STUDIES ON LITTLE BEE (*Apis florea* Fabricius) POPULATION OF TAMIL NADU

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DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

CENTRE FOR PLANT PROTECTION STUDIES

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Thesis submitted in part fulfilment of the requirements for the award of the degree of

MASTER OF SCIENCE (AGRICULTURE) IN ENTOMOLOGY

to the Tamil Nadu Agricultural University, Coimbatore – 641 003

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CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON LITTLE BEE (*Apis florea* Fabricius) POPULATION OF TAMIL NADU**" submitted in part fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) IN AGRICULTURAL ENTOMOLOGY** to the Tamil Nadu Agricultural University, Coimbatore, is a record of bonafide research carried out by **Mr. P. ELAYARAJA,** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine

Place: Coimbatore **Dr. S. RAGURAMAN**

Date: 27.09.2011 **Chairman of the Advisory Committee**

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Members: Dr. M. MUTHURAMAN

Dr. D. SUDHAKAR

Affectionately Dedicated to
My Parents and Brothers

ACKNOWLEDGEMENT

"Gratitudes are the Memories of Heart"

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(Elayaraja. P)

ABSTRACT

STUDIES ON LITTLE BEE (*Apis florea* Fabricius) POPULATION OF TAMIL NADU

By

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Degree : Master of Science (Agriculture) in Agricultural Entomology

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The red dwarf honey bee, *Apis florea*, is one of the native honey bees of India. Little is known about its biodiversity and population variation in Tamil Nadu. The present study has been taken up to fill the above two research gaps. Distribution of *A. florea* was widely found in plains. *A. florea* colonies were patchily distributed in high altitude zone and high rainfall zone. *A. florea* colonies were also recorded even at an altitude of 1502 m above mean sea level at Puthukadu near Coonoor in Nilgris hills.

All the 16 morphometric characters used in the study significantly contributed for structural variations in *A. florea* population of Tamil Nadu. Both standard morphometrics and geometric morphometrics analyses clearly revealed the existence of only a single morphocluster in *A. florea* population in different agro-climatic zones of Tamil Nadu. *A. florea* population of Tamil Nadu was structurally different from the *A. florea* population of Nagaland. Little bees in North East India were much larger in size than in South India. Geometric morphometrics of hind wing was found to be a powerful tool for differentiating castes in *A. florea*. A distinct radio-medial cross vein was present only in the hind wing of queen. The study has validated and documented for the first time the

existence of a new ecotype of little bee which is named as little comb little bee. The newly discovered ecotype showed distinct variations both in body size and nesting behaviour. The structural variations between the normal little bee and little comb little bee were confirmed through standard morphometrics.

Synadenium grantii, *Prosopis juliflora* and *Musa spp* were often preferred for nesting by *A. florea.* Arboreal nesting is more common than terrestrial nesting. Any part of the plant *i.e.*, stem, leaf margin, leaf lamina, leaf sheath, peduncle, inflorescence rachis formed a support in arboreal nests. Terrestrial nests of *A. florea* were also found on wall, wooden rafter, cot frame, wire fence, discarded chair frame, corner of iron shelf and concrete beam. The size of the mature comb of little comb little bee never exceeded the size of a human palm. The colour of the comb gradually changed from white, yellow and dark brown from its inception to maturity. The apical portion of comb constituted honey crest in terrestrial nests. Sometimes terrestrial nests of *A. florea* were found too closer to ground.

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CHAPTER – I

INTRODUCTION

Red dwarf honey bee, (*Apis florea* Fabricius) is both a moderate honey hoarder and a potential pollinator widely distributed in India. It has found its ecological niche in the stratum of dense bushes and small trees of the tropics. It is endemic to South Asia. It is a non-gregarious plain species occurring all over India up to an altitude of 300 m above sea level, though occasionally it has been recorded at higher elevations. It is the smallest species in the genus *Apis*, building exposed single-comb fastened to tree branch. Generally it is regarded as a species on relatively low phylogenetic level.

A. florea is the most peculiar honey bee species in several ways (Free, 1981). One of the outstanding traits of *A. florea* is its ability to survive in very hot and dry climates, in spite of its open nesting habit. It can equally adapt well to cold periods. No other honey bee occurs in such regions with extremes of temperatures (Ruttner, 1988). It is found in the arid region of Kutch in coastal Gujarat in India, with summer temperatures going up to 50˚C and winter temperatures dipping to less than 5˚C. (Soman and Chawda, 1996). In Persian Gulf it tolerates a summer temperature of 50^0C and more (Whitcombe, 1984).

It builds new nests by the combination of new wax secreted from their wax glands and old wax collected from the discarded nests. Combs though exposed to light are found in shady places. The workers coat the ends of the nest support with sticky strips of propolis or plant gum or insect lime to ward off ant attacks. It is the only honey bee species that uses this kind of defensive technique (Lindauer, 1956; 1957).

A very conspicuous characteristic of *A. florea* is the striking difference in size between worker bees and sexuals. Its workers are exceptionally small when compared with its drone and queen. This inequality is much smaller in the other species. Differences in body size are reflected also in the diameter of brood cells (Ruttner *et al.*, 1985). The hind basitarsus of drones has a peculiar thumb like clasper organ.

These bees are very much prone to swarming and a colony may send out as many as five or more swarms. The swarming process often leads to the dissolution of the colony (Whitcombe, 1984). They disperse regionally to utilize different microclimates during winter and summer (Tirgari, 1971). Foragers dance on the top surface of the honey crest and point to the food source directly. They can be very aggressive towards larger bees when foraging which may be due to its restricted foraging range (Free, 1981).

There is a good scope to collect honey from *A. florea* without harming the bees or destroying the nest by following simple techniques. It is also possible to transport the colonies and keep their nests at chosen locations, like agricultural and horticultural farms by adopting simple techniques both for honey production and planned crop pollination. The honey of *A. florea* has higher dextrin content and less tendency to granulate than the honey of other *Apis* sp. The Gujarat State Forest Development Corporation procured about 167 metric tonnes of little bee honey collected by local honey hunters from these bees during 1995-96 (Soman and Chawda, 1996). Nests are used widely for food in Thailand. Both the top honey crest and the lower brood area are eaten, the latter being served after lightly roasted (Nakao, 1964).

A. florea is an excellent pollinator of orchard and field crops. It is reported as a main pollinator of hog plum, litchi, mango and tomato. It also plays an important role in seed production of radish, cauliflower and fennel (Wongsiri *et al.*, 1996). Due to its smaller size, its energy demand is low. Hence, they are better pollinators in crops with smaller flowers and narrow corolla tubes. Further, their mild temper and accessible nests increase their utility in planned cross pollination. Since this species is a natural pollinator of several plant species, it plays a crucial role in the maintenance of the natural ecosystem with its critical biodiversity.

Little bees have long been ignored due to their smaller size and poor honey gathering capacity. Nests of this species are collected more easily. Colonies of *A. florea* have been kept in Oman traditionally. In Oman the part of a branch or twig that carries a nest is cut off and its two ends are given new supports and kept in a sheltered place – a tree, wall or other construction. *A. florea* with its single exposed comb and relative gentleness provide good opportunities for apicultural research. Colonies are amenable for partial domestication experimentation and can be managed easily (Kaliyamoorthy, 2010).

Research studies on the biodiversity of little bee fauna is limited especially in South India. Red dwarf honey bee alone is found in South India. Tamil Nadu is physiographically and climatically diverse with seven agro-climatic zones. The possible impact of climatic and geographical factors on little bee population has not been studied so far. Of late the existence of two distinct types of comb building little bees came to lime light during the interactions with tribals and wild honey collectors while taking up bee fauna survey about bee biodiversity in forest ecosystem in Tamil Nadu. Hence, this research study was taken up to find out the new ecotypes of little bees through a systematic research approach with the following objectives.

- To study the structural variations in the population of *A. florea* in Tamil Nadu.
- To find out the existence of new ecotypes of *A. florea.*
- To document the peculiar nesting behaviour of *A. florea.*

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CHAPTER – II

REVIEW OF LITERATURE

Dwarf honey bee is the smallest species of honeybee, both in the body size of its workers and in the size of its nest. There are two distinct species *viz.*, *A. florea* and *A. andreniformis*. They are almost of the same size. They build an exposed single comb nest most frequently in a shaded location. The bibliography on *A. florea* was compiled by Hepburn and Hepburn (2005). The literature pertaining to the chosen research topic *i.e.*, species, population variations and nesting behaviour of dwarf honey bees is reviewed here.

2.1. Distribution of dwarf honey bees

A. florea is mainly distributed in South East Asia. Its distribution is generally confined to warm climates or equatorial area. It is considered to be both tropical and oriental. This species is present in the warmer parts of Oman, Iran, Pakistan, Indian sub-continent and Sri Lanka. It has been found from Indonesia in the east, north to the Himalayan mountains and west to Iran and Oman. Colonies of *A. florea* have been found at temperate zone and at higher altitudes up to 1900 m in Iran (Ruttner *et al*., 1995). Occurrence of *A. florea* colony was first reported in Africa in Sudan (Lord and Nagi, 1987). It has been the most successful honey bee emigrant. It has travelled from Persian Gulf to Sudan and Saudi Arabia (Hepburn *et al.*, 2005).

A. andreniformis is a rare and patchily distributed throughout the tropical and subtropical regions of Asia, especially in South China, India, Burma, Laos, Vietnam, Malaysia, Indonesia, and the Philippines. Wherever two species of dwarf bees occur sympatrically as in Southern China, Assam and greater part of Indo-China, *A. florea* is the low land species and replaced at higher elevations by *A. andreniformis* (Maa, 1953). However, in areas where only one species occurs either of the species can be found up to 1300 m above sea level.

2.2. Species of dwarf honey bees

Two dwarf honey bee species *A. florea* and *A. andreniformis* are superficially similar and were not generally recognized as separate species until Wu and Kuang (1987) reinstated the name *A.(Micrapis) andreniformis* (Smith, 1858). The most reliable characters useful for differentiation of *A. florea* and *A. andreniformis* are tabulated below.

S.No	Character	*A. florea*	*A. andreniformis*	Reference
1	Colour of scutellum in worker	Black	Orange	Woyke, 1977
2	Jugal-Vannal ratio of hind wing in worker	More	Less	Wu and Kuang, 1987
3	Cubital index	Smaller (Above 2)	Higher (about 6)	Wu and Kuang, 1987
4	Marginal setae on hind tibia	Entirely white usually	Dark brown to blackish	Wu and Kuang, 1987
5	Thumb on hind basitarsus of drone	Longer	Shorter	Ruttner, 1988
6	Colour of first and second abdominal tergites	Reddish	Blackish	Thapa and Wongsiri, 1994
7	Second abdominal tergite	Not punctate	Deeply punctate	Wu and Kuang, 1987
8	Endophallus	Fimbriate lobe has six protrusions and straight	Fimbriate lobe has only three protrusions with curved terminals	Wu and Kuang, 1987; Wongsiri *et al.* 1990; Koeniger *et al.* 1991; Chen, 1993
9	Comb midrib	Present only in brood area	Present in both honey crest and brood area	Rinderer *et al.* 1996
10	Defensive behaviour	Defend against intruders	Deserts the nest often when disturbed	Thapa and Wongsiri, 1994
11	Head position of workers while fanning	Held up	Held down	Thapa and Wongsiri, 1994
12	Drone flight time	Just after the sun passes its zenith	Later in afternoon	Rinderer *et al.* 1995

2.3. Morphological variations in dwarf honey bees

A. florea is widely distributed over a stretch of 7000 km. It occupies rainforests, savannas, subtropical steppes and semi deserts (Hepburn *et al.,* 2005). The population of *A. florea* is subjected to both geographical and climatological influences which lead to structural variations. Such variations are studied through both standard morphometrics and geometric morphometrics.

2.3.1. Standard morphometrics

Morphometrics is the measurement and statistical analysis of morphological structures of organism. It includes both genetic and environmentally induced variation (Daly, 1985). Morphometric methods are based on multiple measurements of many individuals. Morphometric analyses could be used in the programme for phenotypic characterization. The use of morphometrics in bee classification has been improved with multivariate analyses (Dupraw, 1965). The multivariate approach, including principal component analyses has also been applied to separate between genetic lines, eoctypes within a race and geographic races (Ruttner *et al.,* 1978). Ruttner *et al.* (1978) choose 42 characters for analysis of honeybee workers from a wide range of geographic locations. This set of characters, called standard morphometry, stood the test of time and continues to be the method most often used in a wide range of studies of geographic variation (Ruttner *et al.,* 2000). Ruttner (1988) obtained three morphoclusters (1) South India and Sri Lanka, (2) Thailand and (3) Oman, Pakistan and Iran from a principal components analysis and factor analysis of 20 characters of 18 samples of *A. florea*. More recently Tahmasebi *et al.* (2002) analyzed the *A. florea* of Iran and defined two morphoclusters. The only comparative, multivariate morphometric study of *A. florea* in Thailand is that of Chaiyawong (2001). The results of her factor and cluster analyses using 22 characters revealed only a single morphocluster. Rinderer *et al.* (1995) reported a comparison of *A. florea* and *A. andreniformis* for 44 morphometric characteristics for colonies from south eastern Thailand. Thirty colonies of *A. andreniformis* were sampled for morphometric analysis. For morphometric analysis, 20 informative characters were used to determine the variation. After plotting the factor scores, *A. andreniformis* from across Thailand were found to belong to one group, a notion further supported by a cluster analysis generated dendrogram.

Narayaranan *et al.* (1960) studied tongue length variation in worker bees of *A. florea*. The mean tongue length was found to be 3.27 mm. The average tongue length in five colonies ranged from 3.21-3.29 mm. The geographic variation of *A. florea* has been explained with multivariate analysis based on characters of fore wing, hind leg, abdomen, wing angles and the number of hamuli and antenna (Ruttner *et al.,* 1995; Chaiyawong *et. al.*, 2004; Hepburn *et. al.*, 2005). Makhmoor and Hafeez Ahmad (1998) reported the utility of 16 morphological characters to distinguish *A. florea, A. mellifera, A. cerana* and *A. dorsata* from the Jammu region of India.

Ruttner (1988) reported that *A. florea* samples from different regions vary not only in size characters, but also in others, especially wing venations. Angles of hind wing were first used for multivariate morphometric study of *A. florea* colonies. Ruttner (1988) used the cubital index as an important character to distinguish *A. florea* and he found that it was 2.89 in southern Iran. Ozkan *et al.* (2009) used both multivariate and spatial autocorrelation analyses to determine the morphometric variations in *A. florea* colonies in Iran. They have proved the utility of hind wing related characters (hind wing length, hind wing width, and hind wing angles) in studying the structural variation of *A. florea*. Seven out of the eight hind wing variables displayed statistically significant differences among populations. Narayanan *et al.* (1960) found significant differences in the average number of hooks on the left and the right hind wing. The mean number of hooks was 10.96 per hind wing. Inter-colony variation was 0.37 hooks. No correlation was found between the tongue length and the number of hooks. Hamuli numbers are often useful in the classification of honeybee populations.

Based on the multivariate analysis of morphometric data, *A. florea* populations from several countries were investigated regionally (Ruttner, 1988; Mossadegh, 1993; Rinderer *et al.,* 1995; Ruttner *et al*., 1995; Makhmoor and Hafeez Ahmad, 1998; Tahmasebi *et al.,* 2002; Chaiyawong *et al.,* 2004; Hepburn *et al*., 2005). The first morphometric research on *A. florea* demonstrated that clinal type of geographical variability exists in *A. florea,* larger bees found in the north and smaller ones in the south (Ruttner, 1988). He also demonstrated that a western group of larger bees found at a higher latitude (29°-34°) and eastern group of smaller bees found at lower latitude (<29°) in Principal Components Analysis in Iran. *A. florea* colonies from lower latitude were morphologically distinguishable from higher latitude. Canonical Variates Analysis also supported morphological differentiation in *A. florea* colonies. Morphoclusters were changed clinally with latitude in a much wider geography extending from Vietnam and southeastern China to Iran and Oman (~7000 km) (Hepburn *et al.*, 2005). Rattanawannee *et al.* (2007) carried out morphometric analysis of *A. andreniformis* population from across Thailand and found out that they belonged to one group through a cluster analysis generated dendrogram. He also found out the existence of clinal patterns by linear regression analysis. The body size of bees increases from South to North but decreases from West to East, although this may reflect altitude rather than longitude.

Both geography-related variability and climate-related variability were reported for *A. florea* (Ruttner *et al.,* 1995). The effects of geographical and climatic conditions on the morphological variation and separation of Iranian small honey bee (*A. florea*) were investigated by Tahmasebi *et al.* (2002). Their study showed the existence of two distinct groups of small honey bee population in Iran. Latitude had a positive correlation with size of body, wings and legs. Amount of precipitation had a negative correlation with size of body, wings and legs. Temperature, wind speed, relative humidity, freezing days and other parameters did not show any correlation with size of the body. The results from this study prove that the more northern areas with high altitude and more precipitation have bigger honey bees.

The standard morphometry characters apart from distances and angles include discrete classes of pigmentation. *A. florea* workers are of the yellow body colour type with yellow-orange bands on the abdomen. The queens are of the same type and drones are black. *A. andreniformis* workers have orange scutellum and narrow orange bands on the abdomen. Queens and drones are black. The gene responsible for body colour in *A. florea* is designed as *Fl*, in *A. andreniformis* as An. Expression of body colour in three castes of *A. florea* depends upon the sex (males black, females yellow) and in *A. andreniformis*, upon the sexuality (workers with yellow-orange bands, sexuals - queens and drones brown or brownish-black). Expression of body colour in the three castes of Asian honeybees may be considered as an adaptation to the environmental living conditions (Woyke, 1977).

2.3.2. Geometric morphometrics

Geometric morphometrics is based on a well established theory of shape. Instead of distances and angles it uses the coordinates of points called landmarks. The landmarks are superimposed by translation, scaling and rotation. After superposition the landmark configurations differ only in shape, and can be analyzed by multivariate statistical methods (Zelditch *et al.,* 2004). Geometric morphometrics can be used as a powerful tool in population structure studies. This method is much faster and more reliable.

In recent years, modern morphometric methods such as geometric morphometric analysis has been used to classify and identify *A. mellifera* subspecies (Francoy *et al.,* 2009; Tofilski, 2008) as well as heritability of wing shape (Monteiro *et al.,* 2002) and influence of hybridization on fluctuating asymmetry (Smith *et al.,* 1997; Schneider *et al.,* 2003).

Francoy *et al.* (2006) used wing shape (discoidal cell) in order to differentiate European and Africanized honey bees. Later Francoy *et al.* (2008) and Tofilski (2008) used wing shape (landmarks on fore wings) in order to identify Africanized honey bees and to discriminate honey bee subspecies respectively.

The population structure of *A. florea* was studied by using wing shape which showed significant differences among *A. florea* populations in Iran. Out of 40 cartesian coordinates, 23 were significantly different among the group. Discriminant function analysis resulted in clear separation of groups when fore wing shape were used, but hind wings failed to separate the groups (Kandemir *et al.,* 2000).

2.4. Nesting behaviour of dwarf honey bees

A. florea nests are found generally in dense foliage, but occasionally nests are also seen in extremely exposed sites. Colonies are normally found in sites offering substantial but not complete shade. The most frequent nesting site of *A. florea* is a thin branch of a bush or small tree. They build their nests on lower sides of small horizontal branches of twigs of trees or bushes or thickets of thorny creepers providing protection and partial shade. To attach the comb to this narrow support, the comb encircles the branch completely. Beneath the support the comb is abruptly reduced in diameter.

Nests of dwarf bees are never as high and inaccessible as in the case of *A. dorsata.* The nests are naturally within the reach of a person. The height of nesting places from the ground level ranges from two to ten meters though the average height is around three meters. The comb of dwarf bees is very much smaller than *dorsata*. The size of the *A. florea* comb varies greatly. The brood comb is 35 cm in length, 18 cm in height and 2 cm in thickness and the upper part of the comb which forms the honey crest is about 5-7 cm thick (Singh, 1962). *A. florea* and *A. andreniformis* colonies construct a band of sticky resin around the branch that supports their nests to protect their nests against ants and other crawling arthropods (Duangphakdee *et al.,* 2004).

A. florea and *A. andreniformis* choose similar nest sites. These colonies may avoid areas containing nests of the other *Apis* species. The tendency of colonies of these species to establish their nest sites near existing nest sites of colonies of the same species may increase the probability that the newly selected nest sites are near suitable floral resources capable of supporting the survival and reproduction of the newer arrivals to the area. More importantly, spatial clumping probably helps in the availability of potential mates for their colony's future reproductive within their mating range. Avoiding close association with colonies of conspecifics may help in diminishing inter specific interference with mating that may arise from the species having similar sex pheromones (Rinderer *et al*., 2002).

Nesting sites are usually arboreal and very rarely terrestrial. *A. florea* also nests in small caves or in sheltered areas of buildings, or even in wells far below ground surface. Here the comb support can be any kind of niche in the wall, caves and boxes. In treeless regions in Oman they also use rock cliffs as nesting sites. In these situations the attachment of the comb to the support differs from that to a branch, the construction type, however, remaining unchanged. In any case, a hexagonal cell pattern is started on the support. On vertical wall, the comb is attached laterally to it, the honey crest thus becoming unilateral. If on a horizontal ceiling, a vertical comb is brought about by gradually shifting the axis of adjacent cells in opposite directions. In this case the honey cap is positioned on the side of the brood comb immediately attached to the ceiling (Rinderer *et al.,* 1996).

A. florea builds single comb exposed to sun, air and rains. Lindauer (1957) recorded 34^{o}C to 36^{o}C as the nest temperature even if external temperature was 42^{o}C.

In addition to fanning and spreading of water on combs, other factors are probably involved which enable such thermal conditioning of their nests. Exposed colonies of *A. florea* can withstand torrential rains by virtue of their peculiar clustering habit all over the comb surface like sloping tiles. This along with their waxy body coats help to drain off water.

The comb is suspended in open vertically. The vertical part of the nest is expanded both upward and downward. The upper honey comb begins to expand upwards at the top. The upper part of the comb forms a thicker crest above and surrounding the supporting branch, and is used for storing honey. Honey cells are one to three times deeper than brood cells and are oversized. Though they usually tend to be hexagonal, many of them are irregular especially along transitions. Cells in the centre of the crest are two or three times as deep as those below. The upper cells may face slightly upward, and the lower cells slightly downward, with many irregularities. The honey cells have their opening downwards in the natural position of comb. (Thakar and Tonapi, 1962). The storage capacity of the honey cap or crest is 500 g up to 1000 g or more of honey (Muttoo, 1956; Tirgari, 1971).

Underneath is the area used for storing pollen and rearing brood. The cells storing pollen form an arch between the honey crest and brood area. The brood region in a mature colony alone has brood cells of all the three castes. Smaller worker cells are found just beneath the pollen arch and they occupy a larger area and just below them are larger drone cells. The queen cells are usually found in the lower comb margin. The comb is covered usually by three to six layers of bees forming a protective curtain.

The combs of *A. andreniformis* and *A. florea* are different. The nests of *A. florea* are larger than the nests of *A. andreniformis*. The comparative sizes of cells are generally in accord with the smaller size of *A. andreniformis.* The depth and width of worker cells and the width of drone cells are all significantly larger in nests of *A. florea*. When *A. florea* combs are extended they may incorporate other secondary branches within the comb structure. This has not been reported for an *A. andreniformis* nest. In *A. florea,* the crowns of the comb do not contain a comb midrib, the structure formed by the bases of the cell on the opposing sides of the comb. The cells are characteristically irregular in arrangement *A. andreniformis,* in contrast, builds a midrib up from the supporting branch, which permits the construction of regular cells (Wongsiri *et al.*, 1996).

CHAPTER - III

MATERIALS AND METHODS

The present study was taken up mainly to understand and gather more information on little bee (*A. florea)* population in Tamil Nadu. The materials utilized and methodologies followed in the present study are described below in detail.

3.1. Population variation studies

3.1.1. Collection of bee samples

A. florea workers were collected from seven agro-climatic zones (Fig.1) of Tamil Nadu to find out the possible influence of geographical and climatic effects on the population of *A. florea*. In each agro-climatic zone, worker bees were collected from five different locations (Table 1). The worker bees were collected at random from the nest by placing a polythene bag just beneath the nest and shaking the twig bearing the nest. The bees which crawled into the bag were collected. Twenty bees were collected at random from each nest. The foraging workers were collected from flowers by using a sweep net wherever it was not possible to locate the nest. The collected bees were preserved in 70 per cent ethanol until the morphometric examinations were carried out. The reference specimens were deposited in the Department of Entomology bee collections. In addition, bee samples were also collected from Nagaland from three locations *viz.*, Meriama, Chanesunmokaa and Yimkhawokha for verifying Bergman's rule and to find out the existence of any population variation between North India (Nagaland) and South India (Tamil Nadu).

3.1.2. Morphometrics

Bees preserved in ethanol were dissected out first to separate the tagmata. Later head, wings, hind leg, tergites, sternites and wax plates were dissected out for making morphometric measurements.

3.1.2.1. Standard morphometrics

In head region, head length (HL), head width (HW), proboscis length (PL), antennal length (AL), distance between lateral ocelli (DO) and ocello-ocular distance were measured (Plate 1). In thorax, hind leg was amputated and length of femur (FL), tibia (TIL), length and width of metatarsus (LMT, WMT) were measured (Plate 2). Both the right wings were removed to measure wing length (WL), wing width (WW), length of cubital cross vein A (CuA) and B (CuB) and number of hamuli (NH) were taken (Plate 3). In abdominal region, length of third tergite (TEL), length and width of third sternite (SL, SW), length and width of wax plate (WPL, WPW), distance between wax plates DWP) (Plate 4) and length and width of tomentum (LT, WT) were measured. All measurements were taken by using Leica M 165C high end research stereo microscope with high speed digital fire wire live camera and LAS measurement module and data transfer. The images were analyzed and the measurements were recorded into a computer.

A total of 16 morphometric characters were measured (Table 2). The morphometric characters were chosen based on the morphometric studies carried out by Ruttner, 1988; Rinderer *et al.*, 1995 and Hepburn *et al.*, 2005. In order to overcome body size variations induced by the cell size variations of comb, for certain characters *viz.*, head length and width (HL:HW), length and width of metatarsus (LMT:WMT), fore wing length and width (WL:WW), third sternite length and width (SL:SW), wax plate length and width (WPL:WPW) and length and width of tomentum (LT:WT) ratios were worked out and used. The cubital index (CI) was calculated by finding out the ratio between the length of CuA and CuB.

3.1.2.2. Geometric morphometrics

Structural variations existing in population were also studied by geometric morphometrics. Right fore and hind wings were used for this study to avoid any problems due to asymmetry. The wing images were captured by Leica M 165C stereo microscope with image analyzer and each image was saved as jpg file. The jpg images were converted to tps format by using the software tpsUtil 1.40 (Rohlf, 2008). Twenty landmarks on fore wing and five landmarks on hind wing were plotted using tpsDig2 version 2.04 (Rohlf, 2005a) (Fig. 2 & 3). Then Cartesian coordinates of the landmarks were aligned and partial warp analysis was done using the software tpsRelw 1.42 (Rohlf, 2005b). A total of 175 worker bee wings of *A. florea* belonging to 35 colonies collected from seven agro-climatic zones were analyzed. Both geometric morphometrics and hind wing venation studies were carried out in three castes of *A. florea* to bring out inter caste structural variations in wings.

3.2. Ecotype studies

This study was carried out to find out the existence of any new ecotypes of little bee in the study area. In order to find out ecotypic variations, standard morphometrics techniques were used. A total of 200 worker bee samples were collected from two kinds of nests *i.e.,* normal nest and little nest from ten different locations in Western agro-climatic zone. The procedure described earlier was followed to study the population variations among little bees.

Nest architecture variations were studied to document the deviations from the normal nesting behaviour. Various measurements like diameter of twig, height of honey crest, width of honey crest, thickness of honey crest, depth of honey cell, width of ten honey cells, vertical length of brood comb, maximum width of brood comb, thickness of brood comb, depth of worker cell, width of ten worker cells, height and width of pollen arch were made in five nests of each ecotype and averages were calculated. These individual nest averages were used to calculate means, standard deviations and coefficient of variations of the studied characteristics for ecotypes. Paired t-tests were performed to estimate the significant differences among ecotypes.

3.3. Documentation of nesting behaviour

Details were collected on nest location, nature of substratum, method of attachment to substratum, location of resin ring, shape and colour of comb, location and shape of honey crest, distribution of brood cells in nest and growth pattern of comb to ascertain any deviation from normal nesting behaviour.

3.4. Statistical analysis

Zone sample mean, standard deviation and coefficient of variation were computed for each morphometric character for each agro-climatic zone. Multivariate statistical analysis *i.e.,* analysis of variance, factor analysis, principal components analysis and discriminant function analysis were used to detect population variations. Analysis of variance was carried out to find out the impact of morphometric characters used in the study on population variations of little bees. Factor analysis was done to find out inter zonal or inter colonial variations. Principal components analyses using colony data were done to detect the presence of possible clusters among the scatter scores from a plotted plane graph of the first two high loading factors. Stepwise discriminant analysis using principal components clusters was carried out to determine the most discriminatory variables to enter into the discriminant functions. The discriminant functions were used to classify the zones / colonies. The same statistical techniques were used both for standard morphometics and geometric morphometrics. All statistical analyses were done by using SPSS 16.0 statistical package (SPSS, 2004).

CHAPTER - IV

EXPERIMENTAL RESULTS

Studies were conducted to find out population variations in *A. florea* in Tamil Nadu. The results of the above studies are presented below.

4.1. Population variations studies through standard morphometrics

4.1.1. Population variations of *A. florea* in Tamil Nadu

Standard morphometrics analyses were performed to find out population variations found in 700 *A. florea* worker bee samples collected from 35 localities representing seven agro-climatic zones of Tamil Nadu. The means, standard deviations and coefficients of variations of 16 informative morphometric characters are presented in Table 3. The proboscis length was varying from 2.80 to 2.96 mm. The range of cubital index was 2.66 to 3.12 mm. The mean number of hamuli was 11.0 (Table 3). Analysis of variance of morphometric characters showed that all the 16 morphometric characters significantly contributed for structural variations ($P < 0.05$) (Table 4).

In a principal components analysis using 16 morphometric characters six factors with eigenvalues greater than one were extracted (Fig. 4): factor 1 includes antennal length, proboscis length, third tergite length; factor 2 comprises length and width of tomentum and distance between lateral ocelli; factor 3 includes ratio of length and width of third abdominal sternite and ratio of length and width of wax plate; factor 4 includes ocello-ocular distance and ratio of length and width of fore wing; factor 5 comprises ratio of length and width of metatarsus, cubital index and factor 6 includes ratio of length and width of head. Factor 1 and factor 2 accounted for 13.50 per cent and 8.97 per cent of total variance in the data respectively. The scatter plot was obtained by using factor 1 and factor 2, which gave a non-separable single morphocluster (Fig. 5).

In stepwise discriminant analysis, ratio of length and width of head, antennal length, ratio of length and width of metatarsus, ratio of length and width of fore wing, ratio of length and width of third abdominal sternite, ocello-ocular distance and distance between two wax plates did not enter the discriminant functions due to lack of discriminatory power in these characters for the discrimination of *A. florea* of Tamil

Nadu. The eight morphometric characters *i.e.,* femur length, ratio of length and width of tomentum, proboscis length, ratio of length and width of wax plate, cubital index, distance between two lateral ocelli, tibial length and number of hamuli entered into the discriminant functions in order of their discriminatory power (Table 5). Scatter plot of discriminant function analysis also showed similar results as obtained from PCA (Fig. 6). These multivariate morphometric analyses clearly revealed the existence of only a single morphocluster in *A. florea* population of Tamil Nadu.

4.1.2. Comparison of *A. florea* populations

Standard morphometrics analyses were performed to find out the population variations found in *A. florea* worker bee samples representing North East India (Nagaland) and South India (Tamil Nadu). For comparison of *A. florea* populations of South India and North East India, 700 and 60 worker bees were used respectively. The means, standard deviations and coefficients of variations of 16 informative morphometric characters pertaining to Tamil Nadu and Nagaland are presented in Table 6. Out of 16 morphometric characters measured 15 characters had greater values for Nagaland population than Tamil Nadu population. Analysis of variance of morphometric characters showed that all the 16 morphometric characters significantly contributed for structural variations ($P < 0.05$) (Table 7).

In a principal components analysis four factors with eigenvalues greater than one were extracted (Fig.7): factor 1 includes proboscis length, antennal length, third tergite length, tibial length, femur length, distance between lateral ocelli, distance between wax plates, ratio of length and width of head, ratio of length and width of wax plate and ratio of length and width of fore wing; factor 2 comprises ratio of length and width of third abdominal sternite, ratio of length and width of tomentum; factor 3 includes ratio of length and width of metatarsus, cubital index and ocello-ocular distance; factor 4 includes number of hamuli. Factor 1 and factor 2 accounted for 40.79 per cent and 12.61 per cent of total variance in the data respectively. The scatter plot obtained by using factor 1 and factor 2, gave two distinct non-overlapping morphoclusters (Fig. 8).

In stepwise discriminant analysis distance between lateral ocelli, ocello-ocular distance, antennal length, femur length, tibial length, distance between wax plates and ratio of length and width of metatarsus did not enter the discriminant functions due to lack of discriminatory power in these characters for the discrimination of *A. florea* populations of South India and North India . The nine morphometric characters *viz.*, ratio of length and width of third abdominal sternite, proboscis length, ratio of length and width of wax plate, ratio of length and width of head, cubital index, ratio of length and width of fore wing, tergite length, number of hamuli and ratio of length and width of tomentum entered into the discriminant functions in order of their discriminatory power (Table 8). Scatter plot of discriminant function analysis also showed similar results as obtained from PCA (Fig. 9). These multivariate morphometric analyses clearly distinguished *A. florea* population of South India from North East India. The bees in the north east were bigger than in the south.

4.2. Population variations studies through geometric morphometrics

4.2.1. Population variations of *A. florea* in Tamil Nadu

Geometric morphometrics analyses were performed to find out the population variations found in 700 *A. florea* worker bee samples collected from 35 localities representing seven agro-climatic zones of Tamil Nadu. PCA of Cartesian coordinates extracted from the fore and hind wings of *A. florea* gave four (Fig. 10) and three eigenvalues (Fig. 12) greater than one respectively.

In fore wing, 4X, 10X, 9X, 11X, 3X, 2X, 20X , 5X, 14X, 1X, 7X, 8X, 16X, 15X, 12X, 17X and 18X were the variables that influenced the first factor; alone it explained 39.1 percent of the variability among the groups. Variables 17Y, 12Y, 18Y, 19Y, 13Y, 8Y, 16Y, 6Y, 1Y and 7Y influenced the second factor most; it explained 30.4 percent of the variability. Variables 4Y, 14Y, 20Y, 5Y, 10Y, 9Y, 11Y, 15Y, 3Y and 2Y were the variables that influenced the third factor; alone it explained 19.4 per cent of the variability among the groups. Variables 13X, 6X and 19X influenced the third factor which contributed 9.3 per cent of total variability among groups. Mean configuration of 20 landmarks plotted in *A. florea* fore wings is presented in Table 9.

In hind wing, 2Y, 4Y, 5Y, 3Y and 1X were the variables that influenced the first factor; alone it explained 43.2 per cent of the variability among the groups. Variables 4X, 5X and 3X influenced the second factor most; it explained 38.8 per cent of the variability. Variable 1Y influenced the third factor; it explained 10.2 per cent of the variability. Mean configuration of five landmarks plotted in *A. florea* hind wings is presented in Table 10.

Fore wing variables 11X, 19Y, 13Y, 17Y, 6X, 19X, 20X, 7X, 16X, 18X, 13X, 1X, 17X, 10Y, 1Y and 4Y entered in discriminant function analysis. Variables 11X and 19Y alone accounted 74.5 per cent of total variance in discriminant function analysis. Hind wing variables 1Y, 4X and 4Y entered in discriminant function analysis. The scatter plots obtained through discriminant function analysis gave a non-seperable single morphocluster both in fore wings (Fig. 11) and hind wings (Fig. 13). These geometric morphometric analyses also clearly revealed the existence of only a single morphocluster *A. florea* population in Tamil Nadu.

4.2.2. Caste differentiation in *A. florea*

Geometric morphometrics analyses of hind wings of queen, worker and drone of *A. florea* clearly differentiated the castes based on hind wing landmarks. Principal component analysis of Cartesian coordinates gave three eigenvalues greater than one (Fig. 14). The first factor was influenced by the variables 4Y, 2Y, 1X, 5Y, 3Y, 5X, 4X and 2X which alone explained 72.7 per cent of total variability among the groups. Variable 1Y influenced the second factor which contributed 14.6 per cent of total variability. The third factor was influenced by a single variable *viz.*, 3X which accounted 11.8 per cent of total variability.

Hind wing variables 1Y, 2X and 4Y entered in discriminant function analysis. Variables 1Y and 2X were the two variables which contributed 99.9 per cent of total variance in discriminant function analysis. The scatter plot obtained through discriminant function analysis gave three distinct non-overlapping morphoclusters (Fig. 15). In addition, in the hind wings of queen a small distinct radio-medial cross vein (Plate 5) was found which was lacking both in drone and worker.

4.3. Ecotype studies

4.3.1. Variations in adult populations

The structural variations found between the adult workers of normal comb little bee and little comb little bee are given in Table 11. Out of 16 structural characters measured, both the bees significantly differed with respect to 12 characters. There were no significant variations in the size of head, wing and tergite. However, other important morphometric characters like length of antenna, lapping tongue, femur, tibia, tarsus, wax plate, sternite, tomentum; number of hamuli and cubital index showed market variations in these two groups of bees.

Standard morphometrics analyses were performed with 200 bees collected from ten colonies comprising of five normal comb building colonies and five little comb building colonies to confirm the existence of any new ecotype of *A. florea*. The means, standard deviations and coefficients of variations of 16 informative morphometric characters are presented in Table 11. Analysis of variance of morphometric characters showed that all the 16 morphometric characters significantly contributed for structural variations ($P < 0.05$) (Table 12).

In a principal components analysis, four factors with eigenvalues greater than one were extracted (Fig. 16): factor 1 includes tibial length, antennal length, femur length, distance between ocelli, number of hamuli, ratio of length and width of wax plate, ratio of length and width of metatarsus, proboscis length and ratio of length and width of third abdominal sternite; factor 2 comprises tergite length, ocello-ocular distance and ratio of length and width of head; factor 3 includes ratio of length and width of fore wing, ratio of length and width of tomentum and factor 4 includes cubital index and distance between wax plates. Factor 1 and factor 2 accounted for 13.50 per cent and 8.97 per cent of total variance in the data respectively. The scatter plot was obtained by using factor 1 and factor 2, which gave two distinct non-overlapping morphoclusters (Fig. 17).

In stepwise discriminant analysis ratio of length and width of head, distance between ocelli, ocello-ocular distance, femur length, ratio of length and width of metatarsus, tergite length, ratio of length and width of third abdominal sternite, distance between two wax plates and ratio of length and width of tomentum did not enter the discriminant functions due to lack of discriminatory power in these characters for the discrimination of *A. florea* ecotypes. The seven morphometric characters *i.e.*, tibial length, ratio of length and width of fore wing, cubital index, antennal length, ratio of length and width of wax plate, number of hamuli and proboscis length entered in discriminant functions analysis in order of their discriminatory power (Table 13). Scatter plot of discriminant function analysis also showed similar results as obtained from PCA (Fig. 18). These multivariate morphometric analyses gave two distinct non-overlapping morphoclusters in *A. florea* which clearly revealed the existence of a new little comb building ecotype.

4.3.2. Variations in nesting behaviour

The metric data related to the nest of two ecotypes are presented vide Table 14. The honey crest of the little comb little bee was unique. It was more globular than the normal comb little bee (Plate 7). The size variations observed in the honey crest and pollen arch between these two groups were significant. Brood cell dimensions were smaller in little comb little bees.

4.3. Documentation of nesting behaviour

Peculiarities in nesting behaviour of *A. florea* were photographically documented (Plate 6 to 24). The peculiar comb building behaviour was observed in both arboreal and terrestrial nests (Table 15). *Synadenium grantii* (Plate 7 & 8), a greenish, latex yielding hedge plant, *Prosopis juliflora* (Plate 14) and *Musa* spp (Plate 13) were often preferred for nesting. In certain nests, size of mature comb was substantially smaller (Plate 7) and never exceeded the size of a human palm. In such combs the honey crest was globular. All types of brood cells were very well accommodated within the small brood zone. This study has documented for the first time this type of unique comb building behaviour, and named such nest building bees as little comb little bees. The comb of normal comb little bee was usually rounded (Plate 6). As the comb grew it became elliptical in shape (Plate 12). Similarly the colour of the comb also varied from white (Plate 8, 10 & 11) to yellow (Plate 6) and then became darker. Concentric rings of a few layers of open and sealed brood cells alternated in colonies (Plate 9) led by an active young comb. Any part of the plant *i.e.*, stem, leaf margin, leaf lamina, peduncle, inflorescence rachis formed a support in arboreal nests. Though the attachment of arboreal nests to substratum is usually parallel, in rare situation it was also perpendicular (Plate 9). The comb usually encircled the plant part.

Terrestrial nests of *A. florea* were also found on wall (Plate 17 & 18), wooden rafter (Plate 19), cot frame (Plate 20), wire fence (Plate 21), discarded chair frame (Plate 22), corner of iron shelf (Plate 23) and concrete beam (Plate 24). In such situations the comb attachment was unique and it did not encircle the support usually except on barbed wire fence. In all these nests honey crest formed the apical region of the nest. The nest attachment to the substratum also differed and was lateral in position. The resin ring was built around the nest in a semi circular fashion in the nest attached to a concrete beam (Plate 24).

CHAPTER – V

DISCUSSION

Studies were conducted on population variations of little bees (*A. florea*) collected from different agro-climatic zones of Tamil Nadu. The results obtained from this study are discussed here under various heads in detail with appropriate supporting evidences.

5.1. Population variations

Little bees (*A. florea*) are widely distributed in Tamil Nadu. Their distribution was found to be more common in plains during bee faunal survey. However, they were patchily distributed in high altitudes. Usually *A. dorsata* is widely found in high altitudes. Little bees do not build nests in close association with conspecifics mainly to avoid inter specific competition for floral resources and inter specific interference for mating since all species of *Apis* have similar type of sex pheromone.

During the present study, little bees were rarely found upto an altitude of 1502 m in Pudhukadu, Nilgris (Table 1). Though *A. florea* is an inland species, its nests were found even at an altitude of 1900 m in Iran (Ruttner *et al.*, 1995) supports the present finding. Distribution of *A. florea* colonies was not widespread in high rainfall zones. The possible reason can be attributed to the dominance of *A. cerana* in those areas where commercial *cerana* keeping is widely prevalent.

The proboscis length of little bees collected from different agro-climatic zones of Tamil Nadu ranged from 2.80 to 2.96 mm (Table 3). However, earlier workers like Malathi (2005) reported a mean tongue length of 3.57 mm for bees collected from Karnataka and Makhmoor and Hafeez Ahmad (1998) 4.00 mm for bees collected from Jammu. Since Tamil Nadu is found in the southern most end of India, the tongue length values are lower. Further the above three data agree with Bergman's rule that geographic races of one species increase in size with increasing altitude or distance from the tropics (Ruttner, 1988). Such clinal variations in the length of proboscis from the Baltic Sea to the Caucasus were earlier reported for six geographical races of *A. mellifera* (Ruttner, 1988).

The lowest mean cubital index (2.66) was found in high rainfall zone and the highest mean cubital index (3.12) was recorded in north western zone (Table 3; Fig.1).

This variation is also in agreement with Bergman's rule. Cubital index was first used for the morphological discrimination of *A. mellifera* subspecies (Ruttner, 1988). He also used the same to distinguish the *A. florea* population of South Iran which was found to be 2.89.

The mean number of hamuli found in *A. florea* population of Tamil Nadu was 11 (Table 6). However, Ruttner (1988) reported a slightly lower value (10.5) for *A. florea* population of South India. Earlier workers reported that the distribution and the number of hamuli varied significantly within *A. andreniformis* and *A. florea* in different countries (Hepburn *et al.,* 2005).

A. florea population of Tamil Nadu is almost uniform with very little variations. This was confirmed in the present study by using 16 morphometric characters by employing standard morphometrics techniques. Similarly geometric morphometrics studies revealed the existence of a single morphocluster which may be due to low level of population variations. These bees also do not migrate to far off places like *A. dorsata*. In addition, since these bees are not totally domesticated gene flow due to transhumance is also totally ruled out. Earlier studies by Ruttner (1988) grouped all *A. florea* populations of South India and Sri Lanka into one morphocluster supports the present findings. In addition, the earlier reports regarding the occurrence of a single morphocluster of *A. florea* population in Thailand (Chaiyawong, 2001) and *A. andreniformis* in Thailand and Malaysia (Rattanawannee *et al.,* 2007) indicated country wide lack of variations in *A. florea* populations. The homogenous nature of *A. florea* populations both in Thailand and South India through mtDNA studies also confirms the present findings (Smith, 1991). However, Ozkan *et al.* (2009) demonstrated the existence of two distinct non overlapping morphoclusters of *A. florea* in Iran, which clearly indicated the existence of two distinct populations. Similarly two distinct non-overlapping morphoclusters (Fig.8 & 9) were obtained when *A. florea* population of Tamil Nadu was compared with Nagaland. Out of 16 morphometric characters used for comparison, Nagaland population recorded greater values for 15 characters. Nagaland bees were relatively larger than Tamil Nadu bees. The first morphometric study on *A. florea* brought out the existence of clinal variations in *A. florea i.e.,* larger bees found in north and smaller bees in south. It adds support to the present study.

In the present study apart from conventional morphometrics, geometric morphometrics was also utilized to differentiate *A. florea* populations collected from Tamil Nadu using 20 landmarks in fore wing (Fig. 2) and five landmarks in the hind wing (Fig.3). Ozkan *et al.,* (2009) also showed the utility and worth of hind wing length, hind wing width and hind wing angles for differentiating North Iranian *A. florea* population.

In the present study, hind wings were also used for caste differentiation apart from finding out population variations. They were found to be better suited to differentiate castes of *A. florea* through discriminant function analysis (Fig.15). In addition, in the hind wings of queen a small distinct radio-medial cross vein (Plate 5) was found which was lacking both in drone and worker. The present study brought put the value of wing morphology in caste differentiation in *A. florea*. Similarly Francoy *et al.,* (2009) demonstrated the utility of fore wing in differentiating the genders and species of stingless bees. Likewise, the use of geometric morphometry was used in resolving taxonomic problem in bumble bees. Recently Higgs *et al.*,(2009) found out an unequivocal test to differentiate both the species of dwarf honey bees and also castes and immature stages utilizing the mitochondrial large sub unit of ribosomal RNA gene.

Of late molecular studies have become a vital tool to discriminate species, sub species and ecotypes. Some work has been already done to discriminate both *A. florea* and *A. andreniformis* populations (Smith, 1991). Hence, molecular tools should also be intensified and applied along with size dependent and size independent characters to elucidate population differentiation of *A. florea*. Both morphometrics and molecular studies have to be complementary to each other, to draw valid conclusions

5.2. Ecotype studies

Multivariate morphometric analyses demonstrated a high degree variability of size related characters between normal comb little bee and little comb little bee. Out of 16 morphometric characters studied 12 characters varied significantly between these two groups (Table 11). Structurally little comb little bees were smaller than normal comb little bees. Similarly spectacular variations were found in their nest building behaviour (Table 14). Out of 13 nest characters studied eight nest characters varied significantly between these two groups of bees. The most spectacular nest character is the size of the mature comb. Like the size of the new, the mature comb is also smaller. Both principal component analysis (Fig. 17) and discriminant function analysis (Fig.18) clearly revealed the existence of two distinct non-overlapping morphoclusters, since these bees markedly small and also build a unique type of small sized comb with a globular honey crest.The bee find out through this study has been appropriately named as little comb little bee, a new ecotype of *A. florea*. The colonies of little comb little bee are scantly distributed. They occur sympatrically along with normal comb little bees. Khan as early as in 1947 reported the existence of two strains of little bees *i.e.,* one smaller and another bigger from Bhopal. Interaction with local honey collecting tribals also revealed the existence of distinct varieties of little bees. More research has to be done on this new ecotypes of little bee. Molecular studies are essential to establish their genetic uniqueness. Studies on its ethology, developmental biology, reproductive biology and social biology are essential to know more about the exact taxonomic categoty *i.e.,* either a new sub species or a new ecotype.

5.3. Nesting behaviour

Dwarf bees always select shaded secretive sites for nesting. They select sites offering substantial but not complete shade. Such nest site selection provides the bees protection against predators and sun. Some amount of sun light must fall on the comb during some part of the day as the scout bees dance on the honey crest and use sun as a celestial cue.

Nesting behaviour of little bees is unique in certain respects. The comb structure is mainly dictated by the nature of the substratum. In arboreal nesting, the plant support is encircled. A thin branch or twig is always chosen as a nesting site. Unusually other plant parts are also preferred (Plates 10 to 13). In terrestrial nesting, the comb is attached to the substrate usually. Terrestrial nests usually lack a crown which is always present in arboreal nests. In terrestrial nests found on walls, the comb is attached laterally (Plate 17). Nesting behaviour differs between species. The comb built by *A. andreniformis* has a mid rib both above and below the supporting branch. However the comb built by *A. florea* has a mid rib only in the brood area below the supporting branch (Rinderer *et al.,* 1996). These are the only species of *Apis* which go far wax foraging. The foragers collect old wax from the deserted combs and mix them with new wax secreted from their bodies. Similarly they alone apply a band of propolis on either side of the comb to ward off ants. Resin band laying pattern differ in terrestrial nesting. It is applied as a band partially surrounding the comb (Plate 24).

Little bees peacefully coexist with human beings. They often build their nests on or inside buildings (Plates 17, 18 & 24). Even the materials like cot, discarded chair and kitchen shelf (Plates 20, 22 & 23) offer nesting sites for these bees.

Nests of dwarf bees are reported to be found in caves (Thakar and Tonapi, 1961). Although the nest sites of these species are exposed the comb itself is normally hidden by a bee curtain (Plate 12 & 13) and thus protected from rain, sun and wind. The nests are usually not far away from ground. But occasionally they are built very close to ground (Plate 17 & 18).

A peculiar type of comb building behaviour has been documented during this study. This comb often does not expand and grow huge in size. Such a comb builder identified in this study has been aptly named as little comb little bee (Plate 7). The mature comb is smaller and it comprises of all nest components *viz.*, a globular honey crest, pollen arch, worker brood, drone brood and queen cells. Worker cells are smaller in this type of comb (Table 14). Attachment of nest to substratum (Plate 18) and disposition of drone brood become unusual (Plate 15) to adjust with the existing conditions.

This study has brought out the existence of a new ecotype of little bee which is an important contribution to bee science. Further, the lack of variations in the population of *A. florea* in Tamil Nadu has been found out. The following essential future thrusts needed for better understanding of the biodiversity of the most valuable honey bee species.,

- Molecular studies
- Much more intensive survey for the new ecotype
- Extensive use of geometric morphometrics

CHAPTER – VI

SUMMARY

The salient findings of the study on population variations in *Apis florea* are summarized below.

- ❖ Distribution of *A. florea* was widely found in plains.
- ❖ *A. florea* colonies were patchily distributed in high altitude zone and high rainfall zone.
- ❖ *A. florea* colonies were also recorded at an altitude of 1502 m above mean sea level at Puthukadu near Coonoor in Nilgris.
- ❖ All the 16 morphometric characters used in the study contributed for structural variations in *A. florae* population of Tamil Nadu.
- ❖ Both standard morphometrics and geometric morphometrics analyses clearly revealed the existence of only a single morphocluster in *A. florea* population in different agro-climatic zones of Tamil Nadu.
- ❖ *A. florea* of Tamil Nadu was structurally different from the *A. florea* of Nagaland.
- ❖ Little bees in North India were larger than in South India.
- ❖ A distinct radial medial cross vein was present only in the hind wing of queen.
- ❖ Geometric morphometrics of hind wing was found to be a powerful tool for differentiating castes in *A. florea*.
- ❖ The study has validated and documented for the first time the existence of a new ecotype of little bee which is named as little comb little bee.
- ❖ The newly discovered ecotype showed distinct variations both in body size and nesting behaviour.
- ❖ The structural variations between normal little bee and little comb little bee were confirmed through standard morphometrics.
- ❖ *Synadenium grantii*, *Prosopis juliflora* and banana were often preferred for nesting by *A. florea*.

- Arboreal nesting is more common than terrestrial nesting.
- Stem, leaf sheath, leaf margin, leaf lamina, peduncle and inflorescence rachis were the common supports used for arboreal nesting.
- Terrestrial nests of *A. florea* were also found on wall, wooden rafter, cot frame, wire fence, discarded chair frame, corner of iron shelf and concrete beam.
- The size of the mature comb of little comb little bee never exceeded the size of a human palm.
- The colour of the comb gradually changed from white to yellow and later to dark brown.
- The apical portion of the comb constituted the honey crest in terrestrial nests.
- Terrestrial nests of *A. florea* were occasionally found too close to ground.

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Table 1. Sampling locations of *Apis florea* in seven agro-climatic zones of Tamil Nadu

Agro Climatic Zone No.	Name of Agro Climatic Zone	Location	N	Source	Latitude	Longitude	Altitude (m)
I	North Eastern Zone	Kalavai	20	Flowers	12° 46' N	79° 25' E	138
		Arcot	20	Flowers	12° 54' N	79° 20' E	164
		Gudiyatham	20	Nest	12° 56′ N	78° 51′ E	172
		Chidambaram	20	Nest	11° 24' N	79° 44' E	3
		Cuddalore	20	Nest	11° 43' N	79° 49' E	1
II	North Western Zone	Pennagaram	20	Nest	12° 80' N	77° 54' E	493
		Theerthamalai	20	Nest	12° 50' N	77° 59' E	350
		Hogenakkal	20	Flowers	12° 07′ N	77° 46′ E	228
		Kallakurichi	20	Nest	11° 44′ N	78° 58′ E	185
		Mettur	20	Nest	11° 52' N	77° 50' E	238
III	Western Zone	Nellithurai	20	Flowers	11° 17' N	76° 53' E	326
		Bhavanisagar	20	Flowers	11° 28′ N	77° 70′ E	280
		Annur	20	Flowers	11° 14′ N	77° 06′ E	338
		Siruvani	20	Flowers	10° 93' N	76° 68' E	411
		Pollachi	20	Nest	10° 40′ N	77° 01′ E	293
IV	Cauvery Delta Zone	Vamban	20	Nest	10° 22' N	78° 55' E	88
		Trichy	20	Nest	10° 50' N	78° 46' E	88
		Thanjavur	20	Nest	10° 47' N	79° 10' E	77
		Anavayal	20	Nest	10° 19' N	79° 70' E	88
		Veppankulam	20	Flowers	8° 46' N	77° 41' E	5

Table 1. Sampling locations of *Apis florea* in seven agro-climatic zones of Tamil Nadu (Continued…)

Agro Climatic Zone No.	Name of Agro Climatic Zone	Location	N	Source	Latitude	Longitude	Altitude (m)
V	Southern Zone	Madurai	20	Flowers	9° 58' N	78° 10' E	8
		Aruppukottai	20	Flowers	9° 31' N	78° 08' E	97
		Kovilpatti	20	Nest	9° 10′ N	77° 52′E	106
		Vallanadu	20	Flowers	11° 15' N	76° 17' E	83
		Thirunelveli	20	Nest	8° 44' N	77° 44' E	47
VI	High Rainfall Zone	Innaiyam	20	Nest	8° 44' N	77° 27' E	16
		Pechiparai	20	Nest	8° 44' N	77° 31' E	19
		Thiruvattaru	20	Nest	8° 20′ N	77° 16′ E	11
		Vadakkankulam	20	Nest	8° 15' N	77° 36' E	13
		Thuckalay	20	Nest	08° 20′ N	77° 26′ E	17
VII	High Altitude Zone	Omalur	20	Nest	11° 48' N	78° 13' E	715
		Thalavady	20	Flowers	9° 21′ N	76° 32′ E	1098
		Puthukadu	20	Nest	11° 21′ N	76° 49′ E	1502
		Thadiyankudisai	20	Nest	10° 18' N	77° 45' E	1123
		Asanur	20	Flowers	11° 37' N	79° 11' E	1075

N - Number of worker bees collected

Table 2. List of morphological characteristics of *A. florea* worker bees studied

S. No.	Characteristics	Abbreviation
I. Head		
1	Ratio between head length and width	HL:HW
2	Proboscis length (mm)	PL
3	Distance between two lateral ocelli (mm)	DO
4	Ocello - ocular distance (mm)	OOD
5	Antennal length (mm)	AL
II. Thorax		
6	Femur length (mm)	FL
7	Tibial length (mm)	TIL
8	Ratio between length and width of metatarsus	LMT:WMT
9	Ratio between fore wing length and width	WL:WW
10	Cubital index	CI
11	Number of hamuli	NH
III. Abdomen		

12	Tergite length (mm)	TEL
13	Ratio between third sternite length and width	SL:SW
14	Ratio between wax plate length and width	WPL:WPW
15	Distance between wax plates (mm)	DWP
16	Ratio between length and width of tomentum	LT:WT

Table 3. Mean, SD and CV of 16 morphometric characters in *A. florea* population from seven agro-climatic zones of Tamil Nadu

Characters	North Eastern Zone (NEZ) n=100		North Western Zone (NWZ) n=100		Western Zone (WZ) n=100		Cauvery Delta Zone (CDZ) n=100		Southern Zone (SZ) n=100		High Rainfall Zone (HRZ) n=100		High Altitude Zone (HAZ) n=100	
	Mean ± SD	**CV**	**Mean ± SD**	**CV**	**Mean ± SD**	**CV**	**Mean ± SD**	**CV**	**Mean ± SD**	**CV**	**Mean ± SD**	**CV**	**Mean ± SD**	**CV**
HL:HW	0.55 ± 0.02	3.91	0.52 ± 0.03	4.89	0.55 ± 0.01	2.20	0.53 ± 0.03	5.01	0.53 ± 0.02	4.23	0.52 ± 0.02	2.52	0.54 ± 0.03	5.55
PL	2.85 ± 0.08	2.67	2.80 ± 0.16	5.85	2.85 ± 0.04	1.57	2.81 ± 0.07	2.32	2.96 ± 0.15	5.03	2.86 ± 0.11	3.98	2.82 ± 0.17	5.88
DO	0.43 ± 0.02	5.84	0.40 ± 0.02	5.13	0.41 ± 0.01	3.51	0.40 ± 0.02	4.82	0.39 ± 0.02	4.62	0.39 ± 0.01	0.81	0.35 ± 0.01	3.82
OOD	0.29 ± 0.01	5.03	0.30 ± 0.02	5.88	0.30 ± 0.02	6.30	0.33 ± 0.01	3.48	0.30 ± 0.01	3.81	0.29 ± 0.02	0.67	0.29 ± 0.02	5.47
AL	2.94 ± 0.08	2.58	2.97 ± 0.18	5.97	2.95 ± 0.14	4.59	2.89 ± 0.04	1.39	2.96 ± 0.05	1.73	2.92 ± 0.13	5.75	2.91 ± 0.07	2.30
FL	1.54 ± 0.03	2.25	1.50 ± 0.09	5.76	1.52 ± 0.08	5.10	1.51 ± 0.10	6.80	1.54 ± 0.09	5.80	1.94 ± 0.09	4.44	1.64 ± 0.03	2.09
TIL	1.98 ± 0.04	2.18	1.98 ± 0.03	1.72	1.97 ± 0.10	5.02	1.91 ± 0.08	4.19	1.94 ± 0.07	3.67	1.96 ± 0.07	3.43	1.97 ± 0.05	2.56
LMT:WMT	2.14 ± 0.09	4.32	2.21 ± 0.14	6.30	2.18 ± 0.06	2.88	2.17 ± 0.15	6.70	2.16 ± 0.11	5.07	2.37 ± 0.09	3.92	2.10 ± 0.08	3.76
WL:WW	2.94 ± 0.09	2.93	2.93 ± 0.08	2.64	2.84 ± 0.06	2.01	2.97 ± 0.03	0.95	2.91 ± 0.09	3.00	2.92 ± 0.07	2.95	2.88 ± 0.09	3.29
CI	2.74 ± 0.07	2.65	3.12 ± 0.18	5.91	2.68 ± 0.08	2.95	2.70 ± 0.14	5.01	2.81 ± 0.13	4.75	2.66 ± 0.14	5.26	2.77 ± 0.09	3.27
NH	11.0 ± 0.10	0.91	11.0 ± 0.50	4.49	11.0 ± 0.35	3.08	11.0 ± 0.24	2.17	11.4 ± 0.60	5.25	11.0 ± 0.40	4.49	11.0 ± 0.51	4.42
TEL	1.23 ± 0.03	2.07	1.24 ± 0.03	2.47	1.25 ± 0.02	1.62	1.22 ± 0.03	2.10	1.22 ± 0.03	2.72	1.21 ± 0.04	3.42	1.29 ± 0.07	5.60
SL:SW	0.55 ±0.02	3.95	0.56 ± 0.02	2.79	0.52 ± 0.02	3.03	0.54 ± 0.02	4.54	0.53 ± 0.01	1.51	0.53 ± 0.01	1.87	0.52 ± 0.02	3.84
WPL:WPW	0.58 ± 0.04	6.52	0.62 ± 0.03	4.83	0.58 ± 0.03	5.23	0.58 ± 0.02	3.63	0.54 ± 0.02	3.88	0.96 ± 0.02	1.84	0.53 ± 0.02	4.60
DWP	0.12 ± 0.01	6.49	0.11 ± 0.01	5.11	0.12 ± 0.00	3.43	0.11 ± 0.01	4.64	0.11 ± 0.01	5.11	0.10 ± 0.01	1.18	0.11 ± 0.00	2.31
LT:WT	0.53 ± 0.05	9.63	0.46 ± 0.02	5.28	0.54 ± 0.03	4.92	0.43 ± 0.02	4.33	0.46 ± 0.02	5.23	0.46 ± 0.10	4.75	0.43 ± 0.03	5.98

n - Number of worker bees analyzed SD – Standard Deviation CV – Coefficient of Variation

Table 4. Test of equality of means of 16 morphometric characters in *A. florea* population from seven agro-climatic zones of Tamil Nadu

Characters	Wilks' Lambda	F	df1	df2	Significant
HL:HW	0.850	3.441	34	665	0.000*
PL	0.758	6.231	34	665	0.000*
DO	0.749	6.558	34	665	0.000*
OOD	0.891	2.381	34	665	0.000*
AL	0.843	3.648	34	665	0.000*
FL	0.679	9.248	34	665	0.000*
TIL	0.832	3.936	34	665	0.000*
LMT:WMT	0.878	2.711	34	665	0.000*
WL:WW	0.842	3.683	34	665	0.000*
CI	0.793	5.101	34	665	0.000*
NH	0.813	4.500	34	665	0.000*
TEL	0.831	3.982	34	665	0.000*
SL:SW	0.844	3.625	34	665	0.000*
WPL:WPW	0.744	6.734	34	665	0.000*
DWP	0.853	3.374	34	665	0.000*
LT:WT	0.713	7.861	34	665	0.000*

* Significant ($P < 0.05$)

Table 5. Canonical discriminant functions of *A. florea* of seven agro-climatic zones of Tamil Nadu

Function	Characters	Eigenvalue	% of Variance	Cumulative %	Canonical correlation
1	FL	0.848 [a]	33.2	33.2	0.677
2	LT:WT	0.455 [a]	17.8	51.0	0.559
3	PL	0.333 [a]	13.0	64.1	0.500
4	WPL:WPW	0.293 [a]	11.5	75.6	0.476
5	CI	0.237 [a]	9.3	84.9	0.438
6	DO	0.211 [a]	8.3	93.1	0.417
7	TIL	0.129 [a]	5.1	98.2	0.338
8	NH	0.046 [a]	1.8	100.0	0.211

a. First eight canonical discriminant functions were used in analysis.

Table 6. Mean, SD and CV of morphometric characters in *A. florea* population from North East India (Nagaland) and South India (Tamil Nadu)

Characters	Nagaland		Tamil Nadu	
	Mean* ± SD	**CV**	**Mean** ± SD**	**CV**
HL:HW	0.57 ± 0.03	4.84	0.53 ± 0.01	2.38
PL	3.00 ± 0.12	4.08	2.85 ± 0.05	1.88
DO	0.42 ± 0.03	7.5	0.40 ± 0.02	6.17
OOD	0.33 ± 0.01	4.53	0.30 ± 0.01	4.71
AL	3.08 ± 0.10	3.24	2.93 ± 0.03	0.98
FL	1.64 ± 0.07	4.32	1.54 ± 0.05	3.01
TIL	2.07 ± 0.07	3.59	1.96 ± 0.03	1.30
LMT:WMT	2.07 ± 0.13	6.42	2.19 ± 0.09	3.95
WL:WW	2.99 ± 0.08	2.6	2.91 ± 0.04	1.45
CI	2.91 ± 0.25	8.62	2.78 ± 0.16	5.66
NH	12.0 ± 0.92	7.65	11.0 ± 0.15	1.37
TEL	1.25 ± 0.05	4.35	1.24 ± 0.03	2.17
SL:SW	0.55 ± 0.03	5.44	0.54 ± 0.02	2.82
WPL:WPW	0.92 ± 0.03	2.95	0.58 ± 0.03	5.48
DWP	0.13 ± 0.02	13.61	0.11 ± 0.01	6.19
LT:WT	0.60 ± 0.08	13.14	0.47 ± 0.04	9.44

* Mean of 60 worker bee samples

** Mean of 700 worker bee samples

Table 7. Test of equality of means of 16 morphometric characters in *A. florea* population from North East India (Nagaland) and South India (Tamil Nadu)

Characters	Wilks' Lambda	F	df1	df2	Significant
HL:HW	0.227	73.793	7	152	0.000*
PL	0.119	160.020	7	152	0.000*
DO	0.482	23.371	7	152	0.000*
OOD	0.870	3.236	7	152	0.003*
AL	0.269	59.065	7	152	0.000*
FL	0.587	15.248	7	152	0.000*
TIL	0.397	32.990	7	152	0.000*
LMT:WMT	0.826	4.559	7	152	0.000*
WL:WW	0.453	26.219	7	152	0.000*
CI	0.643	12.065	7	152	0.000*
NH	0.889	2.714	7	152	0.011*
TEL	0.192	91.189	7	152	0.000*
SL:SW	0.010	2.251	7	152	0.000*
WPL:WPW	0.119	160.575	7	152	0.000*
DWP	0.513	20.585	7	152	0.000*
LT:WT	0.501	21.653	7	152	0.000*

* Significant ($P < 0.05$)

Table 8. Canonical discriminant functions of *A. florea* of North East India (Nagaland) and South India (Tamil Nadu)

Function	Characters	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	SL:SW	124.195[a]	88.8	88.8	0.996
2	PL	13.364[a]	9.6	98.3	0.965
3	WPL:WPW	1.368[a]	1.0	99.3	0.760
4	HL:HW	0.900[a]	0.6	99.9	0.688
5	CI	0.087[a]	0.1	100	0.283
6	WL:WW	0.020[a]	0.0	100	0.141
7	TEL	0.002[a]	0.0	100	0.046
8	NH	0.002[a]	0.0	100	0.046
9	LT:WT	0.002[a]	0.0	100	0.046

a. First nine canonical discriminant functions were used in the analysis.

Table 9. Mean configuration of the 20 landmarks plotted in *A. florea* fore wing from seven agro-climatic zones of Tamil Nadu

Coordinates	Agro-climatic zones						
	NEZ	NWZ	WZ	CDZ	SZ	HRZ	HAZ
1X	0.01054	-0.03890	-0.10488	0.26027	-0.06992	-0.11950	0.23922
1Y	-0.01644	0.07628	-0.00259	-0.03972	0.00113	0.02954	-0.06466
2X	0.01078	-0.03284	-0.11289	0.24645	-0.06680	-0.11084	0.23037
2Y	-0.00189	0.09265	0.01383	-0.00205	0.00865	0.04392	-0.02891
3X	0.01062	-0.02830	-0.09562	0.20806	-0.05770	-0.09424	0.19565
3Y	0.00163	0.08183	0.01405	0.00478	0.00920	0.03870	-0.01779
4X	0.00651	-0.01282	-0.07602	0.13015	-0.03972	-0.06012	0.12525
4Y	0.02009	0.08075	0.02757	0.05129	0.01162	0.04810	0.03232
5X	0.00963	-0.00414	-0.07470	0.10720	-0.03268	-0.04309	0.10958
5Y	0.03780	0.09259	0.04260	0.08955	0.02577	0.05929	0.06964
6X	0.00349	0.03812	-0.02963	-0.00434	-0.00193	0.00221	0.00620
6Y	0.04231	0.06035	0.04204	0.09783	0.02294	0.04088	0.09416
7X	-0.02214	0.05950	0.15558	-0.38534	0.10453	0.18409	-0.34503
7Y	0.02226	-0.11204	0.00302	0.05835	-0.00192	-0.04316	0.09187
8X	-0.00522	0.02224	0.05002	-0.15042	0.03827	0.07281	-0.13343
8Y	0.02441	-0.01887	0.01652	0.05982	0.00761	0.00195	0.07330
9X	-0.00951	0.02837	0.11490	-0.22329	0.06510	0.10059	-0.21479
9Y	-0.01467	-0.11036	-0.03134	-0.04088	-0.01776	-0.06076	-0.01497
10X	-0.01035	0.02103	0.10411	-0.19877	0.05833	0.08770	-0.19243
10Y	-0.01767	-0.10417	-0.03072	-0.04453	-0.01973	-0.05840	-0.01968
11X	-0.00256	0.01605	0.06138	-0.13130	0.03654	0.05810	-0.12351
11Y	-0.00209	-0.05564	-0.00849	-0.00715	-0.00466	-0.02562	0.00940
12X	0.00100	0.01695	0.00967	-0.06413	0.01645	0.03240	-0.05636
12Y	0.02746	0.01502	0.02287	0.06063	0.01088	0.01863	0.06760
13X	-0.00021	0.00841	-0.01109	-0.02175	0.00539	0.00889	-0.01362
13Y	0.03140	0.03593	0.03002	0.07075	0.01337	0.02968	0.06912
14X	0.00491	-0.00373	-0.04076	0.05697	-0.01928	-0.02584	0.05578
14Y	0.01788	0.04902	0.02236	0.04688	0.01412	0.03004	0.03585
15X	0.00282	0.00013	-0.00628	0.00927	-0.00167	-0.00402	0.00917
15Y	0.00468	0.01196	0.00570	0.01008	0.00217	0.00730	0.01038
16X	0.00137	-0.01009	-0.01874	0.05830	-0.01536	-0.02531	0.04917
16Y	-0.01112	0.00673	-0.00822	-0.02450	-0.00359	-0.00085	-0.02831
17X	-0.00099	-0.01573	-0.01075	0.07251	-0.01759	-0.03536	0.06214
17Y	-0.03113	-0.01545	-0.02589	-0.06795	-0.01403	-0.01772	-0.07226
18X	-0.00004	-0.02258	-0.01174	0.05902	-0.02619	-0.04644	0.08105
18Y	-0.04168	-0.02460	-0.03781	-0.10550	-0.01928	-0.02787	-0.10386
19X	-0.00229	-0.02031	0.00345	0.07283	-0.01487	-0.03669	0.05589
19Y	-0.04761	-0.04359	-0.04537	-0.07924	-0.02436	-0.04099	-0.11810
20X	-0.00834	0.00867	0.09399	-0.13492	0.03911	0.05468	-0.14028
20Y	-0.04562	-0.11837	-0.05016	-0.10523	-0.02555	-0.07265	-0.08511

Table 10. Mean configuration of the five landmarks plotted in *A. florea* hind wing from seven agro-climatic zones of Tamil Nadu

Coordinates	Agro-climatic zones						
	NEZ	**NWZ**	**WZ**	**CDZ**	**SZ**	**HRZ**	**HAZ**
1X	0.03499	0.06587	0.05728	0.12147	0.01170	-0.09437	0.07449
1Y	0.17914	0.05566	0.07540	-0.03871	-0.09679	0.11749	0.12582
2X	0.24971	0.42554	0.31595	-0.20699	-0.15704	0.23402	0.43316
2Y	-0.04087	-0.11496	-0.02255	-0.47369	0.02749	0.31265	-0.09305
3X	0.04387	0.08096	0.05091	-0.08923	-0.04088	0.09004	0.07318
3Y	-0.06497	-0.04813	-0.01968	-0.09755	0.08224	0.03823	-0.06484
4X	-0.16453	-0.28144	-0.20129	0.11398	0.10418	-0.14240	-0.28222
4Y	0.00536	0.06691	-0.00094	0.30672	-0.00164	-0.20976	0.04003
5X	-0.16406	-0.29093	-0.22285	0.06077	0.08203	-0.08729	-0.29862
5Y	-0.07865	0.04052	-0.03222	0.30323	-0.01130	-0.25861	-0.00795

Table 11. Mean, SD and CV of 16 morphometric characters for *A. florea* ecotypes

S. No	N	Characters	LCLB		N	NCLB		Df	p - value
			Mean ± SD	CV		Mean ± SD	CV		
1	100	HL:HW	0.52 ± 0.02	4.31	100	0.55 ± 0.02	3.20	198	0.099
2	100	PL	2.51 ± 0.07	2.78	100	2.81 ± 0.07	2.64	198	0.006*
3	100	DO	0.35 ± 0.01	3.57	100	0.40 ± 0.01	3.58	198	0.000*
4	100	OOD	0.29 ± 0.01	3.39	100	0.29 ± 0.02	5.25	198	0.662
5	100	AL	2.36 ± 0.08	3.31	100	2.92 ± 0.11	3.77	198	0.001*
6	100	FL	1.29 ± 0.06	4.44	100	1.50 ± 0.03	1.72	198	0.003*
7	100	TIL	1.54 ± 0.05	3.12	100	1.95 ± 0.09	4.73	198	0.001*
8	100	LMT:WMT	1.64 ± 0.03	1.97	100	2.20 ± 0.04	2.03	198	0.000*
9	100	WL:WW	2.82 ± 0.11	3.86	100	2.88 ± 0.08	2.70	198	0.339
10	100	CI	2.97 ± 0.10	3.45	100	2.72 ± 0.11	4.01	198	0.014*
11	100	NH	10.6 ± 0.59	5.54	100	11.5 ± 0.37	3.16	198	0.044*
12	100	TEL	1.22 ± 0.03	2.21	100	1.23 ± 0.03	2.53	198	0.164
13	100	SL:SW	0.64 ± 0.02	2.51	100	0.52 ± 0.01	2.09	198	0.000*
14	100	WPL:WPW	0.57 ± 0.03	5.02	100	0.74 ± 0.02	3.30	198	0.001*
15	100	DWP	0.13 ± 0.01	5.25	100	0.11 ± 0.00	4.29	198	0.004*
16	100	LT:WT	0.45 ± 0.03	5.96	100	0.51 ± 0.03	5.45	198	0.034*

* Significant ($P < 0.05$)

N – Number of samples

SD - Standard Deviation

CV - Coefficient of Variation

LCLB - Little Comb Little Bee

NCLB - Normal Comb Little Bee

Table 12. Test of equality of means of 16 morphometric characters for *A. florea* ecotypes

Characters	Wilks' Lambda	F	df1	df2	Significant
HL:HW	0.756	6.824	9	190	0.000*
PL	0.507	20.538	9	190	0.000*
DO	0.689	9.514	9	190	0.000*
OOD	0.910	2.078	9	190	0.033*
AL	0.340	40.913	9	190	0.000*
FL	0.609	13.575	9	190	0.000*
TIL	0.282	53.882	9	190	0.000*
LMT:WMT	0.519	19.592	9	190	0.000*
WL:WW	0.627	12.533	9	190	0.000*
CI	0.830	4.336	9	190	0.000*
NH	0.655	11.129	9	190	0.000*
TEL	0.880	2.865	9	190	0.003*
SL:SW	0.732	7.741	9	190	0.000*
WPL:WPW	0.488	22.178	9	190	0.000*
DWP	0.893	2.537	9	190	0.009*
LT:WT	0.860	3.437	9	190	0.001*

* Significant ($P < 0.05$)

Table 13. Canonical discriminant functions of *A. florea* ecotypes

Function	Characters	Eigen value	% of Variance	Cumulative %	Canonical correlation
1	TL	5.641[a]	82.80	82.8	0.922
2	WL:WW	0.726[a]	10.70	93.5	0.649
3	CI	0.380 [a]	2.08	98.4	0.530
4	AL	0.241[a]	3.50	97.0	0.441
5	WPL:WPW	0.133[a]	2.00	98.9	0.343
6	NH	0.064[a]	0.90	99.9	0.245
7	PL	0.008[a]	0.10	100.0	0.089

a. First seven canonical discriminant functions were used in analysis.

Table 14. Nest architecture of *A. florea* ecotypes

S.No.	Characters	N	LCLB			N	NCLB			df	p-value
			Mean	SD	CV		Mean	SD	CV		
1	Diameter of twig	5	1.15	0.47	40.73	5	1.16	0.09	8.15	8	0.950
2	Height of honey crest	5	3.82	0.40	10.37	5	3.36	0.25	7.47	8	0.045*
3	Width of honey crest	5	7.32	0.37	5.06	5	20.80	6.97	33.51	8	0.015*
4	Thickness of honey crest	5	5.36	0.74	13.87	5	3.50	0.24	7.00	8	0.008*
5	Depth of the honey cell	5	1.90	0.07	3.72	5	1.86	0.11	6.13	8	0.178
6	Width of 10 honey cells	5	3.32	0.20	6.17	5	3.88	0.29	7.60	8	0.057
7	Length of brood comb	5	6.04	0.30	5.05	5	8.00	0.32	4.05	8	0.000*
8	Width of brood comb	5	8.66	0.65	7.47	5	9.38	0.34	3.65	8	0.017*
9	Thickness of brood comb	5	1.82	0.27	14.74	5	2.06	0.05	2.66	8	0.136
10	Depth of worker cell	5	0.82	0.08	10.20	5	1.28	0.08	6.54	8	0.001*
11	Width of 10 worker cells	5	2.98	0.08	2.81	5	3.08	0.08	2.72	8	0.189
12	Height of pollen arch	5	0.56	0.05	9.78	5	1.34	0.09	6.67	8	0.000*
13	Width of pollen arch	5	0.64	0.05	8.56	5	0.26	0.05	21.07	8	0.001*

* - Significant ($P < 0.05$)
N - Number of colonies
SD - Standard Deviation
CV - Coefficient of Variation
LCLB - Little Comb Little Bee
NCLB - Normal Comb Little Bee

Table 15. Different types of nesting behaviour of little bee (*A. florea*)

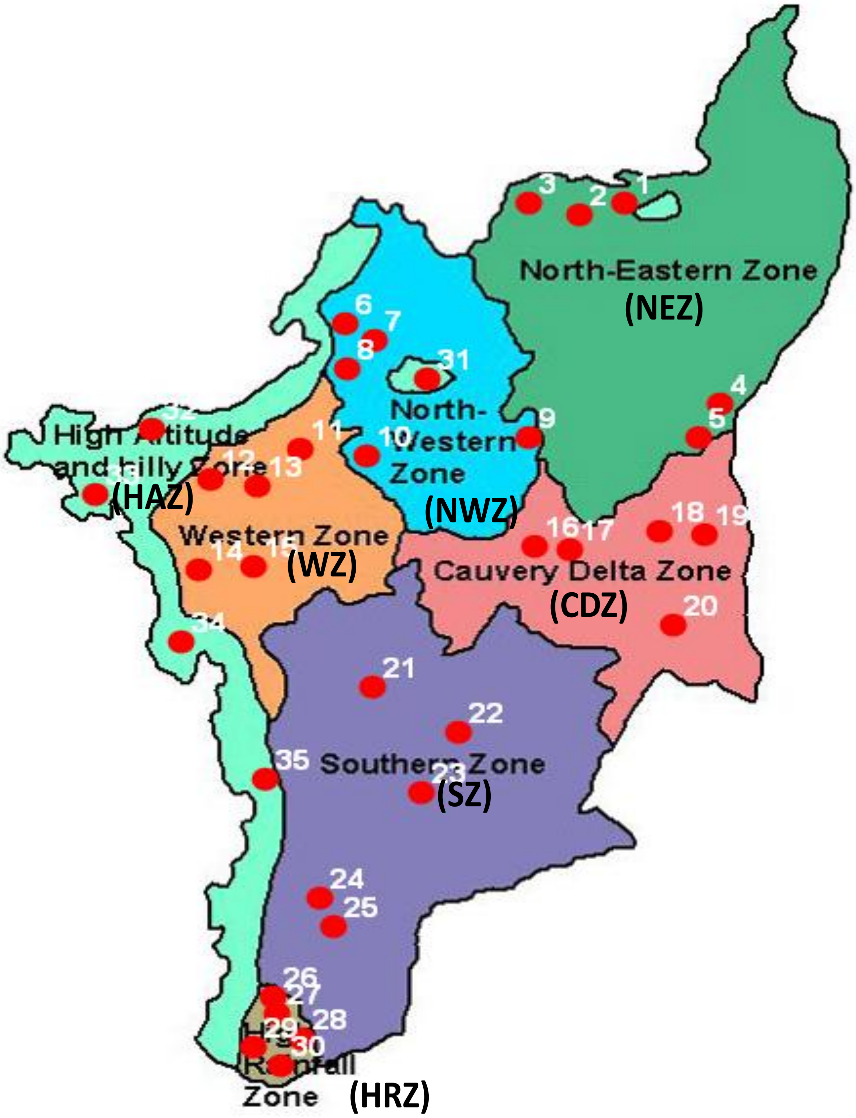
S. No.	Type of nesting behaviour	Substratum	Type of nest attachment	Remarks	Plate No.
1	Arboreal	Twig of *Synadenium grantii*	Comb encircles twig	➢ Comb rounded ➢ Light yellow coloured ➢ Growing comb	6
2	Arboreal	Twig of *Synadenium grantii*	Comb encircles twig	➢ Relatively small sized comb ➢ Globular honey crest ➢ Mature comb ➢ Small brood nest with drone brood	7
3	Arboreal	Thick twig of *Synadenium grantii*	Comb encircles twig	➢ Young comb ➢ White coloured ➢ Prominent resin rings on either side of comb	8
4	Arboreal	Twig	Comb encircles twig	➢ Nest attachment to substratum is peculiar ➢ Comb attachment perpendicular to twig ➢ Worker brood cells in distinct rings ➢ Queen young and active	9
5	Arboreal	Leaf of *Agave sp*	Comb encircles leaf margin and lamina	➢ Young comb ➢ White coloured ➢ Honey crest is found both above and below leaf	10, 11
6	Arboreal	Rachis of *Cocus nucifera* inflorescence	Comb encircles rachis	➢ Elliptical comb attached to rachis of inflorescence	12
7	Arboreal	Peduncle of *Musa sp*	Comb encircles the base of peduncle	➢ Honey crest above peduncle ➢ Comb is also attached to developing fingers	13

Table 15. Different types of nesting behaviour of little bee (*A. florea*) (Continued)

S. No	Type of nesting behaviour	Substratum	Type of nest attachment	Remarks	Plate No.
8	Arboreal	Twig of *Prosopis juliflora*	Comb encircles twig	➢ Comb is irregular in shape ➢ Comb consists of only drone cells ➢ Queenless colony ➢ Drone cells with many eggs ➢ False queen cell with drone larva	14
9	Arboreal	Twig of *Azadirachta indica*	Comb encircles twig	➢ Mature comb ➢ Drone brood laterally placed for want of space ➢ Comb with emergency queen cells	15
10	Arboreal	Twig	Comb encircles twig	➢ Drone brood below worker brood	16
11	Terrestrial	Brick wall	Comb laterally attached to wall	➢ Top of comb forms honey crest ➢ Comb does not encircle the support ➢ Comb touches ground	17
12	Terrestrial	Wall	Comb vertically attached to wall	➢ Top of comb forms honey crest ➢ Comb does not encircle support ➢ Comb very close to ground ➢ Indoor nesting	18
13	Terrestrial	Wooden rafter	Comb is attached to bottom of rafter and also to lateral wall	➢ Top of comb forms honey crest ➢ Comb does not encircle support ➢ Comb is elliptical in shape ➢ Indoor nesting	19
14	Terrestrial	Frame of a cot	Comb encircles iron tubing of cot	➢ Honey crest is above tubing ➢ Comb is rounded ➢ Indoor nesting	20

Table 15. Different types of nesting behaviour of little bee (*A. florea*) (Continued)

S. No	Type of nesting behaviour	Substratum	Type of nest attachment	Remarks	Plate No.
15	Terrestrial	Barbed wire fence	Comb encircles barbed wire	➢ Young comb ➢ White coloured comb ➢ Comb round in shape ➢ Comb is very close to ground ➢ Outdoor nesting	21
16	Terrestrial	Discarded chair frame	Comb encircles iron tubing of the chair	➢ Relatively small sized comb ➢ Honey crest is found above tubing ➢ Comb does not encircle support ➢ Indoor nesting	22
17	Terrestrial	Iron shelf corner	Comb attached to bottom of iron shelf	➢ Top of comb forms honey crest ➢ Comb does not encircle support ➢ Elongated comb ➢ Indoor nesting	23
18	Terrestrial	Concrete beam	Comb mainly attached to bottom of beam	➢ Top of comb forms honey crest ➢ Comb does not encircle the support ➢ Semicircular resin ring on beam	24



1.Kalavai	11.Nellithurai	21.Madurai	31.Omalur
2.Arcot	12.Bhavanisagar	22.Aruppukottai	32.Thalavady
3.Gudiyatham	13.Annur	23.Kovilpatti	33.Puthukadu
4.Chidambaram	14.Siruvani	24.Vallanadu	34.Thadiyankudisai
5.Cuddalore	15.Pollachi	25.Thirunelveli	35.Asanur
6.Pennagaram	16.Vamban	26.Innaiyam	
7.Theerthamalai	17.Trichy	27.Pechiparai	
8.Hogenakkal	18.Thanjavur	28.Thiruvattaru	
9.Kallakurichi	19.Anavayal	29.Vadakkankulam	
10.Mettur	20.Veppankulam	30.Thuckalay	

Fig. 1. Sampling locations of *Apis florea* in seven agro-climatic zones of Tamil Nadu

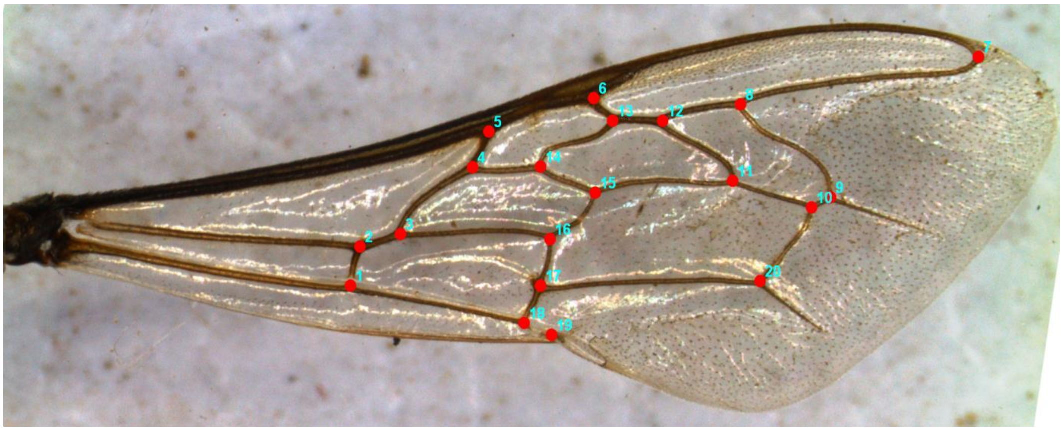


Fig. 2. Distribution of 20 landmarks plotted on fore wing of *A. florea*

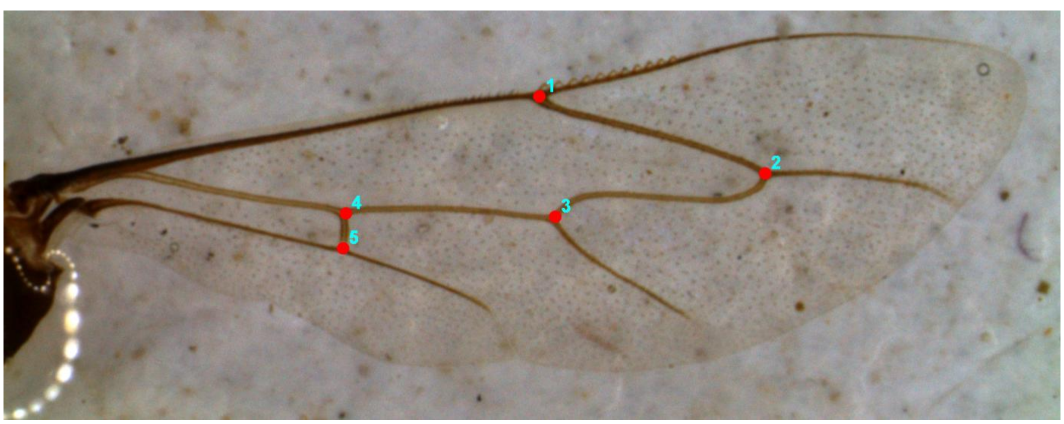


Fig. 3. Distribution of five landmarks plotted on hind wing of *A. florea*

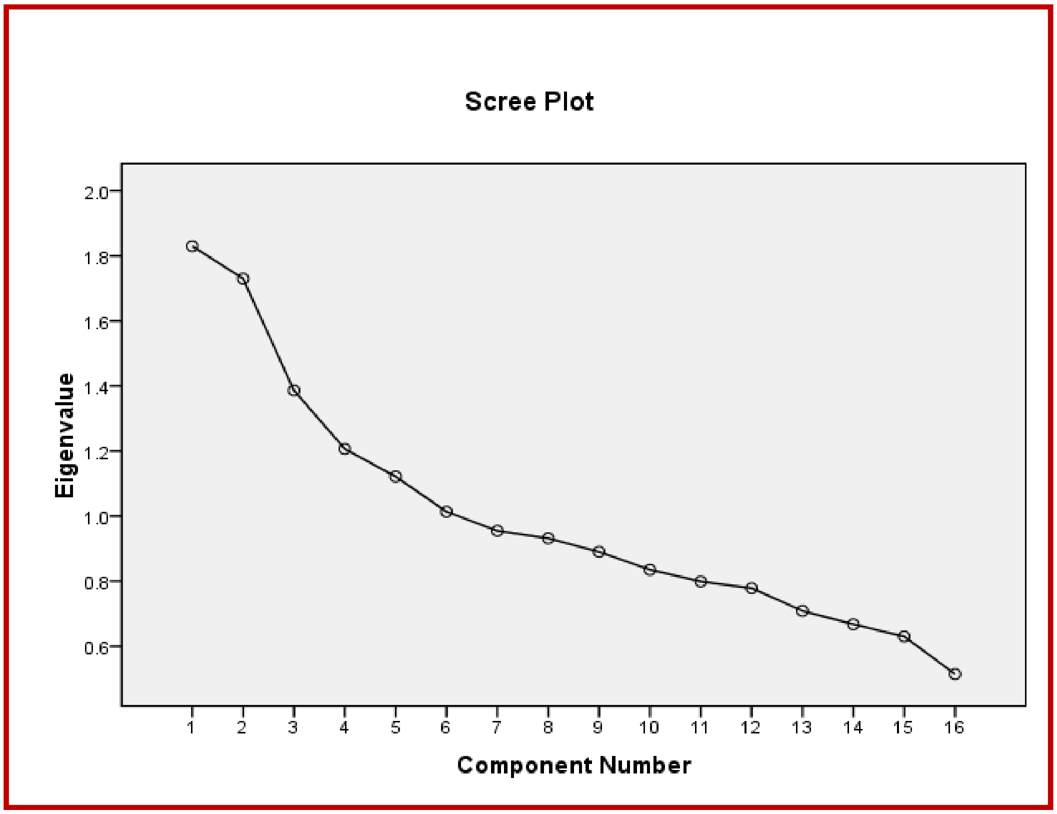


Fig. 4. Scree plot of 35 *A. florea* colonies from seven agro-climatic zones based on PCA

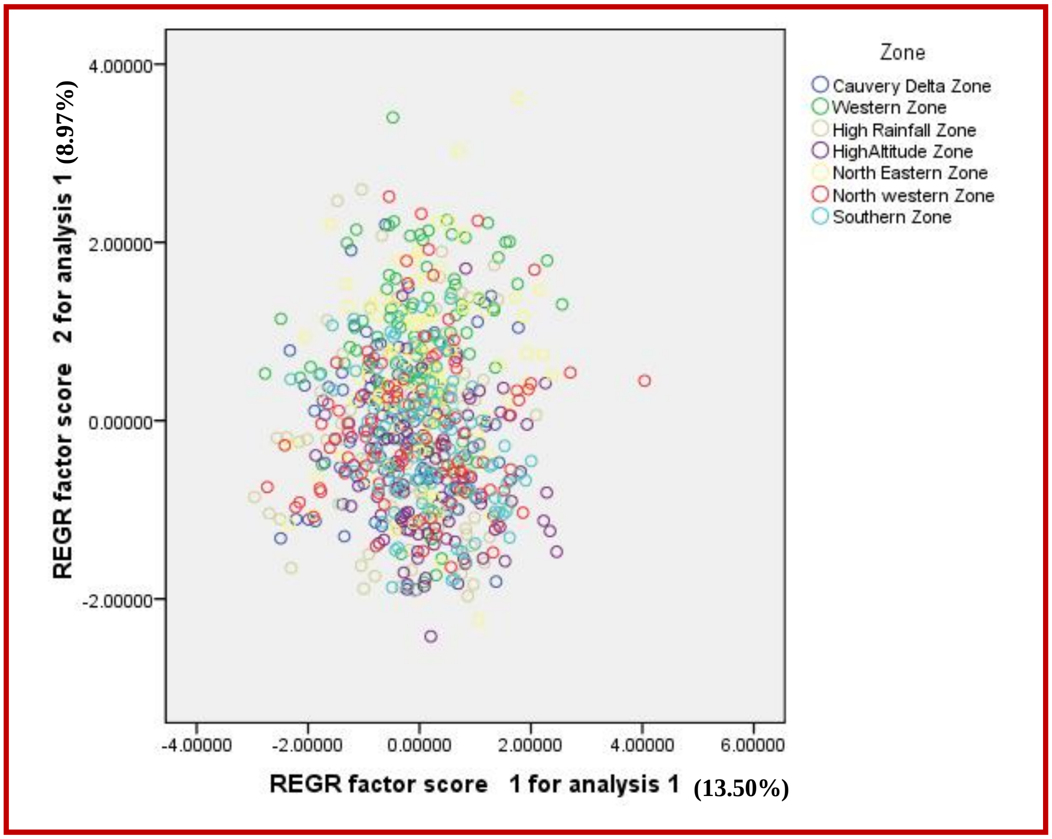


Fig. 5. Scatter plot of 35 *A. florea* colonies from seven agro-climatic zones based on PCA

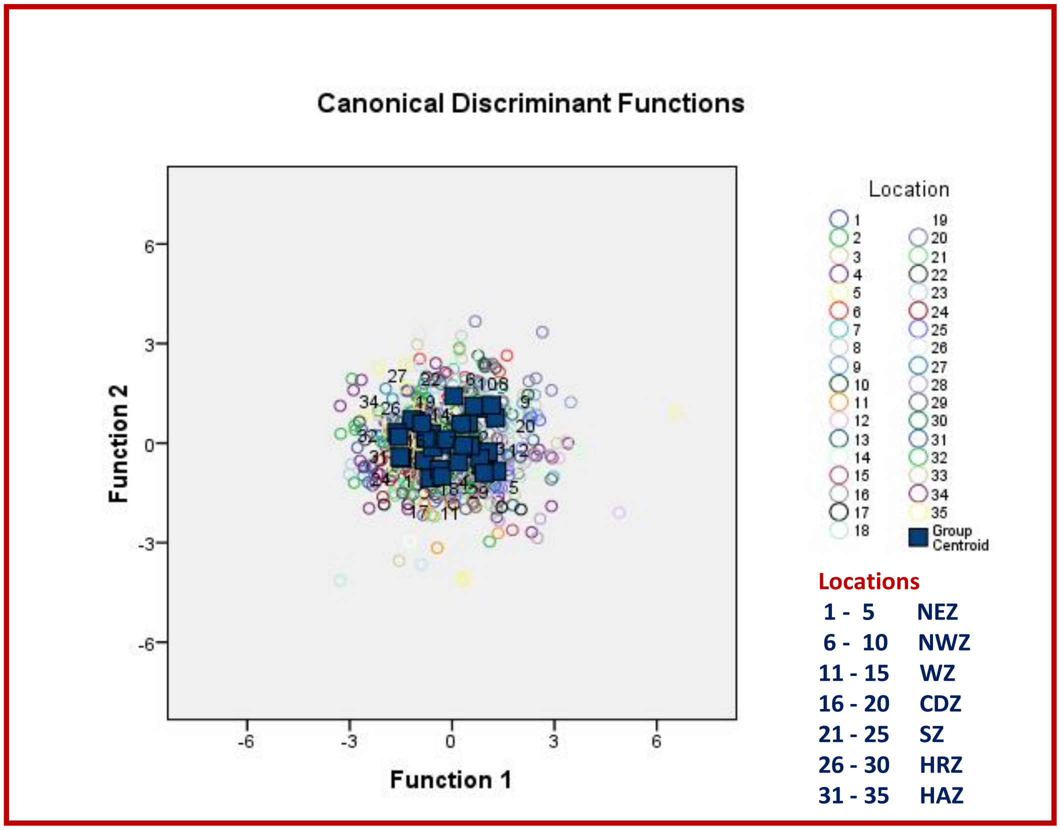


Fig. 6. Scatter plot of 35 *A. florea* colonies from seven agro-climatic zones based on DFA

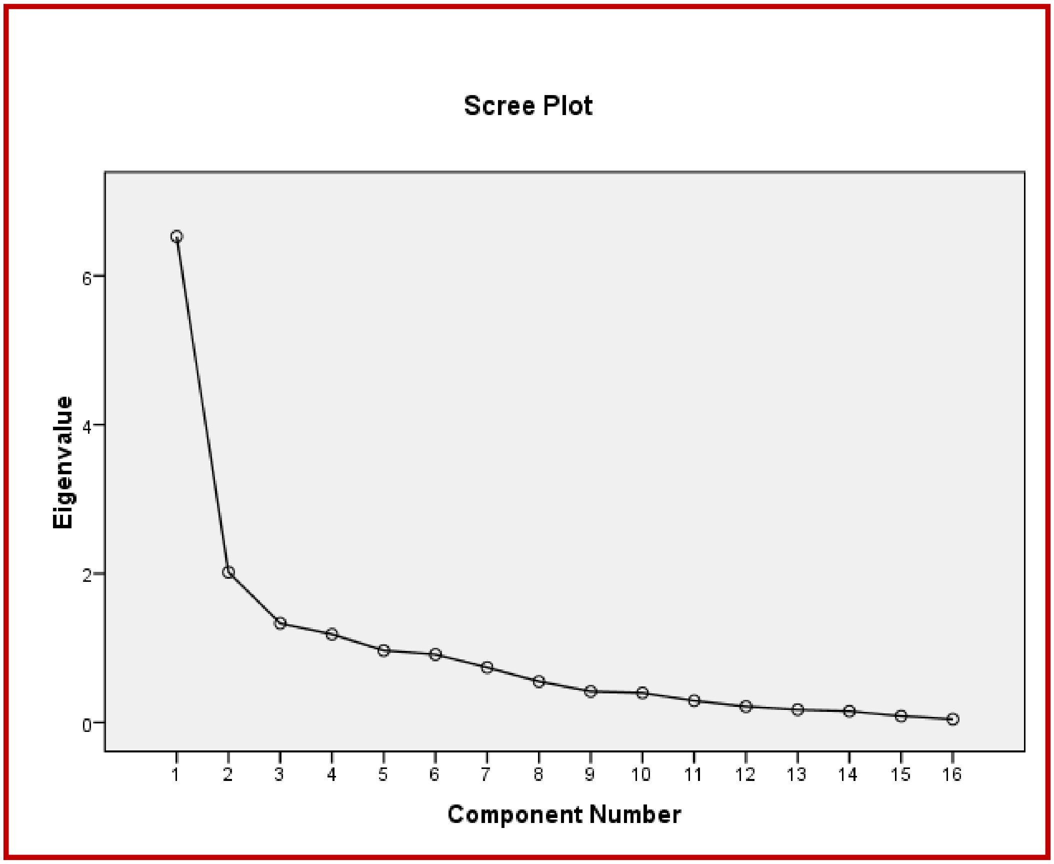


Fig. 7. Scree plot of *A. florea* colonies from North and South India based on PCA

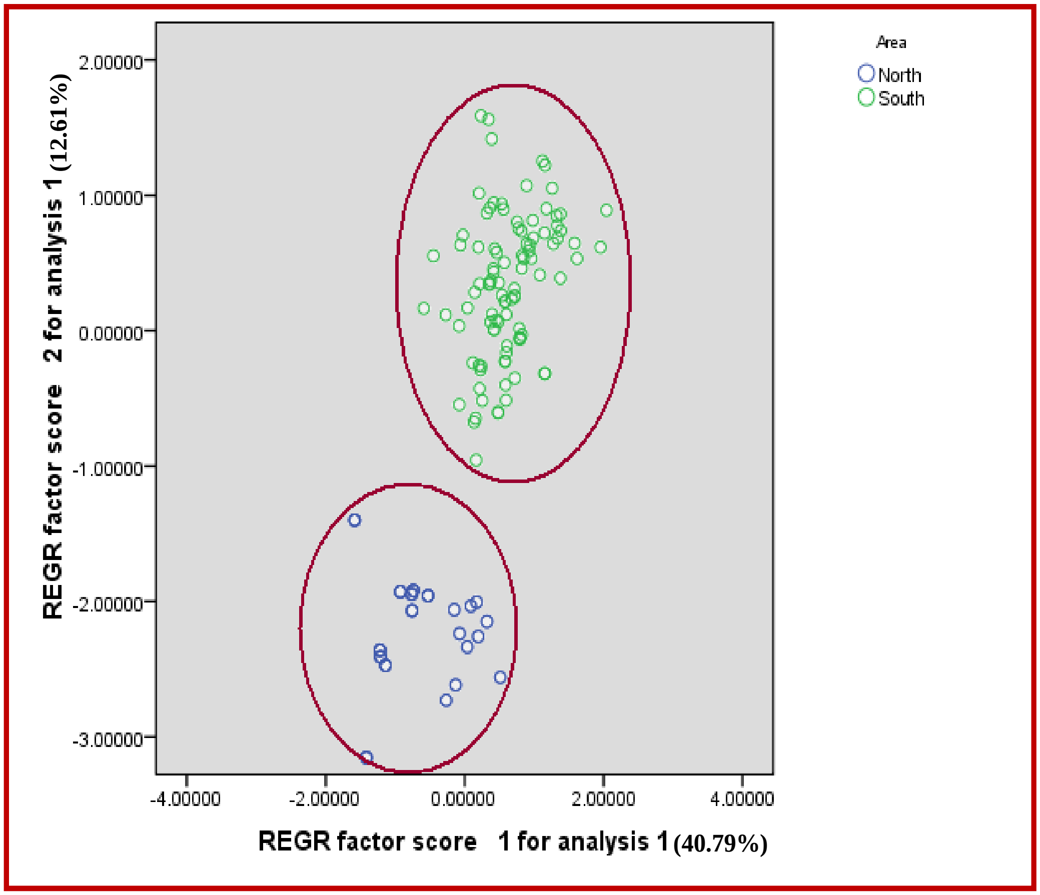


Fig. 8. Scatter plot of *A. florea* colonies from North and South India based on PCA

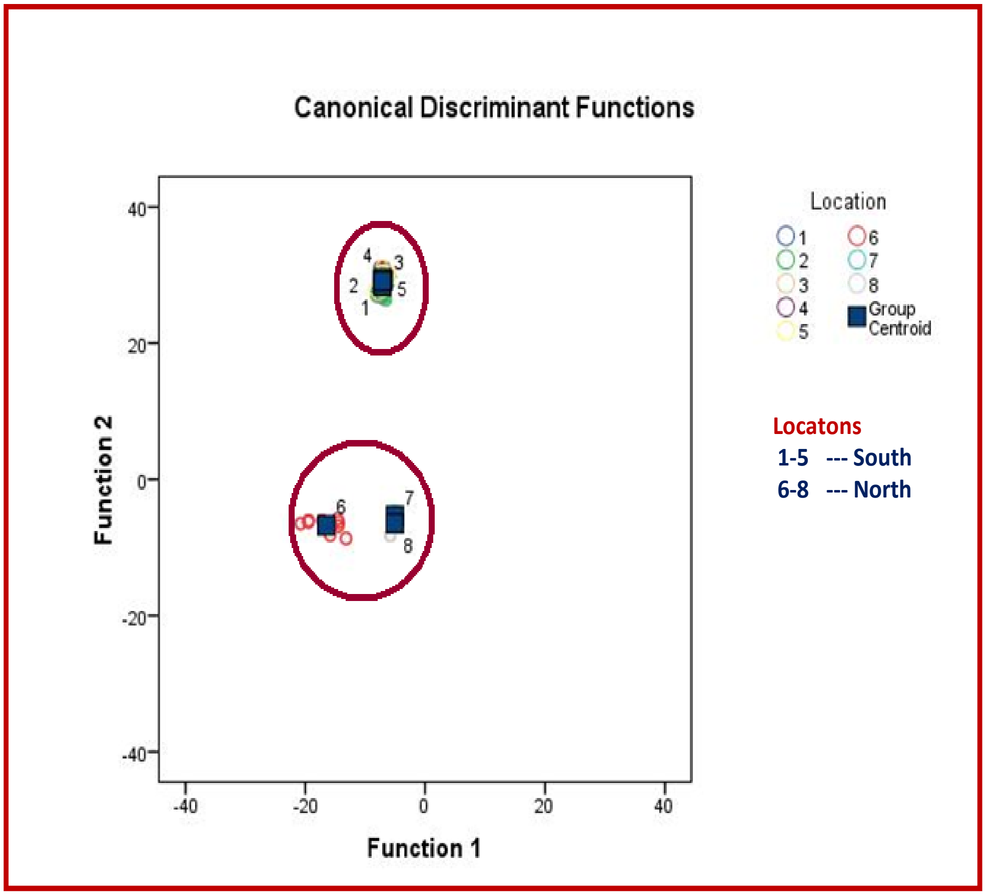


Fig. 9. Scatter plot of *A. florea* colonies from North and South India based on DFA

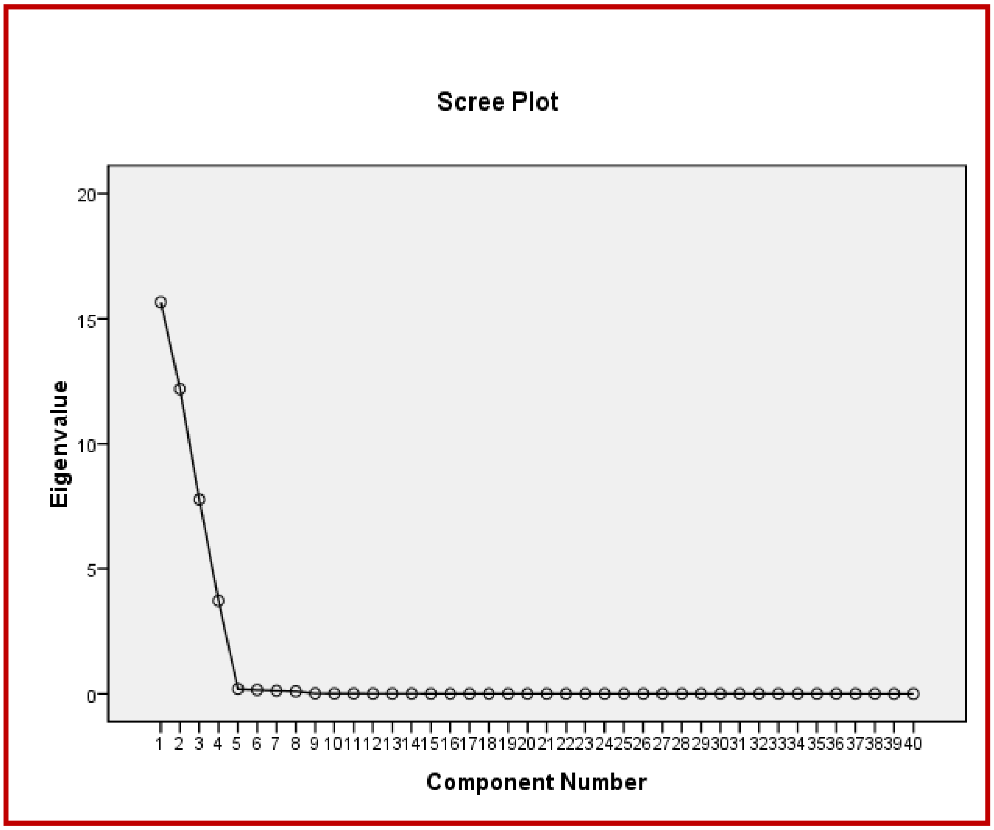


Fig. 10. Scree plot of fore wings of 35 *A. florea* colonies from seven agro-climatic zones based on PCA

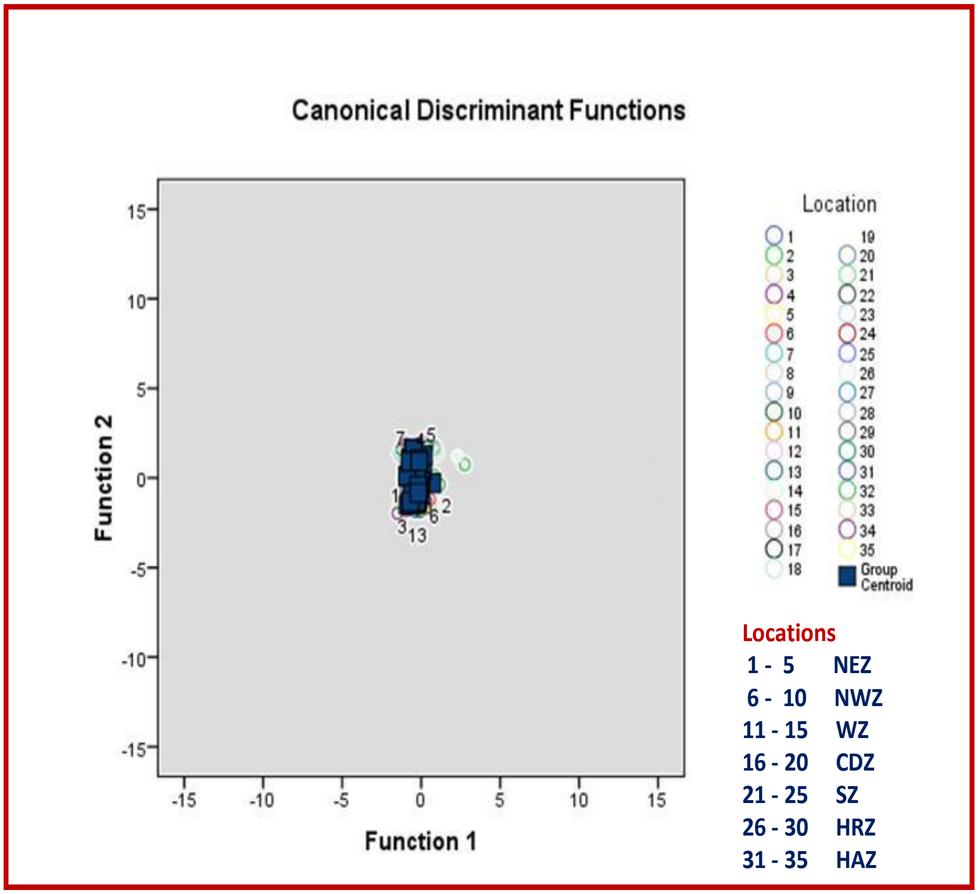


Fig. 11. Scatter plot of fore wings of 35 *A. florea* colonies from seven agro-climatic zones based on DFA

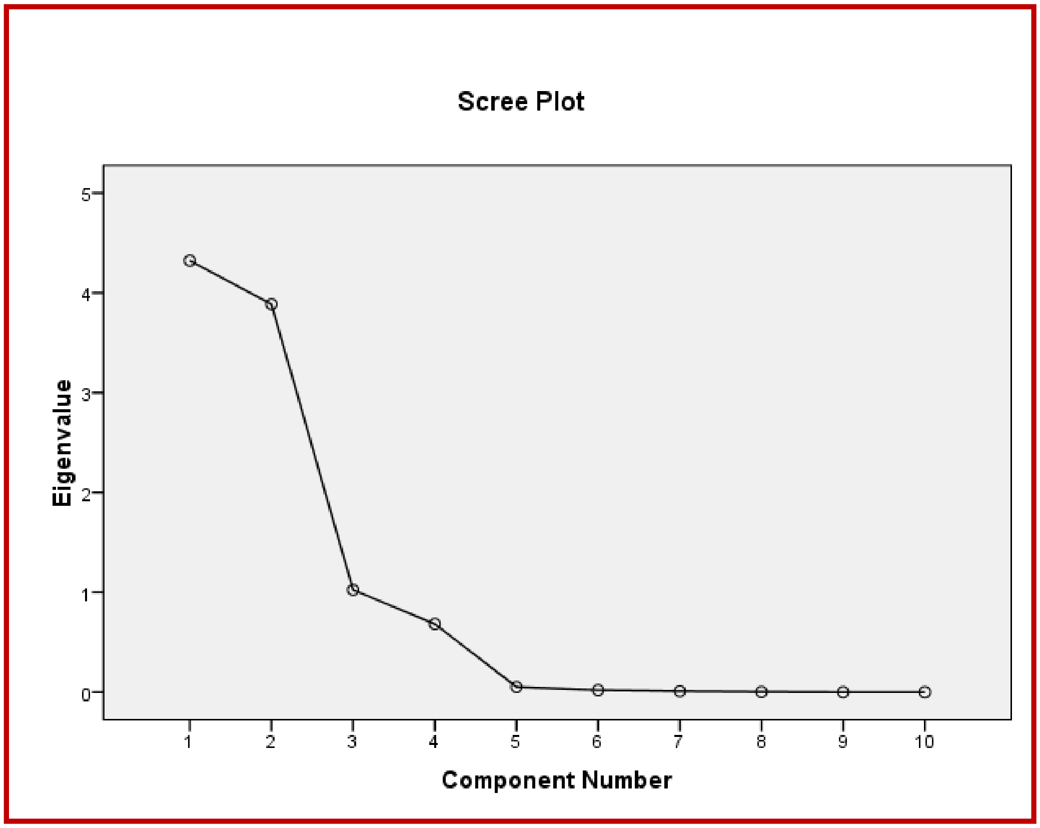


Fig. 12. Scree plot of hind wings of 35 *A. florea* colonies from seven agro-climatic zones based on PCA

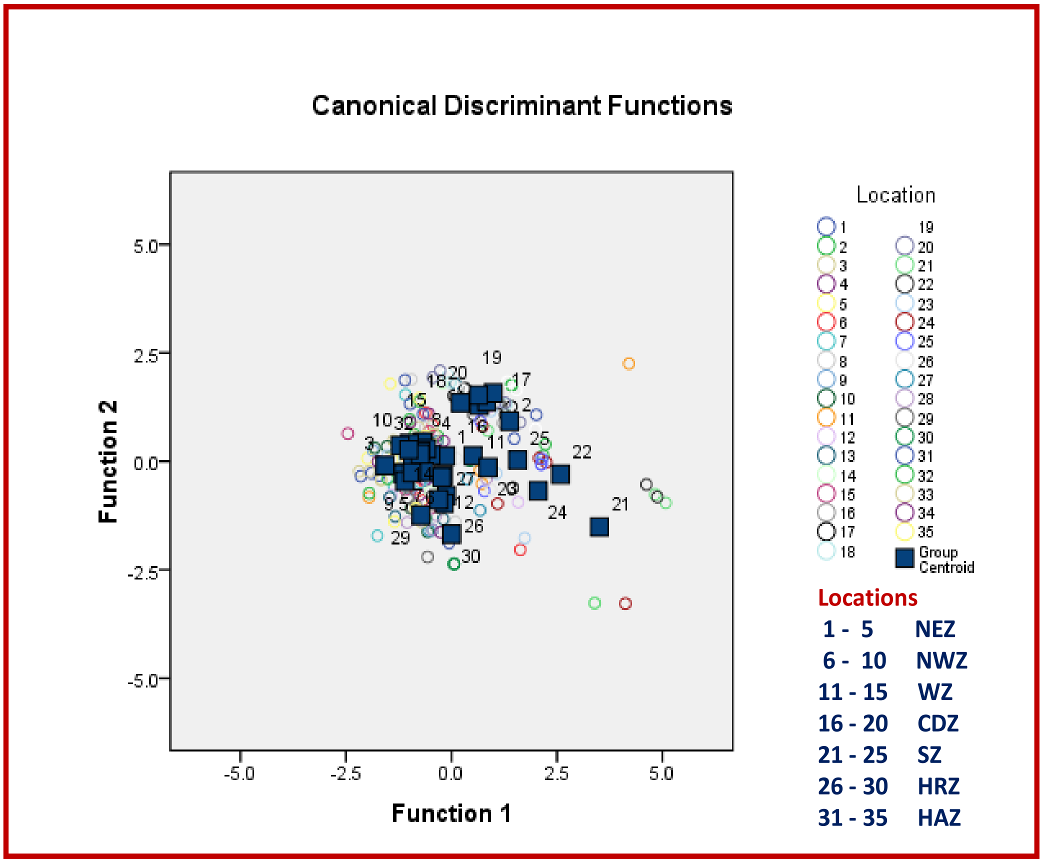


Fig. 13. Scatter plot of hind wings of 35 *A. florea* colonies from seven agro-climatic zones based on DFA

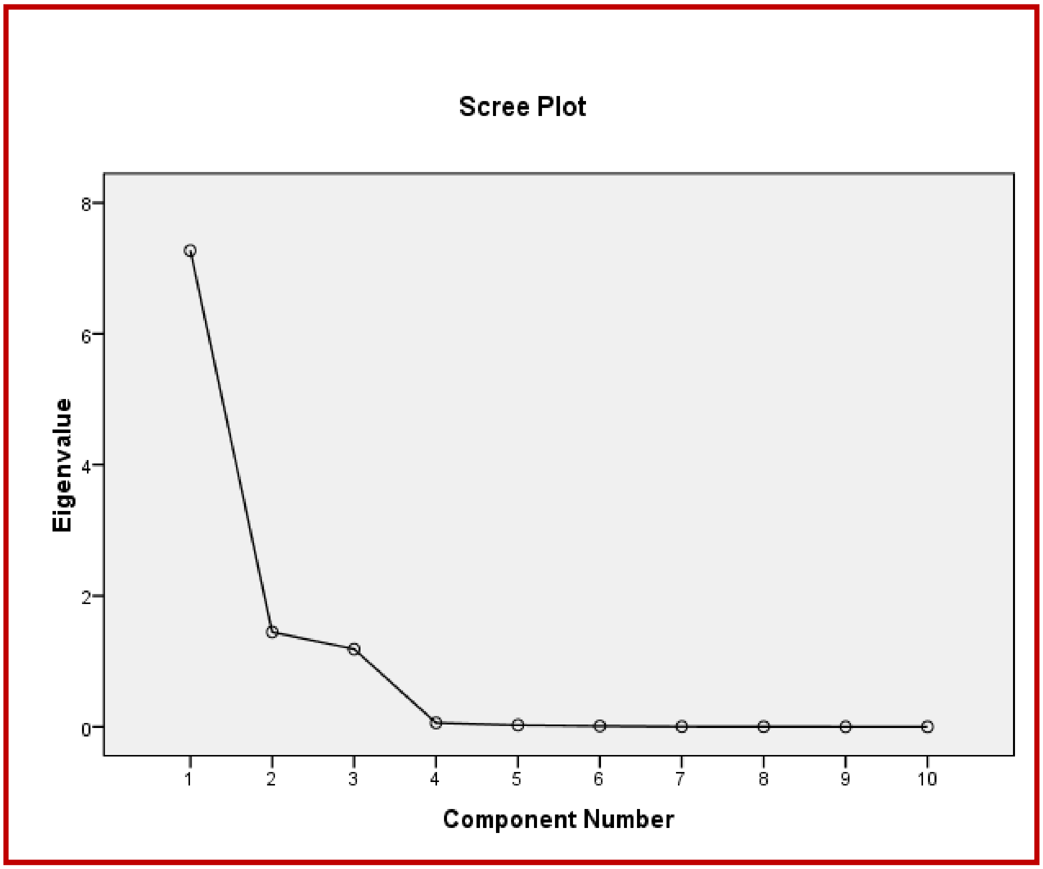


Fig. 14. Scree plot of hind wings of *A. florea* castes based on PCA

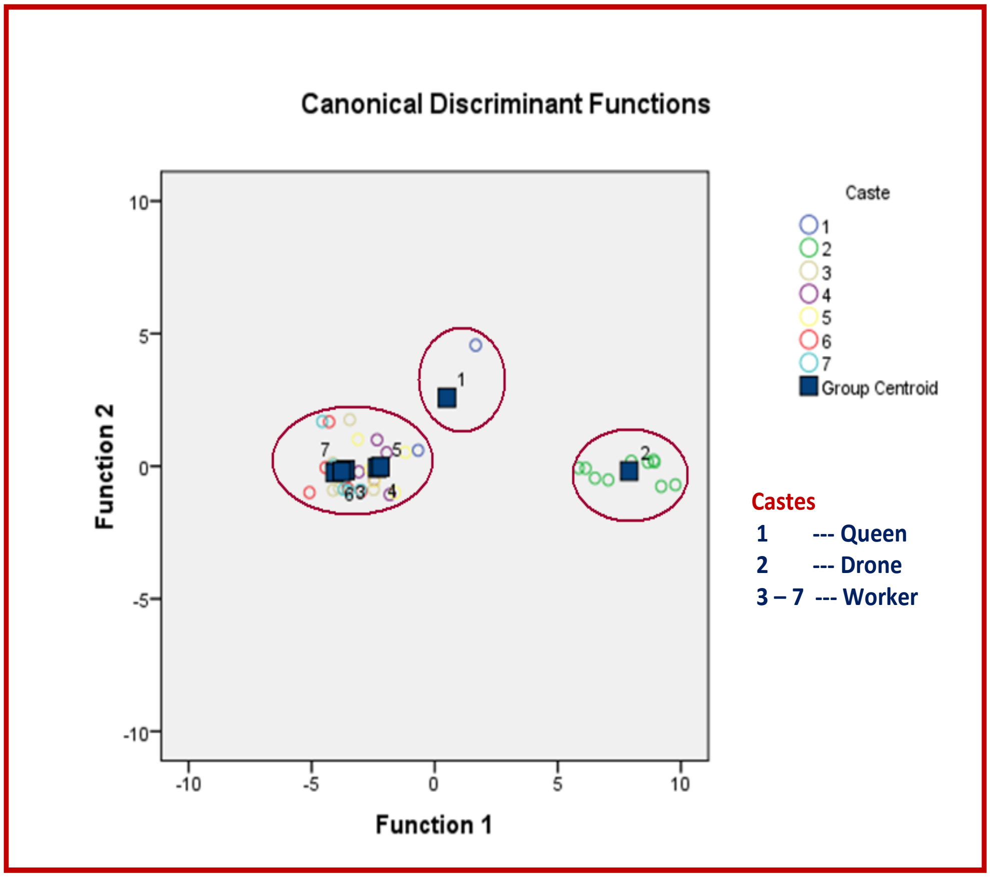


Fig. 15. Scatter plot of hind wings of *A. florea* castes based on DFA

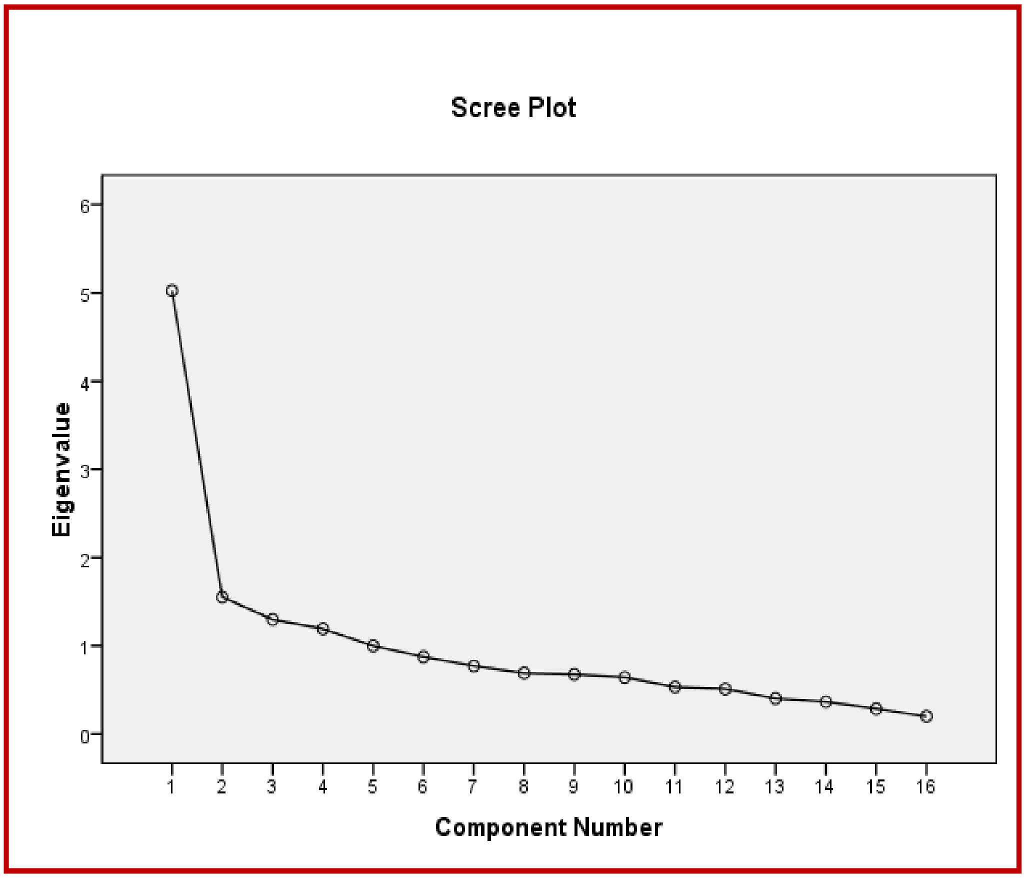


Fig. 16. Scree plot of two ecotypes of *A. florea* colonies based on PCA

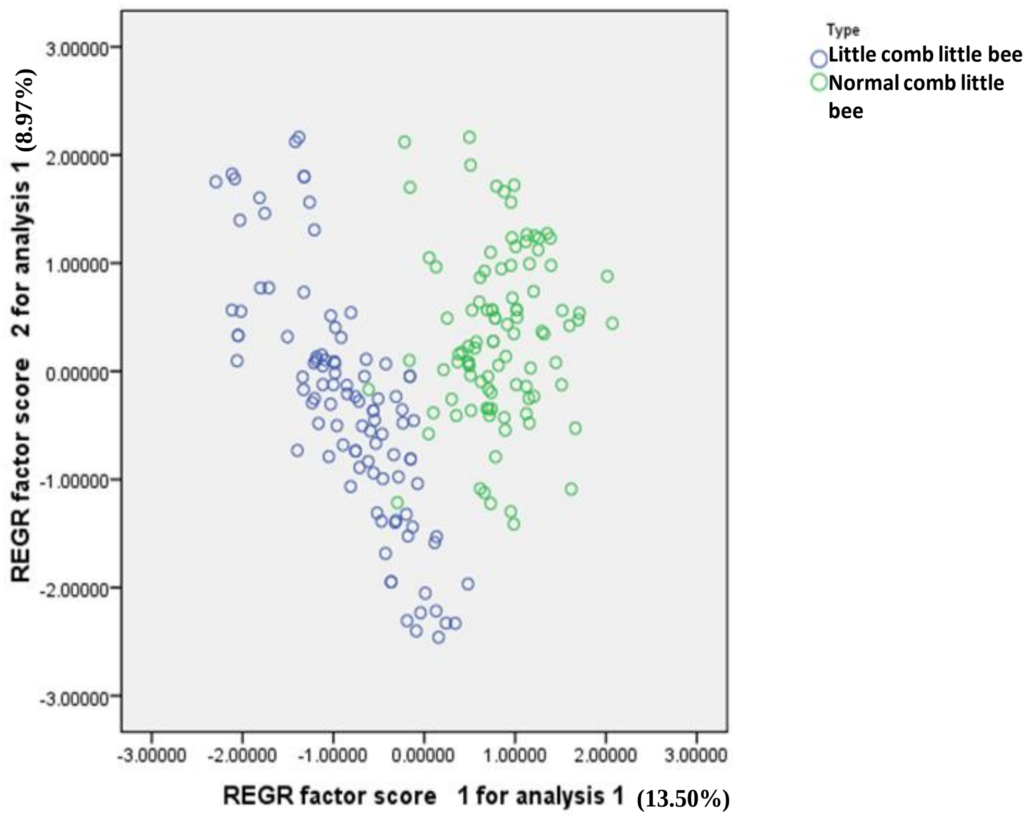


Fig. 17. Scatter plot of two ecotypes of *A. florea* colonies based on PCA

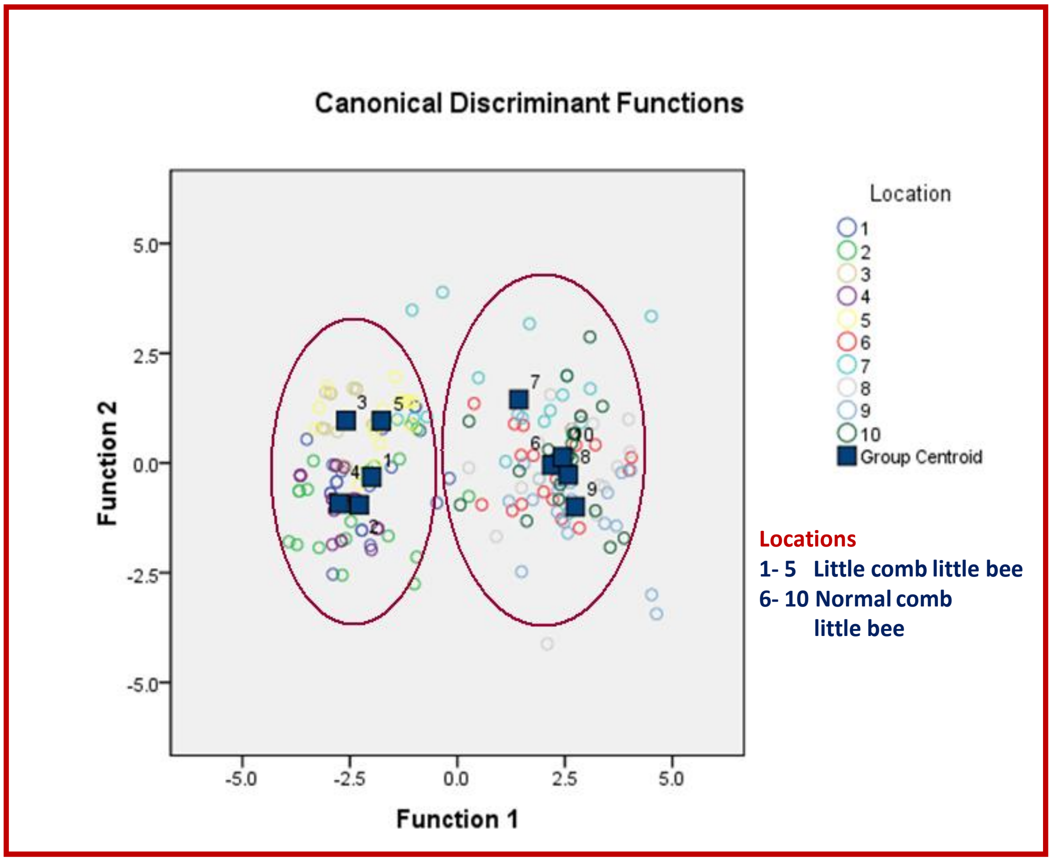


Fig. 18. Scatter plot of two ecotypes of *A. florea* colonies based on DFA

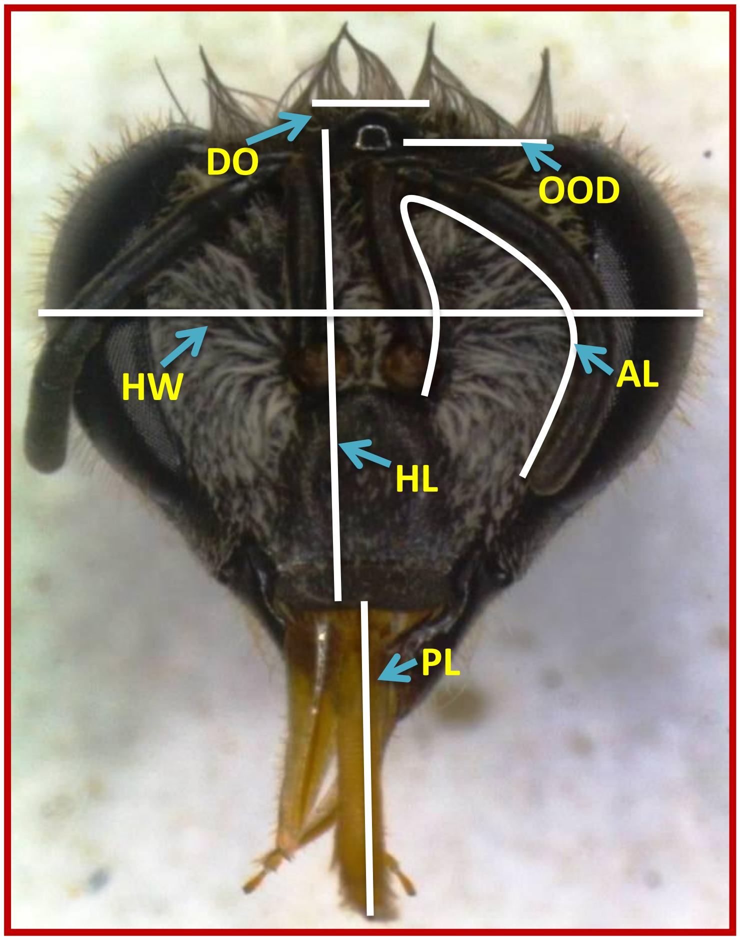


Plate 1. Morphometric characters – Head appendages

HL	-	**Head length**
HW	-	**Head width**
AL	-	**Antennal length**
PL	-	**Proboscis length**
DO	-	**Distance between ocelli**
OOD	-	**Ocello-ocular distance**

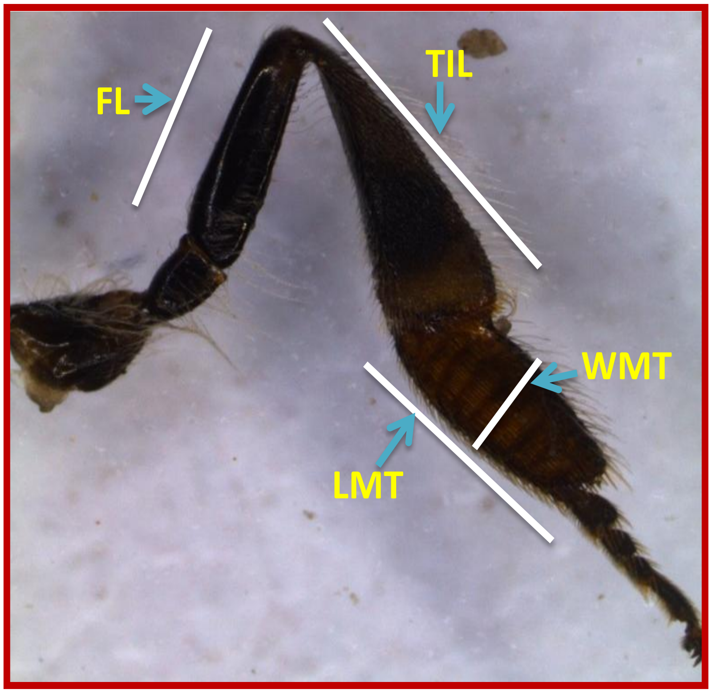


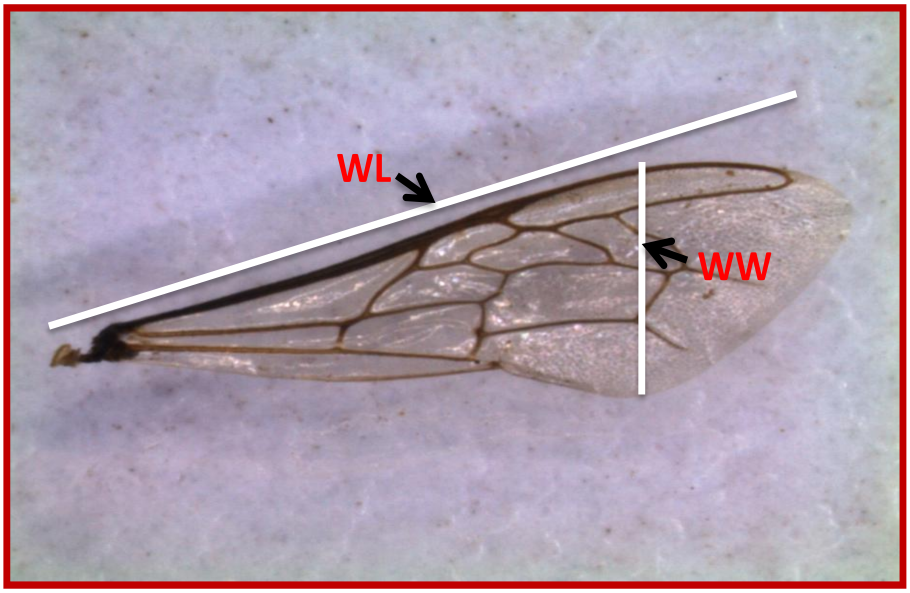
Plate 2. Morphometric characters – Hind leg

FL - Femur length

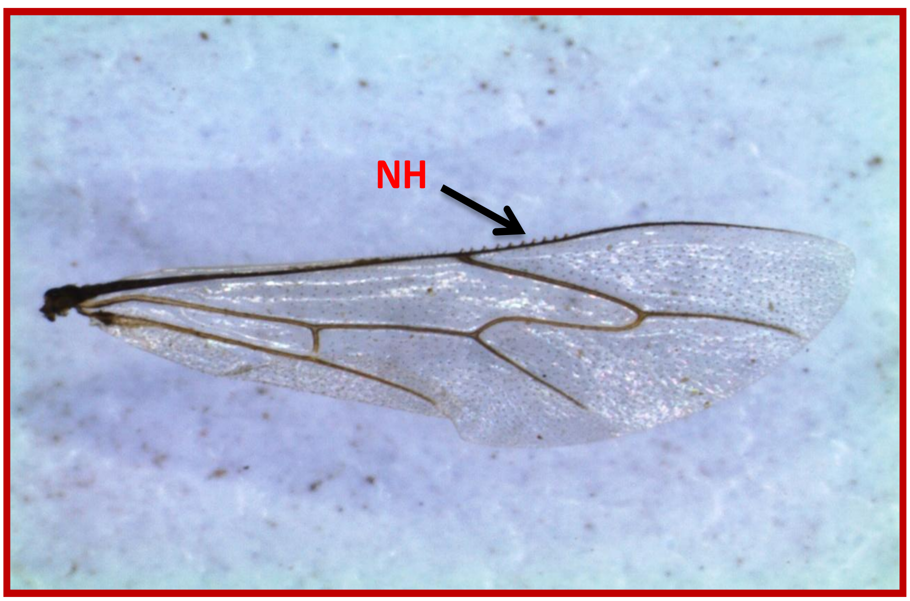
TIL - Tibial length

LMT - Length of metatarsus

WMT - Width of metatarsus



a. Fore wing



b. Hind wing

Plate 3. Morphometric characters – Wings

WL - Fore wing length

WW - Fore wing width

NH - Number of hamuli

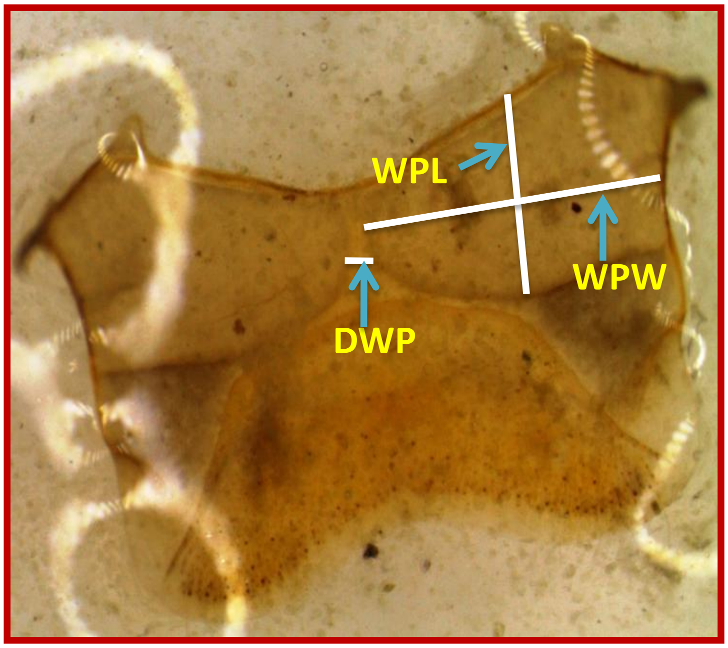


Plate 4. Morphometric characters – Wax plates

WPL - Wax plate length

WPW - Wax plate width

DWP - Distance between wax plates

a. Worker

b. Drone

r-m ---> Radio medial cross vein

c. Queen

Plate 5. Hind wing variations of *A. florea* castes

Plate 6. Nest of normal comb little bee

Plate 7. Nest of little comb little bee

Plate 8. Arboreal nesting – Comb parallel to twig

Plate 9. Arboreal nesting - Comb perpendicular to twig

Plate 10. Arboreal nest encircling leaf margin (Side view)

Plate 11. Arboreal nest encircling leaf margin (Front view)

Plate 12. Arboreal nest encircling inflorescence rachis of coconut

Plate 13. Arboreal nest encircling peduncle of banana

Plate 14. Nest of queenless colony

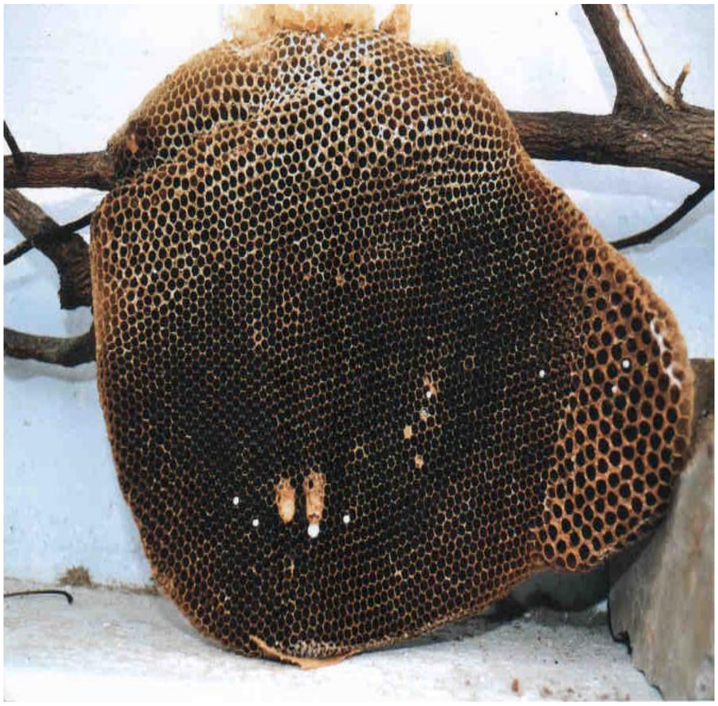


Plate 15. Unusually positioned drone comb

Plate 16. Normally positioned drone comb

Plate 17. Terrestrial nest laterally attached to wall

Plate 18. Terrestrial nest vertically attached to wall

Plate 19. Terrestrial nest on a wooden rafter

Plate 20. Terrestrial nest on a cot frame

Plate 21. Terrestrial nest on barbed wire fence

Plate 22. Terrestrial nest on chair frame

Plate 23. Terrestrial nest on a shelf corner

Plate 24. Terrestrial nest on a concrete beam