

## Symposium 2

# Biodiversity of Dwarf Honey Bees in Thailand

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## ABSTRACT

Two species of dwarf honey bees, *Apis florea* and *Apis andreniformis* has recently been accepted after the re-evaluated and recognized as a valid biological species of *A. andreniformis* based on morphological evidence of unique endophallus, worker wing venation, and drone basitarsus. Both species are native Asian honey bees which coexist in many areas. *A. andreniformis* has been found more than ten Thai provinces from the record in the year 1990 and *A. florea* is distributed throughout Thailand. Biodiversity of these two species in Thailand based on the morphological, ecological, behavioral, and genetics information will be discussed in this presentation to explain how they differ so that they can both be present and how differ among their own species.

**Keywords** : Dwarf honey bees, *A. florea*, *A. andreniformis*

## INTRODUCTION

Dwarf honey bee, *A. florea* (Fabricius 1787) and small (black) dwarf honey bee, *A. andreniformis* (Smith 1858) are native to Thailand (1). Although they are most similar together, the recent studies have re-evaluated and recognized *A. andreniformis* as a valid biological species. Wu and Kuang (2) reported the different characteristics of *A. florea* and *A. andreniformis* in Yunnan Province in China, particularly the different of furcated basitarsus between drones of these both species. Their conclusion were supported by the studies of Wongsiri *et al.* (1990) (3) and Rinderer *et al.* (1993, 1995, 1996) (4, 5, 6).

The both species build nests comprised of a single, exposed comb. *A. andreniformis* is smaller than *A. florea* (3,7,8,9). They appear to utilize some similar resources in the same or similar habitats. They are potential pollinators of tropical forest plants and some tropical fruit crops in Thailand (10,11).

*A. florea* normally is found in orchards and forest habitats. *A. andreniformis* is very rarely found in the central part of Thailand. Their honey and nest products have increased the income of villagers (12). Regrettably, traditional methods often involve burning the nest, which destroys both the brood and adult bees.

Thus traditional harvesting methods are extremely damaging and, together with forest clearing, may threaten the survival of these species.

### Distribution

The natural range of *A. florea* extends from Iran, Arabia, Oman, India, Myanmar, Thailand, Laos, the lower latitudes of China, Cambodia and Vietnam. However, this species has never been found in the Malay Peninsula and elsewhere in the surrounding islands (1, 11). It can be found almost everywhere in Thailand including large cities such as Bangkok and Chiangmai but population densities are lower in the South.

*A. andreniformis* is distributed from Bangladesh in the west through southern China in the north, Vietnam in the west. It is common in the Malay peninsula, Sumatra, Java, Borneo and the Philippines (Palawan) (9, 11). In Thailand, it has been found more than ten provinces and in contrast with *A. florea*, this species has never been found in the central part of Thailand especially in urban areas (11).

### Biology

Although the external morphology of *A. florea* and *A. andreniformis* is very similar, recent studies have confirmed that they are separated species. The colour of scutellum is a distinguishing characteristic which tends toward yellow in *A. andreniformis* whereas in *A. florea* it tends toward black. However, the overall colour of *A. florea* body is yellow whereas in *A. andreniformis* is more black (11). Under field conditions, *A. andreniformis* is best distinguished from sympatric *A. florea* by their smaller size and darker colour.

Comparison of body weight (Table 1) and cell sizes (Table 2) indicate that *A. andreniformis* is slightly smaller than *A. florea* but is more significant in queen size of both species. Wongsiri *et al.* (1990)(3) found that the endophallus of *A. andreniformis* differs substantially from that of *A. florea*. They also reported differences in the drone basitarsis and wing shape. Subsequently Rinderer *et al.* (1995)(5) made a detailed comparison of wing venation and other morphology and concluded that these two species are in fact very different.

### Nesting Behaviour

*A. florea* and *A. andreniformis* construct a single comb in an unenclosed nesting site. They prefer to nest on a single branch of small trees, shrubs or bushes (6). *A. florea* tends to nest on tall trees in order to escape from bee hunters especially in urban area. Moreover, *A. florea* nests are now frequently found on man made structures, such as buildings.

### Nest Architecture

The single combs of *A. florea* and *A. andreniformis* are divide into brood area and honey storage area. Brood area covers the area at the lower part of the comb while pollen cells are between brood and honey areas. Cell sizes of both species are obviously different (Table 3). The honey area or crown is above the supporting branch. Normally, the honey storage cells are extended so that honey cells are longer than the brood cells and thus make this part of the comb larger.

The structure of the honey storage is fundamentally different between *A. florea* and *A. andreniformis*. The honey storage area of *A. florea* lacks a mid-rib structure on the crown whereas *A. andreniformis* combs clearly show this structure (Fig. 1) (6).

### Defensive Behaviour

*A. andreniformis* is more defensive of its nest than *A. florea* and similar to *A. dorsata* in displays a "shimmering curtain" behaviour and defending workers leave protective curtain from its lacks an extended "tail" in *A. florea* (11). To protect the colonies from ants and other predators, *A. florea* and *A. andreniformis* workers make two sticky resin rings round the branch of each side of the comb.

### Colony Migration

Tirgari (1971) (16) published that the colony migration of *A. florea* in Iran is local, within the limits area which is partially influenced by seasonal fluctuation of temperature. This movement is considered to be essential for their brood rearing and survival. In Thailand, colony migration behaviour of *A. florea* and *A. andreniformis* are known as seasonal absconding during dearth periods. *A. florea* absconds in response to increased heat or sunlight and usually relocates a short distance away, permitting the bees to collect wax from

the old comb and use for building the new comb. In northern Thailand, *A. florea* is found for two months in the dry season and five months in the rainy season (7). *A. andreniformis* is generally found at higher elevation in the rainy season and lower elevation in the dry season. Their nests can be found in south-eastern (Chanthaburi province) during January to June and in northern (Chiangmai province) during March to April. They usually abscond from June to October (11).

### Parasitic Mites

*A. florea* and *A. andreniformis* are parasitized by separated species of brood infested mites in the genus *Euvaroa*. *E. wongsirii* is apparently found on *A. andreniformis* and not found on other bees. It is thought to be restricted to *A. andreniformis*. Meanwhile *A. florea* is strictly parasitized by *E. sinhai* (17).

### Host Plants

The plant species which are selected by honey bees for their nesting sites are called as 'host plants'. Host plants may be trees or shrubs. Even where sympatric, each species of bee appears to favour particular host (Table 4). Host plants species are variable among *A. florea* and *A. andreniformis* in Table 4 that can be identified *A. andreniformis* has less favour host plants than *A. florea*. They can not be survived when we removed their nests from the forest habitat to the Bee Biology Research Unit in Bangkok but *A. florea* can be survived and established in Chulalongkorn university for more than 10 years (1989-1999). Some favour particular host plant species of *A. andreniformis* are not common growth in central part of Thailand especially in Bangkok.

### Mating Frequency

In honey bee species, multiple mating by queens (polyandry) is usual. However, the numbers of males mating with a queen varies among species. The anatomical structure of *A. florea* drone's endophallus, especially at the distal end of the tip is very different from that of others species (1). Mating behavior and sperm transfer in *A. florea* in Thailand was investigated by Koeniger et al. (1989)(19), suggested the idea of direct sperm transfer into spermatheca during mating. By the same authors, *A. florea* drone contain about  $0.44 \pm 0.037$  million spermatozoa in it seminal vesicles and the queens were presumably mated by 3-4 drones ( ). Koeniger et al. (1990) (20) reported that a drone of *A. andreniformis* in Johore, Malaysia contains  $0.13 \pm 0.01$  million spermatozoa in their seminal vesicles. These findings suggested that the queens mated by 8 drones. However, using microsatellite analysis, Oldroyd et al. (1995, 1997)(21, 22) concluded that *A. florea* and *A. andreniformis* queens from northern Thailand queens mate with 5-14 drones (mean  $5.65 \pm 1.04$ ) and 10-20 males (mean  $9.1 \pm 2.2$ ) respectively. Note that these estimates are based on limited sample sizes. Investigations underway at the Bee Biology Research Unit, in collaboration with the University of Sydney aim to produce new estimates of mating frequency using much higher sample sizes. These investigations may demonstrate even higher mating frequencies than have so far been reported.

### Reproductive Isolation

In honey bees, mating occurs on the wing in specific areas known as drone congregation areas (DCAs). The queens and drones of all honey bee species are superficially similar, and might be easily confused on mating flights. Perhaps because of this, interesting mechanisms for reproductive isolation have evolved. These include the time of mating flights, the location of DCAs, and modifications to the elaborate genitalia of the males. Observations by Koeniger et al. (1989) showed that mating flight of *A. florea* queens at Bangkok occurred during 14.04-15.32 h and drone flight occurred between 13.45-15.30 h (19). Moreover, drone flight of time of four species of honey bees in south-eastern Thailand was studied by Rinderer et al. (1993) (4). These data showed that drones of *A. florea* have a mating flight time during 12.15-13.15 h meanwhile *A. andreniformis* drones have a mating flight time during 13.45-16.45 h. Because the information of DCAs of *A. florea* and *A. andreniformis* are not yet known, therefor we do not know whether or not there are difference concerning the location where the mating takes place.

### Genetic Variation

Molecular biology technique, particularly mitochondrial DNA variability could be successfully used to provide the genetic variability among honey bees species and showed the rediscovery of forgotten species in

honey bees. Based on restriction analysis of mitochondrial DNA, five different origins of the *A. florea* and *A. andreniformis* were separated into 2 groups by Smith (1991) (23); 1) *A. florea* from India and Thailand and 2) *A. andreniformis* from Malaysia (2 locations), and Borneo. Our preliminary study of genetic variation in the both species collected from different areas of Thailand using PCR-RFLP analysis between COI-CO II region of mt DNA (about 1,040 base pairs in length) showed the different fragment patterns among the both species when digested with *Dra* I. However, we could not find mt DNA variation between the samples of its own species. To explain biodiversity of these bees, the further information about genetic variability of the large sample sizes and more regions of mt DNA will be investigated.

## CONCLUSION

Although *A. florea* and *A. andreniformis* inhabit a single exposed comb, *A. florea* has an excellent adaptive behaviour to the urban areas in Bangkok and in other big cities compare to *A. andreniformis* which is very rare in many provinces in Thailand now. They excel in their ability to pollinate most of the tropical fruit flowers (24).

Thus *A. florea* is economically important *Apis* species in Thailand. However, many colonies of this species are annually harvested. Currently, *A. florea* is abundant throughout the country but *A. andreniformis* populations decrease very fast. Therefore, if harvestings continue or increase, these very important agricultural and natural resources may be threatened. The economic value of *A. andreniformis* is not well documented. However, important segments of the naturally occurring flora in the range of *A. andreniformis* probably depends on this species for pollination. Resource partitioning is fundamental to the sympatric existence of species of honey bees. One mode of resource partitioning is for bee species to specialize in collecting food from separate suits of host plants in the ecosystem (Table 4). The populations of *A. andreniformis* are perhaps more threatened than the populations of *A. florea*. It seems to be gradually declining especially due to habitat loss and forest fire.

It is becoming urgent to find ways to protect both of dwarf honey bees in Thailand. They are more adverse pressure and essential for pollinating cultivated and wild plants. They are important for the agricultural production and the maintenance of the natural ecosystem with its biodiversity.

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**Table 1** Body weight of *A. florea* and *A. andreniformis*.

	<i>A. florea</i>	<i>A. andreniformis</i>
Absolute weight (mg) <sup>a)</sup>		
Worker	25.5	24.3
Drone	77.6	<sup>c)</sup>
Dried weight (mg) <sup>b)</sup>		
Worker	5.53	5.14

(a)= Adapted from 13, b)= Adapted from 14, c)= no data

**Table 2** Comparison of nesting sites and characteristics of *A. florea* and *A. andreniformis*.

	<i>A. florea</i>	<i>A. andreniformis</i>
Nest Site	Branch of shrubs, Building	Branch of shrubs
Height (m.)	0.5-10.43	2.69
Visibility	Hidden	Hidden
Dispersion	Widely spaced	Widely spaced
Colony population	6,000	5,000
Aggressiveness	Low	Low
Movements	Local	Local
Foraging area (km <sup>2</sup> )	Small (<3)	Small (<3)
Mass (kg)	0.2	0.2

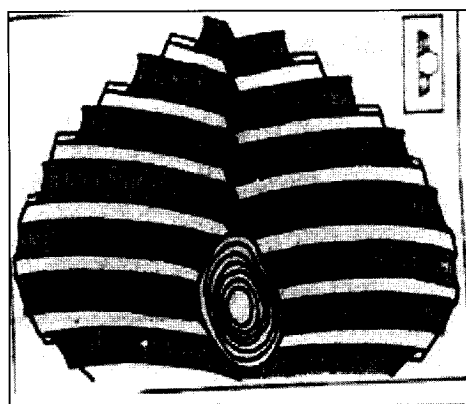
(Adapted from 13 and 15)

**Table 3** Comparison of cell sizes of *A. florea* and *A. andreniformis*

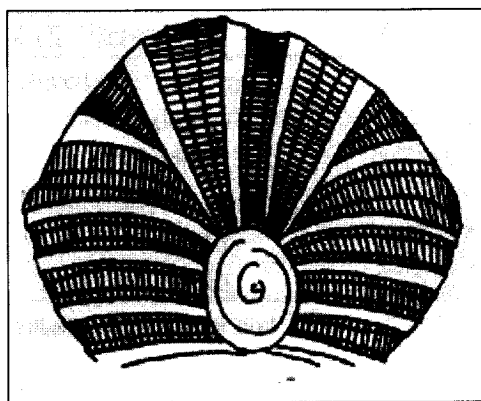
Cell Size		<i>A. florea</i>	<i>A. andreniformis</i>
Queen cell	depth (cm.)	1.41±0.15	1.24±0.26
	width (cm.)	0.47±0.09	0.54±0.08
Worker cell	depth (cm.)	0.93±0.07	0.76±0.14
	width (cm.)	0.29±0.15	0.27±0.11

(Adapted from 5 and 6)

**Figure 1** Cross section view of the honey storage areas of *A. andreniformis* and B) *A. florea*



a



b

Table 4 Host plants of *A. florea* and *A. andreniformis* (Adapted from 18)

Plant Species	Plant Habits	Common Name	<i>A. florea</i>	<i>A. andreniformis</i>
1. <i>Acacia auriculaeformis</i>	T	Wattle	/	-
2. <i>Acacia farnesiana</i>	ST	Sponge tree	/	/
3. <i>Amoora gigantea</i>	T	-	-	/
4. <i>Annona squamoa</i>	S/ST	Custard apple	/	-
5. <i>Antigonon leptopus</i>	C	Chain of love	/	-
6. <i>Artocarpus heterophyllus</i>	T	Jack fruit tree	/	-
7. <i>Atalantia monophylla</i>	ST	-	-	/
8. <i>Bambus arundinacea</i>	B	Bamboo	/	-
9. <i>Bambusa flexuosa</i>	B	Bamboo	/	-
10. <i>Bambusa longispatha</i>	B	-	/	-
11. <i>Bouea macrophylla</i>	T	Plum Mango	/	-
12. <i>Bougainvillea spectabilis</i>	C	-	/	-
13. <i>Broussonetia papyrifera</i>	ST	Paper mulberry	/	-
14. <i>Cassia agnes</i>	T	Cassia	/	-
15. <i>Casuarina junghuhniana</i>	T	Pine tree	/	-
16. <i>Ceiba pentandra</i>	T	White silk Cotton tree	/	-
17. <i>Citrus hystrix</i>	ST	Leech lime	/	-
18. <i>Cocos nucifera</i>	P	Coconut palm	/	-
19. <i>Delonix regia</i>	T	Flame of the forest	/	-
20. <i>Dimocarpus longan</i>	T	Longan	/	-
21. <i>Durio zibethinus</i>	T	Durian	/	-
22. <i>Eugenia cumini</i>	T	Waa	/	-
23. <i>Eugenia malaccensis</i>	ST	Malay apple	/	-
24. <i>Garcinia mangostana</i>	T	Mangosteen	/	/
25. <i>Ixora ebarbata</i>	S	West indian Jusmine	-	/
26. <i>Lagerstroemia calyculata</i>	T	Myrtle	/	-
27. <i>Leucaena leucocephala</i>	S/ST	-	/	-
28. <i>Licuala spinosa</i>	P	-	-	/
29. <i>Litchi chinensis</i>	T	Litchi	/	-
30. <i>Mammea siamensis</i>	T	-	-	/
31. <i>Mangifera indica</i>	T	Mango	/	-
32. <i>Memecylon geddesianum</i>	S	-	-	/
33. <i>Mimusops elengi</i>	T	Bullet wood	/	-
34. <i>Murraya paniculata</i>	S/ST	China box tree	/	-
35. <i>Peltophorum dasyrachis</i>	T	-	-	/
36. <i>Peltophorum pterocarpum</i>	T	-	/	-
37. <i>Pisonia grandis</i>	ST	-	/	-
38. <i>Psidium guajava</i>	ST	Guava	/	-
39. <i>Pithecellobium dulce</i>	T	Manila tamarind	/	-
40. <i>Pterocarpus macrocarpus</i>	T	-	/	-
41. <i>Samanea saman</i>	T	East Indian walnut	/	-
42. <i>Streblus taxoides</i>	S/ST	Brush tree	-	/
43. <i>Tabebuia chrysantha</i>	T	-	/	-
44. <i>Tabebuia rosea</i>	T	-	/	-
45. <i>Tamarindus indica</i>	T	Tamarind	/	-
46. <i>Tecoma stans</i>	S	Yellow bell	/	-
47. <i>Terminalia catappa</i>	T	Bengal almond	/	-
48. <i>Thyrsostachys siamensis</i>	B	Bamboo	/	-
49. <i>Zizyphus mauritiana</i>	ST	Indian plum	/	-

/ = found , - = not found

Plant habits : B = Bamboo, C = Climber, P = Palm, S = Shrub, S/T = Shrub/Tree, S/ST = Shrub/ Shrubby Tree