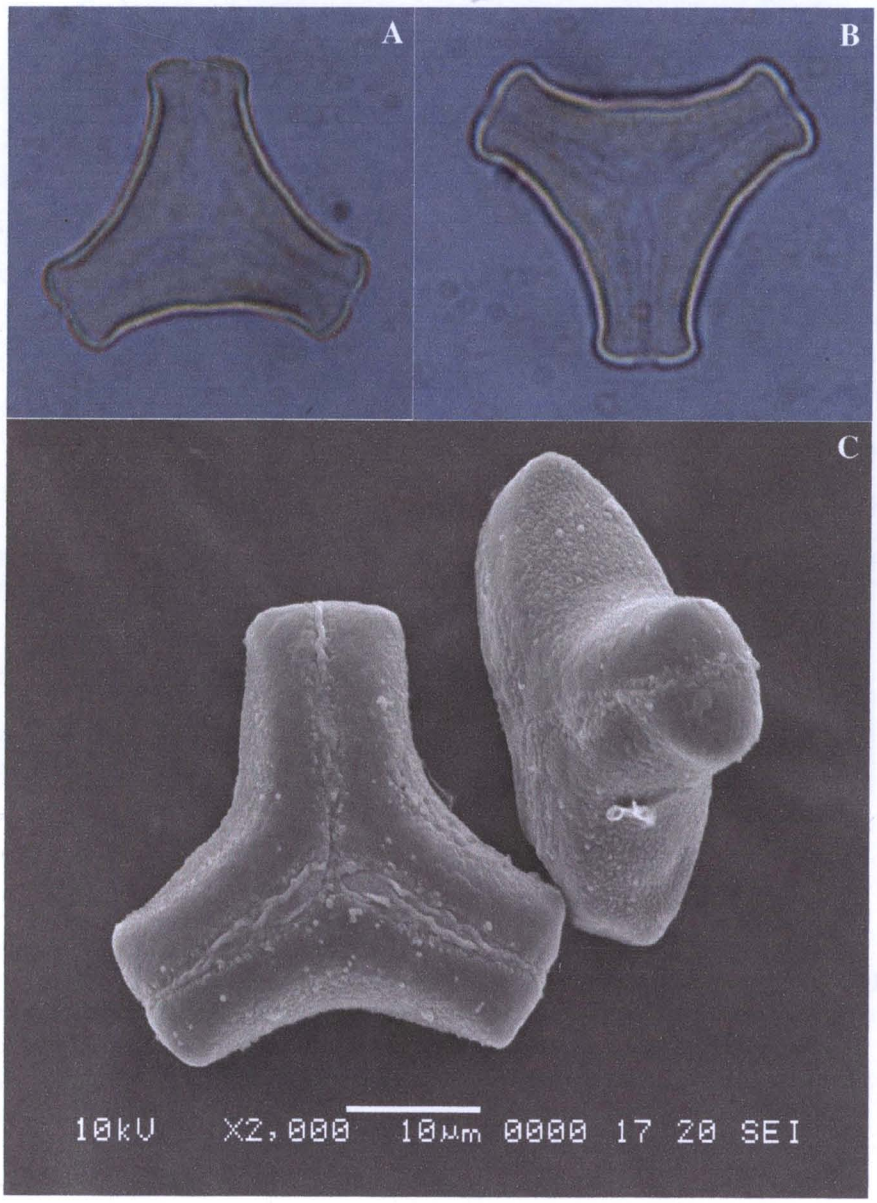

Figure. 18 Kaafaak-like *(Scurrula sp.)* pollen (TC16), Loranthaceae, LM 100x : A) equatorial view, B) tricolpate aperture, SEM 2,000x : C) polar and equatorial view. **Pollen description** : Monad, tricolpate ; isopolar and radial symmetry, polar view : peroblate, equatorial view : (amb.) lobate, small grain, polar axis 12.0-15.2 µm, equatorial axis 23.3 µm, exine thickness 3 µm

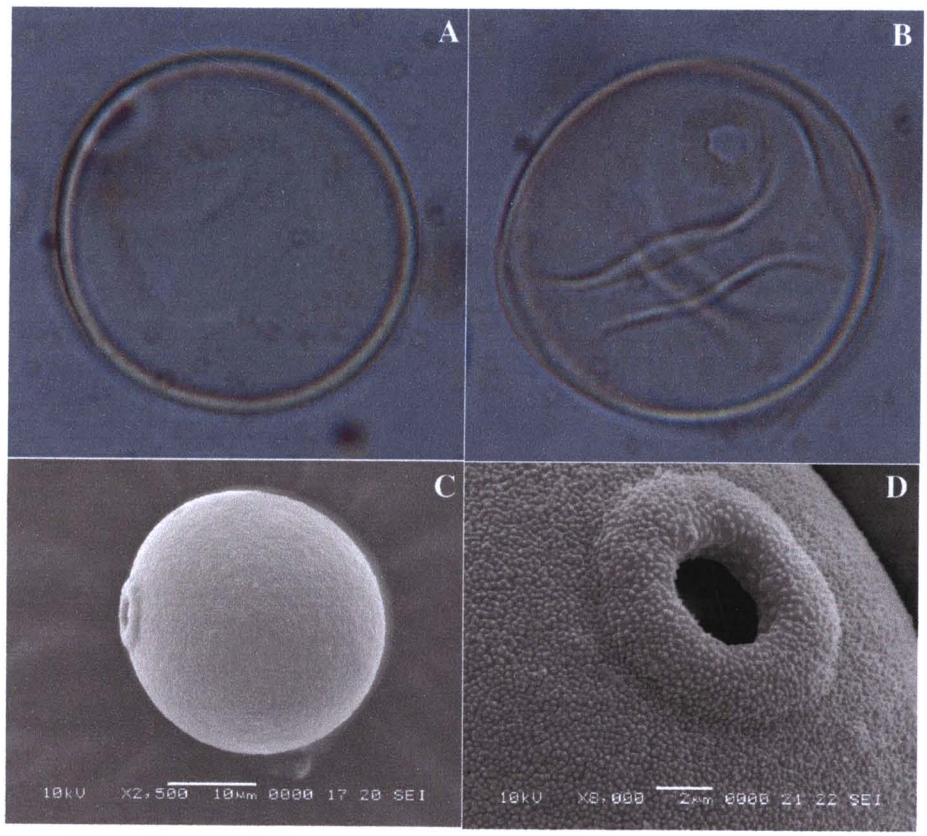

Figure 20. Gramineae pollen (code TC17), LM 100x : A) polar view, B) equatorial view, SEM : C) monoporate aperture (2,500x) and D) scabrate exine sculpturing with annulus (8,000x). **Pollen descriptions** : Monad, monoporate, heteropolar, bilateral symmetry, polar view : prolate-spheroidal, equatorial view : (amb.) circular, medium grain, polar axis 32.9 µm, equatorial axis 29.4 µm, exine thickness 1.5 µm, scabrate exine sculpturing

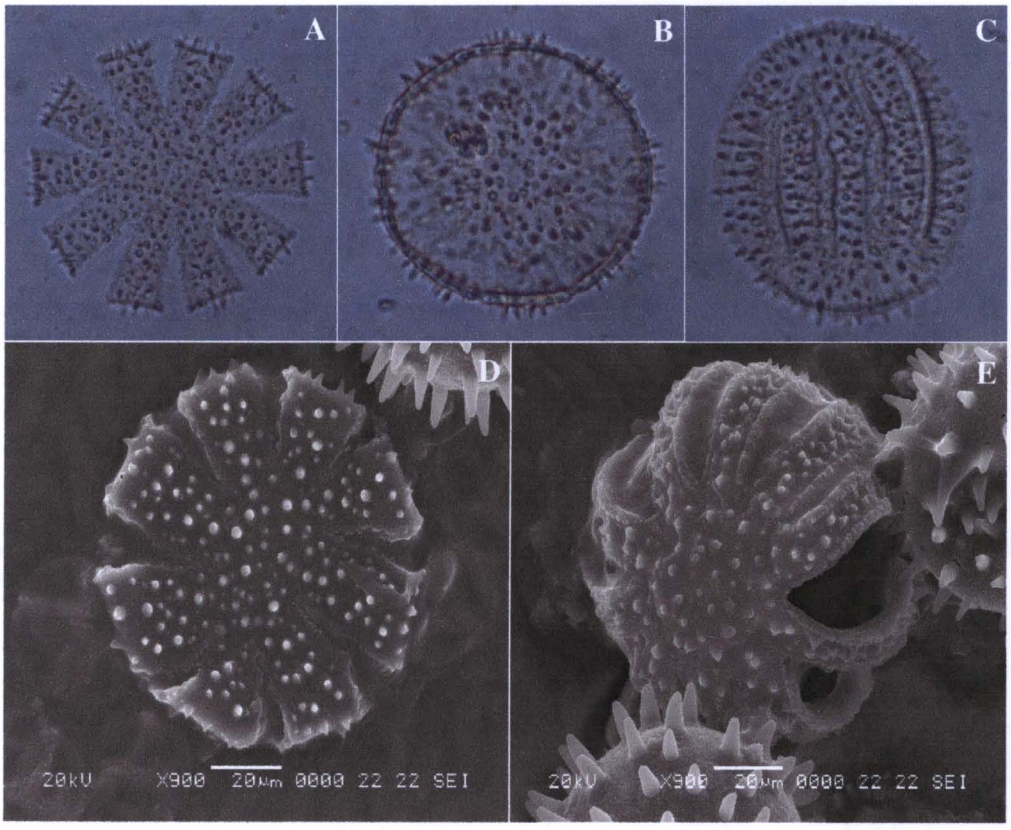

Figure. 21 Verbenaceae pollen (code TC18), LM 40x : A, B) polar view, C) equatorial view, SEM 900x : D, E) stephanocolpate aperture and echinate exine sculpturing. **Pollen descriptions** : Monad, stephanocolpate, isopolar, radial symmetry, polar view :spheroidal, equatorial view : (amb.) circular, large grain, polar axis 50.1 µm, equatorial 49.9 µm, exine thickness 2 µm, exine sculpturing echinate (1.8x4.1 µm)

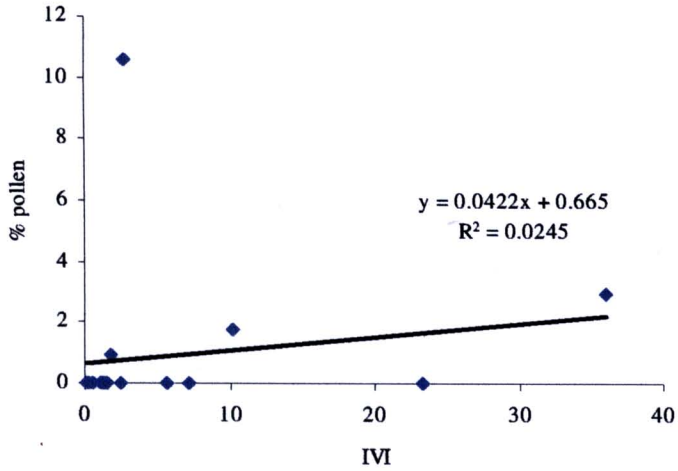

Figure 22. Correlation between Importance value index (IVI) and percent of pollen of the same species from 18 plant species found in pollen load of *Trigona collina* Smith stingless bee

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Behav Ecol Sociobiol (2006) 61:91–97
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ORIGINAL ARTICLE

The reproductive dilemmas of queenless red dwarf honeybee (*Apis florea*) workers

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Abstract Honeybee (*Apis*) workers cannot mate, but retain functional ovaries. When colonies have lost their queen, many young workers begin to activate their ovaries and lay eggs. Some of these eggs are reared, but most are not and are presumably eaten by other workers (worker policing). Here we explore some of the factors affecting the reproductive success of queenless workers of the red dwarf honeybee *Apis florea*. Over a 2-year period we collected 40 wild colonies and removed their queens. Only two colonies remained at their translocated site long enough to rear males to pupation while all the others absconded. Absconding usually occurred after worker policing had ceased, as evidenced by the appearance of larvae. Dissections of workers from eight colonies showed that in *A. florea*, 6% of workers have activated ovaries after 4 days of queenlessness, and that 33% of workers have activated ovaries after 3 weeks. Worker-laid eggs may appear in nests within 4 days and larvae soon after, but this is highly variable. As with *Apis mellifera*, we found evidence of unequal reproductive success among queenless workers of *A. florea*. In the two colonies that reared males to pupation and which we studied with microsatellites, some subfamilies had much higher proportions of workers with activated ovaries than others. The significance of absconding and internest reproductive parasitism to the alternative reproductive strategies of queenless *A. florea* workers is discussed.

Keywords Reproductive competition · *Apis florea* · Red dwarf honeybee · Social parasitism

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Introduction

Honeybee (*Apis*) colonies have been described as being "superorganisms," in which reproduction is channeled exclusively through the queen and selection on workers is restricted to maximizing their efficiency as part of a coordinated whole (Seeley 1989; Moritz and Southwick 1992). Yet, workers have ovaries that are capable of producing male-producing eggs via parthenogenesis (Dzierzon 1845). In the presence of a queen, however, any eggs that workers do lay will almost certainly be removed ("policed") by other workers (Ratnieks 1988; Ratnieks and Visscher 1989).

Worker policing is an adaptive conflict-reducing strategy of many social insect species in which colonies are headed by a single, multiply mated queen (Ratnieks 1988; Barron et al. 2001; Ratnieks et al. 2006). All workers are equally related to the male-destined eggs laid by the queen [relatedness (r)=0.25], but each individual worker is variably related to eggs laid by herself (r=0.5), her full

sisters (r=0.375), and her half sisters (r=0.125) (Ratnieks 1988). This means that the only stable evolutionary compromise for workers is to raise the eggs laid by the queen and to remove all eggs laid by workers. Because worker policing is effective, the benefits of personal reproduction by workers are minimal (Wenseleers et al. 2004a,b; Ratnieks et al. 2006). This probably explains why, in most *Apis* species, the ovaries of workers in queenright colonies are inactive (*Apis mellifera*, Ratnieks 1993; *Apis florea*, Halling et al. 2001; *Apis dorsata*, Wattanachaiyingcharoen et al. 2001), a curious and unexplained exception being *Apis cerana*, where 2–5% of queenright workers have ovaries containing eggs (Blanford 1923; Bai and Reddy 1975; Oldroyd et al. 2001).

Despite functional sterility when colonies are queenright, when a colony becomes queenless many of the workers that have not yet begun foraging begin to activate their ovaries (Lin et al. 1999) and lay eggs and may produce a final batch of males before the colony perishes (Page and Erickson 1988; Robinson et al. 1990; Halling et al. 2001; Oldroyd et al. 2001). To rear the eggs, the colony must undergo a fundamental behavioral shift and cease worker policing (Miller and Ratnieks 2001; Châline et al. 2004; Martin et al. 2005), for without successful worker reproduction, the fitness of a queenless colony is zero (Page and Erickson 1988; Barron et al. 2001; Miller and Ratnieks 2001).

In contrast to queenright nests, when colonies become queenless, the egalitarian queen-laid eggs are no longer present, leading to the prediction of overt reproductive conflict among queenless laying workers (Robinson et al. 1990; Martin et al. 2004), and to two alternative reproductive strategies.

In the first strategy, a worker can remain with the natal nest, cease worker policing, attempt to lay her own eggs, and help rear both her own eggs and those of other workers. This strategy appears to be the usual one in *A. mellifera* (Page and Erickson 1988; Miller and Ratnieks 2001; Martin et al. 2004; Martin et al. 2005). In queenless colonies, each worker would "prefer" that the colony's nurturing effort were directed at her own sons or to the sons of her full sisters than to the sons of her half sisters. Thus, competition is predicted among subfamilies over their relative share of the drones that the queenless colony is able to rear. As predicted, strongly unequal reproductive success has been observed in queenless colonies of *A. mellifera* (Page and Erickson 1988; Martin et al. 2004). Differential reproductive success probably arises from workers preferentially rearing or cannibalizing eggs and from different rates of ovary activation among workers (Robinson et al. 1990; Martin et al. 2004).

In the second strategy, a worker in a queenless colony can abandon her natal nest and attempt to be accepted by another colony. If a worker is successful in being adopted, she will be completely unrelated to the resident workers, and is predicted to parasitize her host colony with her eggs if she can. However, this strategy is only likely to be fruitful if she can be adopted by a queenless colony, for queenright colonies should police worker-laid eggs.

Although workers with activated ovaries are very rare in queenright *A. florea* colonies (Halling et al. 2001), workers activate their ovaries after only a few days of queenlessness (Nanork et al. 2005). This contrasts with temperate ecotypes of *A. mellifera*, in which ovary activation and successful oviposition does not occur for about 3 weeks after dequeening (Page and Erickson 1988; Miller and Ratnieks 2001). A colony of *A. florea* comprises multiple subfamilies (Palmer and Oldroyd 2001), and so, reproductive competition among workers from different subfamilies is predicted as in *A. mellifera* (Martin et al. 2004).

The cessation of worker policing in a queenless colony renders it vulnerable to parasitism by egg-laying workers from other colonies, and such parasitism has now been documented in *A. mellifera capensis* (Neumann et al. 2001b) and *A. florea* (Nanork et al. 2005). Honeybee workers may actively seek queenless colonies to parasitize with their eggs, and similar phenomena have been seen in bumblebees (Birmingham et al. 2004; Lopez-Vaamonde et al. 2004).

Here we examine patterns of absconding (which may be associated with parasitism) and the appearance of eggs and larvae in 40 queenless colonies of *A. florea* with a view to understanding how such colonies respond to the twin necessity of ceasing worker policing so that eggs can be reared and minimizing parasitism by workers from other colonies. We also report on the probable existence of reproductive competition among subfamilies within two queenless colonies. This report extends our findings of reproductive parasitism in *A. florea* (Nanork et al. 2005).

Materials and methods

Experimental colonies and sample collection

Between December 2002 and October 2004, we relocated 40 colonies of wild *A. florea* from coconut plantations in Samutsongkram province, Thailand, to the grounds of Chulalongkorn University, Bangkok. We tied the translocated colonies (usually 4–5 at a time) in convenient locations on the branches of small trees, at least 5 m apart from each other. For each relocated nest we collected approximately 100 adult workers and then removed the queen to induce ovary activation in the workers. Any queen cells that subsequently developed were removed. One hundred workers were collected again after 4 days, 1 week, and 4 weeks of queenlessness. Samples were kept at −20°C.

From a subset of eight randomly selected colonies, we dissected adult workers (n=100 per colony) according to Oldroyd et al. (2001) to determine the level of ovary activation from various sampling times. We classified ovaries as either inactive (ovarioles not discernable or no eggs present) or active (eggs present) (we find this to be a more objective measure of ovary activation than various gradations that have been used by some authors). For the two colonies that successfully reared pupae, we used microsatellite loci to determine the parentage of the dissected workers. We did not genotype early-appearing larvae because there were insufficient numbers for an adequate sample, and we wished to allow them to develop to pupae.

As part of another experiment (Duangphakdee 2006), eight additional colonies were translocated and these were left queenright. These colonies were used to determine if absconding rates differ between queenright and queenless nests.

Paternity analysis of dissected workers

DNA was extracted by boiling the crushed hind leg of a bee in 500 µl Chelex® 100 solution (5% w/v in $TE_{0.1}$) (Walsh et al. 1991; Estoup et al. 1997). Samples were boiled for 15 min and then centrifuged at 1,200 rpm for 15 min. The supernatants were diluted 1:2 with sterile distilled water and stored at 4°C.

Six microsatellite loci (Table 1) were used to determine the subfamily of workers. Diluted DNA (1 µl) was used in 5-µl PCR reactions [1× PCR buffer, 1.5 mM $MgCl_2$, 0.625 mM of each deoxyribonucleotide triphosphate, 0.4 mM of forward and reverse primer (reverse primer Hex labeled), and 0.25 units of *Taq* polymerase; see also Table 1]. PCR products were electrophoresed in 5% urea/polyacrylamide on an automated DNA fragment analyzer (Corbett Research, Sydney, Australia) at 1,400 V and 38°C. Lengths of microsatellite alleles were determined in base pairs using the software package OneDscan (Scananalytics, Fairfax, VT, USA).

For each sample we compared rates of ovary activation among worker subfamilies using G tests (Zar 1996). Where a large number of cells of a contingency table have expected values <5, the G test can produce levels of significance that deviate from the actual. Therefore, a sampling modification of Fisher's exact test (Lewontin and Felsenstein 1965) was also performed using the program Monte Carlo RxC (W. Engels, University of Wisconsin).

Results

Because not all colonies were observed every day (but always more than twice a week), all time-dependent data in this section are approximate by up to ±3.5 days.

About a quarter of the queenless colonies absconded within 2 weeks of being made queenless, and all but two queenless colonies absconded within 1 month (Fig. 1). Absconding was not a gradual process over several days or weeks; most workers left together as a group. In all but two cases, absconding occurred before pupae appeared. The mean number of days to absconding was 20.4 (SE=3.6) days. Although the mean time to absconding in queenright nests (24.2±2.9 days) was similar (Mann–Whitney U=152.0, P=0.82) to that of queenless nests, absconding was much more bimodal; colonies either left as soon as the last brood emerged (five colonies) or remained for 34, 46, or 67 days.

In the queenless nests the proportion of workers with activated ovaries was 6.0% (SE=1.6%) after 4 days. The proportion of workers with activated ovaries increased across time, so that after 3 weeks, about 33.7% (SE=3.2%) of workers had activated ovaries (Fig. 2). In the 32 colonies in which eggs were produced, eggs were first observed an

Table 1 Primer sequences and PCR conditions for six microsatellite loci used to detect paternity in *A. florea*

Locus	Primer sequences	$MgCl_2$ conc. (mM)	Annealing temp. (°C)	Number of cycles	References
A8	5′CGAAGGTAAGGTAAATGGAAC 5′GGCGGTTAAAGTTCTGG	1.5	55	35	Estoup et al. 1994
A76	5′GCCAATACTCTCGAACAATCG 5′GTCCAATTCACATGTCGACATC	1.5	55	35	
A88	5′CGAATTAACCGATTTGTCG 5′GATCGCAATTATTGAAGGAG	1.5	55	35	
A107	5′CCGTGGGAGGTTTATTGTCG 5′GGTTCGTAACGGATGACACC	1.5	55	35	
B124	5′GCAACAGGTCGGGTTAGAG 5′CAGGATAGGGTAGGTAAGCAG	1.5	55	35	
Ap249	5′CGCGCGACGACGAAATGT 5′CAGTCCTTTGATTCGCGCTACC	1.5	57 55 52 49	9 9 9 15	Solignac et al. 2003

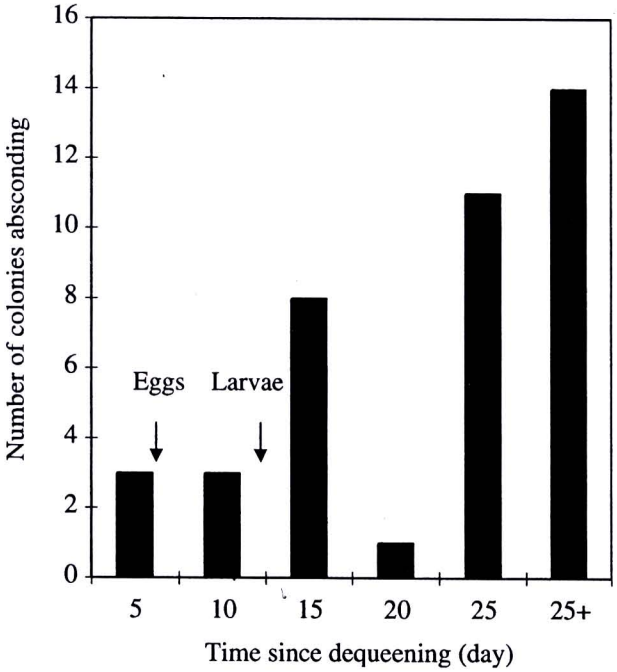

Fig. 1 The numbers of *A. florea* colonies absconding per 5-day period after dequeening. The figure also shows the average time of the first appearance of eggs and larvae

average of 6.1 (SE=0.3) days after dequeening. The shortest period to the appearance of eggs was 3 days, and the longest was 8 days. Only 14 colonies produced larvae. In these colonies, larvae appeared 12.1 (SE=0.9) days after queen removal. The shortest period for larvae to appear was 3 days after eggs appeared and 7 days after dequeening. As the development time of an egg is 3 days in *A. florea* (reviewed in Oldroyd and Wongsiri 2006), this suggests that policing behavior was curtailed as soon as oviposition commenced. In another colony, larvae did not appear until 16 days after eggs first appeared, suggesting that policing behavior in this colony continued long after eggs were being laid. There was no significant correlation between the time to the appearance of the first eggs and the time before larvae appeared (r=−0.18, n=14 colonies P=0.54).

Only two colonies (hereafter 1 and 2, and the same colonies as in Nanork et al. 2005) remained long enough to rear pupae. Microsatellite analysis based on 299 and 282 workers from colonies 1 and 2, respectively, indicated that there were 20 subfamilies in colony 1 and 24 subfamilies in colony 2.

As previously reported, after colonies were made queenless, they showed high rates of reproductive parasitism, with an average 4.5% of workers coming from other colonies (Nanork et al. 2005). Excluding nonnatal workers, there was no significant difference in the proportion of workers with activated ovaries among subfamilies 1 week after dequeening (Table 2, Figs. 2 and 3). However, after 4 weeks, some subfamilies had a much higher proportion of workers with activated ovaries than others (Table 2, Figs. 2 and 3). In colony 2, the subfamilies with activated ovaries were different between the two sample times (significant heterogeneity, Table 2), whereas in colony 1 the reproductively dominant subfamilies did not change over time. The microsatellite loci available were not sufficiently variable to be able to assign the maternity of offspring males to different worker patrilines, but were sufficient to demonstrate whether males arose from natal or nonnatal workers.

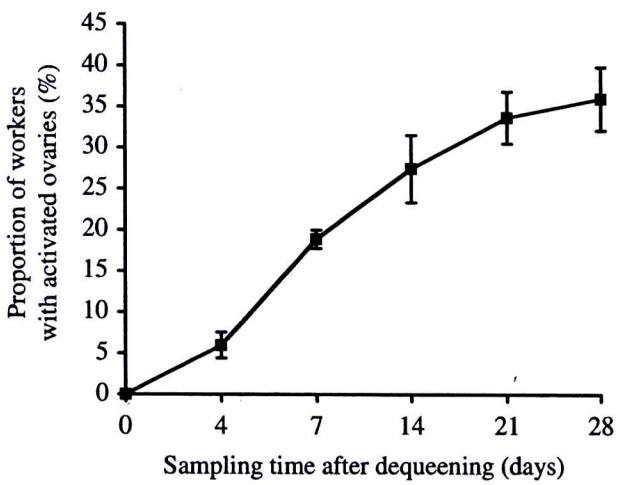

Fig. 2 Average proportion (%) of *A. florea* workers with activated ovaries (n=8 colonies) following queen removal (including nonnatal workers). The *bars* indicate SEs of the mean

Discussion

This study has shown that the reproductive behaviors of queenless *A. florea* workers are complex and variable. In all colonies workers begin to activate their ovaries within 4 days of queen loss, even while female larvae (from which

Table 2 Contingency table analyses of the proportions of adult workers with activated ovaries among different subfamilies in *A. florea* colonies

Colony	Sampling time	G	df	P	P_{Fisher}
1	Week 1	12.55	12	0.4	0.778
	Week 4	33.92	13	0.001	0.002
	Totals	32.26	15	0.006	0.012
	Heterogeneity	32.26	10	0.161	
2	Week 1	20.45	18	0.308	0.491
	Week 4	37.69	19	0.006	0.015
	Totals	58.14	21	0.112	0.163
	Heterogeneity	20.07	16	0.023	

P_{Fisher} is the P value from a Monte Carlo approximation of the Fisher's exact test and P is the probability associated with the presented G test. The table is based on 100 bees per colony per sample period

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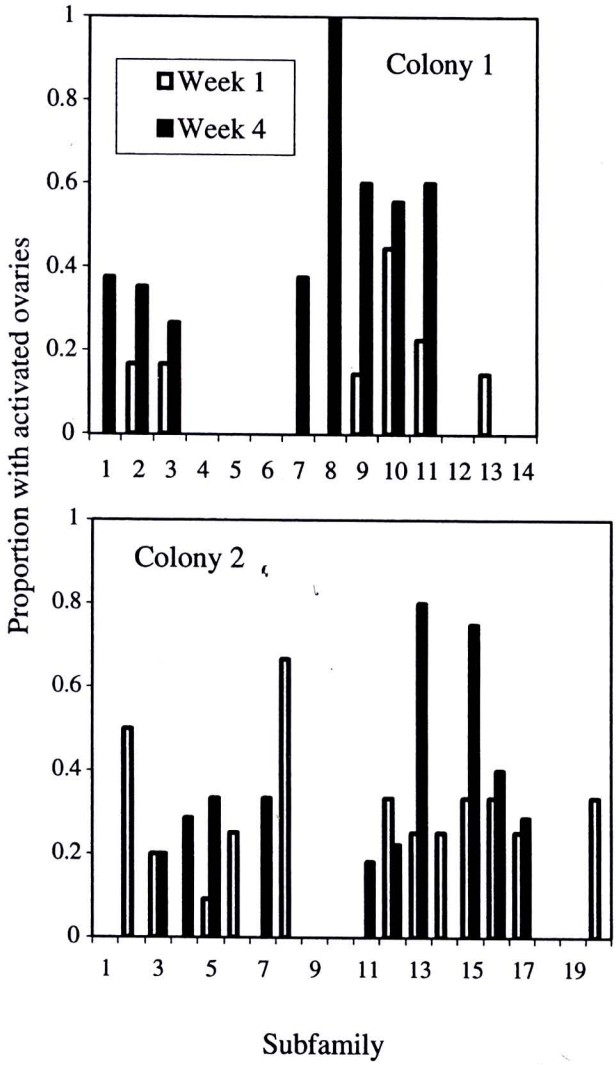

Fig. 3 Proportion of workers of *A. florea* with activated ovaries among different subfamilies after 1 and 4 weeks of dequeening

a replacement queen could be reared) are still present. Worker oviposition occurs soon after ovary activation, as worker-laid larvae can be present within 7 days of dequeening. However, in some colonies, larvae do not appear for at least 2 weeks after the first worker oviposition. This suggests that in these colonies worker policing continues well after oviposition begins, as it does in *A. cerana* (Oldroyd et al. 2001) and temperate *A. mellifera* (Miller and Ratnieks 2001; Martin et al. 2005). After policing stops and larvae appear, almost all colonies abscond before drone pupae appear.

In the first 3 weeks after transfer, queenless and queenright nests have similar rates of absconding. After 3 weeks, however, queenright nests may remain in situ for several months, whereas all queenless colonies abandon their nests within a month. We suggest that nest relocation per se is not a factor in absconding after 3 weeks and that, therefore, queenlessness is a causal factor in absconding. If a queenright nest does abscond, it does so in an orderly fashion, waiting until all brood has emerged (Woyke 1976; Akratanakul 1977; Seeley 1985). This contrasts with the behavior of queenless nests, which often abandon their brood when they abscond.

Queenless *A. mellifera* colonies of temperate subspecies rarely abscond, and this seems an adaptive strategy. If a queenless colony reestablishes at another site, it must first build a new comb before it can commence brood rearing anew, wasting the inherited comb and male pupae that are the orphaned workers' sole heirs. Why, then, should *A. florea* colonies that are about to produce male pupae commonly abscond? We can think of four plausible explanations, but none of these are completely satisfactory, and we do not advocate any one of them at this time.

First, the orphaned workers might benefit from a comb comprising solely of drone cells (Page and Erickson 1988; Neumann et al. 2000; Halling and Oldroyd 2003). We have previously reported a small colony of queenless *A. florea* workers that had built a comb comprised entirely of drone cells (Oldroyd and Wongsiri 2006). However, it would seem that abandoning a comb just to build a new one would reduce the average reproductive success of an orphaned colony, and that it would be more efficient to remodel the existing comb to include more drone cells. Furthermore, if comb remodeling is the goal of absconding, it is difficult to understand why it does not occur more or less immediately after queen loss and before ovary activation.

Second, an absconding swarm may attempt to combine with a queenright colony, as has been observed in *A. m. capensis* (Hepburn et al. 1999; Neumann et al. 2001a). However, because *A. florea* have strong nestmate recognition, and reject nonnestmates that are introduced to their colony (Free and Williams 1979), it seems unlikely that an entire swarm of orphaned workers would be accepted by another colony. There is an obvious reason why a queenright colony should not adopt orphaned workers: such workers are unrelated to the queenright nest, and because so many of the orphaned workers have activated ovaries, they have both the motive and ability to parasitize their adoptive hosts.

Third, absconding swarms might attempt to combine with another queenless nest, but again, there is no obvious advantage to the host colony, which is highly vulnerable to parasitism (Nanork et al. 2005) because policing is no longer effective. Thus, the host colony is predicted to reject the immigrants.

Fourth, the high rates of parasitism by workers from other colonies (Nanork et al. 2005) may drive orphaned *A. florea* colonies to abscond. Workers that continue to contribute to the welfare of a queenless colony may be committing the Concorde fallacy (Dawkins and Carlisle 1976)—further investment in a colony in which reproductive returns are very low due to high rates of parasitism. We

speculate that the open nest that characterizes *A. florea* means that queenless colonies are more vulnerable to worker parasitism than cavity nesting species such as *A. mellifera*, and it is for this reason that queenless *A. florea* are likely to abscond in response to high rates of parasitism, whereas European *A. mellifera* subspecies are less likely to.

Although only two of our 40 dequeened colonies produced male pupae, this study provides evidence for overt reproductive competition among subfamilies. As with queenless *A. mellifera* colonies (Martin et al. 2004), workers of some subfamilies have a significantly higher proportion of individuals with activated ovaries than others. Thus, it seems likely that certain subfamilies have the ability to respond to the lack of queen and brood pheromones earlier than others (Moritz et al. 2000) and to activate their ovaries.

We conclude that queenless *A. florea* nests are highly unstable and vulnerable to reproductive parasitism. Many workers from queenless nests may join other nests. Host nests are vulnerable to reproductive parasitism by such workers, and worker policing may be an important defense against such parasitism.

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Original article

Reinforcing a barrier – a specific social defense of the dwarf honeybee (*Apis florea*) released by the weaver ant (*Oecophylla smaragdina*)[1]

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Abstract – In the arboreal habitat of *Apis florea* one of the dominant insectivorous predators is the weaver ant, *Oecophylla smaragdina*. The main mechanism of *A. florea* to protect its nest against ants and other crawling arthropods are "barriers" of sticky material (sticky bands) which the bees build around the branches and all structures which connect the comb to the outside. We studied whether the presentation of an *O. smaragdina* ant on the comb releases a specific behavioral response of the bees. After the exposure of a living *O. smaragdina* worker, held by a forceps on the top of the *A. florea* comb, the number of bees at the sticky band zone increased and remained on higher level for 2 hours compared to control experiments (presentation of an empty forceps, *Tenebrio molitor* larva or another arboreal ant species, *Crematogaster rogenhoferi*). Further, more sticky material was deposited by the bees after exposure of a weaver ant. This behavior seems to be a specific reaction of *A. florea* to its most important predator *O. smaragdina*.

Apis florea* / *Oecophylla smaragdina* / colony defense / predator-prey relationship / *Crematogaster rogenhoferi

1. INTRODUCTION

Apis florea Fabricius is widespread throughout Asia, where it builds its single comb around small branches of bushes and shrubs (Akratanakul, 1977). The exposed position of the comb with the honey storage, bees and brood attracts a range of predatory species, so it is not surprising that *A. florea* posses a wide array of specific social defence mechanisms among which shimmering (Butler, 1954; Pirk et al., 2002) and hissing are most conspicuous. Shimmering behaviour is released by optical stimuli and for example a butterfly – apparently attracted by the odour of honey – releases "shimmering behaviour" (a specific movement) of a few bees hanging in the curtain and which otherwise does not interfere with colony activities. Hissing behaviour is released by mechanical stimuli or by "piping" of forager bees disturbed on their return to the nest (Sen Sarma et al., 2002). Hissing alerts the whole colony and results in steep decrease of foraging activities. The hissing sound per se may repel mammals and even large Asian bears (Koeniger and Fuchs, 1973) or initiate a full stinging defence of the colony (Koeniger and Fuchs, 1975).

A different permanent threat in the arboreal habitat of *A. florea* is posed by the weaver ant *Oecophylla smaragdina* (Hölldobler and Wilson, 1983), which ranks among the most important predators of *A. florea* (Seeley, 1983).

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In direct fighting, the *A. florea* workers, because of their diminutive size, are no match for the large weaver ants. Colonies rely instead on sticky bands of plant resins plastered around the branch carrying the nest. Thus the comb with the brood, the honey storage and the bees are sealed off completely by sticky barriers on all structures connecting to other parts of the canopy. In a survey in northern Thailand, Seeley et al. (1982) examined 76 *A. florea* colonies. Only 28 of them had sticky bands, but 24 of those were under ant attack. This indicated a strong correlation between sticky bands and the presence of ants. Experimental data on the question how the presence of *Oecophylla smaragdina* ants is related to *A. florea*'s construction of sticky bands are not yet available.

In our experiments we provoked *A. florea* colonies by exposing a weaver ant on the top of the colony and observed the reaction of the bees in regard to the sticky band. In comparison to the weaver ant the reaction after presentation of an empty forceps, a *Tenebrio molitor* larva and another arboreal ant species (*Crematogaster rogenhoferi* Mayr) was recorded. Our aim was to assess whether the presence of a weaver ant inside the barriers of the sticky bands causes an immediate and specific social response of *A. florea* colonies.

2. MATERIALS AND METHODS

2.1. The bees

Observations and experiments were conducted at the campus of Chulalongkorn University (Bangkok) in March to August, 2003 and in January to April 2004.

Eighteen colonies of *A. florea* were collected in a coconut estate near Maeklong, Samut Songkram Province. The *A. florea* nests were detected in shrubs and trees at heights of 3 m to 5 m. Climbing up smoothly during day time the colony was gently smoked and afterwards sprayed with water. Surrounding leaves and twigs were carefully removed. Finally both sides of the nest branch were smoothly removed by sharp garden cutter. The colony was hung at one end of the nest branch and was brought down avoiding accelerations and jerks. Both ends of the nesting branch were tightly fixed in a wooden box for transportation to Bangkok. There, the colonies were hanged on small trees in front of the entomology laboratory (in CU campus) maintaining the vertical position of the comb. The bees started foraging pollen and nectar at the new location. Additionally, we offered 50 g candy (icing sugar mixed with honey) daily on the top of the comb.

2.2. The ants

O. smaragdina was also collected at Maeklong. We selected a fairly large (40 cm × 30 cm) leaf nest in a Mango tree and carefully clipped the surrounding twigs leaving only the branch with the nest. After the ants had calmed down and the main force of them had returned into the nest again (15 min) we cut the nesting branch and swiftly put the nest into a transport box which was closed immediately. In front of the entomology laboratory we fixed the *O. smaragdina* nest to a small potten Mango tree. The pot of this tree was kept in container filled with detergent water to prevent ants from escaping. The ants were fed with 10 g of *T. molitor* (Coleoptera: Tenebrionidae) larvae, 5 g of tinned mackerel fish and 10 mL of honey syrup per day.

Presentation of different objects to the *A. florea* colony

An *O. smaragdina* forager was collected from the feeding dish of the ant nest. A *C. rogenhoferi* worker was caught from a foraging path at a nearby tree. We held the ant by a forceps at its petiolus and put it for 1 min at the center of the top of the *A. florea* comb. Further a *T. molitor* larva of about 1.5 cm length was presented to the colony at a similar position also for 1 min. As a control we took an empty forceps and left it also for 1 min on the *florea* comb.

2.3. Number of bees in sticky band zone

As a parameter for the colony reaction we counted the bees present in the sticky band zone, which was the surface of the nest branch extending from where the branch protruded from the comb to a distance of 4 cm. We counted the number of bees at each colony 5 minutes before the presentation of the ant (see below), directly after (within 2 minutes), 1 hour after and 2 hours later each count was repeated 5 times at each of the sticky bands. We took the mean of the counts for further analysis. Our observation position was 50 cm away and was maintained constant for every colony.

2.4. Deposition of material in sticky band zone

A double layer of plastic bands (Prihimo, made in Japan) of known weight were fixed in the sticky zone. After 2 hours they were carefully removed. Because of the inner layer's contamination by some

sticky materials that were present before the experiment, only the outer layers were used to calculate the weight of the newly deposited materials from increased weight of the band, determined by an 0.001 mg accuracy balance (Sartorius CP2245).

3. RESULTS

3.1. Behavior of bees towards different objects presented on the upper side of the comb

The reaction of the bees towards the *O. smaragdina* worker held in the forceps happened without delay. The bees shied away from the ant and formed – head towards the ant – a multi layered wall around it. The distance to the ant was 4–7 cm. At the same time, many bees from the comb rushed on the upper side joining the group of defending workers. After half a minute some worker bees launched counter attacks against the ant. The attacking bee seized the antenna or a leg of the ant by mandibles and tried to fly off with it. Since the ant was held by the forceps the bee did not succeed at carrying the ant away. In some cases at the end of the 1 min exposure period more bees joined the attack and stinging behavior occurred. Regularly, when we removed the ant, a few bees were attached by their mandibles to the ant so tightly that they were removed together with the ant from the comb. At the end, after we had released the ant from the forceps at the ant arena the bees let the ant go and flew back to the colony which was about 2 m away.

The reaction of the bees to a worker of *C. rogenhoferi* or a larva of *T. molitor* were limited to the group of bees which came into direct contact or which happened to be within a narrow range of it. The bees turned the head toward to the ant or mealworm immediately. With 3 to 5 s after presentation, a few bees attacked by biting and stinging. In comparison to the behavior released by a weaver ant, the reaction was different. While at least 20–50 bees responded to *O. smaragdina* not more than 5–15 bees attacked *C. rogenhoferi* or *T. molitor* larva. In addition, we did not notice bees from the comb rushing up and joining the defense. In summary, the reaction of the *A. florea* colony to presentation of *C. rogenhoferi* or *T. molitor* remained locally restricted while the response released by an *O. smaragdina* worker spread over the whole colony or at least a large part of it.

An "empty" forceps did not increase the activity level on the comb. The bees moved towards to forceps and started "head-pushing" behavior (Sen Sarma et al., 2000). In a few experiments one or two bees bit the tip of the forceps with the mandibles. The relatively low level of activity caused by the empty forceps was strikingly different from the "excitement" and fast movements released by an *O. smaragdina* worker.

3.2. Number of bees at the sticky band zone

Under our experimental conditions *O. smaragdina* workers had no access to the *A. florea* colonies and we did not observe much activity at sticky band zones. In several scans not even a single bee was detected. On average we found 2.3 ± 1.3 bees ($n = 24$ observations). The bees remained motionless facing outside for a few minutes until they turned around and disappeared in curtain covering the comb. One minute after presenting a *O. smaragdina* worker on the comb the number of bees at the sticky band zone increased by a factor of 15.2 ± 7.5. The relative increase of bees was 7.5 after 1 h and 5.6 2 h after the presentation of *O. smaragdina.* The difference compared to the control (empty forceps) was highly significant (Wilcoxon, $P < 0.0005$) after one minute (Fig. 1). Even after 2 h the relative increase was still significant (Wilcoxon, $P < 0.001$). However the number of bees decreased significantly between 1 min and 1 h after the ant stimulus was presented (Wilcoxon, $P < 0.002$). The difference between 1 h and 2 h was not significant (Wilcoxon, $P < 0.122$).

In control colonies, 1 min after presentation of a *Crematogaster* worker, a *Tenebrio* larva or of an empty forceps on the comb, we did not observe an increase of bees in the sticky band zone (Fig. 1). Apparently the increase of bees was a specific reaction to *O. smaragdina.*

3.3. Behavior at sticky band after presentation of *O. smaragdina*

Ant presentation on the comb resulted first in enforcement of the guard bees at the comb

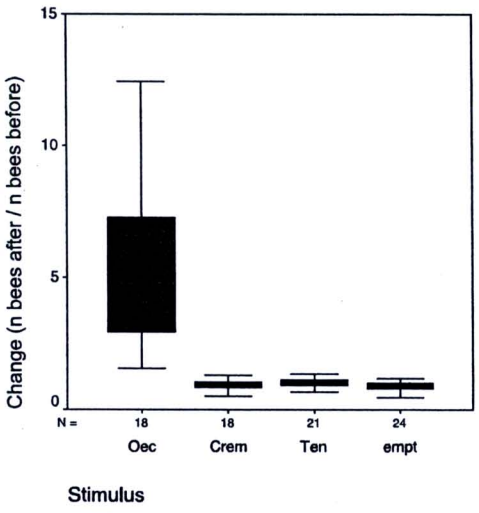

Figure 1. Increase of bees at the sticky band zone 1 minute after stimulation.
Y axis: boxplot of bee numbers divided by number of bees before the experiment.
X axis: Stimulus = presentation of
Oec = *Oecophylla smaragdina* forager,
Crem = *Crematogaster r.*,
Ten = *Tenebrio molitor* larva,
empt = empty forceps,
on the top of *Apis florea* comb.

side of the sticky bands. A large group of densely packed bees (n = 12–25) settled with their heads towards the sticky band in a 2 or 3 fold layer. Looking along the branch over the sticky band towards the comb we saw two or three rows of worker bee heads, comparable to bricks in a wall. A small number of bees went beyond these guard bees and started to "work" on the sticky band. These bees continuously scraped the surface of the branch by their mandibles. The antennae were constantly moving back and forth. The tips of both antennae touched the surface and/or the mandibles and then swung back. The movements looked similar to the behavior of *A. mellifera* workers sealing wooden surfaces inside the hive with propolis (Meyer, 1954). The work on the sticky band went on for 3 to 10 min. Then the bees (when we succeeded in keeping track of them) moved on to the comb and returned within 1 or 2 min and to continue working at the sticky band.

We did not see bees carrying material in the pollen baskets to the sticky band. Further, we did not notice that any larger particles were brought by the mandibles. However, we could not watch the head of these bees constantly during their excursions on the combs. As soon as the bee reached the nest it dived into the layer of bees. So we can not exclude that the bees did collect some smaller parts of material from the comb and fixed this to the sticky band.

3.4. Depositions in sticky band zone

On average the increased weight of deposits on the plastic band after ant exposure was 2.7 mg with a standard deviation of ± 2.73 (6 colonies, 18 experiments). In the control colonies (without ant presentation) we measured a mean of 1.4 mg ± 0.91 mg. The difference was highly significant (Mann-Whitney; $P = 0.0005$) while there was no difference within the control group. So the presentation of an *O. smaragdina* worker on the colony resulted not only increasing numbers of bees but also in a larger amount of deposited material.

4. DISCUSSION

The use of sticky or repellent barriers to protect colonies against ants is wide spread among different taxonomic groups of social insects. In several species of social wasps (Polistinae and Stenogastrinae) the pedicel, by which the nest is fixed to substrate, is regularly impregnated by repellent pheromones from sternal glands (Espelie et al., 1994). The nest entrance of many species of Meliponinae consists of a tube built out of plant resins and sticky materials (Wilson, 1971). So the "invention" of barriers against crawling arthropods has arisen independently several times during evolution.

Generally, the nest branch is the only surface connection of the *A. florea* comb to the "outside world" and it disembogues just under the platform into the comb. This platform serves as location of the bee dances and functions as an information center of the foragers (Lindauer, 1956; Koeniger et al., 1982; Seeley, 1985; Dyer, 1985).

At the platform the bees must deal with mainly two different kind of interferences. Old leaves or debris from the higher portion of the vegetation will fall on the platform and are

removed by the recently described "head pushing" (Sen Sarma et al., 2000). We observed this behavior when we put the empty forceps on the platform. So bees "classified" the forceps alone as "debris". Afterwards we did not notice any increase of activity at the sticky bands.

The second kind of interferences which must be expected at the upper side of comb comes from crawling arthropods or other intruders which succeed in surpassing the sticky band. When we presented the forceps with the *O. smaragdina* worker we saw the latter kind of defensive behavior. The direct reactions of the bees to the ant were aimed to expel the intruder. Though the reactions to *C. rogenhoferi* or *T. molitor* involved fewer bees their intention to remove the presented insects was obvious. Presentation of *C. rogenhoferi* or *T. molitor* did not result in increasing numbers of bees working at the sticky bands. Interestingly, the significant long term effects were restricted to the presentation *O. smaragdina:* The "effect" of this behavior is clear. Increasing the number of guard bees and reinforcing the sticky band were means of precaution and would enable the colony to more effectively repel further intruders.

The weaver ant *O. smaragdina* is a dominant species in areas where *A. florea* occurs (Hölldobler and Wilson, 1990). Their efficient recruitment system enables weaver ants to fast communal foraging and to large prey items (Hölldobler, 1983). Therefore the existence and survival of an *A. florea* colony within the territory of *O. smaragdina* depends on an effective protective system. A main requirement of such defense against *O. smaragdina* is to prevent any foraging success of a weaver ant scout. As we have witnessed several times, an odor trail of a successful scout ant can recruit larger numbers of ants which will overpower defending bees and cause absconding of the colony (Wongsiri, 1989). At the end of such raids, the honey storage, the brood and quite a number of worker bees will lost to the weaver ants.

The reinforcement of the sticky barriers seems to be a specific behavioral response of *A. florea* to its most prominent predator *O. smaragdina.* A comparable phenomenon termed enemy specification (Wilson, 1975) is known to operate between several ant species. These species recognize their most dangerous adversary species and fight against them without delay by specific mass attacks (Hölldobler, 1983).

The question of how *A. florea* recognizes the weaver ant remains open. The experiments, however, with *C. rogenhoferi* and larvae of *Tenebrio* point to possible cues or semiochemicals specific to *O. smaragdina.*

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Résumé – Renforcement d'une barrière – une défense sociale spécifique de l'Abeille naine (*Apis florea*) déclenchée par la Fourmi tisserande *Oecophylla smaragdina.* L'Abeille naine, *Apis florea* Fabricius, largement répandue en Asie, construit son unique rayon à l'air libre, suspendu dans des buissons, des arbustes ou des arbres. Dans cet habitat le principal prédateur insectivore est la Fourmi tisserande *Oecophylla smaragdina* Fabricius. Confrontées individuellement à cette fourmi de grande taille, les petites ouvrières d'*A. florea* ont le dessous. Par contre les colonies se protègent par des bandes collantes, à base de résines de plantes, qui entourent la branche à laquelle est accroché le nid. Le rayon est ainsi complètement barricadé par ces ceintures-pièges et isolé de toutes les structures qui le relient aux autres parties de la canopée. Lors d'une étude dans le nord du Vietnam, Seeley et al. (1982) examinèrent 76 colonies d'*A. florea.* Seules 28 d'entre elles possédaient des ceintures-pièges, mais 24 de celles-ci étaient attaquées par les fourmis, ce qui indique une forte corrélation entre les ceintures-pièges et la présence de fourmis. On ne sait pas à l'heure actuelle de quelle façon la présence d'*O. smaragdina* agit sur la construction de ceintures-pièges par *A. florea.* Nos expériences ont consisté à provoquer une colonie d'*A. florea* en présentant une fourmi tisserande maintenue dans une pince au-dessus d'elle et en observant les réactions des abeilles. Le nombre d'abeilles présentes près des ceintures-pièges et l'augmentation du poids de résine ont été déterminés. Les mêmes observations ont été faites avec un ver de farine (larve de *Tenebrio molitor*) et une autre espèce de fourmi arboricole (*Crematogaster rogenhoferi*) à titre de témoin.

Suite à la présentation d'une ouvrière vivante d'*O. smaragdina* au sommet du rayon d'*A. florea*, le nombre d'abeilles près de la ceinture-piège a augmenté de façon significative par rapport aux témoins (Fig. 1). Il est resté à ce niveau élevé durant 2 h. Le matériel collant a lui aussi augmenté significativement. Le renforcement des ceintures-pièges semble être une réaction comportementale spécifique d'*A. florea* à son prédateur principal. Des comportements comparables, désignés par Wilson (1975) par le terme de spécification de l'ennemi, ont été décrits pour plusieurs espèces de fourmis. Ces espèces reconnaissent leur adversaire le plus dangereux et déclenchent immédiatement leur défense par des attaques massives spécifiques contre lui (Hölldobler, 1983). La question de savoir comment *A. florea* reconnaît la Fourmi tisserande reste ouverte. Les expériences avec *C. rogenhoferi* et les larves de *T. molitor* laissent supposer qu'il s'agit de composés sémiochimiques spécifiques à *O. smaragdina*.

Apis florea* / *Oecophylla smaragdina* / défense de la colonie / relation prédateur-proie / *Crematogaster rogenhoferi

Zusammenfassung – Verstärkung einer Barriere, – eine spezifische soziale Verteidigung der Zwerghonigbiene (*Apis florea*), ausgelöst durch die Weberameise (*Oecophylla smaragdina*). Die Zwerghonigbiene *Apis florea* Fabricius ist in Südasien weit verbreitet, wo sie ihre einzige Wabe frei hängend an Ästen von Büschen, Unterholz und Bäumen baut. In diesem Lebensraum ist die Weberameise *Oecophylla smaragdina* Fabricius ein dominanter Räuber von Insekten. In einer direkten Konfrontation mit dieser großen räuberischen Ameise ist die einzelne *Apis florea* Biene unterlegen. Die Bienenvölker dagegen schützen sich mit Leimringen aus Pflanzenharzen. Auf diese Weise wird die Wabe vollständig verbarrikadiert und alle Strukturen abgesichert, die das Nest mit anderen Zweigen verbindet. In einer Untersuchung in Nordthailand überprüfte Seeley et al. (1982) 76 *A. florea* Nester. Nur 28 von ihnen hatten einen Leimring, bei 24 von ihnen bestand eine Bedrohung durch *Oecophylla smaragdina*. Das deutet auf ein enge Verknüpfung zwischen der Bedrohung durch die Ameisen und den Klebringen hin. Bisher ist nicht bekannt, wie sich die Präsenz von *O. smaragdina* Ameisen auf die Anlage und den Bau von Leimringen durch *A. florea* auswirkt.
In unseren Versuchen wurde eine Weberameise mit einer Pinzette oben auf das *A. florea* Volk gehalten. Dann wurde die Reaktionen der Bienen auf den Leimring beobachtet. Die Änderung der Bienenzahl an den Ringen und die Gewichtszunahme des Klebharzes wurden bestimmt. Als Kontrolle wurde die Reaktion der Bienen auf eine leere Pinzette, einen Mehlwurm (Larve von *Tenebrio molitor*) und eine andere baumbewohnende Ameisenart (*Crematogaster rogenhoferi*) beobachtet.

Wurde eine lebende *O. smaragdina* Arbeiterin dicht über die Wabe von *A. florea* gehalten, war die Zunahme der Anzahl der Bienen im Bereich der Leimringe im Vergleich zur Kontrolle hoch signifikant (Abb. 1). Die Zahl der Bienen blieb für 2 Stunden auf einem höheren Niveau. Auch wurde hoch signifikant mehr klebriges Material aufgetragen. Die Verstärkung der Leimringe scheint eine spezifische Verhaltensreaktion von *A. florea* auf ihren bedeutendsten Räuber *O. smaragdina* zu sein. Vergleichbare Verhaltensweisen wurden von Wilson (1975) als Feindspezifikation bezeichnet und für mehrere Ameisenarten beschrieben. Diese Arten erkennen ihre gefährlichsten Feinde und beginnen sofort mit spezifischen Massenangriffen gegen sie zu kämpfen (Hölldobler, 1983). Die Frage über den Erkennungsmechanismus der Weberameisen durch *A. florea* ist nicht geklärt. Versuche mit *C. rogenhoferi* und den Larven von *Tenebrio* weisen auf Auslöser hin, die spezifisch für *O. smaragdina* sind.

Apis florea* / *Oecophylla smaragdina* / Volksverteidigung / Räuber-Beute-Beziehung / *Crematogaster rogenhoferi

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ภาคผนวก 4

BUMBLE BEES (HYMENOPTERA, APIDAE, BOMBINAE) OF THE UPPER NORTHERN PARTS OF THAILAND

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ABSTRACT

Bumblebees are social insects that are characterized by black and yellow body hairs, often in bands found in the mountainous areas. We have investigated that the number of species of bumblebees in the northern parts of Thailand particularly from Chiang Mai (Doi Inthanon, Doi Suthep, Doi Pui, Chiang Doi, Doi Inching), Chiang Rai (Doi Mae Salon, Doi Tong, Doi Nygem) and Nan. The specimens were caught and chilled in an ice-cooler box. After 20 minutes of chilling, the specimens were examined. The results show that three species of bumblebees; *B. haemorrhoidalis*, *B. trifasciatus*, and *B. breviceps* were found in the mountainous of the upper northern parts of Thailand.

Key words: Hymenoptera, long-tongued bumblebees, *B. haemorrhoidalis*, *B. trifasciatus*, and *B. breviceps*

INTRODUCTION

Bumblebees, (genus *Bombus*), classified as "long-tongued" bees (Michener, 2000), are large roundest bees sporting coats of bright combinations of yellow, orange, red, or white fur on black (Free and Butler, 1959). Bumblebees are among the most diverse and successful pollinators known. Worldwide (Free and Butler, 1959) over 239 species are known, and in North America at least 50 species are present (Krombein *et al.*, 1979; Williams 1998). They range from the frigid arctic coast to tropical rainforests in Amazonia. They are particularly cold hardy, being able to survive arctic winters. Bumblebees have long life spans and flight seasons, and in their selection of flowers to visit (Heinrich 1979). Bumblebees are capable of pollinating many varieties of flower types, and as pollinators, they have several advantages over honey bees, and sting less bees. Bumblebees, in particular, are among the most important pollinators of temperate zone plants (Proctor *et al.*, 1996). Declines of bumblebee populations have been documented in several parts of the world. There is no such record of bumblebees of Thailand existed. Still it does not know how many species are existed in the northern parts of Thailand. How many species of bumblebees are known in Thailand?

Generally, bumblebees are used to pollinate tomato in greenhouse (Heinrich, 1979). Almost one million colonies of *Bombus terrestris* and *B. impatiens* are reared

annually in commercial facilities and are distributed throughout the world (Velthuis and Doorn, 2006). *B. terrestris* colonies are imported to Japan from Europe (Ono 1997, Thorp, 2003), and Russia (Berezin and Beiko, 1996). In 2004, 99,000 acres of greenhouse tomato production were pollinated worldwide by bumblebees.

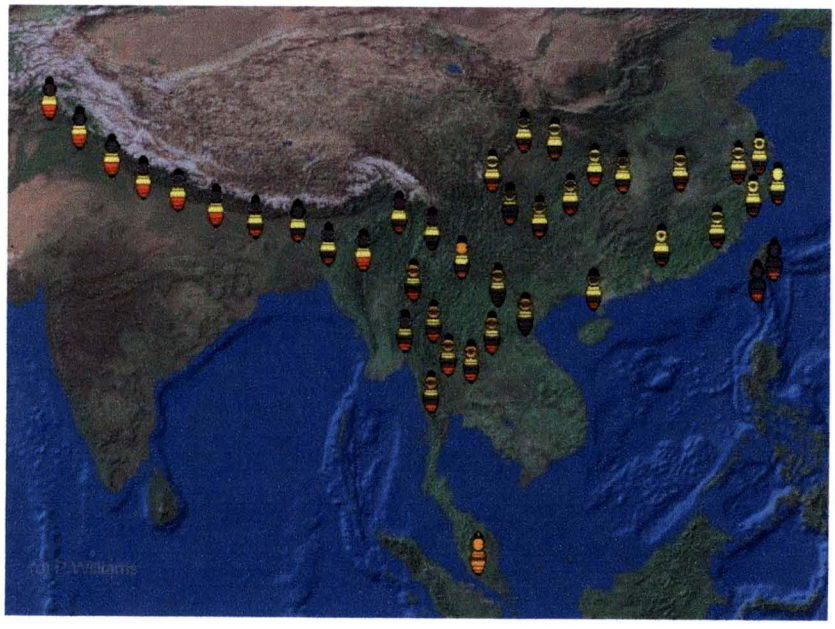

FIGURE 1. Geographical distribution of bumblebees based on color pattern (Source: Williams, 1988).

OBJECTIVE

The main objective of this research was to:-

- collect and record from the upper northern parts of Thailand.

METHODOLGY

Sites of collection

Chiang Mai, Chiang Rai, and Nan provinces were monthly visited to collect bumblebees (Figure 2).

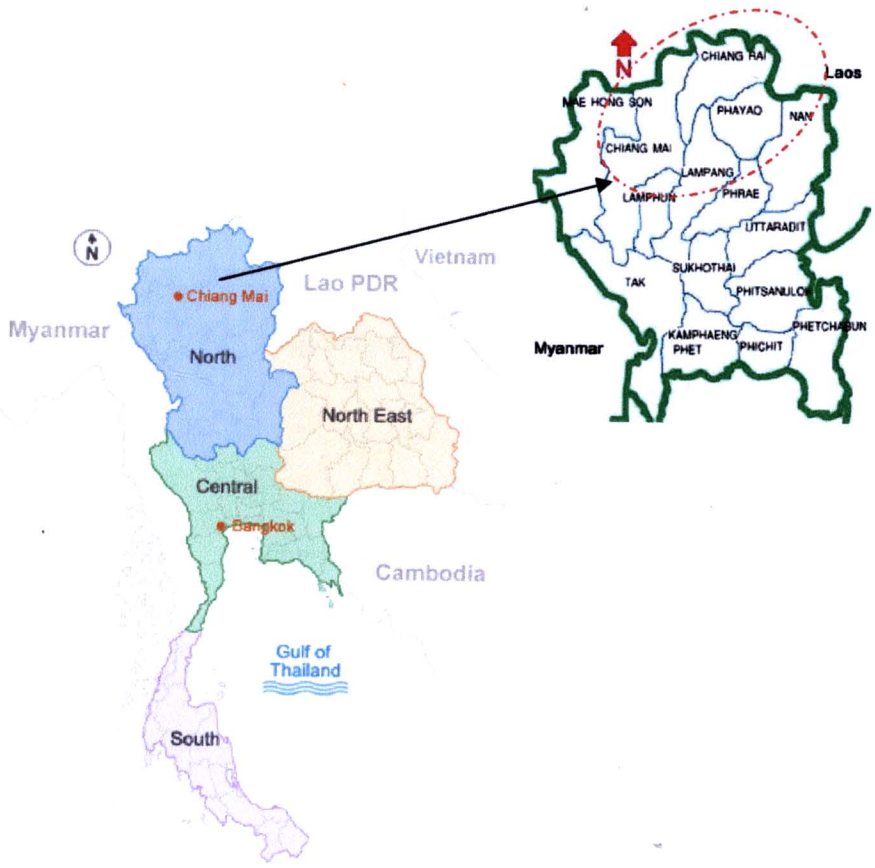

FIGURE 2. Locations of bumblebees collection in the northern Thailand.

Samples collection

The bumblebees were directly collected from the flowers and chilled in an ice box for 20 minutes. The chilled specimens were examined. Firstly, the collected species were killed in a killing jar and preserved for further identification in the Mae Fah Luang Entomology Museum.

RESULTS

The species of bumblebees were recorded from the three provinces are listed in Table 1. All three species of bumble bees were long tongued and buff-tailed bumblebees.

Table 1. List of bumblebees species observed in the northern parts of Thailand.

Species		Location	Altitude (m)
B. haemorrhoidalis	long-tongued species	Doi An Khang, Doi Inthanon, Doi Suthep Doin Tung	900-1476
B. trifasciatus	long-tongued species	Doi An Khang, Doi Inthanon, Doi Suthep Doin Tung	900-1476
B. breviceps	long-tongued species	Doi An Khang, Doi Inthanon, Doi Suthep Doin Tung	900-1476

Figure 3. Long tongued bumblebee, *B. haemorrhoidalis* collected from Doi An Khang, Chiang Mai. 13, March, 2008.

DISCUSSION

The results show that three species of bumblebees; *B. haemorrhoidalis*, *B. trifasciatus*, and *B. breviceps* were found in the mountainous areas of the upper northern Thailand. Three species; *B. haemorrhoidalis*, *B. trifasciatus*, and *B. breviceps* were found between 900-2100 m in Doi Ankang, Doi Suthep, Doi Inthanon (Chiang Mai Province) and Doi Tung (Chiang Rai Province). However, these species were not observed below 850 m. *B. haemorrhoidalis*, and *B. trifasciatus* were observed flying after 10:00 PM. Before 10:00 PM, non of them were observed. All three species were observed foraging on cultivated varieties of spring flowers. *B. haemorrhoidalis* queens emerged in the mid of March. The workers were observed in April and May. *B. breviceps* was observed in June in Doi Tung, Chiang Rai. This species was also foraged on cultivated plants. Previous report indicated that there are six species of bumblebees are existed in Thailand. However, only three species, *B. haemorrhoidalis*, *B. trifasciatus*, and *B. breviceps* were found in the northern parts of Thailand. It may be due to the limited geographic distribution in Thailand (Williams, 1998). Probably, the other species of bumblebees are existed in the middle and southern parts of Thailand, or are already declined or extinct from Thailand is still unclear issue.

CONCLUSION

Three species of long-tongued bumblebees; *B. haemorrhoidalis*, *B. trifasciatus*, and *B. breviceps* are existed in 900-2100 m of the northern parts of Thailand. Bumblebees are increasingly cultured for agricultural use as pollinators because they can pollinate plant species that other pollinators cannot by using a technique known as buzz pollination need urgently study and conservation in order to protect the native plant species.

RECOMMENDATIONS FOR CONSERVATION

(i) Bumblebees habitat should be protected. Habitat should include plentiful food (pollen and nectar resources), abandoned rodent burrows in which to nest, and probably proximity to water sources (lakes, rivers, and streams) for prolongation of flowering plants as food sources for bumblebees.

(ii) Development of checklist of Thai bumblebees.

(iii) Development of quick guide for Thai bumblebee fauna (genus *Bombus*).

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ภาคผนวก 5

ภาพกิจกรรมในการจัดการประชุม

ผู้เข้าร่วมประชุมและเสนอผลงาน

ประธานมอบของที่ระลึกให้ผู้เข้าร่วมเสนอผลงาน

อบรมการผลิตนางพญาให้กลุ่มชาวบ้านผู้สนใจ

ติดถ้วยเพาะนางพญาบนคอน

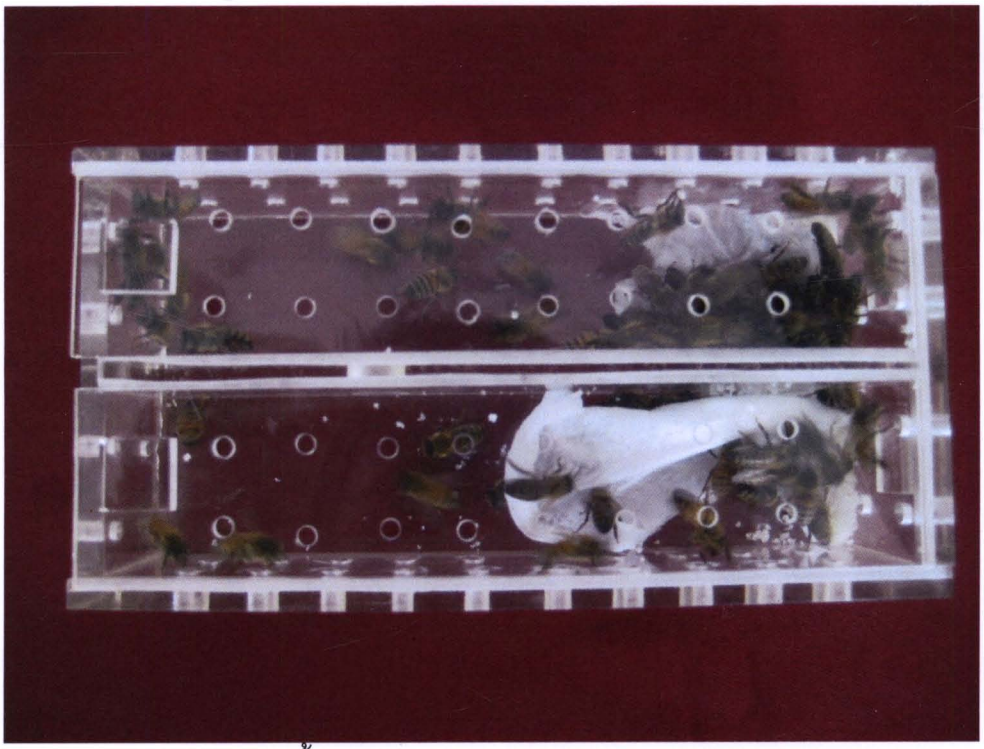
ตรวจหาตัวอ่อน

ย้ายตัวหนอนใส่ในถ้วยเพาะนางพญา

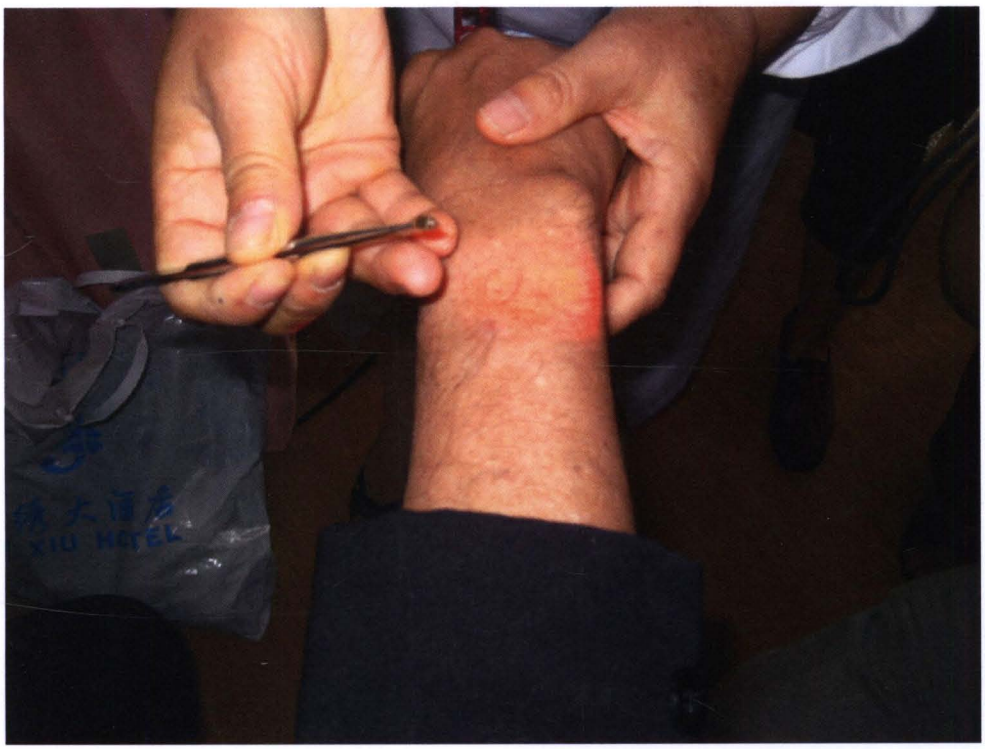
หลอดรวงนางพญาที่ได้

ผู้เข้าร่วมอบรมการผลิตผึ้งนางพญา

การประชุมนานาชาติ 9th International Conference on Apitherapy Health Care



ผึ้งพันธุ์สำหรับ venom apitherapy



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