BARCODING AND BIOSYSTEMATIC STUDIES ON HYMENOPTERAN POLLINATORS OF CUCURBITACEOUS VEGETABLES

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"BARCODING AND BIOSYSTEMATIC STUDIES ON HYMENOPTERAN POLLINATORS OF CUCURBITACEOUS VEGETABLES"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

BLAST	Basic Local Alignment Search Tool
CD (0.05)	Critical difference at 5 % level
COI	Cytochrome c oxidase I
DNA	Deoxyribonucleic acid
DPX	Dibutylphthalate Polystyrene Xylene
et al.	Co-workers/ Co-authors
FAO	Food and Agricultural Organisation
Fig.	Figure
g	Gram
h	Hour
ha	Hectare
IOD	Inter Ocellar Distance
KOH	Potassium hydroxide
M	Molar
mg	Milligram
min.	Minute
mL	Millilitre
mm	Millimetre
mM	Millimolar
MT	Metric tons
m^2	Per meter square
NCBI	National Center for Biotechnology Information
ng	Nanogram

OOD	Ocello Orbital Distance
PCR	Polymerase Chain Reaction
pM	Picometer
RAPD	Random Amplification of Polymorphic DNA
rpm	Revolutions per minute
SE (m)	Standard error of mean
sec.	Seconds
sp.	Species (singular)
sp. nov.	New species
UV	Ultraviolet
V	Volts
viz.,	Namely
µg	Microgram
µl	Microliter
>	Greater than
%	Percent of
@	at the rate of
°C	Degree Celsius

Introduction

1. INTRODUCTION

Insect pollinators play an important role in effecting optimum pollination of several crops and contribute to the raise of their productivity and quality. Their essentiality is more significant in crops like cucurbitaceous vegetables.

Among the vegetable crops, cucurbits are cultivated extensively in India. The cucurbitaceous family comprises of cucumber, culinary melon, pumpkin, chow-chow, bitter gourd, bottle gourd, ridge gourd, ash gourd, watermelon, muskmelon. Globally, the family cucurbitaceae comprises of 118 genera and 825 species. At present in India, cucurbits are cultivated in an area of 555,000 ha with a productivity of 9,912,000 MT (NHB, 2018).

The centre of origin of many edible cucurbit species is The Asia-Pacific region. Many of them are cultivated in both the developing and developed parts of the world. FAO estimates show that, in India about 6% of the total vegetables produced are from eight species of cucurbitaceous vegetables. In India, studies have been done on some of the important cucurbit crops to record the insect visitors and to understand the pollinators diurnal activity.

Cucurbits being monoecious and their pollen grains being large and sticky to be carried by wind depends mainly on insects for pollination. Hence, pollination by insects is essential to bear improved quality of fruits and seeds (Free, 1970; Mc Gregor, 1976).

In cucurbitaceous vegetables, among all the pollinating insects, the honey bees are known to be the most efficient. For maximising the yield of cross pollinated crops, utilization of pollinators particularly honey bees is taken into account in concert of the most affordable and eco-friendly approach (Free, 1970).

Hence, keeping in view of the pollination requirements of cucurbits, bee conservation requires rapid and effective tools for identification and delineation of species. For this purpose, composition, relative abundance, diurnal activity of hymenopteran pollinators is to be studied.

In addition to the above aspects mentioned, morphological and molecular characterization of bees have to be undertaken. For morphological characterization, the specimens have to be processed for the study of external morphological characters of body parts and genitalia following established procedures with the modifications wherever required. In India, on hymenopteran cucurbitaceous pollinators, only very few detailed taxonomic studies are available. Hence, understanding and generation of basic information on molecular identification and diversity is necessary.

In this regard, for complimenting the conventional taxonomy, development of DNA barcode has become a significant tool. DNA barcoding being an emerging global standard, helps in identification of species using a short standardized gene sequence. Hebert *et al.* (2003 a) stated that, "DNA barcoding" is an alternative way to identify species accurately that also complements conventional taxonomy. Across metazoan taxa, DNA barcoding can be considered as a cost-effective reliable, robust, and easy molecular species diagnosis tool.

The present work will strengthen the research efforts in mapping the beneficial pollinators through systematic characterization.

The main objectives of this study are-

1) To study the composition and relative abundance of hymenopteran pollinators of five selected cucurbits *viz.*, culinary melon (*Cucumis melo var. acidulus*), bitter gourd (*Momordica charantia* L.), ash gourd (*Benincasa hispida* Thunb. and Cogn.), pumpkin (*Cucurbita moschata* L.) and ridge gourd (*Luffa acutangula* (Roxb.) L.) in Thiruvananthapuram and few other districts of Kerala.

2) To study their diurnal activity in one selected cucurbit at College of Agriculture, Vellayani.

3) To explore their morphological and molecular diversity.

Review of Literature

2. REVIEW OF LITERATURE

The literature pertaining to the diurnal activity and dynamics of hymenopteran pollinators of cucurbitaceous vegetables and their morphological and molecular diversity aspects are presented here under.

2.1. COMPOSITION AND RELATIVE ABUNDANCE OF FLOWER VISITORS IN CUCURBITACEOUS VEGETABLES

2.1.1. Cucumber (*Cucumis sativus*)

Cervanica and Bergonia (1990) reported that in Philippines, pickling cucumber flowers were frequently visited by *Xylocopa philippinensis* Smith, *Xylocopa chlorine* Cockerell, *Apis dorsata* F. and *Megachile atrata* Smith. In cucumber, among the flower visitors, honey bees (*Apis mellifera* F.) constituted 82.60 % (Nogueira-Coutao and Calmona, 1993).

In Karnataka, on cucumber flowers, more than 82 % of the total floral visitors were constituted by the hymenopterans *viz., A. dorsata, Apis cerana* F., *Apis florea* F. and *Tetragonula iridipennis* Smith (Prakash, 2002). Sajjanar *et al.* (2004) reported that in Karnataka, a total of twenty four insect species were found visiting the cucumber flowers and among bee visitors, *A. dorsata* was the most frequent visitor.

Pateel and Sattagi (2007) observed that in Karnataka during Rabi season on cucumber flowers, *A. florea, A. cerana* and *A. dorsata* were the most abundant insect pollinators. Khaja (2010) recorded that, in Karnataka on cucumber crop during flowering, among the honey bee species more than 84 % of the total floral visitors were constituted by *A. cerana, A. florea* and *A. dorsata.*

Atwal (1970) reported that on cucurbitaceous flowers at Ludhiana, more than twenty three species of insect pollinators were found frequently visiting. Of these species, *A. dorsata* was the most abundant pollinator, followed by *A. florea*, *Ceratina binghami* Cockerell, *Xylocopa pubescens* L., *Nomioides* sp. and halictine bees. The data on the abundance of insect visitors on inbred lines of

cucumber hybrid KH-1 at Solan (H. P.), showed that *A. mellifera* (3.57 bees/m²/10 min) was most abundant followed by *Braunsapis haemorrhoidalis* Smith (3.43 bees/m²/10 min) and *Halictus* sp. (1.81 bees/m²/10 min) (Thakur and Rana, 2008).

Kapil and Chaudhury (1974) reported that, in Hisar (Haryana) on cucurbit flowers from June to September, the floral bee visitors were *A. florea* which constituted 33.10 % followed by *Nomia* sp. (21.30 %), *Halictus* sp. (19.30 %), *Pithitis* sp. (11.30 %), *Nomioides* sp. (8.30 %) and *Xylocopa* sp. (6.00 %). In Hisar (Haryana), cucumber flowers are predominantly pollinated by hymenopterans. Among hymenopterans, four species were from Apidae, where as Vespidae, Eumenidae, Megachilidae and Halictidae represented 3, 2, 2 and 1 respectively, with the dominance of *Ceratina sexmaculata* Smith followed by *A. dorsata*. (Hanh *et al.*, 2014)

Jangaiah (2007) reported that in Kerala, on culinary melon flowers, the frequent visitors were *A. cerana indica, A. mellifera* and *Trigona* sp. Among the bee visitors, *A. cerana indica* was the most dominant one. Dorjay *et al.* (2017) revealed that in Jammu, on cucumber flowers honey bees were the most predominant and comprised more than 74 % of the total insect pollinators. Among all floral visitors *A. mellifera* was the most abundant followed by *A. cerana*, *A. dorsata*, *A. florea*.

2.1.2. Bitter gourd (*Momordica charantia*)

Kumar (2010) observed that in Hisar on bitter gourd flowers, hymenopterans were the major floral visitors comprising of 11 species. Among all insect visitors, *Halictus* sp. was the most frequent visitor (43.88 %) followed by *Megachile* sp. (32.11 %) and *A. dorsata* (24.01 %). Balina *et al.* (2012) reported that in Haryana on bitter gourd flowers, a total of nine bee species of three families (Apidae, Halictidae and Megachilidae) were the most frequent visitors, among which *A. dorsata* was the most efficient pollinator followed by *Halictus* sp. and *Megachile* sp.

In Karnataka, out of 10 species of pollinators in bitter gourd, the most predominant is *A. florea* comprising 43.00 %, followed by *A. cerana* (26.00 %), *A. dorsata* (13.00 %) and other pollinators (18.00 %) (Nidagundi, 2004). Subhakar *et al.* (2011) reported that in Tirupathi on bitter gourd flowers, *T. iridipennis* (86.31 %), *Halictus gutturosus* Vachal (8.68 %) and *A. florea* (3.84 %) were the most frequent and abundant visitors.

Grewal and Sidhu (1978) recorded that in Punjab, the most frequent visitors of bitter gourd flowers were *A. florea* and various species of Anthophoridae and Halictidae with 28, 10 and 5.2 %, respectively. In Jammu, on bitter gourd flowers the most important floral visitors were *A. dorsata A. mellifera* and *A. cerana* which comprised more than 69 % of the total flower visiting pollinators. (Dorjay *et al.* 2017)

2.1.3. Pumpkin (*Cucurbita moschata*)

Alen & Bradley (1966) reported that, the natural pollinators of pumpkin were carpenter bees, bumble bees, honey bees and squash bees. Hemanthkumar (2006) observed that in Karnataka, on pumpkin during flowering stage, eight species of floral visitors belong to Hymenoptera *viz.*, *A. cerana indica* and *A. dorsata* comprised more than 98 % of the total floral visitors. Rachna and Verma (2016) reported that in Meghalaya, on pumpkin flowers *Bombus* sp. being the dominant pollinator with 69.69 % mean relative abundance followed by *A. florea* with 24.51 % and *Anthophora* sp. with 13.83 % relative abundance.

In Bhubaneshwar, on pumpkin flowers, the hymenopterans were the dominant insect pollinators constituting of *A. cerana*, *A. dorsata*, *A. florea*, *Bombus* sp. Among them *A. cerana* was the most frequent and dominant pollinator (Mohapatra and Sontakke, 2012). Lalita and Yogesh (2015) observed that in Haryana on pumpkin flowers, under agro-ecological conditions of Hisar, *A. dorsata* was the most efficient pollinator followed by *A. mellifera*, *A. cerana* and *A. florea*.

2.1.4. Ridge gourd (*Luffa acutangula*)

Ramesh (2007) reported that, in Bangalore varieties Arka Sumeet and Arka Sujath of ridge gourd was pollinated predominantly by hymenopterans. Among hymenopterans six species are from Apidae, two species from Formicidae and one species each from Xylocopidae, Sphecidae and Vespidae. Of the total floral visitors *A. florea*, *A. cerana* and *A. dorsata* comprised 78.90 %.

In Bangalore, on ridge gourd *A. cerana, A. florea, T. iridipennis* constituted 78.09 % of all pollinators (Kuberappa *et al*., 2008). Lakshmi (2013) reported that in Bangalore, on ridge gourd flowers *A. cerana* was the most frequent and dominant pollinator which constituted 76.46 % of total flower visitors.

Hiren and Minam (2016) reported that, on *L. acutangula* among eight species of insect pollinators visited the dominant order was Hymenoptera followed by Coleoptera and Lepidoptera. In Hymenoptera *Xylocopa collaris* L., *Xylocopa tenuiscapa* Westwood , *Xylocopa aestuans* L., *Xylocopa violacea* L. and *Pelopidas mathias* F. visited more flowers per unit time and spent more time on flowers. In Bihar, *A. mellifera* was the most frequent and abundant pollinator on ridge gourd flowers (Gautam *et al*., 2018).

2.1.5. Summer squash (*Cucurbita pepo*)

In 1974 and 1975 at Ludhiana on *C. pepo* flowers, *A. dorsata* and *A. florea* constituted 77.20 and 70.70 % respectively (Grewal and Sidhu, 1979). On summer squash flowers, more than 80 % of the insect pollinators were constituted by *A. cerana, A. florea* and *A. dorsata* (Manjula, 2007). In Hisar, field studies were carried out on summer squash during 2013-14. The data on the abundance of insect visitors showed that *A. mellifera* was most abundant followed by *A. cerana*, *A. dorsata* and *A. florea* (Rani *et al.*, 2016).

2.1.6. Bottle gourd (*Lagenaria siceraria*)

In Hisar, bottle gourd flowers were pollinated frequently by *Xylocopa fenestrata* F. (Sihag, 1990; Sihag, 1993). Srikanth (2012) observed that in Bangalore, *A. dorsata* was the most frequent and dominant pollinator on bottle gourd flowers which comprised more than 23 percent of the total floral visitors.

2.1.7. Watermelon (*Citrullus lanatus*)

In watermelon bees are the chief pollinating agents (Rosa, 1925). Brewer (1974) opined that in USA, honey bees were the adequate pollinators of watermelon. Rao and Suryanarayana (1988) on watermelon, recorded that *A. cerana* (87.00 %) was the dominant floral visitor than *A. florea* and *T. iridipennis*.

2.1.8. Other Cucurbitaceous Vegetables

In Punjab, on muskmelon flowers, *A. florea* was the most frequent floral visitor (Grewal and Sidhu, 1978). Eswarappa (2001) reported that in chow-chow crop, out of 26 insect species visited, 14 belongs to Hymenoptera and four each to Lepidoptera, Coleoptera and Diptera. *A. dorsata, A. cerana, A. florea* and *T. iridipennis* comprised more than 82 % of the total insect pollinators of crop. Thapa (2006) stated that on sponge gourd, the hymenopteran floral visitors were bumble bee *Bombus* sp. oriental wasp *Vespa orientalis* L. and golden wasp *Vespa magnifica* Smith.

Bernard *et al.* (1996) observed that, honey bees, *A. mellifera* was the predominant pollinator of cantaloupe with an average of 54 %, followed by muscid flies. In Kannur, on ash gourd flowers, *T. iridipennis* was the most dominant pollinator followed by *Halictus timidus* Smith, *A. cerana*, *Ceratina hieroglyphica* Smith, *Halictus taprobanae* Cameron (Leena and Nasser, 2015).

2.2. FORAGING BEHAVIOUR OF BEES

In the process of evolution, to attract bees in more numbers, flowers secrete nectar and pollen in large quantities. Foraging activity is the most important factor that aids in pollination. Foraging is a trade between the amount of nectar and pollen expected from a flower and time required to extract it (Inouye, 1980). Floral structure of the crop particularly the corolla depth and foraging behaviour of the insects affects the foraging speed and foraging rate of the pollinators (Gilbert, 1980).

Due to variation in the microclimate around the plants honey bees show variations in their visits between plants sometimes only a few feet apart. This means that some flowers receive more visits than necessary which all flowers are expected to receive an optimum number of visits (Whitaker and Bohn, 1952).

In cucurbit crops, bee activity begins on the flower shortly after it opens, reaches a peak at about 11:00 h and ceases by about 05:00 h (Mc Gregor and Todd, 1952).

2.2.1. Cucumber (*Cucumis sativus*)

Shemetkov (1960) reported that in Russia the active period of visit of pollinators was between 08:00 to 10:00 h on cucumber flowers. Seyman *et al.* (1969) opined that, for the pollination of cucumber crop honey bees are extremely important and during the midday period the major portion of bees pollination activity occurs.

Sanduleac (1959) reported that on cucurbit flowers in Rumania, honey bees showed peak foraging activity between 08:00 to 09:00 h. Rapp (1981) reported that in Israel on cucumber flowers, honey bees started foraging at 06:00 h and reach maximum between 09:00 to 12:00 h and subsequently decreased.

Conner (1969) recorded that, for effective cucumber pollination the best time of the day was from 10:00 h to 15:00 h. For satisfactory fruit set a cucumber flower needs at least 8 to 10 bee visits. Collison (1973) reported that on cucumber,

between 09:00 h and 14:00 h bee visits (80.0 %) were maximum. On an average, each visit and time spent on pistillate flowers was twice as that on staminate flower and the overall foraging rate was 5.30 bees/flower/minute. Cervancia and Bergonia (1990) reported that in Philippines on cucumber, bees showed peak foraging activity from 10:00 to 11:00 h.

Sajjanar *et al.* (2004) reported that on cucumber flowers, at around 06:00 h, *A. dorsata* began peak foraging activity whereas, other pollinators started activity by 08:00 h. Hossain *et al.* (2018) studied the foraging activities of insect visitors on cucumber and recorded that the foraging behaviour of *A. mellifera* was peak at 08:00-09:00 h.

Prakash (2002) reported that in cucumber, the collection of pollen by different honeybee species was found to be maximum between 08:00 and 09:00 h and among the honey bees, for pollen collection maximum time was spent by *A. florea* (13.49 sec), followed by *T. iridipennis* (11.44 sec), *A. cerana* (9.65 sec), *A. mellifera* (8.74 sec) and the least in *A. dorsata* (7.22 sec). In case of cucumber, maximum population of *A. mellifera* was observed between am 09:00-10:00 h (Dorjay *et al.*, 2017).

In Hisar, Kumar (2004) observed that, foraging activity of *A. mellifera* on cucumber flowers during rabi under poly-house conditions was peak during 09:00-10:00 h followed by 10:00-11:00 h and it was low during 13:00-14:00 h of the day. In Hisar, the data on the foraging activity of insect visitors in cucumber hybrids, *viz.*, Evergreen, NBH-Manu, Damini and Rani showed that *A. dorsata* visited more number of flowers (15.18 flowers/min) at 07:00-08:00 h. The mean foraging rate irrespective of different day hours was highest in *A. dorsata* (8.63 flowers/min.) followed by *C. sexmaculata* (5.03 flowers/min.), and it was lowest in *Halictus* sp. (4.38 flowers/min.) (Hanh *et al.*, 2014).

Foraging activities of insect visitors on inbred lines of cucumber hybrid KH-1 at Solan (H.P.), showed that the time spent per flower (foraging speed) was highest in *Halictus* sp. (30.79 sec/flower) followed by *A. mellifera* (11.69

sec/flower), *B. haemorrhoidalis* (5.27 sec/flower) during 09:00-10:00 h. Foraging rate (flowers visited/min) was highest in *B. haemorrhoidalis* (10.95 flowers/min) followed by *A. mellifera* (6.64 flowers/min) and *Halictus* sp. (4.14 flowers/min) during 15:00-16:00 h (Thakur and Rana, 2008).

Studies on the foraging activity of stingless bee, *T. iridipennis* was carried out in cucumber cultivated greenhouses in TNAU, Coimbatore and farmer's field in Srivilliputhur village. From the observations recorded it was noticed that inside the greenhouse of Srivilliputhur village maximum number of foragers were seen between 10:00 h to 12:00 h. In the greenhouse located in TNAU, Coimbatore maximum number of foragers were seen between 09:00 h to 11:00 h. In both the greenhouses the foraging activity of stingless bees was peak in the morning hours than in the afternoon hours due to the high temperatures in the greenhouses compared to afternoon hours (Kishan *et al.*, 2017).

2.2.2. Bitter gourd (*Momordica charantia*)

Nidagundi and Sattagi (2005) reported that on bitter gourd, *A. florea* and *A. dorsata* showed highest diurnal activity at 12:00 h. Subhakar *et al.* (2011) recorded that in bitter gourd, diurnal activity of *A. florea*, *H. gutturosus*, and *T. iridipennis* was maximum at 09:00-10:00 h. On bitter gourd, among the 17 species belonging to 10 families of 4 insect orders *T. iridipennis*, *H. gutturosus* and *A. florea* showed peak foraging activity (Subhakar and Sreedevi, 2015)

2.2.3. Pumpkin (*Cucurbita moschata*)

Hemanthkumar (2006) observed that on pumpkin, at 07:00 h flowers started opening and anther dehisces at 09:00 h, which resulted in the availability of plenty of pollen as a result at 09:00 h *A. cerana indica* showed peak foraging activity. Later at 11:00 h the activity ceased as the flowers were closed.

In Hisar, foraging activity of different honey bee species on pumpkin flowers during August-September showed that *A. dorsata*, *A. mellifera*, *A. cerana*

and *A. florea* initiated their activity during morning hours and their activity ceased between 10:00 to 11:00 h (Lalita and Yogesh, 2015).

2.2.4. Ridge gourd (*Luffa acutangula*)

Ramesh (2007) reported that on Arka Sumeet and Arka Sujath varieties of ridge gourd, *A. cerana* showed the peak foraging activity at 11:00 h. On ridge gourd, *X. fenestrata* and *Xylocopa leucothorax* De Geer showed peak foraging activity from 05:00 to 19:00 h (Rahman and Deka, 2011). In ridge gourd, between 09:00 and 11:00 h of the day the maximum foraging activity was shown by *A. cerana*, *A. florea* and *T. iridipennis* (Lakshmi, 2013).

2.2.5. Summer squash (*Cucurbita pepo*)

Girish (1981) recorded that on summer squash during February *A. cerana* started foraging at 06:00 h, and two hours later by *A. dorsata* and *A. florea*, later in all species foraging ceased at about 12:00 h. Manjula (2007) recorded that on summer squash, the foraging activity of *A. cerana* was maximum compared to *A. florea* and *T. iridipennis*.

Field studies were carried out on summer squash in Hisar and the results showed that among the bee species, *A. mellifera* (5.45 flowers/minute) showed peak foraging rate at 08:00-10:00 h, followed by *A. cerana* (4.38 flowers/minute) and *A. dorsata* 3.21 flowers/minute), and it was lowest in *A. florea* (2.10 flowers/minute) (Rani *et al.*, 2016).

2.2.6. Watermelon (*Citrullus lanatus*)

Bhambure (1958) reported that in Bombay, pollen collection from watermelon started at 08:30 h by *A. cerana, A. florea* and *Melipona* sp. and they showed peak activity at 10:30 h. Fakuda (1987) recorded that on watermelon, honey bee showed peak activity from 08:00 to 10:00 h and female flowers were visited more frequently by bees than male flowers. In watermelon, the foraging activity is initiated earlier by *A. cerana* than other bee pollinators and activity was

peak from 09:00 to 10:00 h and then ceased by 13:00 h gradually (Rao and Suryanarayana, 1988).

2.2.7. Other Cucurbitaceous Vegetables

Many workers on the cantaloupe flowers reported that, the bee activity begins at 07:00 to 08:00 h and it was peak at 11:00 to 12:00 h. (Mc Gregor, 1950). Eswarappa (2001) recorded that, in chow-chow the foraging activity by different species of honey bees was maximum at 10:00 to 11:00 h and lowest at 06:00 h. Among the honey bees, for pollen collection maximum time was spent by *A. florea* (14.63 sec.), followed by *T. iridipennis* (12.89 sec), *A. cerana* (7.59 sec), *A. mellifera* (6.77 sec.) and the lowest in *A. dorsata* (5.77 sec.).

2.3. MORPHOLOGICAL CHARACTERIZATION OF BEE FAUNA

Ruttner (1988) delineated thirty six morphometric characters (such as distances, angles, categories of pigmentation) and these characters are widely utilized in honey bee subspecies classification.

Rasmuseen (2013) observed that through morphometry, the length of the body varies as 4.70 mm in *Lisotrigona arcifera* Cockerell, 3.55 mm in both *T. iridipennis* and *Tetragonula bengalensis* Cameron, 3.45 mm in *Tetragonula ruficornis* Smith, 3.33 mm in *Tetragonula praeterita* Walker and 2.90 mm in *Lisotrigona cacciae* Nurse. Francoy *et al.* (2016) sampled bees from different regions of India and claimed the existence of patterns of variability in stingless bees of the country using wing morphology and opined that, little is known about the stingless bee biology and taxonomy.

Mattu and Verma (1983) from Himachal and Kashmir regions of India, collected workers of the Indian honeybee (*A. cerana indica*) and conducted morphometrics and found that with locality there were comparative significant differences in the Himachal region in the length of postmentum, pedicel, flagellum and antenna but in Kashmir, only in the length of postmentum. Rathore *et al.* (2013) conducted morphological analysis of *Tetragonula gresitti,* and found

that the head is 1.1 times wider than long and compound eyes were 2.7 times longer than wide. Viraktamath and Jose (2017) recorded the differences in mean head width of *Lisotrigona chandrai*, *L. revani*, *L. chandrai* males and queen measured as 1.19 mm, 1.14 mm and 1.18 mm through morphometric studies.

Verma (1994) conducted morphometric studies on Indian honeybee (*A. cerana indica*), hind legs, tergites and sternites and reported significant variation with geographical locality. Morphometric studies conducted in workers of *Tetragonula iridipennis* showed that the wing length including the tegula has found to be ranging from 3.2 to 3.9 mm and wing length of males ranges from 3.1 to 3.8 mm (Vijayakumar and Jeyaraaj, 2014).

Morphometric studies on blue banded bees, *Amegilla zonata* was conducted in Tamil Nadu during 2015-16 and included 25 morphometric characters *viz.*, body length, length and width of head, length and width of compound eye, length of scape and pedicel, length and width of metasoma etc. and reported that both the sexes can be easily differentiated based on size, clypeal markings, compound eyes, number of flagellomeres, mandibles and bluish bands. (Sandeep and Muthuraman, 2018)

2.4. MOLECULAR CHARACTERIZATION OF BEE FAUNA THROUGH DNA BARCODING

In the absence of obvious morphological characters independent assessment of insect species distinctions can be done through recent advances in molecular techniques. DNA sequencing of a standard gene region or ''DNA barcoding'' can be helpful in species diagnosis (Hebert *et al.*, 2003 a). DNA barcoding has great implications for taxonomy, yet, perhaps one of the greatest promises is in the studies of biological diversity within regional and poorly studied habitat-specific biotas (Smith *et al.*, 2005).

For assessing and understanding the extent of diversity in groups that have proven difficult by classical taxonomic techniques, DNA barcoding has shown a great role (Kohler, 2007). Vogler and Monaghan (2007) opined that, for

taxonomic decisions and descriptions, and as a standard method of analysis "DNA taxonomy", is a concept of adopting DNA sequencing as a central criterion.

Hajibabaei *et al.* (2007) advocated that, DNA barcoding play an important role for species-level identifications. Sheffield *et al.* (2009) advocated that, for species-level identification of many animal taxa DNA barcoding is a reliable and rapid method. Syromyatnikov *et al.* (2018) developed a procedure for quick identification of honey bee subspecies by PCR with restriction fragment length polymorphism (RFLP) using mutagenic primers which is fast and inexpensive.

Hebert *et al.* (2003 a) confirmed that in animals, the mitochondrial gene cytochrome c oxidase I (COI) can serve as the core for global bio-identification. For species identification and discovery in large assemblages of life, DNA barcoding employs sequence diversity in short, standardized gene regions. (Hebert *et al.,* 2003 b, Savolainen., 2005). DNA barcoding can also be used for identification of cryptic species, biotypes, haplotypes, etc. in addition to species discovery (Boykin *et al.*, 2012).

Ratnasingham and Hebert (2007) stated that, Barcode of Life Data System (BOLD) has been developed to manage and provide analytical tools for large amounts of data. In order to aid the species identification resources, Consortium for the Barcode of Life (CBOL), Barcode of Life Data Systems (BOLD) were established. Later, in 2010 the International Barcode of Life (IBOL) project was started for efficient and quick barcode data generation (IBOL, 2013).

Barcode of Life Data Systems serves as home to 2,97,859 species with barcodes of which the major contributors are phylum Arthropoda (2,27,296), class Insecta (201,073). In order Hymenoptera, species with barcodes (32,579) includes the families *viz.,* Apidae (2,593), Halictidae (1,658), Megachilidae (1,468), Colletidae (839), Andrenidae (768), Vespidae (552), Melittidae (55) and Stenotritidae (2) (BOLD, 2019).

Several workers based on mitochondrial DNA (mtDNA), conducted molecular analysis of honey bee and wild bee species (Moritz *et al.,*1986). Susnik

et al. (2004) studied the intra-specific genetic variability in *A. mellifera*, using mitochondrial and nuclear DNA analyses. Agra *et al.* (2018) analysed the diversity of *A. mellifera* in Argentina, using mitochondrial (COI-COII region) and nuclear (eight microsatellites) markers. Three European (M4, C1, C2J) and three African (A1, A4, A30) haplotypes were detected.

Materials and Methods

3 .MATERIALS AND METHODS

The current study on Barcoding and biosystematic studies on hymenopteran pollinators of cucurbitaceous vegetables was carried out in the Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, with an objective of studying the composition and relative abundance of hymenopteran pollinators of five selected cucurbits *viz.,* culinary melon (*Cucumis melo var. acidulus*), bitter gourd (*Momordica charantia*), ash gourd (*Benincasa hispida*), pumpkin (*Cucurbita moschata*) and ridge gourd (*Luffa acutangula*) in Thiruvananthapuram and four other districts of Kerala and to study their diurnal activity in one selected cucurbit at College of Agriculture, Vellayani and to explore their morphological and molecular diversity. The materials used, techniques adopted and the observations made for the current study are presented here.

3.1. STUDY OF DIURNAL ACTIVITY, COMPOSITION AND RELATIVE ABUNDANCE OF FLOWER VISITORS

3.1.1. Diurnal Activity

Diurnal activity observations were recorded from the flowers of one selected cucurbit *viz.,* culinary melon in College of Agriculture, Vellayani. Foraging speed and foraging rate of bees were recorded from 06:00 h to 18:00 h.

3.1.1.1. Foraging Speed

The time spent by different species of bees on the flowers of one selected cucurbit *viz.,* culinary melon from landing to till take off was recorded by using stop watch. The observations were recorded from 06:00 h to 18:00 h during flowering period at an hourly interval for five minutes. The mean time spent by each bee was expressed in seconds per flower.

3.1.1.2. Foraging Rate

The number of flowers visited per minute (foraging rate) by different species of bees on one selected cucurbit *viz.*,culinary melon flowers were recorded. The observations were recorded from 06:00 h to 18:00 h during flowering period at an hourly interval for five minutes.

3.1.2. Composition and Relative Abundance of Flower Visitors

The composition and relative abundance of the different hymenopteran pollinators visiting flowers of cucurbitaceous vegetables were recorded in Thiruvananthapuram and four other districts of Kerala *viz.*, Kollam, Pathanamthitta, Alappuzha and Kasaragod.

Observations were taken from the randomly marked one square meter area during flowering period from 06:00 h to 18:00 h of the day at an hourly interval for five minutes and expressed as mean number of pollinators/m^2/5 min.

$$\text{Relative abundance} = \frac{\text{No. of individuals of a species}}{\text{Total number of individuals}} \times 100$$

3.2. COLLECTION OF POLLINATORS

3.2.1. Study Area

Samples were collected from fields of selected cucurbits mainly from Thiruvananthapuram district of Kerala. Few samples were also collected from other four districts of Kerala.

3.2.2. Time of Collection

Specimens were collected in the months of September, October, November and December during the year 2018 and in January, February and March during the year 2019 from 06:00 h to 18:00 h of the day.

3.2.3. Preservation of Specimens

All specimens were preserved either as dry or wet, and were neatly labelled and stored in insect boxes.

3.2.3.1. Wet specimen Preservation

The wet specimens were preserved in 70% ethyl alcohol in O- ring vials.

3.2.3.2. Dry specimen Preservation

Few of the specimens from each sample were pinned using entomological pins and also by carding. Specimens were spread in such a way that all possible diagnostic characters were properly visible. The pinned specimens were kept in a hot air oven at 45^0 C for drying.

3.2.3.3. Labelling

Acid-free paper was used for labelling of the dry specimens. Each specimen was labelled with country, state, district, vegetable name, date of collection, and name of the collector.

3.2.3.4. Storage

Both dry and wet specimens were stored at normal temperature. Vials containing wet samples were kept in cryo cube boxes. Pinned dry specimens were stored in airtight insect boxes.

3.3. IDENTIFICATION OF HYMENOPTERAN POLLINATORS

3.3.1. Morphological Characterization

The collected specimens were processed for the study of external morphological characters of body parts and genitalia following established procedures with the modifications wherever required. Important body regions and structures are measured and their relevant proportions were worked out.

3.3.1.1. Preparation of Permanent Slides

Permanent slides of antennae, mouthparts, legs, wings, and abdominal segments were prepared.

3.3.1.1.1. Clearing of Muscles

The specimens were heated in 10% KOH solution on a heat block at low mode for 30 to 40 minutes.

3.3.1.1.2. Passing Through Alcohol series

After KOH treatment, the specimens were rinsed with distilled water and passed through alcohol series of 70%, 90%, and 100%.

3.3.1.1.3. Dissection of Body Parts

Various body parts such as head, thorax, legs, wings, tergites, and sternites were dissected out in a cavity block containing ethyl alcohol. Fine forceps and pointed needles were used for dissection. Smooth brushes were used for transferring dissected parts to the slides. Proboscis and sternites were stretched with a drop of alcohol on a clean slide and fixed with coverslips to obtain better measurements.

3.3.1.1.4. Mounting

Dissected body parts were carefully aligned and DPX was used as mounting agent, immediately after which, coverslips were placed over it. The coverslip was gently pressed to remove excess DPX.

3.3.2. Molecular Characterization

Selected specimens identified through morphometric characterization were subjected to DNA sequencing.

3.3.2.1. Genomic DNA isolation

Genomic DNA was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions.

Tissues were placed in an 1.5 mL microcentrifuge tube. One hundred and eighty µl of T1 buffer and twenty five µl of proteinase K was mixed and incubated at 56^0 C in a water bath till the tissue was fully lysed. After completion of lysis, five µl of RNase A (100 mg/mL) was mixed and incubated at room temperature for five minutes. Two hundred µl of B3 buffer was added and incubated at 70^0 C for ten minutes. Two hundred and ten µl of 100 % alcohol was added and mixed totally by vortexing. The mixture was pipetted into NucleoSpin® Tissue column placed in a 2 mL collection tube and centrifuged at 11000 x g for one minute. The NucleoSpin® Tissue column was transferred to a new 2 mL tube and washed with five hundred µl of BW buffer. Washing was repeated using six hundred µl of B5 buffer. After complete washing the NucleoSpin® Tissue column was kept in a clean 1.5 mL tube and DNA was eluted out by using fifty µl of BE buffer.

3.3.2.2. Agarose gel electrophoresis for DNA quality check

The standard of the DNA isolated was checked using agarose gel electrophoresis. One µl of 6 X gel-loading buffer was added to five µl of DNA. The samples were loaded to 0.8 % agarose gel prepared in 0.5 X TBE (Tris-Borate-EDTA) buffer having 0.5 µg/mL ethidium bromide. Electrophoresis was conducted with 0.5 X TBE as electrophoresis buffer at 75 V till bromophenol dye reaches to the bottom of the gel. The gels were visualized in UV transilluminator (Genei) and therefore the image was captured under UV light using Gel documentation system (Bio-Rad).

3.3.2.3. PCR analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1 X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 1 µl DNA, 0.2 µl Phire Hotstart II DNA

polymerase enzyme, 0.1 mg/mL BSA and 3% DMSO, 0.5 M Betaine, 5 pM of forward and reverse primers.

Primers used

Target	Primer Name	Direction	Sequence (5' → 3')
COX1	LCO	Forward	GGTCAACAAATCATAAAGATATTGG
	HCO	Reverse	TAAACTTCAGGGTGACCAAAAAATCA

The PCR amplification was carried out in a PCR thermal cycler.

PCR amplification profile

COX 1

98^0 C	-	30 sec	
98^0 C	-	5 sec	10 cycles
45^0 C	-	10 sec	
72^0 C	-	15 sec	
98^0 C	-	5 sec	30 cycles
50^0 C	-	10 sec	
72^0 C	-	15 sec	
72^0 C	-	60 sec	
4^0 C	-	∞	

3.3.2.4. Agarose gel electrophoresis of PCR products

The PCR products were checked in 1.2 % agarose gels prepared in 0.5 X TBE buffer having 0.5 µg/mL ethidium bromide. One µl of 6 X loading dye was mixed with five µl of PCR products and was loaded and activity was conducted at 75 V with 0.5 X TBE as electrophoresis buffer for 1-2 hours, till the bromophenol blue had migrated to the bottom of the gel. The molecular standard used was a 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.3.2.5. ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) consists of 2 hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

Five micro litres of PCR product is mixed with 2 µl of ExoSAP-IT and incubated at 37^0 C for 30 minutes followed by enzyme inactivation at 80^0 C for 15 minutes.

3.3.2.6. Sequencing using Big Dye Terminator v 3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the Big Dye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems , USA) following manufactures protocol.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated) - 10-20 ng

Primer	-	3.2 pM (either Forward or Reverse)
Sequencing Mix	-	0.28 µl
DMSO	-	0.30 µl
5x Reaction buffer	-	1.86 µl
Sterile distilled water	-	make up to 10 µl

The sequencing PCR temperature profile consisted of a 1^{st} cycle at 96^0 C for 2 minutes followed by 30 cycles at 96^0 C for 30 sec, 50^0 C for 40 sec and 60^0 C for 4 minutes.

3.3.2.7. Post sequencing PCR clean up

1. Master mix I of ten µl milli Q and two µl 125 mM EDTA and master mix II of two µl of 3M sodium acetate pH 4.6 and 50 µl of ethanol were prepared.
2. Twelve µl of master mix I was added to each reaction containing ten µl of reaction contents and was properly mixed.

3. 52 µl of master mix II was added to each reaction.
4. Contents were mixed by inverting and incubated at room temperature for 30 minutes
5. Spun at 14,000 rpm for 30 minutes
6. Decanted the supernatant and added 100 µl of 70% ethanol
7. Spun at 14,000 rpm for 20 minutes.
8. Decanted the supernatant and repeated 70% ethanol wash
9. Decanted the supernatant and air dried the pellet.
10. The cleaned up air dried product was sequenced in ABI 3500 DNA analyzer.

3.3.2.8. Sequence analysis

The sequence quality was checked using Sequence Scanner Software v 1 (Applied Biosystems). By using Geneious Pro v 5.1 sequence alignment and required editing of the obtained sequences were carried out.

DNA sequences were analysed by using CLC sequence viewer software. Specimens were sequenced to know the intra specific variation in the sequences. Phylo-genetic tree was constructed using UPGMA (Un-weighed Pair Group Method with Arithmetic averaging).

Results

4. RESULTS

In the present study, the composition and relative abundance of hymenopteran pollinators in five selected cucurbitaceous vegetables and diurnal activity of hymenopteran pollinators in one selected cucurbit are presented in this chapter. Twenty nine species of hymenopteran pollinators grouped in 17 genera and 10 subfamilies were collected from 34 different locations in Thiruvananthapuram and four other districts of Kerala. These specimens were identified and diagnostic characters were documented with the help of photographs. An illustrated key to the species of hymenopteran pollinators in cucurbitaceous vegetables is provided. The specimens which could not be identified to species were subjected to DNA barcoding and the results are also presented in this chapter.

4.1. STUDY OF DIURNAL ACTIVITY, COMPOSITION AND RELATIVE ABUNDANCE OF FLOWER VISITORS

4.1.1. Diurnal Activity

Observations on diurnal activity were recorded from the flowers of one selected cucurbit *viz.,* culinary melon at College of Agriculture, Vellayani during two crop seasons at weekly intervals for three weeks (Plate 1 A and B).

4.1.1.1. Foraging Speed

The time spent by different bee species on the flowers of one selected cucurbit *viz.,* culinary melon, from landing to take off were recorded from 06:00 h to 18:00 h at an hourly interval for five minutes by using stop watch during two crop seasons in three weeks interval. The mean time spent by each bee was expressed in seconds per flower.

The foraging speed of *A. cerana indica* during two crop seasons is depicted in Table 1. It was found that, the mean time spent by each bee per flower was highest at 10:00-11:00 h in both the seasons with a mean of 10.61 and 10.63 seconds respectively.

Plate A. Season I

Plate B. Season II

Plate 1. Field view of culinary melon during season I & II

Table 1. Foraging speed (seconds) of *Apis cerana indica* in two seasons

Time (h)	Season I (week)				Season II (week)			
	I	II	III	Mean*	I	II	III	Mean*
06:00-07:00	1.20	1.50	1.30	1.33	1.40	1.60	1.80	1.60
07:00-08:00	3.10	3.60	3.30	3.33	2.80	3.80	3.20	3.26
08:00-09:00	4.90	4.20	3.70	4.26	5.00	4.00	3.40	4.13
09:00-10:00	7.90	6.80	8.70	7.80	8.00	7.70	7.60	7.76
10:00-11:00	10.23	10.71	10.89	10.61	10.60	10.80	10.50	10.63
11:00-12:00	7.20	5.40	7.00	6.53	7.80	5.60	6.00	6.46
12:00-13:00	5.60	4.50	5.20	5.10	5.20	4.20	5.00	4.80
13:00-14:00	3.60	3.20	2.50	3.10	3.40	3.00	2.80	3.06
14:00-15:00	4.30	4.10	4.60	4.33	4.20	4.00	4.20	4.13
15:00-16:00	2.30	2.40	2.20	2.30	2.60	2.40	2.40	2.46
16:00-17:00	2.10	1.40	1.60	1.70	2.20	1.60	1.80	1.86
17:00-18:00	1.30	1.20	0.80	1.10	1.20	0.60	1.40	1.06
CD (0.05)				0.84				0.78
SE (m) ±				0.29				0.27

*Mean of 5 observations

The foraging speed of *Tetragonula travancorica* Shanas and Faseeh during two crop seasons is depicted in Table 2. From the observations, it was inferred that, the mean time spent by each bee per flower was highest at 10:00-11:00 h in both the seasons with a mean of 11.23 and 11.46 seconds respectively.

The foraging speed of *C. hieroglyphica* during two crop seasons is depicted in Table 3. It was inferred that, the mean time spent by each bee per flower was highest at 09:00-10:00 h in both the seasons with a mean of 9.02 and 9.11 seconds respectively.

The foraging speed of *Halictus* sp. during two crop seasons is depicted in Table 4. It was found that, the mean time spent by each bee per flower was highest at 10:00-11:00 h in both the seasons with a mean of 10.26 and 10.40 seconds respectively.

The foraging speed of *Lasioglossum* sp. during two crop seasons is depicted in Table 5. From the observations it was confirmed that, the mean time spent by each bee per flower was highest at 09:00-10:00 h in both the seasons with a mean of 11.06 and 11.30 seconds respectively.

Finally, it was observed that, during two seasons, the foraging speed of *A. cerana indica, T. travancorica* and *Halictus* sp. was found to be highest during 10:00-11:00 h. The foraging speed of *C. hieroglyphica* and *Lasioglossum* sp. was found to be highest during 09:00-10:00 h. The foraging speed in the descending order was *T. travancorica* > *Lasioglossum* sp. > *A. cerana indica* > *Halictus* sp.> *C. hieroglyphica*.

4.1.2.2. Foraging Rate

The number of flowers visited per minute (foraging rate) by different bee species on one selected cucurbit *viz.*, culinary melon flowers were recorded from 06:00 h to 18:00 h at an hourly interval for 5 minutes during two crop seasons at three weekly intervals.

Table 2. Foraging speed (seconds) of *Tetragonula travancorica* in two seasons

Time (h)	Season I (week)				Season II (week)			
	I	II	III	Mean*	I	II	III	Mean*
06:00-07:00	1.60	1.90	1.40	1.64	1.60	1.50	1.70	1.60
07:00-08:00	3.60	3.90	3.40	3.76	3.30	3.80	3.50	3.53
08:00-09:00	5.20	5.40	5.10	5.23	5.50	5.60	5.30	5.43
09:00-10:00	8.10	8.50	8.20	8.26	8.40	8.60	8.30	8.43
10:00-11:00	11.10	11.40	11.20	11.23	11.70	11.20	11.50	11.46
11:00-12:00	7.90	8.10	7.60	7.86	7.40	8.10	7.60	7.70
12:00-13:00	5.20	5.80	5.30	5.43	5.60	5.30	5.70	5.53
13:00-14:00	4.70	4.30	4.80	4.61	4.50	4.10	4.90	4.50
14:00-15:00	5.10	5.20	5.10	5.13	5.20	5.1 0	5.50	5.26
15:00-16:00	3.40	3.90	3.50	3.61	3.10	3.70	3.40	3.40
16:00-17:00	2.30	2.80	2.40	2.51	2.10	2.70	2.50	2.43
17:00-18:00	1.60	1.80	1.30	1.56	1.40	1.60	1.20	1.40
CD (0.05)				0.29				0.43
SE (m) ±				0.09				0.55

*Mean of 5 observations

Table 3. Foraging speed (seconds) of *Ceratina hieroglyphica* in two seasons

Time (h)	Season I (week)				Season II (week)			
	I	II	III	Mean*	I	II	III	Mean*
06:00-07:00	2.53	3.01	2.71	2.77	2.41	3.12	2.09	2.54
07:00-08:00	4.21	3.98	4.15	4.11	3.71	4.09	3.92	3.90
08:00-09:00	6.32	5.12	6.03	5.82	7.01	6.92	6.81	6.91
09:00-10:00	8.92	9.12	9.03	9.02	9.22	9.14	8.97	9.11
10:00-11:00	7.21	8.32	7.92	7.81	7.84	8.12	8.02	7.99
11:00-12:00	5.03	4.92	4.12	4.69	5.16	5.04	4.84	5.01
12:00-13:00	3.11	2.98	2.41	2.83	2.71	2.32	2.91	2.64
CD (0.05)				0.78				0.47
SE (m) ±				0.25				0.15

*Mean of 5 observations

Table 4. Foraging speed (seconds) of *Halictus* sp. in two seasons

Time (h)	Season I (week)				Season II (week)			
	I	II	III	Mean*	I	II	III	Mean*
06:00-07:00	2.76	2.25	1.98	2.33	2.42	2.61	2.03	2.35
07:00-08:00	3.87	3.11	3.01	3.33	3.72	3.09	2.86	3.22
08:00-09:00	5.91	6.02	5.53	5.82	5.82	5.89	6.13	5.94
09:00-10:00	8.11	7.92	8.01	8.04	8.18	8.10	7.84	8.02
10:00-11:00	10.40	9.80	10.60	10.26	10.80	10.00	10.40	10.40
11:00-12:00	6.12	5.73	5.01	5.62	5.84	6.21	6.09	6.04
12:00-13:00	3.31	2.71	3.24	3.08	3.18	3.09	3.12	3.13
CD (0.05)				0.55				0.46
SE (m) ±				0.18				0.16

*Mean of 5 observations

Table 5. Foraging speed (seconds) of *Lasioglossum* sp. in two seasons

Time (h)	Season I (week)				Season II (week)			
	I	II	III	Mean*	I	II	III	Mean*
06:00-07:00	1.34	1.76	1.87	1.65	1.98	1.54	1.65	1.72
07:00-08:00	4.23	4.81	4.92	4.65	4.76	4.51	4.62	4.63
08:00-09:00	7.74	7.91	8.01	7.88	8.11	7.82	7.94	7.95
09:00-10:00	11.02	10.84	11.34	11.06	11.73	11.21	10.97	11.30
10:00-11:00	8.21	8.63	7.54	8.12	8.11	8.32	8.09	8.17
11:00-12:00	5.43	5.82	4.73	5.32	4.93	5.64	5.78	5.45
12:00-13:00	2.76	2.41	2.82	2.66	2.23	2.67	2.44	2.41
CD (0.05)				0.68				0.52
SE (m) ±				0.22				0.16

*Mean of 5 observations

The foraging rate of *A. cerana indica* in two crop seasons is depicted in Table 6. It was found that, the number of flowers visited by each bee per minute was highest at 11:00-12:00 h in both the seasons with a mean of 10.60 and 10.88 respectively.

The foraging rate of *T. travancorica* in two crop seasons is depicted in Table 7. It was observed that the number of flowers visited by each bee per minute was highest at 10:00-11:00 h in both the seasons with a mean of 9.16 and 9.23 respectively.

The foraging rate of *C. hieroglyphica* in two crop seasons is depicted in Table 8. It was observed that the number of flowers visited by each bee per minute was highest at 10:00-11:00 h in both the seasons with a mean of 5.01 and 5.10 respectively.

The foraging rate of *Halictus* sp. in two crop seasons is depicted in Table 9. It was observed that the number of flowers visited by each bee per minute was highest at 09:00-10:00 h in both the seasons with a mean of 4.03 and 4.13 respectively.

The foraging rate of *Lasioglossum* sp. in two crop seasons is depicted in Table 10. It was observed that the number of flowers visited by each bee per minute was highest at 10:00-11:00 h in both the seasons with a mean of 4.83 and 4.85 respectively.

Finally, it was observed that, during two seasons, the foraging rate of *T. travancorica*, *C. hieroglyphica* and *Lasioglossum* sp. was found to be highest during 10:00-11:00 h. The foraging rate of *A. cerana indica* and *Halictus* sp. was found to be highest during 11:00-12:00 h and 09:00-10:00 h respectively. The foraging rate in the descending order was *A. cerana indica* > *T. travancorica* > *C. hieroglyphica* > *Lasioglossum* sp. > *Halictus* sp.

Table 6. Foraging rate of *Apis cerana indica* in two seasons

Time (h)	Season I (week)				Season II (week)			
	I	II	III	Mean*	I	II	III	Mean*
06:00-07:00	1.40	1.70	1.30	1.46	1.80	1.60	1.53	1.64
07:00-08:00	2.20	2.60	2.40	2.40	2.40	2.30	2.40	2.36
08:00-09:00	3.90	4.10	3.70	3.90	4.20	3.80	3.86	3.95
09:00-10:00	6.50	6.80	6.10	6.46	6.20	6.60	6.20	6.33
10:00-11:00	9.60	9.20	8.90	9.23	9.20	9.40	9.20	9.26
11:00-12:00	10.90	10.60	10.30	10.60	11.20	10.40	11.60	10.88
12:00-13:00	6.50	7.40	6.80	6.90	7.40	6.80	6.93	7.04
13:00-14:00	5.70	5.40	5.60	5.56	5.60	5.80	5.60	5.66
14:00-15:00	6.40	6.30	6.20	6.30	6.80	6.20	6.46	6.48
15:00-16:00	5.10	5.20	5.30	5.20	5.20	5.60	5.33	5.37
16:00-17:00	3.30	3.60	2.40	3.10	4.00	3.40	3.33	3.57
17:00-18:00	1.20	1.70	1.30	1.40	1.60	1.00	1.46	1.35
CD (0.05)				0.45				0.46
SE (m) ±				0.15				0.15

*Mean of 5 observations

Table 7. Foraging rate of *Tetragonula travancorica* in two seasons

Time (h)	Season I (week)				Season II (week)			
	I	II	III	Mean*	I	II	III	Mean*
06:00-07:00	1.11	1.22	1.31	1.21	1.16	1.20	1.60	1.32
07:00-08:00	1.56	2.12	1.89	1.85	2.01	1.97	2.00	1.99
08:00-09:00	3.42	3.21	3.18	3.27	3.68	3.35	3.40	3.47
09:00-10:00	5.14	5.23	5.41	5.26	5.05	5.13	5.42	5.20
10:00-11:00	9.08	9.14	9.27	9.16	9.12	9.10	9.48	9.23
11:00-12:00	8.12	8.23	8.56	8.30	8.46	8.52	8.61	8.53
12:00-13:00	7.03	6.12	6.09	6.41	6.04	6.19	6.92	6.38
13:00-14:00	5.04	5.11	5.29	5.14	5.02	5.13	5.22	5.12
14:00-15:00	6.11	6.14	6.23	6.16	6.13	6.08	6.26	6.15
15:00-16:00	4.16	4.51	4.20	4.29	4.95	4.48	4.60	4.67
16:00-17:00	3.21	3.09	3.16	3.15	3.35	3.12	3.20	3.22
17:00-18:00	1.02	1.05	1.07	1.04	1.03	1.02	1.40	1.15
CD (0.05)				0.37				0.30
SE (m) ±				0.12				0.10

*Mean of 5 observations

Table 8. Foraging rate of *Ceratina hieroglyphica* in two seasons

Time (h)	Season I (week)				Season II (week)			
	I	II	III	Mean*	I	II	III	Mean*
06:00-07:00	0.58	0.66	0.33	0.52	0.43	0.54	0.23	0.40
07:00-08:00	0.75	1.06	0.56	0.79	0.81	1.23	0.67	0.90
08:00-09:00	2.50	2.87	2.37	2.58	2.94	2.61	2.18	2.57
09:00-10:00	3.37	3.43	3.12	3.30	3.74	3.52	3.06	3.44
10:00-11:00	4.82	5.03	5.12	5.01	4.91	5.16	5.23	5.10
11:00-12:00	2.81	3.06	2.56	2.81	2.62	2.94	3.02	2.86
12:00-13:00	1.31	1.56	1.06	1.31	1.14	1.42	1.24	1.26
CD (0.05)				0.23				0.43
SE (m) ±				0.07				0.14

*Mean of 5 observations

Table 9. Foraging rate of *Halictus* sp. in two seasons

Time (h)	Season I (week)				Season II (week)			
	I	II	III	Mean*	I	II	III	Mean*
06:00-07:00	0.15	0.11	0.08	0.11	0.06	0.19	0.14	0.13
07:00-08:00	0.39	0.24	0.19	0.27	0.21	0.42	0.34	0.32
08:00-09:00	1.42	1.36	1.28	1.35	1.63	1.54	1.82	1.66
09:00-10:00	3.91	4.12	4.08	4.03	4.31	3.98	4.12	4.13
10:00-11:00	2.44	2.16	2.05	2.21	2.32	2.08	2.21	2.20
11:00-12:00	1.20	1.12	1.07	1.13	1.04	1.19	1.11	1.11
12:00-13:00	0.24	0.16	0.13	0.17	0.21	0.12	0.09	0.14
CD (0.05)				0.16				0.21
SE (m) ±				0.05				0.06

*Mean of 5 observations

Table 10. Foraging rate of *Lasioglossum* sp. in two seasons

Time (h)	Season I (week)				Season II (week)			
	I	II	III	Mean*	I	II	III	Mean*
06:00-07:00	0.05	0.08	0.02	0.05	0.04	0.06	0.03	0.04
07:00-08:00	0.18	0.24	0.32	0.24	0.16	0.21	0.29	0.22
08:00-09:00	1.02	1.12	1.46	1.20	1.34	1.28	1.30	1.30
09:00-10:00	2.13	2.48	2.32	2.31	2.83	2.41	2.64	2.62
10:00-11:00	5.01	4.92	4.56	4.83	4.81	4.63	5.12	4.85
11:00-12:00	3.41	3.18	3.05	3.21	3.23	3.31	3.02	3.18
12:00-13:00	1.52	1.44	1.36	1.44	1.61	1.32	1.24	1.39
CD (0.05)				0.31				0.28
SE (m) ±				0.10				0.09

*Mean of 5 observations

4.1.2. Composition and Relative Abundance of Flower Visitors

To determine the composition and relative abundance of different hymenopteran pollinators visiting the flowers of five selected cucurbitaceous vegetables, net sweepings were made throughout the blooming period at different locations in Thiruvananthapuram and four other districts of Kerala from 06:00 h to 18:00 h of the day by a cone type hand net.

The number of hymenopteran pollinators visiting the flowers of five selected cucurbitaceous vegetables were observed closely and recorded. The composition and relative abundance of hymenopteran pollinators is presented crop wise below.

4.1.2.1. Culinary melon (Cucumis melo var. acidulus)

Hymenopteran pollinators documented from culinary melon from five districts are listed in Table 11. The major floral visitors documented comprise *A. cerana indica, T. travancorica, Ceratina* sp., *A. dorsata, Nomia* sp., *A. florea, Amegilla zonata* L., *Xylocopa verticals* (Apidae), *Lasioglossum* sp., *Halictus* sp. (Halictidae) and wasps (Vespidae and Scoliidae) (Plate 2). Among the floral visitors, *A. cerana indica* (42.15 %) was the most dominant pollinator followed by *T. travancorica* (18.56 %) and *Ceratina* sp. (11.38 %).

4.1.2.2. Bitter gourd (Momordica charantia)

Hymenopteran pollinators documented from bitter gourd from five districts are listed in Table 12. The major floral visitors documented comprise *T. travancorica, A. cerana indica, Ceratina* sp., *A. dorsata* (Apidae), *Lasioglossum* sp., *Halictus* sp. (Halictidae), *Megachile lanata* F., *Megachile disjuncta* F. (Megachilidae). Among the floral visitors, *T. travancorica* (31.86 %) was the most dominant pollinator followed by *A. cerana indica* (29.90 %) and *Ceratina* sp. (11.76 %).

Table 11. Composition and Relative Abundance of Different Hymenopteran Pollinators in Culinary melon (*Cucumis melo var. acidulus*)

Pollinator	Thiruvananthapuram	Kollam	Pathanamthitta	Alappuzha	Kasaragod	Total	% Relative abundance
Apis cerana indica	167	21	13	4	8	213	42.51
Tetragonula travancorica	77	5	6	-	5	93	18.56
Ceratina sp.	46	6	5	-	-	60	11.38
Apis dorsata	44	2	4	-	1	51	10.18
Lasioglossum sp.	10	4	5	-	4	23	4.59
Nomia sp.	12	-	-	5	2	19	3.79
Halictus sp.	5	3	3	3	-	20	2.79
Apis florea	7	1	2	-	-	10	1.96
Xylocopa verticals	6	-	-	-	3	9	1.80
Amegilla zonata	5	-	-	2	-	7	1.40
Wasps	3	-	1	1	-	5	1.00
Total	382	42	39	15	23	501	

A. *Apis cerana indica*

B. *Apis dorsata*

C. *Apis florea*

D. *Tetragonula travancorica*

E. *Ceratina binghami*

F. *Ceratina hieroglyphica*

G. *Halictus* sp.

H. *Lasioglossum* sp.

Plate 2. Hymenopteran pollinators in culinary melon

Table 12. Composition and Relative Abundance of Different Hymenopteran Pollinators in Bitter gourd (*Momordica charantia*)

Pollinator	Thiruvananthapuram	Kollam	Pathanamthitta	Alappuzha	Kasaragod	Total	% Relative abundance
Tetragonula travancorica	34	7	8	6	10	65	31.86
Apis cerana indica	39	6	7	4	5	61	29.90
Ceratina sp.	19	-	2	3	-	26	11.76
Lasioglossum sp.	8	-	-	1	3	17	8.00
Megachile sp.	5	4	4	-	2	15	7.35
Apis dorsata	7	2	-	3	1	16	6.37
Halictus sp.	6	3	1	2	-	12	5.88
Total	118	24	22	19	21	204	

4.1.2.3. Pumpkin (Cucurbita moschata)

Hymenopteran pollinators documented from pumpkin from five districts are listed in Table 13. The major floral visitors documented comprise *A. cerana indica, T. travancorica, Ceratina* sp., *A. dorsata* (Apidae), *Halictus* sp. and *Lasioglossum* sp. (Halictidae). Among the floral visitors, *A. cerana indica* (38.76 %) was the most dominant pollinator followed by *T. travancorica* (24.03 %) and *Ceratina* sp.(10.85 %).

4.1.2.4. Ash gourd (Benincasa hispida)

Hymenopteran pollinators documented from ash gourd from five districts are listed in Table 14. The major floral visitors documented comprise *T. travancorica, A. cerana indica, Ceratina* sp., *A. dorsata, Nomia* sp.,(Apidae), *Lasioglossum* sp., *Halictus* sp. (Halictidae), *M. lanata, M. disjuncta* (Megachilidae), Wasps (Scoliidae). Among the floral visitors, *T. travancorica* (33 %) was the most dominant pollinator followed by *A. cerana indica* (26.73 %) and *Ceratina* sp.(9.41 %).

4.1.2.5. Ridge gourd (Luffa acutangula)

Hymenopteran pollinators documented from ridge gourd from five districts are listed in Table 15. The major floral visitors documented comprise *A. cerana indica, X. verticals, A. dorsata, A. zonata* (Apidae), *Lasioglossum* sp., (Halictidae) and wasps (Vespidae). Among the floral visitors, *A. cerana indica* (35.16 %) was the most dominant pollinator followed by *X. verticals* (18.68 %) and wasps (15.38 %).

4.2. COLLECTION OF POLLINATORS

Samples were collected from the fields of five selected cucurbits in 34 locations from Thiruvananthapuram and four other districts of Kerala *viz.*, Kollam, Pathanamthitta, Alappuzha and Kasaragod. The places covered during collection are presented in Table 16. During the collection, 29 species of hymenopteran pollinators recorded in five cucurbitaceous vegetables (Table 17).

Table 13. Composition and Relative Abundance of Different Hymenopteran Pollinators in Pumpkin (*Cucurbita moschata*)

Pollinator	Thiruvananthapuram	Kollam	Pathanamthitta	Alappuzha	Kasaragod	Total	% Relative abundance
Apis cerana indica	28	7	6	5	4	50	38.76
Tetragonula travancorica	15	4	4	3	5	31	24.03
Ceratina sp.	8	3	-	-	3	14	10.85
Apis dorsata	7	-	1	3	2	13	10.08
Lasioglossum sp.	2	5	-	1	-	11	8.53
Halictus sp.	5	1	2	4		10	7.75
Total	65	20	13	17	14	129	

Table 14. Composition and Relative Abundance of Different Hymenopteran Pollinators in Ash gourd (*Benincasa hispida*)

Pollinator	Thiruvananthapuram	Kollam	Pathanamthitta	Alappuzha	Kasaragod	Total	% Relative abundance
Tetragonula travancorica	43	6	7	4	6	66	33.00
Apis cerana indica	39	4	5	2	4	54	26.73
Ceratina sp.	10	-	4	-	3	17	9.41
Halictus sp.	8	3	-	5	-	16	8.42
Nomia sp.	7	-	2	-	2	13	6.44
Lasioglossum sp.	6	-	2	-	1	9	5.45
Wasps	-	5	2	4	-	11	5.10
Apis dorsata	6	1	-	-	-	7	3.47
Megachile sp.	4	-	-	-	-	4	2.04
Total	123	19	24	15	16	197	

Table 15. Composition and Relative Abundance of Different Hymenopteran Pollinators in Ridge gourd (*Luffa acutangula*)

Pollinator	Thiruvananthapuram	Kollam	Pathanamthitta	Alappuzha	Kasaragod	Total	% Relative abundance
Apis cerana indica	11	6	5	4	6	32	35.16
Xylocopa verticals	5	5	-	6	1	17	18.68
Wasps	5	-	4	3	2	14	15.38
Apis dorsata	3	4	1	-	4	12	13.19
Lasioglossum sp.	3	-	-	2	4	9	9.89
Amegilla zonata	1	3	3	-	-	7	7.69
Total	28	18	13	15	17	91	

Table 16. Localities of sample collection

District	Localities covered	Number of locations
Thiruvananthapuram	Vellayani, Karamana, Kulathoor, Karyavattom, Karode, Venkulam, Balaramapuram,Vellarada, Pangode, Parassala, Idinjar, Pallichal, Mukkola, Oorutukaala, Azhicode, Melvettoor, Kalliyoor, Muttakkad, Venganoor, Vizhinjam, Athiyanoor, Pothencode, Puliyancode, Vattiyoorkavu, Perumkadavila	25
Kollam	Edamon, Kottarakkara, Karunagappally	3
Kasaragod	Padannakkad, Nileshwar	2
Alappuzha	Moncompu, Kavalam	2
Pathanamthitta	Padam, Thiruvalla	2
Total		34

Table 17. List of hymenopteran pollinators in cucurbitaceous vegetables

Family	Common name	Scientific name	Vegetable
Apidae	Indian bee	*Apis cerana indica* F.	Culinary melon, Bitter gourd, Pumpkin, Ash gourd, Ridge gourd
	Rock bee	*Apis dorsata* F.	Culinary melon, Bitter gourd, Pumpkin, Ash gourd, Ridge gourd
	Little bee	*Apis florea* F.	Culinary melon
	Stingless bee	*Tetragonula travancorica* Shanas and Faseeh	Culinary melon, Bitter gourd, Pumpkin, Ash gourd
		Tetragonula sp. nov.1	Pumpkin
	Small carpenter bee	*Ceratina hieroglyphica* Smith, *Ceratina simillima* Smith, *Ceratina binghami* Cockerell, *Ceratina unimaculata javanica* van der Vecht	Culinary melon, Bitter gourd, Pumpkin, Ash gourd, Ridge gourd
	Blue- banded bee	*Amegilla zonata* L.	Culinary melon, Ridge gourd
	Carpenter bee	*Xylocopa verticals* L.	Culinary melon, Ridge gourd

Family	Common name	Scientific name	Vegetable
Halictidae	Sweat bee	*Lasioglossum* sp.	Culinary melon, Bitter gourd, Pumpkin, Ash gourd
		Halictus sp. 1, *Halictus* sp. 2, *Halictus* sp. 3	Culinary melon, Bitter gourd, Pumpkin, Ash gourd, Ridge gourd
	Alkali bee	*Nomia eliotti* Smith, *Nomia westwoodi* Gribodo *Nomia curvipes* F., *Nomia* sp.	Culinary melon, Ashgourd
Megachilidae	Leaf- cutter bee	*Megachile lanata* F., *Megachile disjuncta* F.	Bitter gourd, Ash gourd
Vespidae	Paper wasp	*Ropalidia brevita* Das &Gupta	Culinary melon, Ridge gourd
	Potter wasp	*Eumenes* sp.	Ridge gourd
		Anterhynchium abdominale abdominale Illiger	Culinary melon
Sphecidae	Mud dauber	*Sceliphron madraspatanum* F.	Culinary melon
	Blue mud dauber	*Chalybion bengalense* Dahlbom	
Scoliidae	Scoliid wasp	*Phalerimeris phalerata phalerata* de Saussure	Ash gourd
		Campsomeriella annulata annulata F.	
Crabronidae	Mole cricket hunters	*Larra maura* F.	Culinary melon

4.3. IDENTIFICATION OF HYMENOPTERAN POLLINATORS

4.3.1. Morphological Characterization

All the collected specimens were observed under Leica stereo microscope (Model M-165 C) to identify the hymenopteran pollinators in five selected cucurbitaceous vegetables. Twenty nine species of hymenopteran pollinators were depicted family wise (Plate 3 to 9).

A check list of the species recorded during the study is given below.

Superfamily Apoidea

Family Apidae

Subfamily Apinae

Genus *Apis*

1) *A. cerana indica* F.

2) *A. dorsata* F.

3) *A. florea* F.

Genus *Tetragonula*

1) *T. travancorica* Shanas and Faseeh

2) *Tetragonula* sp. nov.1

Genus *Amegilla*

1) *A. zonata* Linnaeus

Subfamily Xylocopinae

Genus *Ceratina*

1) *C. hieroglyphica* Smith

2) *C. unimaculata javanica* van der Vecht

3) *C. binghami* Cockerell

4) *C. simillima* Smith

A. *Apis cerana indica*

B. *Apis dorsata*

C. *Apis florea*

D. *Tetragonula travancorica*

E. *Ceratina hieroglyphica*

F. *Ceratina simillima*

G.*Ceratina binghami*

H. *Ceratina unimaculata javanica*

I. *Amegilla zonata*

J. *Xylocopa verticals*

K. *Tetragonula* sp. nov.1

Plate 3. Hymenopteran Pollinators of Cucurbits: Family - Apidae

Genus *Xylocopa*

1) *Xylocopa verticals Smith*

Family Halictidae

Subfamily Halictinae

Genus *Halictus*

1) *Halictus* sp. 1

2) *Halictus* sp. 2

3) *Halictus* sp. 3

Genus *Lasioglossum*

1) *Lasioglossum* sp. 1

Subfamily Nomiinae

Genus *Nomia*

1) *Nomia eliotti* Smith

2) *N. westwoodi* Gribodo

3) *N. curvipes* Fabricius

4) *Nomia* sp.

Family Megachilidae

Subfamily Megachilinae

Genus *Megachile*

1) *M. lanata* F.

2) *M. disjuncta* F.

Family Vespidae

Subfamily Polistinae

Genus *Ropalidia*

1) *R. brevita* Das & Gupta

Subfamily Eumeninae

Genus *Eumenes*

1) *Eumenes* sp.

A. *Nomia eliotti*

B. *Nomia westwoodi*

C. *Nomia curvipes*

D. *Nomia* sp.

E. *Halictus* sp. 1

F. *Halictus* sp. 2

G. *Halictus* sp.3

H. *Lasioglossum* sp.

Plate 4. Hymenopteran Pollinators of Cucurbits: Family - Halictidae

A. *Megachile lanata*

B. *Megachile disjuncta*

Plate 5. Hymenopteran Pollinators of Cucurbits: Family - Megachilidae

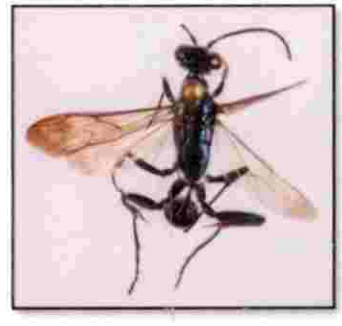
A. *Ropalidia brevita*

B. *Eumenes* sp.

C. *Anterhynchium abdominale abdominale*

Plate 6. Hymenopteran Pollinators of Cucurbits: Family - Vespidae

A. *Sceliphron madraspatanum*



B. *Chalybion bengalense*

Plate 7. Hymenopteran Pollinators of Cucurbits: Family - Sphecidae

Genus *Anterhynchium*

1) *Anterhynchium abdominale abdominale* Illiger

Family Sphecidae

Subfamily Sceliphrinae

Genus *Sceliphron*

1) *Sceliphron madraspatanum* F.

Genus *Chalybion*

1) *Chalybion bengalense* Dahlbom

Family Scoliidae

Subfamily Scoliinae

Genus *Phalerimeris*

1) *Phalerimeris phalerata phalerata* de Saussure

Genus *Campsomeriella*

1) *Campsomeriella annulata annulata* F.

Family- Crabronidae

Subfamily Crabroninae

Genus *Larra*

1) *Larra maura* F.

Illustrated key to the hymenopteran pollinators recorded on cucurbitaceous vegetables during the study.

1. First segment of hind tarsus wider than other segments, body hairs branched ..... Apiformes ..... 2 (Plate 10 A and B)

   First segment of hind tarsus as wide as other segments, body hairs not branched ..... Spheciformes ..... 5 (Plate 34 A and B)

2. Forewings with three submarginal cells; scopa on hind leg 3

A. *Phalerimeris phalerata phalerata*

B. *Campsomeriella annulata annulata*

Plate 8. Hymenopteran Pollinators of Cucurbits:Family - Scoliidae

A. *Larra maura*

Plate 9. Hymenopteran Pollinators of Cucurbits:Family - Crabronidae

(Plate 10 A and C)

Forewings with two submarginal cells; scopa on ventral aspect of abdomen ..... Megachilidae (Plate 29 A and 30 D)

Dorsum of T6 of female usually with hairs,pygidial plate usually absent but,if present, then represented by a narrow mid apical process .....Megachilinae (Plate 29 F)

Arolia absent ..... *Megachile* (Plate 29 B)

A. Abdomen with basal segment clothed with pale yellow long pubescence, rest of abdomen with sparse black pubescence; Pollen brush entirely jet-black ..... *M. disjuncta* (Plate 30 B and E)

B. Abdomen with basal one or two segments with fulvous pubescence, remaining banded with white pubescence; Pubescence with rich fulvous red ..... *M. lanata* (Plate 29 E and F)

3. Episternal groove present ..... 4 (Plate 28 B)

Episternal groove absent ..... Apidae (Plate 10 G)

Scopa of female, forming a corbicula on posterior tibia; pygidial, basitibial plates and hind tibial spurs absent; jugal lobe of hind wing present although notch delimiting it shallow; arolia present ..... Apinae (Plate 10 C and D)

All veins well developed, marginal cell closed by strong vein ..... *Apis*

C. Body size

a. Of large size; Head, thorax, and the basal three segments of the abdomen black, apical three segments of the abdomen honey yellow ..... *A. dorsata* (Plate 11 A)

b. Smaller; Head, thorax and apical abdominal segments black, the scutellum and basal five segments of the abdomen testaceous yellow ..... *A. cerana indica* (Plate 10 H)

c. Still smaller; Head, thorax and abdomen dull and opaque, basal two abdominal segments more or less red ..... *A. florea* (Plate 12 A)

Submarginal cross veins and second recurrent vein weak compared to other veins ..... *Tetragonula* (Plate 13 E)

Clypeus is reddish brown with yellowish margin and apical black border, prominently light yellowish throughout ..... *Tetragonula* sp. nov.1 (Plate 14 A)

Clypeus black without yellowish margin and apical black border ..... *T. travancorica* (Plate 13 A)

Scopa present on legs; scutellum not as above ..... *Amegilla* (Plate 15 B and E)

D. Abdomen with distinct transverse pubescent fasciae above, clypeus without a carina.

a. Hair of metasomal terga metallic blue; Pubescence on thorax dull rufofulvous clypeus has an each side at base an broad quadrate black mark ..... *A. zonata* (Plate 15 C and D)

Scopa of female not forming a tibial corbicula; pygidial and basitibial plates present; eyes very rarely hairy and jugal lobe of hind wing always present ..... Xylocopinae (Plate 16 E, F ,G and H)

Metallic blue or green integument, arolia present ..... *Ceratina* (Plate 18 A and C)

E. Black, variegated with yellow

a. A perpendicular shaped yellow macula on clypeus; Abdominal segments 1-5 with a yellow transverse band ..... *C. heiroglyphica* (Plate 20 A and F)

b. Clypeus with a transverse yellow line slightly broadened in the middle; Space at base of median segment minutely punctured ..... *C. simillima* (Plate 19 A and B)

Integument non metallic; arolia vestigial or absent, robust bees
..... *Xylocopa* (Plate 16 H)

F. With yellow and black pubescence
Occiput, thorax sometimes basal abdominal segment also with yellow pubescence; Yellow pubescence spreading to thorax under wings
..... *X. verticals* (Plate 16 A, B C and D)

4. Basal vein strongly arcuate, arolia well developed; Scopa present on tibia, nearly always absent on abdominal sterna ..... Halictidae (Plate 23 F and 25 F)

Episternal groove distinct and directed strongly downward below scrobal groove ..... Halictinae (Plate 28 B)

Episternal groove below scrobal groove absent or represented by weak depression ..... Nomiinae (Plate 24 B)

Abdominal terga with shining green, yellow or white integumental bands
..... *Nomia* (Plate 22 D)

G. Postscutellum with two teeth or spines in the middle posteriorly (Plate 21 A)
a. posterior legs black with pale glittering light pubescence ..... *Nomia eliotti* (Plate 21 E)

b. posterior legs pale rufo- testaceous ..... *N. westwoodi* (Plate 22 E)

H. Postscutellum unarmed posteriorly (Plate 23 C)

a. Legs rufo-fulvous or ferruginous; First abdominal segment with a transverse fasciae ..... *N. curvipes* (Plate 23 D, E)

Abdominal terga with apical pubescent hair bands, inferior basal process of penis valve rounded at apex; distal wing veins strong ..... *Halictus*
(Plate 25 C, D and G)

Abdominal terga with basal hair bands, inferior basal process of penis valve broad, truncate or obliquely truncate; distal wing veins weak
..... *Lasioglossum* (Plate 28 D, F and G)

5. Claws simple ..... Crabronidae (Plate 38 B)

Midtibia with 2 apical spur; forewing with fused submarginal and discoidal cells ..... Crabroninae

Forewing with stigma ..... *Larra* (Plate 38 F)

I. Propodeal enclosure round; Propodeum without punctations
..... *L. maura*

Claws with one, two or three inner teeth ..... Sphecidae (Plate 35 B)

Mandible simple ..... Sceliphrinae

Propodeal enclosure prominent..... *Sceliphron*

Propodeal enclosure absent..... *Chalybion*

J. Propodeum with medial longitudinal furrow; petiole slender with hairs; Thorax less pubescent; femora and tibia of posterior legs partly yellow with black marking ..... *S. madraspatanum* (Plate 34 D and E)

Clypeus finely punctuate; ocelli present; antennal sockets present above the clypeus; thorax hairy with deep punctures; abdomen with petiole
..... *C. bengalense* (Plate 35 A and B)

6. First abdominal segment not narrowed into a petiole, Claws of tarsi simple ..... Vespidae (Plate 31 E and F)

Hind coxa without dorsal carina, metasoma variable in shape, petiolate to subsessile or funnel shaped in dorsal view ..... Polistinae

Postscutellum with an apical triangular smooth and shiny area in middle, apical margin usually truncate; first gastral segment petiolate ..... *Ropalidia* (Plate 31 D)

K. Propodeum at base with pair of carina, propodeal orifice very narrow; Abdominal terga 2 with a broad apical yellow band, with its width near the middle more than one- fourth length of the tergum, clypeus wider than long ..... *R. brevita* (Plate 31 A)

First abdominal segment long and narrowed into a petiole, Claws of tarsi bifid or dentate ..... Eumeninae (Plate 32 E)

Petiole not narrowed at apex, mandibles long pointed without distinct teeth, antennae inserted high up in the middle of the face ..... *Eumenes* (Plate 32 A and C)

Metanotum without tubercles, scutum and scutellum punctate, metanotum angled, propodeal dorsum not at same level, propodeum crenulated or toothed laterally ..... *Anterhynchium*

Basal two thirds of second gastral tergite very finely and shallowly punctate ..... *A. abdominale*

Gaster mostly red except the following black markings, a narrow transverse band at the apex of abdominal terga 1, a spot on the middle of posterior apex of the abdominal terga 2 and sterna 2 ..... *A. abdominale abdominale* (Plate 33 C)

7. Posterior lateral angles of pronotum produced back to the base of wings ..... Scoliidae

Inner eye margin medially incised, reniform; middle tibia with a single spur; fore wing without a break or articulation between pterostigma and prestigma

..... Scoliinae

Lateral carina of propodeum abbreviated or extended up to spiracle; dorso-median area of propodeum triangularly protruded posteriorly, Wings entirely fuscous to yellowish hyaline ..... *Campsomeriella*

Body entirely black, white vestiture except on last abdominal segment black, wings hyaline except apical third of forewing dark brown

.....*C. annulata annulata* (Plate 37 C and D)

Lateral carina of propodeum extended beyond the spiracle; fore wing with first marginal cell almost entirely setose. Basal abdominal tergites with yellow or reddish-brown apical bands ..... *Phalerimeris*

Wings yellowish, forewing with a dark well defined subapical mark Abdominal terga 1-3 with narrow yellow apical bands ..... *P. phalerata phalerata* (Plate 36 A and C)

Diagnostic characters of hymenopteran pollinators in cucurbitaceous vegetables recorded during the study

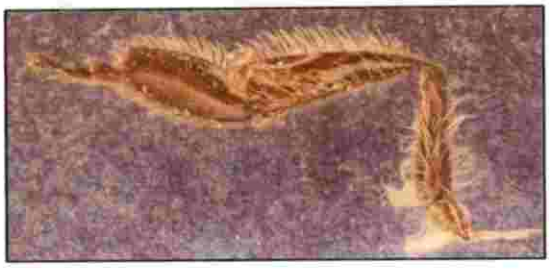
Genus- *Apis*

1. *Apis cerana indica*

Head, thorax and abdomen smooth and shining, Head, thorax and apical abdominal segments black, the scutellum and basal five segments of the abdomen testaceous yellow, legs rufo fuscous (Plate 10).

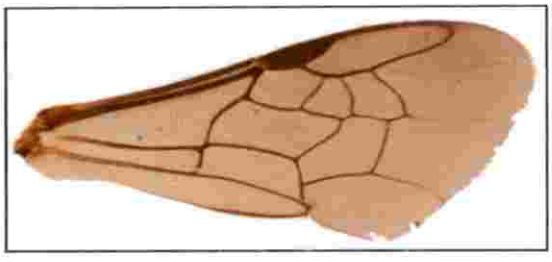
2. *Apis dorsata*

Head, thorax, and the basal three segments of the abdomen black, apical three segments of the abdomen honey yellow, legs beneath especially the posterior tibia and tarsi with short ferruginous pubescence (Plate 11).



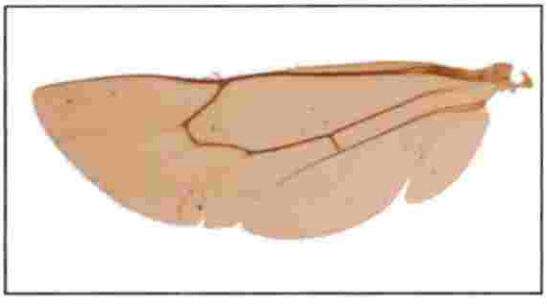
A. Hind leg

B. Body hairs



C. Forewing

D. Posterior tibia and tarsi



E. Hindwing

F. Head

G. Thorax

H. Abdomen

Plate 10. Diagnostic characters of *Apis cerana indica*

3. *Apis florea*

Head, thorax and abdomen dull and opaque, slightly pubescent, the pubescence on the head and thorax white, on the posterior tarsi ferruginous gold, the basal two abdominal segments more or less red (Plate 12).

Genus *Tetragonula*

1. *T. travancorica*

Integument, head, neck, gena, clypeus, thorax black. Labrum yellow; mandibles golden brown with black apex; compound eyes red brown; ocelli brown; It is distinguished from other Indian species by a strongly nebulous radial vein on the hind wings and dark brown erect setae on the margin of mesoscutellum. Metasoma darker towards apex; first two abdominal segments red brown, remaining ones darker; apical segment yellow to brown at apex, dark brown basally (Plate 13).

2. *Tetragonula* sp. nov.1

Black integument. Head is black. Face black with minute punctuation. Clypeus is reddish brown with yellowish margin and apical black border, prominently light yellowish throughout. Abdomen is darkening towards the apex. First two abdominal segments red brown and remaining segments are darker. Apical segment is yellowish at the tip and basally brownish (Plate 14).

Genus- *Amegilla*

1. *Amegilla zonata*

Arolia absent; body with metallic white, blue, and black markings Pubescence on thorax dull rufofulvous, yellow marking along clypeus and medial line across head (Plate 15).

A. Abdomen

B. Hind leg

Plate 11. Diagnostic characters of *Apis dorsata*

A. Abdomen

B. Hind leg

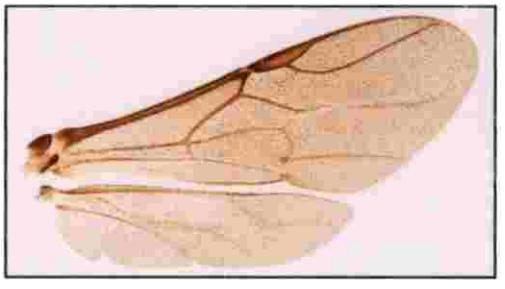
Plate 12. Diagnostic characters of *Apis florea*

A. Head

B. Mesoscutellum

C. Mesoepisternum

D. Metasoma



E. Forewing and Hindwing

Plate 13. Diagnostic characters of *Tetragonula travancorica*

A. Head

B. Infraepimeron

C. Mesoscutellum

D. Hind leg

E. Posterior tarsi

Plate 14. Diagnostic characters of *Tetragonula* sp. nov. 1

A. Hind leg

B. Thorax

C. Abdomen

D. Head

E. Tibial scopa

Plate 15. Diagnostic characters of *Amegilla zonata*

Genus- *Xylocopa*

1. *Xylocopa verticals*

Black, the pubescence on the head and face. Occiput, thorax sometimes basal abdominal segment also with yellow pubescence. Yellow pubescence spreading to thorax under wings (Plate 16)

Genus- *Ceratina*

1. *Ceratina binghami*

Bright blue-green species often with brassy reflection; well developed yellow marking on clypeus, with transverse bar apically; punctures on median portions of genal areas usually markedly smaller than those on upper and lower portions (Plate 17).

2. *Ceratina unimaculata javanica*

Body entirely greenish blue; Pronotal tubercles white or yellowish white; Legs with yellow markings. By a flat, smooth interspacing, punctures on upper portions of genal areas and lateral portions of mesoscutum were separated very well from each other (Plate 18).

3. *Ceratina simillima*

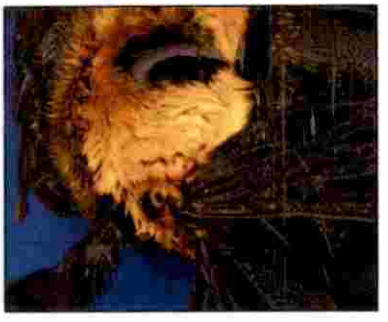
Clypeus with a transverse yellow line slightly broadened in the middle, transverse fasciae on the apical margin of abdominal segments 2-5 yellow, the fasciae on the 2nd and 3rd segment very widely, and on the 4th narrowly interrupted in the middle, on the 5th segment the fasciae is entire and broader than the others, two short parallel longitudinal lines on the disc of mesonotum and a yellow line reduced to two spots on the apical margin of basal segments of abdomen (Plate 19).

4. *Ceratina hieroglyphica*

Black, a perpendicular shaped mark on clypeus a lunate spot above it, a stripe on each side broadened below, a spot above each antenna, a broad stripe on each

A. Head

B. Abdomen



C. Yellow pubescence

D. Thorax

E. Hind leg

F. Hindwing

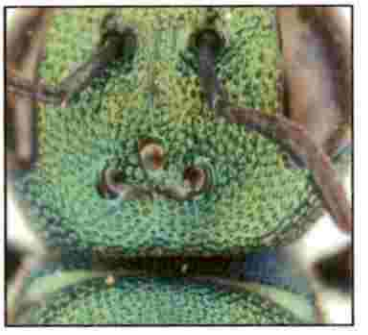
G. Pygidial plate

H. Posterior tarsi

Plate 16. Diagnostic characters of *Xylocopa verticals*

A. Lateral view

B. Clypeus



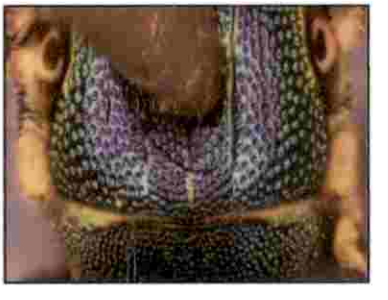
C. Head

Plate 17. Diagnostic characters of *Ceratina binghami*

A. Dorsal view

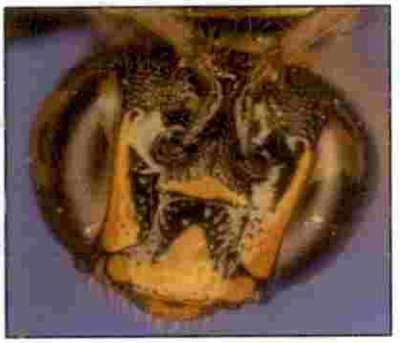
B. Head

C. Hind leg



D. Thorax

Plate 18. Diagnostic characters of *Ceratina unimaculata javanica*



A. Head

B. Thorax

C. Yellow lines

D. Abdomen

E. Scutellum

Plate 19. Diagnostic characters of *Ceratina simillima*

cheek, two parallel longitudinal lines on the mesonotum, a broad squarish mark in the middle of the scutellum (Plate 20).

Genus- *Nomia*

1. *Nomia eliotti*

Head and thorax closely and finely punctured, the postscutellum with two teeth or spines in the middle posteriorly, the basal four abdominal segments with bright emerald green transverse fasciae on their apical margins, Black the head and thorax with a white and somewhat griseous thin pubescence, the posterior legs black with pale glittering light pubescence (Plate 21).

2. *Nomia westwoodi*

This species closely resembles *Nomia eliotti* in having the postscutellum armed with two spines or teeth in the middle posteriorly, but it is constantly smaller, and in colour too it differs in having the posterior legs pale rufo-testaceous (Plate 22).

3. *Nomia curvipes*

Black the antennae, and the legs testaceous brown, the scape of the antennae pale the front and the legs with a pale glittering pubescence. Postscutellum unarmed posteriorly, thorax with more or less fulvous pubescence, first abdominal segment with a transverse fasciae, the apical margins of abdominal segments 1-4 bright greenish-yellow, smooth and shining (Plate 23).

4. *Nomia* sp.

Head and thorax with golden yellow pubescence closely and finely punctured; Postscutellum unarmed posteriorly and covered with light pale fulvous pubescence; the apical margins of abdominal segments 1-5 covered with pale fulvous pubescence; the posterior femora slightly swollen (Plate 24).

A. Head

B. Thorax

C. Mesonotum

D. Face

E. Antennae

F. Abdomen

Plate 20. Diagnostic characters of *Ceratina hieroglyphica*

A. Postscutellum

B. Head

C. Abdomen

D. Pronotal lobe

E. Legs

Plate 21. Diagnostic characters of *Nomia eliotti*

A. Head

B. Tegula

C. Thorax

D. Abdomen

E. Hind leg

Plate 22. Diagnostic characters of *Nomia westwoodi*

A. Antenna

B. Head

C. Postscutellum

D. Abdomen

E. Hind leg

F. Tibial spur

Plate 23. Diagnostic characters of *Nomia curvipes*

A. Head

B. Thorax

C. Scutellum

D. Tegula

E. Abdomen

F. Posterior tibia

Plate 24. Diagnostic characters of *Nomia* sp.

Genus *Halictus*

1. *Halictus* sp. 1

Head and thorax thickly pubescent, abdomen smooth slightly shining; median segment at the enclosed semicircular space at base large; golden yellow pubescence on the legs, abdomen very finely punctured; the basal segments of the abdomen rufo-fuscous; tegula pale testaceous (Plate 25).

2. *Halictus* sp. 2

Head and thorax black, the thorax and legs are lightly covered with hairy pubescence; abdomen smooth and shining, scutellum slightly pubescent, tegula testaceous, abdomen with narrow transverse bands of white pubescence (Plate 26).

3. *Halictus* sp. 3

Brassy green, head and thorax closely and finely punctured, the face covered with short white pubescence, the enclosed space at the base of metathorax reticulate, the postscutellum covered with short downy white pubescence (Plate 27).

Genus *Lasioglossum*

1. *Lasioglossum* sp. 1

Head and thorax black; Abdominal terga with basal hair bands, inferior basal process of penis valve broad, truncate or obliquely truncate; distal wing veins weak; tibial spurs with more than five teeth (Plate 28).

Genus- *Megachile*

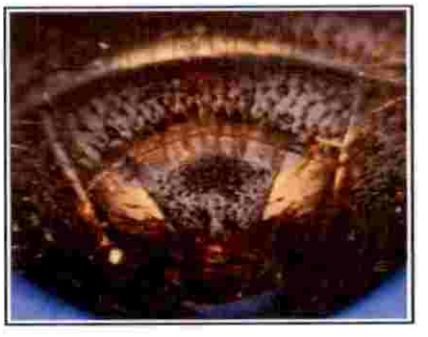
1. *Megachile lanata*

Abdomen with basal one or two segments with fulvous pubescence, remaining banded with white pubescence, pubescence with rich fulvous red (Plate 29).

A. Head

B. Scutellum

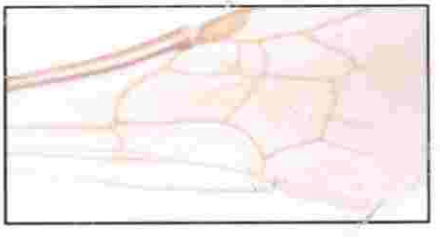
C. Abdomen



D. Penis valve

E. Tibial spur

F. Posterior tarsi



G. Forewing

Plate 25. Diagnostic characters of *Halictus* sp. 1

A. Head

B. Thorax

C. Scutellum

D. Abdomen

Plate 26. Diagnostic characters of *Halictus* sp. 2

A. Head

B. Thorax

C. Abdomen

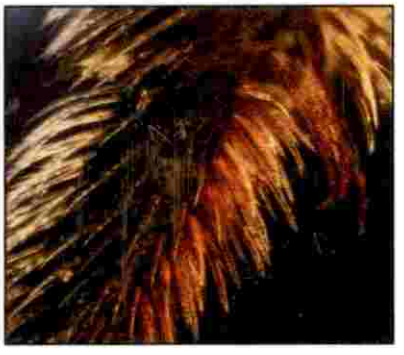
Plate 27. Diagnostic characters of *Halictus* sp. 3

A. Head

B. Lateral view

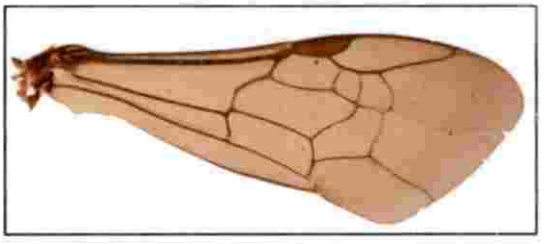
C. Thorax

D. Abdomen



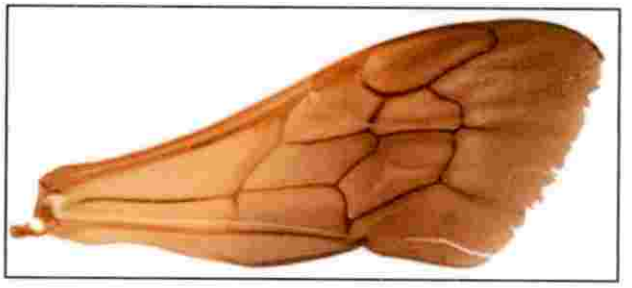
E. Tibial spur

F. Penis valve



G. Forewing

Plate 28. Diagnostic characters of *Lasioglossum* sp.



A. Forewing

B. Tarsi

C. Hind leg

D. Head

E. Thorax

F. Abdomen

Plate 29. Diagnostic characters of *Megachile lanata*

2. *Megachile disjuncta*

Abdomen with basal segment clothed with snow-white sometimes, pale yellow long pubescence, rest of abdomen with sparse black pubescence. Pollen brush entirely jet-black (Plate 30).

Genus- *Ropalidia*

1. *Ropalidia brevita*

First metasomal tergum proportionally wider, nearly half as wide as that of second tergum. Male terminal antennal flagellomere less strongly curved and bluntly pointed at apex, approximately two fold as long as its basal width. Aedeagus not spatulate apically (Plate 31).

Genus *Eumenes*

1. *Eumenes* sp.

First abdominal segment long and narrowed into a petiole, Claws of tarsi bifid or dentate Petiole not narrowed at apex, mandibles long pointed without distinct teeth, antennae inserted high up in the middle of the face (Plate 32)

Genus- *Anterhynchium*

1. *Anterhynchium abdominale abdominale*

Head and mesosoma black; metasoma dull orange-red with variegated black markings as follows: the basal segment with a transverse black apical band, Abdominal terga 2 with or without a transverse black spot in the middle of its apical margin, the remaining segments usually orange-red except last segment black, Wings dark fuscous with purple reflections (Plate 33).

Genus *Sceliphron*

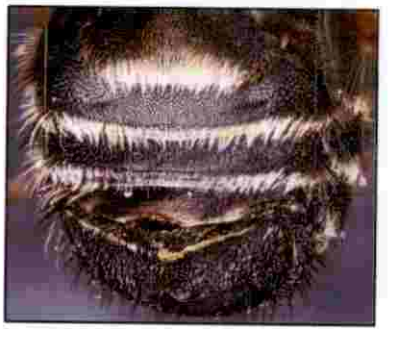
1) *Sceliphron madraspatanum*

Body black with yellow pattern on thorax; narrow slender waist yellow in colour; scape below except base, tegulae, part of post scutellum, petiole, nearly apical half of fore and mid femora and basal half of hind femora, whole of fore and mid tibiae and basal two thirds of hind tibiae, first tarsomere of hind legs

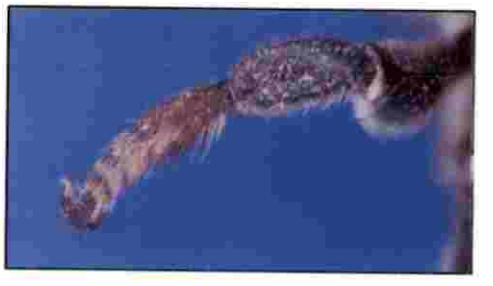
A. Head

B. Yellow pubescence

C. Abdomen



D. Abdominal sterna



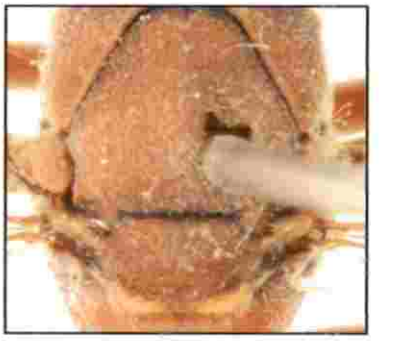
E. Pollen brush

Plate 30. Diagnostic characters of *Megachile disjuncta*

A. Head

B. Eyes

C. Antenna



D. Thorax

E. Abdomen

F. Posterior tarsi

Plate 31. Diagnostic characters of *Ropalidia brevita*

A. Head

B. Thorax

C. Propodeum

D. Abdomen

E. Claws

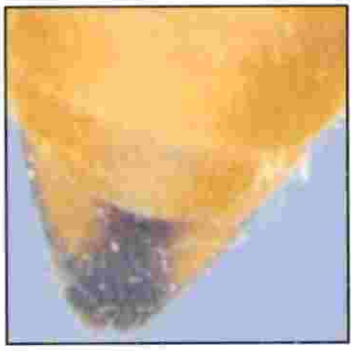
Plate 32. Diagnostic characters of *Eumenes* sp.

A. Thorax

B. Head

C. Abdomen

D. Forewing



E. Pygidial plate

Plate 33. Diagnostic characters of *Anterhynchium abdominale abdominale*

except base and apex above, and trochanter yellow; wings hyaline with a brownish tint (Plate 34).

Genus- *Chalybion*

1) *Chalybion bengalense*

Clypeus broad, bears three to five projections; clypeus finely punctuate; ocelli present; antennal sockets present above the clypeus; thorax hairy with deep punctures; propodeal enclosure carinated; abdomen with petiole; claws simple (Plate 35).

Genus- *Phalerimeris*

1) *Phalerimeris phalerata phalerata*

Group of deep punctures in front of anterior ocellus, Scapulae without shallow longitudinal grooves, forewing with submarginal cell almost entirely setose; Abdominal terga 1-3 with narrow yellow apical bands, Legs black with tibia and tarsi pale red, Wings yellowish, forewing with a dark well defined subapical mark (Plate 36).

Genus- *Campsomeriella*

1) *Campsomeriella annulata annulata*

Antennal flagellum black; with black integument, white vestiture except on last abdominal segment black, wings hyaline except apical third of forewing dark brown (Plate 37).

Genus- *Larra*

1) *Larra maura*

Head and thorax minutely and closely punctured and thinly pubescent, head shining, clypeus much broader than long, thorax bearing a medial longitudinal shallow furrow, abdomen smooth and shining with transverse bands of silky pale, which are broadened laterally on the posterior margins of first to fifth segments

A. Hind leg

B. Body hairs

C. Head

D. Thorax

E. Abdomen

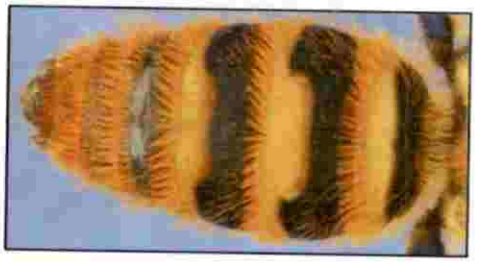
Plate 34. Diagnostic characters of *Sceliphron madraspatanum*

A. Head

B. Abdomen

C. Thorax

Plate 35. Diagnostic characters of *Chalybion bengalense*



A. Abdomen

B. Hind leg

C. Forewing

Plate 36. Diagnostic characters of *Phalerimeris phalerata*

A. Head

B. Abdomen

C. Pygidial plate

D. Forewing

Plate 37. Diagnostic characters of *Campsomeriella annulata annulata*

above, these bands being only visible in certain lights, the scape of the antennae in front rufo-fuscous, posterior femora bright red (Plate 38).

Among the pollinators, five species *viz*., *P. phalerata phalerata, C. annulata annulata* from ash gourd, *M. disjuncta* from bitter gourd, *C. similima,* and *C. unimaculata javanica* from culinary melon were reported for the first time pollinating cucurbitaceous vegetables.

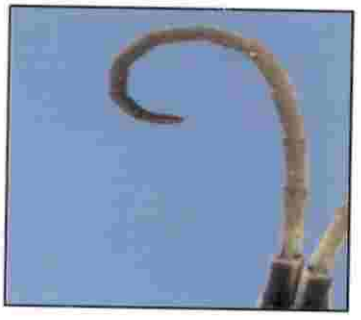
The stingless bee *Tetragonula* sp. nov.1 has been recorded from Thiruvananthapuram district. The specimen was collected from a pumpkin flower and identified as new species with the help of morphometric characters and key provided by Shanas and Faseeh (2019) and was verified with DNA barcoding. The specimens collected have been deposited in Travancore insect collection, College of Agriculture, Vellayani, Thiruvananthapuram. It was morphologically characterised as given below.

Holotype : Female (worker): Head width= 1.815 mm, Head length= 1.551 mm, Length of scape = 0.656 mm, diameter of scape= 0.102 mm, Length of 3rd flagellomere= 0.141 mm, Length forewing including tegula= 4.5 mm, Length of marginal cell= 1.44 mm, Length of mesoscutum = 1.1 mm, Length of tibia = 1.71 mm, Width of tibia = 0.651 mm, Length of basitarsus = 0.54 mm, Length of body = 4.3 mm. Bees are large in size

Color: Black integument. Head black. Face black with minute punctuation. Clypeus reddish brown with yellowish margin and apical black border, prominently light yellowish throughout. Mandibles yellowish except at the base and apical region, apical ¼ th which is brown. Labrum yellow with short white hairs and long golden hairs. Labrum with black spot. Compound eyes yellow brown. Ocelli white. Hairs on vertex stout and black. The lower area of eyes (area between compound eyes and mandible) slightly brown, but malar area black. Antenna yellowish ventrally and brownish dorsally with depression on each flagellomere except on the first flagellomere. First flagellomere more yellowish.

A. Head

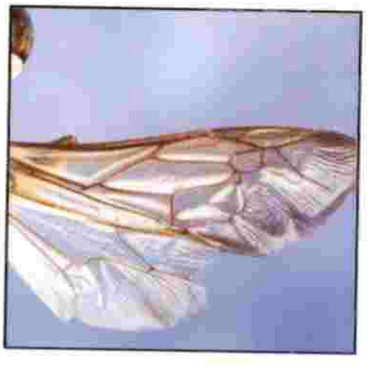
B. Abdomen



C. Antenna

D. Thorax

E. Hind leg



F. Wings

Plate 38. Diagnostic characters of *Larra maura*

Scape ventrally yellow and dorsally brown. Antennifer milky white. Neck and gena black.

Thorax black. Tegula and wing sclerites brown. Veins on the wing are brown. Pterostigma light brown. Wings hyaline and iridescent. Legs reddish brown with tarsal segments brown. Trochanter yellow brown with white hairs. Forelegs lighter than middle and hind legs. Mesotibial spur yellow. Brown spot on the black basitarsus. Arolium yellow brown with distally golden yellow color. Scutellum black. Metanotum lighter. Propodeum black.

Abdomen darkening towards the apex. First two abdominal segments red brown and remaining segments darker. Apical segment yellowish at the tip and basally brownish. Hairs absent on the first two abdominal segments.

Pilosity: Dense plumose hairs present on the region below the antennifers, and above which hairs comparatively less and not dense. Hairs on vertex dark and stout. Malar space have hairs in some bees. Labrum with white and black hairs. Fine hairs present in the gena and few of the hairs behind the vertex plumose. Hairs present on the neck. Area behind the vertex with long plumose hairs. Hairs on gena light brown. Neck with simple white hairs.

Mesoscutum with long, dull brown, less branched plumose hairs. Stout black hairs found more anteriorly. Stout white hairs also distributed over mesoscutum. No distinct hair bands, hairs cover mesoscutum evenly. Scutellum with both dark and dull brown simple hairs, along with dull brown plumose hairs. Hairs on scutellum and mesoscutum almost similar in size. Metanotum with short and dull brown hairs.

Anterior mesopleuron with white plumose hairs. Plumose hairs at the base of hind wing (metapleuron) more denser than hairs on anterior region. Trochanter with white spurs. Femur and tibia with black stout hairs on it. Posterior region of hind basitarsus with simple hairs on the medial line (Plate 14).

Males: Unknown

Material examined: Holotype: F: INDIA, KERALA: Thiruvananthapuram, Kulathoor, Erra Harisha, 28-X-2018; **Paratypes**: 3 F: Same data as that of Holotype.

Distribution: INDIA (Kerala, Thiruvananthapuram).

Out of 50 different measurements taken, the mean values of the new species was distinct with respect to six parameters. The measurements taken were, Head width, Intertentorial distance, Clypeo ocellar distance, Length of pedicel+flagellomere, Length of forewing excluding tegula, Length of forewing including tegula, Wing diagonal (WL2), Width of forewing, Length of marginal cell, Length of abscissa of Cu, Length of abscissa of M. The following ratios are also distinct in these bees, Ratio between length and diameter of scape, Length and width of mandible, Length and width of pterostigma, Alveolar orbital distance and alveolar diameter.

The overall measurements of all body parts were larger in *Tetragonula* sp. nov. 1. The width of head, ratio between length and diameter of scape, ratio between length and width of pterostigma, IOD/OOD, length of mesoscutum/width of mesoscutum and length of eye/scape length gave distinct values which can clearly differentiate *Tetragonula* sp. nov.1 from *Tetragonula calophyllae* Shanas and Faseeh.

4.3.2. Molecular Characterization

Samples which were unidentified through morphological characterization were given for DNA barcoding. The sequence of 2 samples *viz.*, *Tetragonula* sp. nov.1 and *T. travancorica* were obtained. Among these, the species (*Tetragonula* sp. nov.1) of stingless bee, based on adult worker specimen is described. Differences in morphology and genetic analysis based on partial sequences of the mitochondrial COI gene barcode region support the recognition of the new species.

>*Tetragonula travancorica*

ATTATTGGATCATCTTTTAGTATGCTAATTCGAATAGAACTTAACAGTC
CTGGAATATGGATCAATAACGATCAAATTTACAACTCAATTATTACTA
GCATGCATTTCTAATAATTTTTTTCATAGTTATACCTTTCATAATTGGG
GGGTTTGGAAATTTTTTGATCCCCATAATGCTTGGATCTCCCGATATGG
CTTTTCCACGTATAAATAATGTTAGGTTTTGACTTCTACCACCATCTCT
TTTACTCCTTCTCCTAAGAAACTTTTTATTTCCTAGATCAGGAACTGGG
TGAACAATCTATCCTCCATTGTCATCCTATCTGTACCATTCATCTCCTTC
AGTTGACTTAACTATTTTTTCTATTCACATGACTGGAATCTCATCAATT
TTAGGATCTCTAAATTTCATTGTAACAATTTTTATAATAAAAAATTTTT
CTCTTAATTACGATCAGATCAGACTCTTCTCTTGATCTATTTCAGTTAC
TGTGATTCTTCTTATTGTTTCCCTACCTGTTTTGGCAGGAGCAATTACA
ATACTCTTGTTTGATCGAAACTTCAACACTTCATTCTTTGATCCAATAG
GAGGAGGAGATCCAATTCTTTATCAGCACCTTTTCTGATTC

> *Tetragonula* sp. nov. 1

CAAAATTCTTGGAGGGTGCGGCATTCATGATCAGGATTATCGGATCTT
CTTTCAGAATAATTATTCGAATAGAATTGAATAATCCAGGTATATGAA
TTAATAATGACCAAATTTATAATTCAATTATTACTAGGCATGATTTCTA
ATAATTTTTTTTATAGTTATACCTTTTATAATTGGAGGTTTTGGAAATTT
TCTTGTTCCTTTAATGCTTGGAGCTCCTGATATAGCTTTTCCACGAATG
AATAATATTAGATTTTGATTACTTCCACCTTCATTATTTCTTTTATTAAT
TAGAAGTTTTATTTTTCCTAGGTCAGGGACAGGATGAACTGTTTATCCT
CCTTTATCTTCATTCATATTCAGCCGTCCCCTTCTGTAGATTTTACCATT
TTTTCTATTCATATAAAGGGATTCATCTAATTTAAGGACAATAAATATT
ATTGTAAAATTTTTATAATAAAAATATATCTCTAAATAATGATCAAATT
AGTTATTTCCTTGATCAATTCAAGTTACTGTAATTCTATTAATTTGTATC
TTTACCTGGATGGGGCGGGAGCAATTAACAATACTTTTATTTTGACCG
AAATTTTAAATACCTCTTTTTTTTGATCCAATGGGAAGGGGGGGTTCCA
ATTCTTTATCAACATCTTTTTTTGATTTTTTTGGCCCCCCCAGAAAAAA

AAAGCCTTCTCCGGAATTGGTTTCAATTTTTTTTTTGATTTGTTGCACA
AATTTTTTTTTATTTTGTTG

The results of DNA sequencing also support the previous observations (Morphological characterization). The partial sequencing of mitochondrial COI gene showed 27% variation between the new species, *Tetragonula* sp. nov.1 and *T. travancorica* indicating that this species does not belong to the subgenus *Tetragonula*. However, when compared with the already known sequence of *T. calophyllae*, the new species differed only 11% which confirms that the new species falls under the subgenera *Flavotetragonula*. Hence the species was designated as new to science.

The COI DNA sequence identity as revealed by a nucleotide BLAST search indicates that, the new species has 82.68% sequence similarity to *Paratrigona* sp. (Accession MK397276.1). Hence, the 17.32% COI sequence divergence observed, supports the distinctness of the new species (Plate 39).

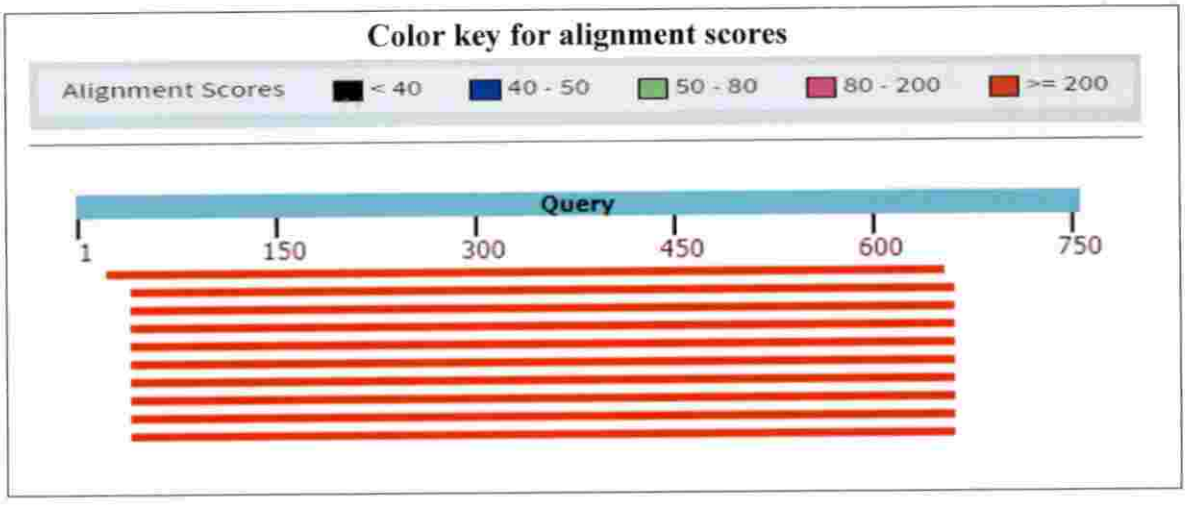
Job title: Nucleotide Sequence

RID MWKYZPP9015 (Expires on 08-11 13:02 pm)

Query ID	lclQuery_47591	**Database Name**	nr
Molecule type	dna	**Description**	Nucleotide collection (nt)
Query Length	756	**Program**	BLASTN 2.9.0+
Description	None		

Graphic Summary

Distribution of the top 10 Blast Hits on 10 subject sequences



Sequences producing significant alignments Manage Columns Show 10

select all *10 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession
Paratrigona sp. BdM1887 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial	549	549	83%	6e-152	82.68%	MK397276.1
Partamona bilineata isolate C1-4A1 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial	544	544	81%	3e-150	82.97%	KX433186.1
Partamona bilineata isolate F09-4A28 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial	544	544	81%	3e-150	82.97%	KX433184.1
Partamona bilineata isolate E04-8B22 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial	544	544	81%	3e-150	82.97%	KX433183.1
Partamona bilineata isolate F05-4A10 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial	538	538	81%	1e-148	82.81%	KX433185.1
Partamona bilineata isolate E05-8B26 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial	538	538	81%	1e-148	82.81%	KX433182.1
Partamona bilineata isolate H04-14A41 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial	538	538	81%	1e-148	82.81%	KX433180.1
Partamona bilineata isolate E12-9B23 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial	538	538	81%	1e-148	82.81%	KX433179.1
Partamona bilineata isolate D6-12B8 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial	538	538	81%	1e-148	82.81%	KX433175.1
Partamona bilineata isolate G03-12A26 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial	538	538	81%	1e-148	82.81%	KX433174.1

Plate 39. NCBI Blast showing matching of COI gene of *Tetragonula* sp. nov.1 with other COI genes from NCBI gene bank

Discussion

5. DISCUSSION

The results of an extensive study on hymenopteran pollinators in cucurbitaceous vegetables (2017-2019), with an objective to know the diversity of hymenopteran pollinators in five selected cucurbitaceous vegetables in Thiruvananthapuram and four other districts of Kerala and their morphological and molecular characterization are discussed in this chapter.

5.1. STUDY OF DIURNAL ACTIVITY, COMPOSITION AND RELATIVE ABUNDANCE OF FLOWER VISITORS

The current study shows the composition of different hymenopteran pollinators visiting the flowers of five selected cucurbitaceous vegetables. The results reveal that the family Apidae was the most frequent floral visitor, which is similar to the results obtained in studies of Kapil and Chaudhury (1974), Nogueira-Coutao and Calmona (1993), Khaja (2010), Dorjay *et al.*, (2017).

In our study it was observed that *A. cerana indica* was the most frequent pollinator followed by *T. travancorica.* Jangaiah (2007) reported that in Kerala, on culinary melon flowers, *A. cerana indica* was the most dominant and frequent floral visitor.

In our study we recorded that *T. travancorica* (31.86 %) was the dominant pollinator on bitter gourd flowers followed by *A. cerana indica* (29.90 %) and *Ceratina* sp.(11.76 %). Subhakar *et al.* (2011) observed that in Tirupathi on bitter gourd flowers, *T. iridipennis* (86.31 %) was the most frequent visitor. The abundance of bees depends on anthesis, weather parameters, competing flora, nectar concentration and its volume (Free, 1970). At peak flowering, the availability of flowers is more than commencement and cessation of flowering, and maximum number of insects would visit the crop during this period to increase the pollination process. Therefore, the flower number clearly influences the pollinator abundance, and in turn, the level of pollination.

The current study showed that *A. cerana indica* (38.76 %) was the frequent floral visitor followed by *T. travancorica* (24.03 %). Hemanthkumar (2006) and Mohapatra and Sontakke (2012) observed that on pumpkin flowers *A. cerana* was the most abundant floral visitor followed by *A. dorsata.*

In the present study it was observed that *A. cerana indica* (35.16 %) was the most frequent floral visitor on ridge gourd followed by *Xylocopa verticals* (18.68 %). Kuberappa *et al*., (2008) and Lakshmi (2013) reported that, on ridge gourd flowers *A. cerana* was the most frequent and dominant pollinator.

In our study it was observed that *T. travancorica* (33 %) was the most dominant pollinator on ash gourd followed by *A. cerana indica* (26.73 %). Leena and Nasser (2015) reported that, on ash gourd flowers, *T. iridipennis* was the most abundant pollinator followed by *H. timidus*, *A. cerana*, *C. hieroglyphica* and *H. taprobanae* in Kannur (Kerala).

In the current study on culinary melon flowers, *A. cerana indica, T. travancorica* and *Halictus* sp. recorded the highest foraging speed during 10:00 h to 11:00 h. *C. hieroglyphica* and *Lasioglossum* sp. recorded highest foraging speed during 09:00 to 10:00 h. *T. travancorica*, *C. hieroglyphica* and *Lasioglossum* sp. recorded the highest foraging rate during 10:00 h to 11:00 h. *A. cerana indica* and *Halictus* sp. recorded the highest foraging rate during 11:00 to 12:00 h and 09:00 to 10:00 h. Sanduleac (1959) reported that on cucurbit flowers, honey bees showed peak activity between 08:00 to 09:00 h. Rapp (1981) reported that, on cucumber flowers, honey bees started foraging at 06:00 h and was maximum from 09:00 to 12:00 h and was found decreasing in the afternoon hours. The peak foraging activity during morning hours can be correlated with the abundant availability of pollen and nectar due to which bees took more time to collect the forage during this period.

In the current study the maximum time was spent by *T. travancorica* (11.23 sec and 11.46 sec) followed by *Lasioglossum* sp. (11.06 sec and 11.30 sec), *A. cerana indica* (10.61 sec and 10.63 sec), *Halictus* sp. (10.26 sec and 10.40 sec)

and *C. hieroglyphica* (9.02 sec and 9.11 sec) during two seasons for pollen collection. Prakash (2002) reported that in cucumber, among the honey bees, for pollen collection, maximum time was spent by *A. florea* (13.49 sec), followed by *T. iridipennis* (11.44 sec), *A. cerana* (9.65 sec), *A. mellifera* (8.74 sec) and *A. dorsata* (7.22 sec). The difference in the foraging speed of bee species, may be due to different climatic conditions, type of crop , geographic location and species specific differences and variation in the availability of foraging source.

In our study on culinary melon flowers, the mean foraging rate was highest in *A. cerana indica* (10.60 & 10.88 flowers/min) followed by *T. travancorica* (9.16 and 9.23 flowers/min), *C. hieroglyphica* (5.01 and 5.10 flowers/min), *Lasioglossum* sp. (4.83 and 4.85 flowers/min) and *Halictus* sp. (4.03 and 4.13 flowers/min) during the two seasons. In Hisar, the data on the foraging activity of insect visitors in cucumber hybrids, *viz.*, Evergreen, NBH-Manu, Damini and Rani showed that the mean foraging rate irrespective of different day hours was highest in *A. dorsata* (8.63 flowers/min.) followed by *C. sexmaculata* (5.03 flowers/min.), and it was lowest in *Halictus* sp. (4.38 flowers/min.) (Hanh *et al.*, 2014). Fluctuation in visits of insect pollinators on culinary melon flowers reveals that the visits were low at the time of commencement and cessation of flowering but these remained high during mid flowering period. This difference might be due to variation in the floral density during the span of blooming and changes in climatic conditions.

5.2. IDENTIFICATION OF HYMENOPTERAN POLLINATORS

In our study, a total of twenty-nine species of different hymenopteran pollinators from five selected cucurbitaceous vegetables. They include the families Apidae, Halictidae, Megachilidae, Vespidae, Sphecidae, Scoliidae and Crabronidae. It was observed that, among all the hymenopteran pollinators, family Apidae was the most dominant. In Hisar, cucumber flowers are pollinated by twenty-four insect species, twelve species belong to Hymenoptera, six to Lepidoptera, three to Diptera, two Hemiptera and one Coleoptera. Among hymenopterans, four species are from Apidae, three species from Vespidae, two

species from Eumenidae and Megachilidae, one from Halictidae (Hanh *et al.*, 2014).

5.2.1. Morphological Characterization

The present study introduces one new stingless bee (*Tetragonula* sp. nov.1) new to science, to the existing bee fauna of the India. The stingless bees of Indian subcontinent are not much explored unlike the neo-tropical stingless bees. The studies of Mohan and Devanesan (1999), Jayalakshmi (2015), Rahman *et al.*,(2013), mentioned the presence of *T. iridipennis* in Kerala which was challenged by Shanas and Faseeh (2019). They also described three new species of stingless bees from Kerala.

The requirement of additional collection of stingless bee from Indian subcontinent is mentioned by Rasmussen (2013), the author also stated the group has the potential to rise the species from Indian sub continent and more native workers are needed to solve the problem of this group. More extensive study can come across with new species from Indian sub continent (Rathore *et al.*, 2013). The geometric morphometric analysis on wing patterns clearly showed the existence of various clusters within the *Tetragonula* complex (Francoy *et al.*, 2016) and the novel taxonomic tools, morphological and molecular data (Francoy *et al.*, 2016) will be useful for discovery of new species.

Identification of the *Tetrgonula* species through morphological characterization of workers is difficult due to rarity of distinct morphological characters in this group and most of the previous workers used body ratios, size, color, and hair pattern to distinguish these bees (Sakagami, 1978; Rasmussen *et al.*, 2008; Rasmussen, 2013; Rahman *et al.*, 2013; Viraktamath and Jose, 2017; Engel *et al.*, 2017; Silva *et al.*, 2018). The *Tetragonula* complex regarded as the notorious group without a proper key to identify them at species level (Rasmussen *et al.*, 2008). Rasmussen (2013) reviewed the stingless bees of Indian sub continent without giving a species level key to *T. iridipennis* complex and mentioned lack of comprehensive publication to solve the species complex of the

Tetragonula bees of India. The availability of literature from India is scarce in stingless bees (Vijayakumar and Jeyaraaj, 2014). Many of the bees in the genus *Tetragonula* are very close in external appearance, so authors have to relay up on the characters such as color, size and setation on the body to distinguish the stingless bees from this group (Engel *et al.*, 2017).

All the body measurements and body ratios were taken by following previous works of Sakagami (1978), Rasmussen (2013), Viraktamath and Jose (2017) and Engel *et. al*, (2017).

The new species comes most near to *T. calophyllae* which has a yellow band on the clypeus. The new species in the present study shows yellow shade throughout its clypeus. The difference in the measurements of *T. calophyllae* and *Tetragonula* sp. nov.1 is depicted in Table 18. Width of head in the new species is 1.98 mm when compared to 1.72 mm in *T. calophyllae*. Ratio between length and diameter of scape 7.13 mm in the new species compared to 6.95 in *T. calophyllae*. Length of mesoscutum/width of mesoscutum is 1.06 when compared to 0.8 in *T. calophyllae* and length of eye/scape length is 1.96 mm when compared to 1.88 mm in *T. calophyllae*. Remaining measurements recorded in the present study is similar to the ones documented by Rasmussen (2013) except slight variations.

5.2.2. Molecular Characterization

The attempt of partial sequencing of Mitochondrial COI gene of *Tetragonula* sp. resulted in the considerable variation in their nucleotide base pairs. Molecular works on *Trigona* species are not known from India (Sriram *et al.*, 2004). Sriram *et al.*, (2004) concluded the possibility of existence of two different stingless bee species based on morphometry and molecular analysis (the results of RAPD-PCR analysis of two population of stingless bees from Tamil Nadu showed much variation at genetic level). A genetic difference 11% was observed when the new species was compared to *T. calophyllae* and 27%

Table. 18. Morphometric variation in *Tetragonula* sp.

Measurements	***Tetragonula* sp. nov.1**	***T. calophyllae***	**Difference**
Width of head	1.98	1.72	0.26
Ratio between length and diameter of scape	7.13	6.95	0.18
Ratio between length and width of pterostigma	4.22	4.06	0.17
Inter Ocellar Distance / Ocello Orbital Distance	2.00	1.70	0.30
Length of mesoscutum / width of mesoscutum	1.06	0.80	0.26
Length of eye/scape length	1.96	1.88	0.08

sequence variation was observed when compared to *T. travancorica* which confirms that the new species falls under the subgenera *Flavotetragonula*.

Molecular studies on stingless bees have never been attempted in India till date. Koch (2010) resolved the taxonomy of *Lisotrigona* sp. in Switzerland through combined studies on morphology and DNA barcoding. In this study also through morphological characterization and molecular analysis *Tetragonula.* sp. nov.1 was described as new species.

The new species has 82.68% sequence similarity to *Paratrigona* sp. (Accession MK397276.1) as revealed by a nucleotide BLAST search. Hence, the 17.32% COI sequence divergence observed, which supports the distinctness of the new species. According to Gurney *et al.* (2000), closely related species have 90% similarity in the standardized DNA sequence but distantly related species have less than 90% similarity in the same genes sequence.

Summary

6. SUMMARY

The investigation on "Barcoding and biosystematic studies on hymenopteran pollinators of cucurbitaceous vegetables" was implemented in three stages- collection of hymenopteran pollinators from five cucurbitaceous vegetables in Thiruvananthapuram and other four districts of Kerala *viz.*, Kollam, Pathanamthitta, Alappuzha and Kasaragod; composition and relative abundance of hymenopteran pollinators in five cucurbitaceous vegetables, diurnal activity study on one selected cucurbit *viz.*, culinary melon; identification of specimens through morphological characterization, micrometry and molecular analysis. A total of 29 species of hymenopteran pollinators collected were critically examined for morphological differences.

The results of the study are summarized as follows. The study on composition and relative abundance of hymenopteran pollinators from Thiruvananthapuram and four other districts of Kerala revealed that, *A. cerana indica* (42.51 %) was the dominant pollinator followed by *T. travancorica* (18.56 %), *Ceratina* sp. (11.38 %) in culinary melon. *A. cerana indica* (38.76 %) was the dominant pollinator followed by *T. travancorica* (24.03 %) and *Ceratina* sp. (10.85 %) in pumpkin. *A. cerana indica* (35.16 %) was the dominant pollinator followed by *X. verticals* (18.68 %) and wasps (15.38 %) in ridge gourd whereas, *T. travancorica* (31.86 %) was the dominant pollinator followed by *A. cerana indica* (29.90 %) and *Ceratina* sp. (11.76 %) in bitter gourd; and *T. travancorica* (33 %) was the dominant pollinator followed by *A. cerana indica* (26.73 %) and *Ceratina* sp.(9.41 %) in ash gourd.

Observations on diurnal activity were carried out at College of Agriculture, Vellayani in culinary melon during two crop seasons for 3 weeks at weekly intervals. During two seasons, the foraging speed of *A. cerana indica, T. travancorica* and *Halictus* sp. was found to be highest during 10:00-11:00 h (10.61 and 10.63, 11.23 and 11.46, 10.26 and 10.40 seconds) respectively. The foraging speed of *C. hieroglyphica* and *Lasioglossum* sp. was found to be highest during 09:00-10:00 h (9.02 and 9.11, 11.06 and 11.30 seconds) respectively. The

foraging rate of *T. travancorica*, *C. hieroglyphica* and *Lasioglossum* sp. was found to be highest during 10:00-11:00 h (9.16 and 9.23, 4.83 and 4.85 flowers/m^2/5 min) respectively. The foraging rate of *A. cerana indica* and *Halictus* sp. was found to be highest during 11:00-12:00 h and 09:00-10:00 h (10.60 and 10.88, 4.03 and 4.13 flowers/m^2/5 min) respectively.

A total of 29 species of different hymenopteran pollinators from five selected cucurbitaceous vegetables were sampled from Thiruvananthapuram and four other districts of Kerala *viz.*, Kollam, Pathanamthitta, Alappuzha and Kasaragod. They include the families Apidae, Halictidae, Megachilidae, Vespidae, Sphecidae, Scoliidae and Crabronidae. It was observed that, among all the hymenopteran pollinators, family Apidae was the most dominant.

The observed diversity of hymenopteran pollinators include 29 species *viz.*, *A. cerana indica*, *A. dorsata*, *A. florea*, *Tetragonula* sp. nov.1, *T. travancorica*, *A. zonata*, *C. hieroglyphica*, *C. simillima*, *C. unimaculata javanica*, *C. binghami* and *X. verticals* under the family Apidae; *Halictus* sp. 1, *Halictus* sp. 2, *Halictus* sp. 3, *Lasioglossum* sp., *N. eliotti*, *N. westwoodi* and *N. curvipes* under the family Halictidae; *M. lanata* and *M. disjuncta* under the family Megachilidae.

R. brevita, *Eumenes* sp., *A. abdominale abdominale*, under the family Vespidae; *S. madraspatanum* and *C. bengalense* under the family Sphecidae; *P. phalerata phalerata* and *C. annulata annulata*, under the family Scoliidae and *Larra maura* under the family Crabronidae were studied.

Among the pollinators, five species *viz.*, *P. phalerata phalerata*, *C. annulata annulata* from ash gourd, *M. disjuncta* from bitter gourd, *C. simillima*, and *C. unimaculata javanica* from culinary melon were reported for the first time pollinating cucurbitaceous vegetables.

The stingless bee *Tetragonula* sp. nov.1. collected from a pumpkin flower in Thiruvananthapuram district was identified as new species with the help of morphometric characters and key provided by Shanas and Faseeh (2019) and was verified with DNA barcoding. The width of head, ratio between length and diameter of scape, ratio between length and width of pterostigma, IOD/OOD, length of mesoscutum/width of mesoscutum and length of eye/scape length gave distinct values which can clearly differentiate *Tetragonula* sp. nov.1 from *T. calophyllae*. The overall measurements of all body parts were larger in *Tetragonula* sp. nov. 1.

The partial sequencing of the Mitochondrial COI gene also supported the identity of new species of stingless bee described, where it shown inter-specific genetic variation of 11% with respect to *T. calophyllae*. Differences in morphology and genetic analysis based on partial sequences of the mitochondrial COI gene barcode region support the recognition of the new species. This study is opening a window towards a new stingless bee species from Kerala. So there is immense scope for conducting researches on the biology, honey production potential and medicinal properties of the honey of the new species. More over the pollination potential of these bees has to be explored as early as possible, so that they can be used to improve the productivity of various cucurbitaceous vegetables.

Future line of work

1. Studies on pollinators in vegetable ecosystem is necessary to understand species status and diversity.
2. Documentation and database generation is necessary.
3. Studies on diurnal activity of pollinators is essential to understand foraging behaviour and to recommend the suitable pollinators for enhanced pollination.
4. DNA barcoding helps for quick and easy identification of species.

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BARCODING AND BIOSYSTEMATIC STUDIES ON HYMENOPTERAN POLLINATORS OF CUCURBITACEOUS VEGETABLES

by

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Abstract of the thesis

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ABSTRACT

The study entitled "Barcoding and biosystematic studies on hymenopteran pollinators of cucurbitaceous vegetables" was conducted during the year 2017-2019 at the Department of Agricultural Entomology, College of Agriculture, Vellayani with an objective to study the diurnal activity and dynamics of hymenopteran pollinators of cucurbitaceous vegetables and to explore their morphological and molecular diversity. To determine the composition and relative abundance of different hymenopteran pollinators visiting the flowers of five selected cucurbitaceous vegetables *viz.,* culinary melon (*Cucumis melo var. acidulus*), bitter gourd (*Momordica charantia* L.), ash gourd (*Benincasa hispida* Thunb. and Cogn.), pumpkin (*Cucurbita moschata* L.) and ridge gourd (*Luffa acutangula* (Roxb.) L.) collections were made throughout the blooming period in Thiruvananthapuram and four other districts of Kerala *viz.,* Kollam, Pathanamthitta, Alappuzha and Kasaragod from 06:00 h to 18:00 h of the day with a cone type hand net. Among the above mentioned vegetables, culinary melon was selected for detailed study on diurnal activity at College of Agriculture, Vellayani.

The study on composition and relative abundance of hymenopteran pollinators revealed that, *A. cerana indica* was the dominant pollinator in culinary melon (42.51 %), pumpkin (38.76 %) and ridge gourd (35.16 %) whereas, *T. travancorica* was the dominant pollinator in bitter gourd (31.86 %) and ash gourd (33 %). Observations on diurnal activity were carried out at College of Agriculture, Vellayani in culinary melon during two crop seasons for 3 weeks at weekly intervals. For foraging rate, the number of flowers visited by each bee for 1 minute and for foraging speed, time spent by each bee per flower were observed respectively. During two seasons, the foraging speed of *A. cerana indica, T. travancorica* and *Halictus* sp. was found to be highest during 10:00-11:00 h (10.61 and 10.63, 11.23 and 11.46, 10.26 and 10.40 seconds) respectively. The foraging speed of *C. hieroglyphica* and *Lasioglossum* sp. was found to be highest during 09:00-10:00 h (9.02 and 9.11, 11.06 and 11.30 seconds) respectively. The

foraging rate of *T. travancorica*, *C. hieroglyphica* and *Lasioglossum* sp. was found to be highest during 10:00-11:00 h (9.16 and 9.23, 4.83 and 4.85 flowers/m^2/5 min) respectively. The foraging rate of *A. cerana indica* and *Halictus* sp. was found to be highest during 11:00-12:00 h and 09:00-10:00 h (10.60 and 10.88, 4.03 and 4.13 flowers/m^2/5 min) respectively.

Samples which were unidentified through morphological characterization were given for DNA barcoding. The sequence of 2 samples *viz.*, *Tetragonula* sp. nov.1 and *T. travancorica* were obtained. Among these, new species (*Tetragonula* sp. nov.1) of stingless bee, based on adult worker specimen is described. Differences in morphology and genetic analysis based on partial sequences of the mitochondrial COI gene barcode region support the recognition of the new species.

The above results revealed that *A. cerana indica* was dominant in culinary melon, pumpkin, and ridge gourd and *T. travancorica* was dominant in bitter gourd and ash gourd. The foraging speed during two seasons in the descending order was *T. travancorica* > *Lasioglossum* sp. > *A. cerana indica* > *Halictus* sp. > *C. hieroglyphica*. The foraging rate during two seasons in the descending order was *A. cerana indica* > *T. travancorica* > *C. hieroglyphica* > *Lasioglossum* sp. > *Halictus* sp. Among the pollinators, five species *viz.*, *P. phalerata phalerata*, *C. annulata annulata* from ash gourd, *M. disjuncta* from bitter gourd, *C. simillima*, and *C. unimaculata javanica* from culinary melon were reported for the first time pollinating cucurbitaceous vegetables. *Tetragonula* sp. nov.1 of stingless bee, collected from pumpkin flower, is the new species report from the study and it is morphologically characterised.