

# Nectar and Pollen Sources for Honeybee (*Apis cerana cerana* Fabr.) in Qinglan Mangrove Area, Hainan Island, China

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## Abstract

In the present study, nectar and pollen sources for honeybee (*Apis cerana cerana* Fabr.) were studied in Qinglan mangrove area, Hainan Island, China, based on microscopic analysis of honey and pollen load (corbicular and gut contents) from honeybees collected in October and November 2004. Qualitative and quantitative melittopalynological analysis of the natural honey sample showed that the honey is of unifloral type with *Mimosa pudica* L. (Mimosaceae) as the predominant (89.14%) source of nectar and pollen for *A. cerana cerana* in October. Members of Araceae are an important minor (3%–15%) pollen type, whereas those of Arecaceae are a minor (<3%) pollen type. Pollen grains of *Nypa fruticans* Wurmb., *Rhizophora* spp., *Excoecaria agallocha* L., *Lumnitzera* spp., *Bruguiera* spp., *Kandelia candel* Druce, and *Ceriops tagal* (Perr.) C. B. Rob. are among the notable mangrove taxa growing in Qinglan mangrove area recorded as minor taxa in the honey. The absolute pollen count (i.e. the number of pollen grains/10 g honey sample) suggests that the honey belongs to Group V (>1 000 000). Pollen analysis from the corbicular and gut contents of *A. cerana cerana* revealed the highest representation (95.60%) of members of *Sonneratia* spp. (Sonneratiaceae), followed by *Bruguiera* spp. (Rhizophoraceae), Euphorbiaceae, Poaceae, Fabaceae, Arecaceae, Araceae, Anacardiaceae, and Rubiaceae. Of these plants, those belonging to *Sonneratia* plants are the most important nectar and pollen sources for *A. cerana cerana* and are frequently foraged and pollinated by these bees in November.

**Key words:** Hainan Island; honeybee; mangrove; nectar and pollen sources.

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Nectar is an important source of the carbohydrate needed for the growth and development of honeybees, whereas pollen is the main protein source (Lin et al. 1993). Previous studies

(Parker 1926; Chaturvedi 1973) have proved that the honeybee collects pollen of entomophilous and anemophilous plants. Analyzing the pollen taxa in the honey and the pollen load (including corbicular and gut contents) reveals information on nectar and pollen source plants foraged by the honeybee, the preference for each of these sources, and the biological and environmental factors affecting the pollen-gathering activity of the honeybee (Lin et al. 1993).

So far, some melittopalynological studies have been undertaken in mainland China (Zhang and Wang 1965, 1966; Liu 1994,

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1998, 2001; Wang 1995; Yan et al. 2001; Shi and Yun 2005), as well as in different parts of Taiwan (Chen and Wu 1976; Chen 1979; Chen et al. 1984; Jeng et al. 1986; Lin et al. 1993). However, Hainan Island has good prospects for apiculture, with a favorable climate and flowering nectariferous plants available for honeybees (Zen 2004, 2005a, 2005b). In particular, the mangrove taxa of the nature reserve in Qinglan Harbor, Wenchang City (Figures 1, 2) include 28 species from 13 families that account for 90% of the total mangrove species in China (Mo et al. 1999); however, there are no melissopalynological studies reported for this area.

The aim of the present study was to determine the pollen spectrum from honey sample and pollen load (corbicular and gut contents) from the honeybees *Apis cerana cerana* Fabr. in Qinglan mangrove area, as well as to provide some information regarding nectar and pollen sources in October and November for beekeepers because they are dependent on the

production of honey and pollen loads for their income. The other major aim is to recognize the local vegetation through microscopic analysis of honey and bee pollen load.

## Results

### Honey sample

Twenty pollen types (Figures 3, 4) belonging to 15 families were identified in the 2 837 pollen grains counted from six prepared slides, made from 0.1 mL sediments. *Mimosa pudica* L. (Mimosaceae) was found to be the predominant pollen type. The relative frequency of *M. pudica* reached 89.14% of total pollen (Figure 3). Araceae pollen ranked as the second-most frequent pollen type, representing 6.87%. Arecaceae pollen accounted for 2.08%. Other pollen types, namely *Cocos* spp.,

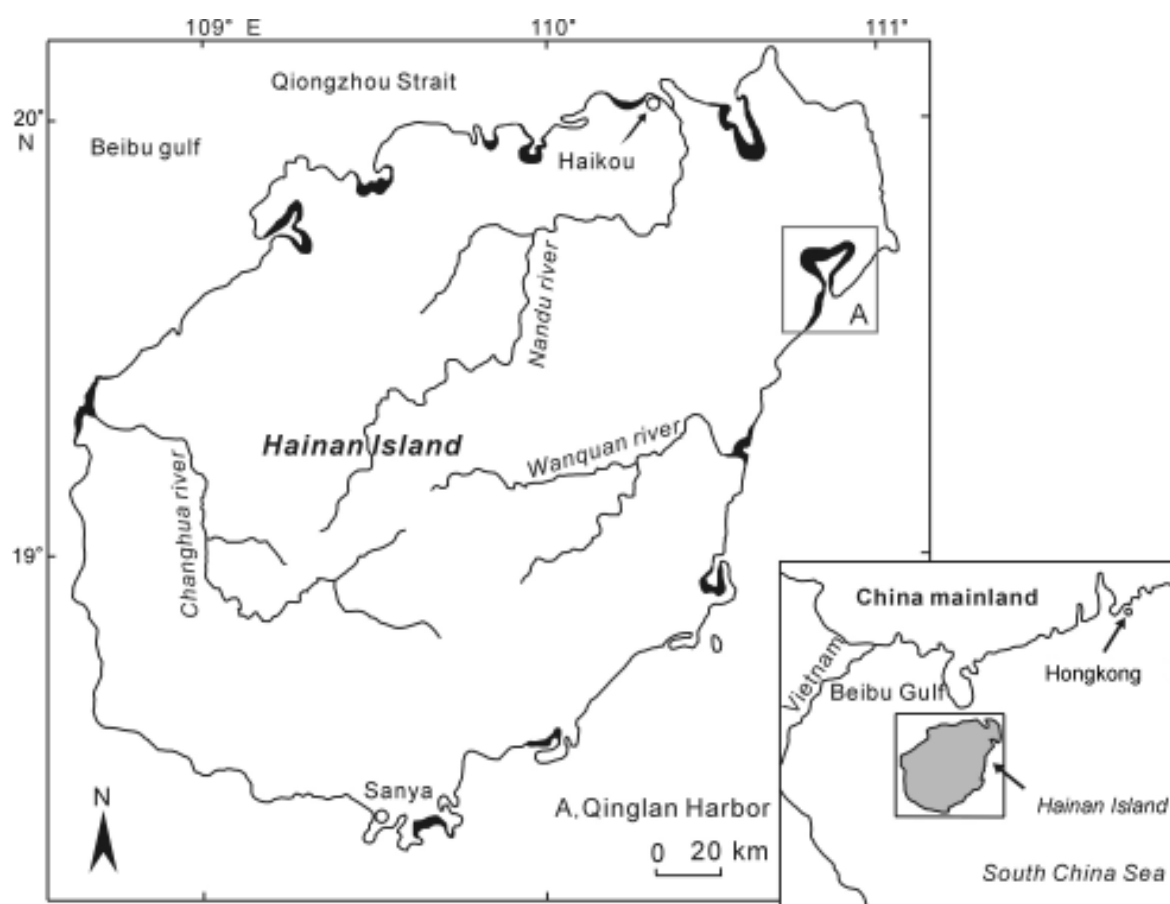
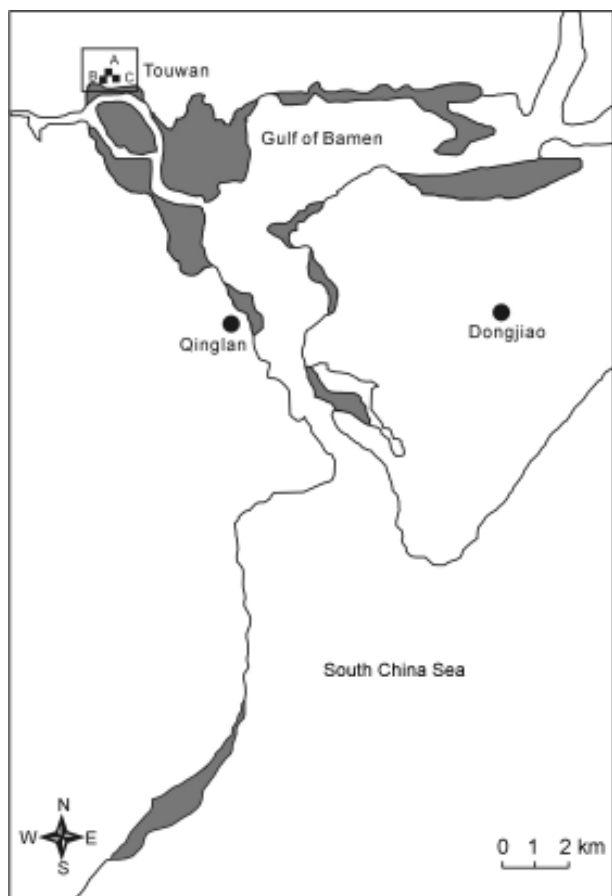


Figure 1. Location of Qinglan Harbor in Hainan Island, China (Modified from Mao et al. 2003).

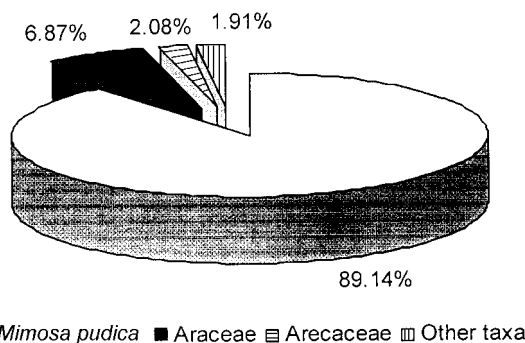


■ Sampling location  
 A, Honey sample and honeybees from the beehives  
 B, Honeybees from the foraging plant of *Sonneratia ovata*  
 C, Honeybees from the foraging plant of *Bruguiera gymnorhiza*

**Figure 2.** Mangrove distribution in Qinglan Harbor (Modified from Mao et al. 2003).

*Nypa fruticans* Wurm., *Sonneratia* spp., *Rhizophora* spp., *Excoecaria agallocha* L., *Lumnitzera* spp., *Bruguiera* spp., *Kandelia candel* Druce, *Ceriops tagal* (Perr.) C. B. Rob., *Pandanus* spp., *Acacia* spp., Apocynaceae, Chenopodiaceae, Plantaginaceae, Sapindaceae, Rutaceae, and Poaceae, accounted for 1.91% of the total pollen count (Figure 3).

Poaceae and Chenopodiaceae pollens were subtracted from the total number of pollen grains prior to calculating the frequency of pollen from nectar-producing plants. Poaceae is a wind-pollinated plant. Chenopodiaceae is known to be nectarless but is entomophilous (Louveaux et al. 1978). The frequency of pollen grains of anemophilous and nectarless



**Figure 3.** Percentage of pollen grains in the honey sample from Hainan Island.

Note, other taxa found in the honey sample include *Cocos* spp., *Nypa fruticans*, *Sonneratia* spp., *Rhizophora* spp., *Excoecaria agallocha*, *Lumnitzera* spp., *Bruguiera* spp., *Kandelia candel*, *Ceriops tagal*, *Pandanus* spp., *Acacia* spp., Apocynaceae, Plantaginaceae, Sapindaceae, Rutaceae, Poaceae, and Chenopodiaceae.

**Table 1.** Flowering calendar in October and November of major plants near the study area in Qinglan mangrove area, Hainan Island (from Chun et al. 1964, 1965, 1974, 1977)

Plant name	Flowering period
<i>Acacia pennata</i>	Mar.–Oct.
<i>Bauhinia purpurea</i> L.	Sept.–Nov.
<i>Bruguiera gymnorhiza</i>	Jan.–Dec.
<i>B. sexangula</i> Poir.	Sept.–May
<i>Carica papaya</i>	Jan.–Dec.
<i>Cerbera manghas</i> L.	Mar.–Oct.
<i>Ceriops tagal</i>	Sept.–Feb.
<i>Cocos nucifera</i>	Jan.–Dec.
<i>Kandelia candel</i>	Jan.–Dec.
<i>Mimosa pudica</i>	Mar.–Oct.
<i>Rhizophora stylosa</i> Griff.	Sept.–Feb.
<i>R. apiculata</i> Blume	Jan.–Dec.
<i>Sonneratia</i> spp.	Jan.–Dec.
<i>Thespesia lampas</i> Dalzell ex Dalzell & Gibs.	Sept.–Jan.
<i>Xylocarpus granatum</i>	Apr.–Nov.

plants, including Poaceae and Chenopodiaceae, was sporadic in the honey examined in the present study, reaching only 0.1%.

The absolute pollen count of the honey was found to be 2 956 000/10 g honey and, therefore, placed the honey examined in the present study into Group V (including only pressed



**Figure 4.** Major pollen types from the honey sample and corbicular and gut contents of honeybees.

(A, B, C, D) *Excoecaria agallocha*;

(E, F) *Lumnitzera* sp.;

(G, H) *Cocos* sp.;

(I) Combretaceae;

(J, K) *Sonneratia* sp.;

(L) *Acacia* sp.;

(M) *Mimosa pudica*;

(N) *Nypa fruticans*;

(O) Poaceae;

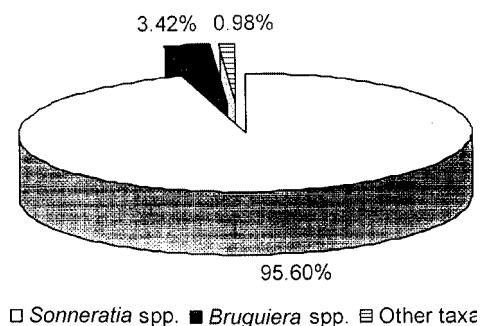
(P) Spindaceae;

(Q) *Rhizophora* sp.;

(R) *Ceriops tagal*;

(S) *Brugueria* sp.

All pollen micrographs are magnified  $\times 1\,000$  except 7, 8 and 12 ( $\times 600$ ).



**Figure 5.** Percentage of pollen grains recovered from the corbicular and gut contents of honeybees.

Note, other taxa found include Anacardiaceae, Arecaceae, Araceae, Euphorbiaceae, Fabaceae, Poaceae, and Rubiaceae.

honey rich in pollen).

#### Pollen load (corbicular and gut contents)

Due to poor pollen content of corbicular and gut of honeybees collected from the beehives, contents from all the honeybees collected from beehives and foraged plants (*Sonneratia ovata* Backer and *Bruguiera gymnorhiza* (L.) Lam.) were mixed together and regarded them as one sample.

The analysis of the corbicular and gut contents of *A. cerana cerana* showed nine pollen types belonging to nine families (Figure 5) of a total of 2 135 pollen grains counted from one prepared slide. This analytical data reveals the highest representation (with a relative frequency of 95.60%) of members of *Sonneratia* spp. (Sonneratiaceae), followed by *Bruguiera* spp. (Rhizophoraceae), Euphorbiaceae, Poaceae, Fabaceae, Arecaceae, Araceae, Anacardiaceae, and Rubiaceae. Of these plants, those belonging to *Sonneratia* are the most important nectar and pollen sources for *A. cerana cerana*, and these plants are frequently foraged and pollinated by the honeybees in November.

## Discussion

#### Nectar and pollen source plants

The honeybee collects many different types of pollen to achieve a nutritional balance (Barbier 1970). It has been found that if the honeybee confines its nourishment to a single species of pollen, its growth, development, and reproductive activity will stop (Lin et al. 1993). The present study has identified 20 pollen taxa from honey sample and nine pollen taxa from the

corbicular load and gut contents of *A. cerana cerana*, revealing that *Mimosa pudica* and *Sonneratia* spp., respectively, are the chief nectar and pollen sources for *A. cerana cerana* during October and November, in Qinglan mangrove area of Hainan Island.

The honey sample is characterized by the absence of any secondary pollen types and only one important minor pollen type (Araceae). All other pollen types were minor pollen types, including some mangrove taxa growing in this area (i.e. *N. fruticans*, *Rhizophora* spp., *E. agallocha*, *Lumnitzera* spp., *Bruguiera* spp., *K. candel*, *C. tagal* etc.).

Records of foraging of mangrove taxa by honeybees as source of nectar and pollen in India have been reported mostly during April and May (Mondal and Mitra 1980; Chaubal and Kotmire 1986; Malakar et al. 1995; Jana and Bera 2004). Species of *Sonneratia*, *Ceriops*, *Bruguiera*, *Acanthus*, *Aegiceras*, *Excoecaria*, *Phoenix* and *Nypa* are the principal pollen types recovered from unifloral honeys collected during March-June from the Sunderbans Mangrove Reserve Forest area, West Bengal, India (Jana 2005). Similar to the present study, analysis of mangrove honeys from Brazil (Barth and Luz 1998; Luz and Barth 2001; Barth 2004) revealed the predominance of different species of *Mimosa*, including *M. bimucronata* Kuntze, *M. caesalpiniaefolia* Benth., and *M. pudica*. In addition, mangrove taxa were also poorly represented in the honey sample in the present study. While examining the pollen content of honeybee loads in Taiwan over a 1-year period, Lin et al. (1993) found that *M. pudica* was an important minor source (6.85%). These authors suggested that wild plants such as *Mimosa*, *Ageratum*, and *Humulus*, may become a dominant source of pollen for honeybees when some of the cultivated plants are not in full bloom.

Mangrove plants are recognized as good honey plants (Jana and Bera 2004; Naskar 2004). Only a few mangrove taxa flower during October in Hainan Island (Table 1). Thus, the honeybees cannot forage on them and are compelled to forage on plants such as *M. pudica* and members of the Chenopodiaceae and Plantaginaceae. The absence of fungal elements, the lack of pollen grains from toxic plants, and the high absolute pollen count of Group V suggests that the local flora in the present study area may be used as a source of good-quality honey.

#### Factors affecting pollen frequency

Pollen productivity, length of the flowering period, and the area of distribution of vegetation can affect pollen frequency in honey and pollen loads (Lin et al. 1993). *Mimosa pudica* is distributed widely and has a long flowering period from March to October in the mangrove area investigated in the present study. As a result, *M. pudica* pollen had the highest frequency (89.14%) in our honey sample. Terrab et al. (2004) mentioned that some

other factors influence the under- or over-representation of pollen in honey, including the size of the pollen grains, monoecious or dioecious plants, insufficiency or lack of pollen, and dominant pollen of nectarless species. In the present study, over-representation of *M. pudica* may also be due to its small size (diameter approximately 10  $\mu\text{m}$ ; Figure 4M) compared with the pollen of other plants.

The distribution area of *Sonneratia* plants, including *S. caseolaris* (L.) Engl., *S. ovata*, *S. hainanensis* W. C. Ko, E. Y. Chen & W. Y. Chen, and *S. alba* Sm., is also very large in the mangrove area investigated in the present study and the flowering period lasts year round (Table 1). In addition, the mostly open perianths of *Sonneratia* spp. make it easier for the honeybee to gather pollen. Consequently, *Sonneratia* spp. (95.60%) dominated in the total pollen count from the corbicular load and gut contents in November.

Generally, environmental factors, such as temperature, light intensity, humidity, and windspeed, also affect the collection activity of the honeybee. In high humidity, the honeybee becomes sluggish and is less likely to venture out to collect pollen (Lin et al. 1993). In the present study, honeybees were collected from the beehives, as well as from foraging plants of *S. ovata* (approximately 20 m away from the beehives) and *B. gymnorhiza* (approximately 40 m away from the beehives) on a drizzly and humid day in November 2004. Most honeybees did not go out to gather pollen, so little pollen was found in the corbicular load and gut contents of the honeybees collected from beehives. In the meantime, honeybees often gather pollen from plants that are located close to the hive rather than venturing too far away (Moezeal et al. 1987), which also contributes to the higher frequency of *Sonneratia* spp. in the corbicular and gut content of honeybees compared with that of other pollen types.

#### Preference of *A. cerana cerana* for certain types of plants

The honeybee will not gather the pollen of certain plants (Moezeal et al. 1987). For instance, *Hibbertia* species generally have numerous exposed stamens but appear to present little pollen and no nectar for collection by bees (Keighery 1975). In the present study, honey and pollen load analysis indicated that none or only very little pollen of certain species of plants in Qinglan mangrove area, namely *Acacia pennata* Willd., *Carica papaya* L., *Cocos nucifera* L., *Xylocarpus granatum* Koen. etc, was present, even though these plants grow in the area and bloom in October and November (Table 1). Of all nectar and pollen source plants recovered from the pollen analysis, *A. cerana cerana* seems to prefer to gather the pollen of *M. pudica* and *Sonneratia* spp. during this season. *Mimosa pudica* is the plant of "first choice" for *A. cerana cerana* in October. When the flowering period of *M. pudica* ends in November, *Sonneratia*

spp. become the plant of "first choice" for the honeybees.

## Conclusions

The present study suggests that *M. pudica* and *Sonneratia* spp. are the most important nectar and pollen sources for *A. cerana cerana* during October and November in Qinglan mangrove area of Hainan Island. The mangrove forest area has considerable potential for medium- to large-scale bee-keeping ventures for the production of good-quality honey. The development of apiaries in the region may result in increased crop production owing to frequent foraging by the honeybees and may improve the socioeconomic situation of the people of Hainan Island, China.

## Materials and Methods

Natural honey sample produced by *A. cerana cerana* was collected directly from a professional beekeeper in October 2004 in Qinglan mangrove area, Touwan Town (19°37' N, 110°47' E), Wenchang City, Hainan Island, China (Figures 1, 2). Twenty-five honeybees were collected from three spots (Figure 2): beehives and foraging plants of *S. ovata* (approximately 20 m away from the beehives) and *B. gymnorhiza* (approximately 40 m away from the beehives) in November 2004 in this mangrove area. Immediately after capture, honeybees were preserved in vials containing 70% alcohol and the vials were labeled with the names of the foraging plants (Bera et al. 2004). Preserved honeybees were dissected carefully to isolate the gut. The gut and corbicular load of 10 honeybees foraging on different plants, as well as those from honeybees from the beehives, were opened and micro-slides were prepared following the acetolysis technique (Erdtman 1960).

For pollen analysis of the honey sample, the method recommended by the International Commission for Bee Botany (Louveaux et al. 1978) was adopted. Honey (10 g) was dissolved in 20 mL warm (40 °C) water. The solution was centrifuged for 10 min at 2 500 r/min, the supernatant solution was decanted, and the sediments were collected into a conical tube and treated with the acetolysis mixture (Erdtman 1960) for approximately 30 min at room temperature. After treatment with the acetolysis mixture, the sediments were rinsed with distilled water, centrifuged for 5 min at 2 500 r/min, and preserved for study.

The absolute pollen count of the honey sample (i.e. the number of pollen grains/10 g honey) was made determined according to the methods of Maurizio (1939). Accordingly, the sample was categorized under various groups in accord with the universally followed grading parameters provided by Louveaux et al. (1978) as follows: Group I, <20 000; Group II, 20 000–

100 000; Group III, 100 000–500 000; Group IV, 500 000–1 000 000; and Group V, >1 000 000. The types of pollen were allocated to one of four frequency classes: (i) predominant pollen types (>45% of the total pollen complement of nectariferous taxa); (ii) secondary pollen types (16%–45%); (iii) important minor pollen types (3%–15%); and (iv) minor pollen types (<3%). The honey sample is unifloral if it contains a predominant pollen type, otherwise it is considered to be multifloral honey.

Pollen types were identified by comparison with reference slides of pollen collected directly from the plants in the present study area. In addition, some monographs and references (Institute of Botany and South China Institute of Botany, Chinese Academy of Sciences 1982; Liu and Tang 1989; Wang et al. 1995; Mao et al. 2003) were also used.

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