

MORPHOMETRY AND GENITALIA OF DRONES OF APIS HONEY BEE SPECIES FROM INDIA

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ABSTRACT

Morphometry of drones of *Apis cerana* (N - 158), *A. dorsata* (N - 35) and *A. florea* (N - 33) collected from seven states viz. Andhra Pradesh (AP), Assam (AS), Jammu & Kashmir (JK), Karnataka (KA), Kerala (KL), Maharashtra (MH) and Tamil Nadu (TN), was studied for 19 morphological and one genitalial parameters. The data were subjected to factor, principal component and canonical discriminant analysis. *Apis cerana* drones from Assam were the largest with body length of 11.47 mm while drones from Tamil Nadu were the smallest with body length of 9.61 mm. However, *A. dorsata* drones from Kerala measured 15.95 mm in length as against the drones from Assam which measured only 12.45 mm. Drones of *A. florea* from Karnataka, Assam and Andhra Pradesh were larger with body length ranging from 11.04 to 11.36 mm while the drones from other states varied from 10.43 to 10.77 mm. Principal component and canonical discriminant analysis of morphometric data of *A. cerana* drones from seven states resulted in formation four clusters (drones from JK + AS; AS alone; JK + AS + KA; and AP + KA + KL + MH + TN). *A. dorsata* drones also formed four overlapping clusters (AS + KA + KL + MH; AS + KA + KL + MH + TN; KA alone and KA + MH) while *A. florea* drones formed 5 clusters in factor analysis (drones from AS alone; KA + TN; AP + KA + MH + TN; AP + KA + TN and KL + TN) and 3 clusters in canonical discriminant analysis (Drones from AS alone; AP + KA + KL and MH + TN). Genitalia of *A. cerana*, *A. dorsata* and *A. florea* from India have been described and illustrated for the first time. It is concluded that the drones of *A. cerana* and *A. dorsata* from seven states formed overlapping clusters indicating extensive migration and interbreeding among the populations while *A. florea* drones of Assam are distinct from the southern states. The genitalia of drone of each species of honey bee are distinct but the genitalial structures within the species varied.

INTRODUCTION

India is blessed with three native species of honey bees viz. *Apis dorsata* F., *A. florea* F. and *A. cerana* F. which occur throughout the country. The geographical and climatic conditions of India greatly vary. Similarly there is also great variation in the size, colour, behavior, biological traits among these bees. Several studies have been made to document the morphological variation among the worker bees of these species by using many morphometric parameters in India as well as in other countries which is reviewed by Mishra and Sihag (1987), Ruttner (1988), Abrol (2009) and Radloff *et al.*, (2011).

Determination of the bees at the species level has been made by the study of male genitalial structures (Ruttner, 1988). However, descriptions and variations within the species are based mainly on the morphological characters of worker bees. Ruttner (1983, 1988) studied morphometry of drones of *A. mellifera* and *A. cerana* to identify the subspecies. In his studies of *A. cerana* drones only six samples were included from Afghanistan, Pakistan, north China and south India. Genitalia of *Apis florea* is studied by Bahrmann (1961), Ruttner (1988), that of *A. dorsata* by Bahrmann (1961), Simpson (1970), Ruttner (1988), Koeniger and Koeniger (1990) and *A. cerana* by Ruttner (1988). Koeniger *et al.* (1991) has given a detailed account of

comparative anatomy of genitalia of *Apis* species of Malaysia.

Though study of male genitalia is an important tool in determination of species and subspecies in honey bees, no such studies have been made to describe the genitalia and variations in the drones of honey bee species occurring in India. Hence, studies were made on detailed morphological variations in the drones of *A. cerana*, *A. dorsata* and *A. florea* occurring in seven states of India and how the drones of each species across these states are related, for the first time in India and the results are presented in this paper along with descriptions and illustrations of genitalia of each species of honey bee.

MATERIALS AND METHODS

Samples of drones of *A. cerana* were collected from the colonies in different locations of seven states (Andhra Pradesh, Assam, Jammu and Kashmir, Karnataka, Kerala, Maharashtra and Tamil Nadu) while that of *A. dorsata* (Assam, Karnataka, Kerala, Maharashtra and Tamil Nadu) and *A. florea* (Andhra Pradesh, Assam, Karnataka, Kerala, Maharashtra and Tamil Nadu) in five and six states, respectively. Ten drones of each species from each location were preserved in 75 per cent ethyl alcohol vials separately. Remaining drones were killed immediately in potassium cyanide poison tubes and later pinned, labeled

and preserved dry in insect boxes. The drones preserved in alcohol from each locality were studied for morphometry. To study the morphometry of the drones, the methodology given by Alpatov (1929), Goetze (1964) and Ruttner (1988) was followed. Nineteen standard morphometric parameters (modified from Ruttner, 1988) like body length (BL), length of vertex (VL), width of head including eyes (HW), ratio between length of vertex and head width (HLW), distance between two dorsal ocelli (DBO), distance between lateral ocellus and eye (DOOD), length of hind femur (HFL), length of hind tibia (HTL), length of metatarsus (ML), width of metatarsus (MW), ratio of length and width of metatarsus (MLW), length of forewing (FWL), width of forewing (FWW), ratio of length and width of forewing (FWLW), cubital index (CI), number of hamuli (Ham), length of fourth abdominal tergum (4thTL), width of fourth abdominal tergum (4thTW) and ratio of length and width of 4th abdominal tergum (4thTLW) were measured. However, in the drones of *A. florea* additional parameters like length of thumb like process from the hind basitarsus (TbPL), width of the process (TbPW) and ratio of length and width of the process (TbPLW) were measured. All these parameters were recorded under the Leica stereoscopic microscope (Model M 165 C) fitted with a digital camera and Leica measurement software.

Representative samples of dry preserved drones were used for studying the genital structure. For dissection and study of genitalia the method described by Koeniger *et al.* (1991) was followed with a few modifications. From each pinned drone, the abdomen was separated by gently pressing between the thorax and abdomen with a fine forceps. Care was taken to keep the remaining part of the body intact. The separated abdomen was transferred to a corning test tube containing 10 per cent potassium hydroxide solution. The solution was gently boiled for 60 seconds over a spirit lamp. Later, the abdomen was taken out from potassium hydroxide solution

and washed in distilled water and then transferred to a cavity block containing distilled water. Male genitalia was dissected under the Leica stereoscopic microscope and then photographed. The number of folds was recorded on the cervix of *A. cerana* (CF) while in *A. dorsata* the number of hairy patches on cervix (HP) was recorded. These parameters were included in the analysis of morphometric parameters.

Analysis of morphometry was done as per the methodology given by Ruttner (1988). All the morphometry data of the respective species was subjected to factorial analysis separately to study the influence of different morphometric parameters on the variation followed by Principal Component and Discriminant analysis to find out the related groups.

RESULTS AND DISCUSSION

Apis cerana

Variation in 19 standard morphometric parameters and one genital character among the 158 drones from different states is presented in Table 1.

Length of body was maximum (11.47 mm) in the drones from Assam followed by Maharashtra (10.82 mm) and Jammu & Kashmir (10.68 mm). Drones from Tamil Nadu were the smallest with body length of 9.61 mm.

The length and width of vertex was more in the drones from Assam (0.85 and 3.86 mm respectively) and Jammu & Kashmir (0.87 and 4.06 mm). Drones from Maharashtra had next higher length and width of vertex while the drones from other states were more or less equal size of vertex and head. The ratio of length and width of vertex and head followed the same pattern.

The distance between the two lateral ocelli was more in the drones of Assam, Jammu and Kashmir and Maharashtra (0.30 to 0.33 mm) while in other drones it varied from 0.22 to 0.25

Table 1: Morphometry of drones of *Apis cerana* from different states

State	Andhra Pradesh		Assam		Jammu & Kashmir		Karnataka		Kerala		Maharashtra		Tamil Nadu	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BL	10.31	0.35	11.47	0.52	10.68	0.29	10.42	0.18	10.31	0.34	10.82	0.29	9.61	0.36
VL	0.61	0.06	0.85	0.04	0.87	0.08	0.66	0.02	0.60	0.02	0.80	0.02	0.54	0.04
HW	3.31	0.16	3.86	0.07	4.06	0.28	3.43	0.03	3.36	0.04	3.68	0.04	3.18	0.00
HLW	0.18	0.01	0.22	0.01	0.21	0.01	0.19	0.01	0.18	0.01	0.22	0.00	0.17	0.01
DBO	0.25	0.04	0.30	0.03	0.33	0.01	0.25	0.01	0.22	0.01	0.31	0.00	0.22	0.05
DOOD	0.08	0.01	0.09	0.01	0.05	0.00	0.07	0.01	0.06	0.00	0.10	0.00	0.08	0.01
HFL	2.13	0.11	2.48	0.17	2.63	0.23	2.22	0.14	2.20	0.06	2.16	0.02	2.13	0.08
HTL	2.82	0.09	3.24	0.13	3.38	0.34	2.90	0.08	2.97	0.07	2.98	0.27	2.80	0.21
ML	1.78	0.07	2.05	0.11	2.24	0.20	1.86	0.00	1.88	0.04	1.94	0.08	1.77	0.20
MW	0.95	0.05	1.10	0.08	1.06	0.04	0.99	0.11	1.01	0.06	0.97	0.04	0.99	0.10
MLW	1.88	0.13	1.88	0.20	2.12	0.10	1.90	0.20	1.88	0.07	2.02	0.01	1.79	0.02
FWL	9.25	0.40	9.99	0.51	10.40	0.87	9.34	0.28	9.38	0.04	9.54	0.36	9.12	0.36
FWW	3.00	0.16	3.22	0.19	3.54	0.19	3.10	0.00	3.10	0.08	2.99	0.08	3.00	0.05
FWLW	3.10	0.22	3.11	0.19	2.94	0.09	3.02	0.09	3.03	0.10	3.20	0.03	3.05	0.17
CI	2.53	0.54	2.79	0.43	3.12	0.30	2.71	0.44	2.34	0.04	2.91	0.15	2.09	0.33
HAM	18.30	1.06	19.38	1.74	17.73	0.71	18.86	0.00	20.00	0.71	17.00	0.71	18.69	1.41
4 th TL	1.82	0.10	2.02	0.13	2.04	0.14	1.84	0.13	1.88	0.01	1.78	0.18	1.76	0.11
4 th TW	8.19	0.54	9.48	0.49	9.28	0.10	8.46	0.45	8.74	0.09	8.60	0.79	8.19	0.28
4 th TLW	0.22	0.01	0.21	0.02	0.22	0.01	0.22	0.00	0.22	0.00	0.21	0.00	0.21	0.02
CF	5.00	0.00	6.00	0.59	6.18	0.71	5.97	0.00	5.33	0.00	5.63	0.00	5.17	0.00

BL- Body length; VL- Vertex length; HW- Head width including eyes; HLW- Ratio of vertex length and width; DBO- Distance between dorsal ocelli; DOOD- Distance between right lateral ocellus and eye; HFL- Hind femur length; HTL- Hind tibial length; ML- Metatarsus length; MW- Metatarsus width; MLW- Ratio between metatarsus length and width; FWL- Forewing length; FWW- Forewing width; FWLW- Ratio between forewing length and width; CI- Cubital index; HAM- Number of hamuli; 4th TL- Fourth tergum length; 4th TW- Fourth tergum width; 4thTLW- Ratio of fourth tergum length and width; CF- Number of chitinous folds on cervix

mm. However, there was no definite pattern in the distance between lateral ocellus and the eye.

Drones from Assam and Jammu & Kashmir had longer hind femur as well as hind tibia and metatarsus compared to the drones from other states. However, the ratio between length and width of metatarsus varied from 1.79 (Tamil Nadu) to 2.12 (Jammu and Kashmir). Similarly Ruttner (1988) reported the length of hind leg of the drones from south India was 7.56 mm as against 8.34 mm in the drones from northern countries (Afghanistan, Pakistan and North China).

Longest and widest forewing was recorded in the drones from Jammu & Kashmir (10.4 and 3.54 mm, respectively) followed by the drones from Assam (9.99 and 3.22 mm). But the ratio of length and width of forewing varied from 2.94 to 3.22.

Highest cubital index of 3.12 was recorded in the drones from Jammu & Kashmir followed by Maharashtra (2.91) Assam (2.79) and Karnataka (2.71). In other drones, it varied from 2.09 to 2.53.

The number of hamuli was more in the drones from Kerala (20.00) followed by Assam (19.38) while in other drones hamuli varied from 17.00 to 18.86.

Though the length and width of fourth tergum was more in the drones from Assam (2.02 and 9.48 mm, respectively) and Jammu & Kashmir (2.04 and 9.28 mm), the ratio of length and width of the tergum remained more or less equal among all the drones (0.21 to 0.22). Ruttner (1988) studied six samples

of drones from Afghanistan, Pakistan, south India and north China and found that drones from north were somewhat larger than those from south.

For factorial analysis, 11 morphometric and 1 genitalia parameter were considered and the overall means and standard deviation among 158 drones across seven states is presented in Table 2.

Results of factor analysis and the colony groupings on a scatter plot are presented in Fig.1. Clustering of *A. cerana* drones samples from 158 mean values showed formation of the following four clusters.

Cluster 1, Drones from Jammu & Kashmir and Assam.

Cluster 2. Drones only from Assam.

Cluster 3. Drones from Jammu & Kashmir, Assam and Karnataka

Cluster 4. Drones from Andhra Pradesh, Karnataka, Kerala, Maharashtra and Tamil Nadu.

The Principal Component analysis gave four Eigen values greater than one, which explained 66.30 per cent of the variations among the state groups. Similarly the results of multivariate statistical analysis of 12 characters also yielded four factors with Eigen values greater than one that accounted for 66.30 per cent of the total variations in data.

In the Principal Component 1, head width, body length and distance between ocelli with component loading between 0.81 to 0.941 (Table 4) accounted for 34.73 per cent of variations (Table 3).

In the Principal Component 2, distance between lateral ocellus and eye, length of hind femur and hind tibia with component loading between 0.068 and 0.795 (Table 4) accounted for 12.50 per cent of variations (Table 3).

In the Principal Component 3, ratio of length of metatarsus and forewing and cubital index with component loading from 0.086 to 0.580 accounted for 10.02 per cent of variations.

In the fourth component, number of hamuli, ratio of length and width of fourth tergum and number of folds on cervix of the genitalia with component loading between 0.112 and 0.932 accounted for 9.02 of variation.

In stepwise discriminant analysis, six characters viz. width of head, distance between lateral ocellus and eye, length of body, number of folds on cervix, distance between two lateral ocelli and length of femur entered into the discriminatory functions

Table 2: Mean and standard deviation of twelve morphometric characters in *Apis cerana* drone populations from different states in India

	Mean	Std. Deviation	Analysis N
HW	3.48	0.31	158
BL	10.42	0.71	158
DBO	0.25	0.05	158
DOOD	0.07	0.01	158
HFL	2.25	0.21	158
HTL	2.96	0.25	158
MLW	1.89	0.19	158
FWLW	3.04	0.19	158
CI	2.59	0.64	158
HAM	18.73	1.67	158
CF	5.71	0.70	158
4 th TLW	0.21	0.01	158

Table 3: Total variation among the morphometric parameters in *Apis cerana* drones

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.16	34.73	34.73	4.16	34.73	34.73	4.11	34.25	34.25
2	1.50	12.51	47.25	1.50	12.51	47.25	1.40	11.73	45.98
3	1.20	10.02	57.27	1.20	10.02	57.27	1.32	11.07	57.05
4	1.08	9.02	66.30	1.08	9.02	66.30	1.11	9.24	66.30
5	0.95	7.95	74.25						
6	0.74	6.16	80.42						
7	0.69	5.78	86.20						
8	0.62	5.21	91.41						
9	0.39	3.32	94.74						
10	0.28	2.39	97.14						
11	0.25	2.08	99.22						
12	0.09	0.78	100.00						

(Table 5). Scatter plot of canonical discriminant function analysis presented in Fig. 2 also showed the following four clusters.

Cluster 1. Drones of Assam

Table 4: Rotated component matrix^a

	Component			
	1	2	3	4
HW	0.941	0.069	0.096	-0.121
BL	0.787	0.147	0.100	0.103
DBO	0.810	0.233	0.062	-0.207
DOOD	0.138	0.795	-0.266	0.132
HFL	0.816	-0.209	0.066	0.088
HTL	0.828	-0.068	-0.044	-0.019
MLW	0.325	-0.018	0.580	0.024
FWLW	-0.079	0.735	0.229	-0.200
CI	0.600	-0.139	0.086	0.177
HAM	0.066	-0.046	0.091	0.932
PLATES	0.282	-0.289	0.366	-0.252
4 th TLW	-0.151	0.036	0.830	0.112

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization; a. Rotation converged in 5 iterations.

Table 5: Canonical discriminant functions of drones of *Apis cerana* from seven states of India

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
HW	3.907 ^a	58.0	58.0	0.892
DOOD	1.910 ^a	28.4	86.4	0.810
BL	0.427 ^a	6.3	92.8	0.547
CF	0.349 ^a	5.2	98.0	0.509
DBO	0.113 ^a	1.7	99.6	0.319
HFL	0.025 ^a	0.4	100.0	0.155

a. First 6 canonical discriminant functions were used in the analysis.

Table 6: Morphometry of drones of *Apis dorsata* from different states

State	Assam		Karnataka		Kerala		Maharashtra		Tamil Nadu	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BL	12.45	0.00	14.57	1.24	15.95	0.55	14.02	0.55	13.09	0.00
VL	1.17	0.02	1.12	0.01	1.11	0.01	1.09	0.11	1.08	0.02
HW	4.68	0.18	4.62	0.04	4.65	0.01	4.52	0.08	4.58	0.01
HLW	0.25	0.01	0.24	0.00	0.24	0.00	0.24	0.02	0.23	0.01
DBO	0.26	0.02	0.23	0.01	0.25	0.05	0.25	0.01	0.24	0.00
DOOD	0.10	0.01	0.07	0.01	0.08	0.01	0.10	0.01	0.09	0.02
HFL	2.77	0.44	2.93	0.07	2.97	0.04	2.84	0.01	2.75	0.35
HTL	4.58	0.28	4.57	0.49	4.76	0.16	3.63	0.43	4.40	0.11
ML	2.71	0.14	2.80	0.54	2.73	0.01	2.43	0.10	2.77	0.10
MW	1.44	0.06	1.38	0.01	1.34	0.01	1.14	0.18	1.43	0.00
MLW	1.89	0.02	2.03	0.39	2.05	0.01	2.16	0.26	1.95	0.07
FWL	13.73	0.49	13.54	0.11	13.67	0.52	13.21	0.45	13.20	0.30
FWW	4.33	0.24	4.36	0.16	4.37	0.11	4.25	0.07	4.16	0.15
FWLW	3.17	0.30	3.11	0.15	3.14	0.18	3.11	0.16	3.18	0.19
HAM	25.67	2.83	23.29	0.00	24.29	2.83	21.00	2.12	22.25	0.71
4 th TL	2.52	0.00	2.44	0.09	2.45	0.03	2.26	0.05	2.34	0.04
4 th TW	10.25	0.00	9.86	0.18	10.20	0.04	9.38	0.00	9.75	0.47
4 th TLW	0.25	0.00	0.25	0.00	0.24	0.00	0.24	0.01	0.24	0.01
CI	4.49	0.50	5.57	0.54	5.76	0.54	7.94	1.21	6.81	1.96
HP	8.33	0.71	8.88	0.00	8.57	0.71	8.50	0.00	7.50	0.71

bl- Body length, VL-Vertex length, HW- Head width including eyes, HLW- Ratio of vertex length and head width, DBO- Distance between ocelli, DOOD- Distance between ocellus and eye, HFL- Hind femur length, HTL- Hind tibial length, ML- Metatarsus length, MW- Metatarsus width, MLW- Ratio of length and width of metatarsus, FWL- Forewing length, FWW- Forewing width, FWLW- Ratio of forewing length and width, CI- Cubital index, HAM- Number of hamuli, 4th TL- Length of 4th tergum, 4th TW- 4th tergum width, 4th TLW- Ratio of 4th tergum length and width, HP- Number of chitinous plates on cervix

Cluster 2. Drones from Jammu & Kashmir.

Cluster 3. Drones from Karnataka, Jammu & Kashmir and Assam.

Cluster 4. Drones from Andhra Pradesh, Karnataka, Kerala, Maharashtra and Tamil Nadu.

Apis dorsata

The same 19 morphometric parameters as considered for *A. cerana* and one genitalial character were observed in 35 drones of *A. dorsata* from five different states and the data is presented in Table 6.

Body length varied among the five states with minimum in the drones from Assam (12.45 mm) and maximum in the drones from Kerala (15.95 mm).

The length of vertex and width of head varied from 1.08 to 1.17 mm and 4.52 to 4.68 mm but the ratio between length of vertex and width of head remained more or less uniform varying from 0.23 to 0.25.

Similarly the distance between two lateral ocelli and lateral ocellus to eye did not vary much across the states (0.23 to 0.26 mm and 0.07 to 0.10 mm, respectively).

Length of hind femur and hind tibia was maximum in the drones from Kerala (2.97 and 4.76 mm) closely followed by drones from Karnataka (2.93 and 4.57 mm). However, length and width of metatarsus was more in the drones from Karnataka, Tamil Nadu, Kerala and Assam (2.71 to 2.80 mm and 1.34 to 1.44 mm, respectively). However, the ratio of length and width of metatarsus was least in the drones from Assam (1.89) as against maximum ratio of 2.16 in the drones from Maharashtra.

The length and width of forewing varied from 13.20 to 13.73 and 4.16 to 4.37 mm, respectively. However, the ratio of



Andhra Pradesh



Assam



Kerala



Jammu



Karnataka



Kerala



Kerala

Plate 1: Male genitalia of *Apis cerana* from different states of India

length and width of forewing was more or less uniform (3.11 to 3.18). The cubital index was maximum in the drones from Maharashtra (7.94) followed by Tamil Nadu (6.81). Lowest cubital index was recorded in the drones from Assam (4.49).

Drones from Assam had maximum hamuli (25.67) while least number of hamuli was observed in the drones of Maharashtra (21.00).

Though the length and width of fourth abdominal tergum varied from 2.26 ((Maharashtra) to 2.52 mm (Assam) and 9.38 (Maharashtra) to 10.25 (Assam), respectively, the ratio of length and width of the fourth abdominal was uniform ranging from 0.24 to 0.25 mm.

The number of hairy patches on the cervix of the male genitalia was more in the drones from Karnataka (8.88) and least in the drones from Tamil Nadu (7.50).

Ten morphometric parameters and one genitalia parameter were used for factorial analysis, and the means of observations of these parameters across all the five states is given in Table 7.

Results of factor analysis and the colony groupings on a scatter plot are presented in Fig. 3 Clustering of *A. dorsata* drones

samples from 35 mean values showed formation of the following four clusters.

Cluster 1. Drones from Assam, Karnataka, Kerala and Maharashtra

Cluster 2. Drones from Assam, Karnataka, Kerala and Maharashtra and Tamil Nadu

Cluster 3. Drones from Karnataka

Cluster 4. Drones from Karnataka and Maharashtra.

Drones of Karnataka showed a wide range of variation and were a member of all the four clusters.

The Principal Component analysis also gave four Eigen values greater than one, which explained 64.56 per cent of the variations among the state groups (Table 8). Similarly the results of multivariate statistical analysis of 11 characters also yielded four factors with Eigen values greater than one that accounted for 64.56 per cent of the total variations in data.

In the Principal Component 1, hind tibial length, cubital index, number of hamuli, width of head and length of hind femur with component loading varying between 0.622 to 0.733 (Table 9) accounted for 25.19 per cent of variations (Table 8).

In the Principal Component 2, distance between lateral ocellus and eye and ratio of length and width of fourth abdominal tergum with component loading between 0.462 to 0.805 accounted for 16.26 per cent of variations.

In the Principal Component 3, the number hairy patches on the cervix of male genitalia and ratio between length and width of metatarsus with a component loading from 0.703 to 0.842 accounted for 13.73 per cent of variations.

In the fourth component, ratio of length and width of forewing with component loading of 0.847 accounted for 9.36 per cent of variation.



Assam



Kerala



Tamil Nadu



Karnataka



Kerala



Maharashtra

Plate 2: Male genitalia of *Apis dorsata* from different states of India



Andhra Pradesh



Assam



Karnataka



Kerala



Maharashtra



Tamil Nadu

Plate 3: Male genitalia of *Apis florea* from different states of India

In stepwise discriminant analysis, three characters viz. length of hid tibia, distance between lateral ocellus and eye and the number of hairy patches on cervix entered into the discriminatory functions (Table 10). Scatter plot of canonical discriminant function analysis revealed the presence of the

following four clusters (Fig. 4).

- Cluster 1. Drones from Karnataka, Kerala and Tamil Nadu
- Cluster 2. Drones from Assam, Maharashtra and Tamil Nadu.
- Cluster 3. Drones from Assam and Kerala

Cluster 4. Drones from Maharashtra and Tamil Nadu

Apis florea

For studying the morphometry of the drones of *Apis florea*, 19 morphometric characters (without genitalia parameter) as given under *A. cerana* were recorded from 33 means from six states. In addition, three parameters viz. length and width of thumb-like process from the base of metatarsus, and ratio between length and width of this process were also observed. The variation in the morphometry data in different states is presented in Table 11.

Drones from Karnataka, Assam and Andhra Pradesh were larger with body length of 11.04 to 11.36 mm while the drones from other states varied from 10.43 to 10.77 mm. The length of vertex varied from 0.96 (Maharashtra) to 1.14 mm (Assam). However, the drones from Assam had the widest head (4.12 mm) followed by Andhra Pradesh (3.85 mm), Karnataka and Kerala (3.82 mm). The ratio between length and width of head remained more or less uniform with narrow range of variation (0.26 to 0.28).

The distance between two lateral ocelli was maximum in the drones from Assam (0.42 mm) followed by the drones from Karnataka (0.37 mm). But the distance between lateral ocellus and eye was more or less uniform and varied from 0.08 to 0.12 mm.

The length of hind femur and tibia varied from 1.99 (Karnataka) to 2.29 mm (Assam) and 2.85 (Assam) to 3.26 mm (Kerala). Similarly the length and width of metatarsus varied from 1.62

(Maharashtra) to 1.83 mm (Assam) and 0.71 (Maharashtra) to 0.89 mm (Assam), respectively. However, the ratio between length and width of metatarsus was maximum in the drones from Maharashtra (2.38) followed by Tamil Nadu (2.27).

The length and width of forewing were more in the drones from Assam (9.98 and 3.08 mm) while they varied from 9.00 to 9.32 mm and 2.88 to 3.01 mm, respectively in other drones. The ratio of length and width of forewing was higher in the drones from Karnataka (3.25) and Assam (3.21).

The drones from Andhra Pradesh had highest cubital index (2.66) followed by Karnataka (2.26), Kerala (2.25) and Tamil Nadu (2.22). However, maximum hamuli were observed in the drones from Kerala (14.00) while in other drones their number varied from 12.10 to 13.00.

The fourth abdominal tergum was longer and wider in the drones from Assam (1.98 and 7.25 mm, respectively) and Andhra Pradesh (1.91 and 7.10 mm). However, the ratio of length and width of the tergum remained more or less uniform (0.25 to 0.27).

The drones from Assam and Kerala had longer and wider thumb like process arising from the base of metatarsus (1.31 and 1.32 mm) respectively while the width of the process was higher in the drones from Assam followed by Maharashtra (0.46 mm). But the ratio of length to width of this process was maximum in the drones from Andhra Pradesh (3.32) followed by Karnataka (3.29). Ruttner (1988) has reported that this thumb is distinctly longer in *A. florea* than in *A. andreniformis*. Hence, all the drones in the present study were *A. florea*. It also appears that *A. andreniformis* does not occur in these seven states.

For factorial, principal component and discriminant analysis 12 morphometric parameters were used and the means of observations of these parameters across all the six states is given in Table 12.

Results of factor analysis and the colony groupings on a scatter plot are presented in Fig. 5. Clustering of *A. florea* drones samples from 33 mean values showed formation of the following five clusters.

Cluster 1. Drones from Assam

Cluster 2. Drones from Karnataka and Tamil Nadu

Cluster 3. Drones from Andhra Pradesh, Karnataka, Maharashtra and Tamil Nadu

Table 7: Mean and standard deviation of eleven morphometric characters in *Apis dorsata* drone populations from different states in India

	Mean	Std. Deviation	Analysis N
HW	4.6163	0.10567	35
DBO	.2394	0.02473	35
DOOD	.0803	0.01543	35
HFL	2.8960	0.17914	35
HTL	4.4849	0.40753	35
MLW	2.0282	0.17961	35
FWLW	3.1303	0.17939	35
CI	5.9308	1.48046	35
HAM	23.31	2.246	35
HP	8.57	0.655	35
4 th TLW	0.2445	0.00935	35

Table 8: Total variation in morphometric parameters in *Apis dorsata* drones

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.77	25.19	25.19	2.77	25.19	25.19	2.57	23.36	23.36
2	1.78	16.26	41.45	1.78	16.26	41.45	1.61	14.70	38.07
3	1.51	13.73	55.19	1.51	13.73	55.19	1.59	14.48	52.55
4	1.03	9.36	64.56	1.03	9.36	64.56	1.32	12.00	64.56
5	0.94	8.56	73.13						
6	0.77	7.08	80.21						
7	0.69	6.30	86.51						
8	0.57	5.25	91.77						
9	0.42	3.85	95.62						
10	0.32	2.98	98.61						
11	0.15	1.38	100.00						

Extraction Method: Principal Component Analysis.

Cluster 4. Drones from Andhra Pradesh, Karnataka and Tamil Nadu

Cluster 5. Drones from Kerala and Tamil Nadu

The Principal Component analysis gave five Eigen values greater than one, which explained 68.04 per cent of the

Table 9: Rotated component matrix^a

	Component			
	1	2	3	4
HTL	0.733	-0.249	-0.135	-0.012
CI	-0.716	-0.111	-0.199	-0.060
HAM	0.679	0.045	-0.287	-0.435
HW	0.638	-0.122	-0.085	0.150
HFL	0.622	-0.215	0.354	0.289
DOOD	-0.225	0.805	0.020	0.072
DBO	0.075	0.781	-0.216	-0.072
4 th TLW	0.197	-0.462	-0.256	-0.425
HP	0.195	-0.037	0.842	-0.216
MLW	-0.202	-0.071	0.703	0.258
FWLW	0.303	0.028	-0.071	0.847

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization; a. Rotation converged in 7 iterations.

Table 10: Canonical discriminant functions of drones of *Apis dorsata* from seven states of India

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
HTL	2.369 ^a	56.8	56.8	0.839
DOOD	1.578 ^a	37.8	94.7	0.782
HP	0.223 ^a	5.3	100.0	0.427

a. First 3 canonical discriminant functions were used in the analysis.

Table 11: Morphometry of drones of *Apis florea* from different states in India

State	Andhra Pradesh		Assam		Karnataka		Kerala		Maharashtra		Tamil Nadu	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BL	11.10	0.16	11.04	0.23	11.36	0.40	10.77	0.01	10.84	0.00	10.43	0.00
VL	1.01	0.06	1.14	0.01	1.06	0.13	1.12	0.01	0.96	0.13	1.00	0.05
HW	3.85	0.04	4.12	0.12	3.82	0.16	3.82	0.01	3.76	0.12	3.70	0.13
HLW	0.26	0.01	0.28	0.01	0.28	0.02	0.29	0.01	0.26	0.04	0.27	0.02
DBO	0.35	0.02	0.42	0.08	0.37	0.06	0.35	0.01	0.34	0.01	0.35	0.03
DOOD	0.11	0.01	0.12	0.01	0.11	0.03	0.08	0.01	0.12	0.00	0.11	0.01
HFL	2.14	0.21	2.29	0.16	1.99	0.26	2.20	0.01	2.05	0.01	2.19	0.01
HTL	3.01	0.08	2.85	0.33	2.89	0.15	3.26	0.01	2.96	0.00	2.87	0.12
ML	1.73	0.06	1.83	0.03	1.65	0.04	1.81	0.01	1.62	0.21	1.67	0.04
MW	0.78	0.08	0.89	0.01	0.84	0.03	0.84	0.01	0.71	0.18	0.74	0.10
MLW	2.24	0.28	2.05	0.06	1.98	0.02	2.15	0.01	2.38	0.90	2.27	0.25
FWL	9.00	0.93	9.88	0.05	9.32	0.43	9.35	0.01	9.11	0.74	9.26	0.16
FWW	2.98	0.09	3.08	0.14	2.88	0.17	3.01	0.01	3.00	0.02	3.01	0.01
FWLW	3.03	0.39	3.21	0.17	3.25	0.33	3.11	0.01	3.04	0.22	3.07	0.05
CI	2.66	0.55	1.89	0.10	2.26	0.14	2.25	0.01	1.89	0.14	2.22	0.10
HAM	12.10	0.71	12.50	0.71	12.00	0.00	14.00	0.01	13.00	1.41	12.29	2.83
TbPL	1.20	0.08	1.31	0.16	1.24	0.13	1.32	0.01	1.17	0.23	1.19	0.03
TbPW	0.36	0.00	0.50	0.04	0.39	0.07	0.42	0.01	0.46	0.10	0.39	0.08
TbPLW	3.32	0.24	2.64	0.51	3.29	1.02	3.14	0.01	2.57	0.02	3.08	0.61
4 th TL	1.91	0.14	1.98	0.38	1.76	0.16	1.70	0.01	1.77	0.01	1.77	0.16
4 th TW	7.10	0.21	7.25	0.01	6.82	0.62	6.65	0.01	6.95	0.12	6.91	0.33
4 th TLW	0.27	0.01	0.27	0.01	0.26	0.00	0.26	0.01	0.25	0.00	0.26	0.01

bl- Body length, VL-Vertex length, HW- Head width including eyes, HLW- Ratio of vertex length and head width, DBO- Distance between ocelli, DOOD- Distance between ocellus and eye, HFL- Hind femur length, HTL- Hind tibial length, ML- Metatarsus length, MW- Metatarsus width, MLW- Ratio of length and width of metatarsus, FWL- Forewing length, FWW- Forewing width, FWLW- Ratio of forewing length and width, CI- Cubital index, HAM- Number of hamuli, TBPL- Length of thumb-like process, TBPW- Width of thumb like process, TPPLW- Ratio of length and width of thumb like process 4th TL- Length of 4th tergum, 4th TW- 4th tergum width, 4th TLW- Ratio of 4th tergum length and width,

Cluster 3. Drones from Maharashtra and Tamil Nadu

These morphometric studies clearly show that drones of *Apis cerana* from southern states of India formed overlapping clusters while drones from Jammu and Kashmir and Assam formed separate cluster. Interestingly one cluster included drones from Jammu and Kashmir, Assam as well as from Karnataka indicating the prevalence of a mixed population of *A. cerana* in Karnataka probably due to introduction of colonies from these states in Karnataka in order to increase honey yields.

Similarly drones of *A. dorsata* from Assam also formed two clusters along with the southern states. It appears that *A. dorsata* may migrate for long distance from northern to southern part of India and vice versa. These populations may freely interbreed and there is free exchange of gene pool among the populations.

Contrastingly drones of *A. florea* from Assam formed a non-overlapping separate cluster both in principal component analysis as well as canonical discriminant analysis indicating that Assam population is distinct from that of southern states. However, drones from southern states formed overlapping clusters indicating migration and interbreeding among the populations.

Genitalia studies

A comparative morphometry of all the drones of all the three

species of honey bees occurring in India has been studied for the first time.

All the three species of *Apis* had very distinct genitalia. In all the three species genitalia essentially consisted of three parts viz. 1. a basal vestibulum with cornua and hairy patches, 2. middle cervix with fimbriate lobe dorsally and hairy folds or patches ventrally and 3. a bulbous endophallus continuing into the ejaculatory duct as described by Koeniger *et al.* (1991)

All cornuae were extremely wrinkled which could be stretched easily by pulling. In immature drones cornuae were white to creamy while in matured drones, they were orange because of internal secretions (Koeniger *et al.*, 1991).

A. cerana had one pair of simple ventral cornua and one pair of dorsal cornua each of which had three distinct bulges elongated into tubes (Plate 1). On the ventral side of the vestibulum there was a hairy field varying in pattern. Cervix was twisted spirally with hairy patches both dorsally and ventrally. On the ventral side, there were 6 to 8 folds, some with hairs. The fimbriate lobe arose on the dorsal side very close to apex of cervix and sometimes attached to the endophallus. The fimbriate being membranous greatly varied in the drones from different places. It had a deep notch in the middle forming two separate lobes. Each of these lobes was deeply fringed into varying number of lamellate fringes. In some, each lobe again divided into 2 or 3 lobes which were with or without fringes. The bulbous endophallus was quite large than that of *A. dorsata* and *A. florea*. Often it was filled with hard creamy or orange mucous. Just above the base of the bulb, there was a band of lightly sclerotized area which partially encircled the bulb in some drones (drones from Assam, Jammu, Karnataka, Maharashtra, Tamil Nadu) or completely encircled the bulb in other drones (some drones from Karnataka, Kerala, Maharashtra, Tamil Nadu) or the bulb was without any sclerotized band (drones from Andhra Pradesh). Such sclerotized area is not reported in the drones from Malaysia (Koeniger *et al.*, 1991)

The entire genitalia of *A. dorsata* was much larger than found in *A. cerana* and *A. florea* as also reported by Ruttner (1988). Each ventral cornus was paired and quite long (Plate 2). Similarly each dorsal cornus was also paired but much shorter with several bulges and notches. Vestibulum was with hairy patches ventrally. The cervix was slender compared to *A.*

Table 12: Mean and standard deviation of twelve morphometric characters in *Apis florea* drone populations from different states in India

	Mean	Std. Deviation	Analysis N
HW	3.8324	0.15754	33
BL	10.9642	0.44252	33
DBO	0.3612	0.04037	33
DOOD	0.1127	0.01329	33
HFL	2.1264	0.16384	33
HTL	2.9361	0.15441	33
MLW	2.1819	0.30849	33
FWLW	3.1099	0.22431	33
CI	2.2847	0.42394	33
HAM	12.33	1.137	33
TBPLW	3.0838	0.50033	33
4 th TL	1.8570	0.20395	33

Table 13: Total variation in the morphometric parameters in *Apis florea* drones

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.36	19.67	19.67	2.36	19.67	19.67	2.27	18.97	18.97
2	1.85	15.45	35.13	1.85	15.45	35.13	1.69	14.14	33.12
3	1.67	13.97	49.10	1.67	13.97	49.10	1.57	13.11	46.23
4	1.22	10.22	59.33	1.22	10.22	59.33	1.40	11.70	57.94
5	1.04	8.71	68.04	1.04	8.71	68.04	1.21	10.10	68.04
6	0.91	7.62	75.67						
7	0.79	6.59	82.26						
8	0.65	5.44	87.70						
9	0.59	4.99	92.70						
10	0.42	3.55	96.25						
11	0.26	2.23	98.49						
12	0.18	1.50	100.00						

Extraction method: Principal Component analysis

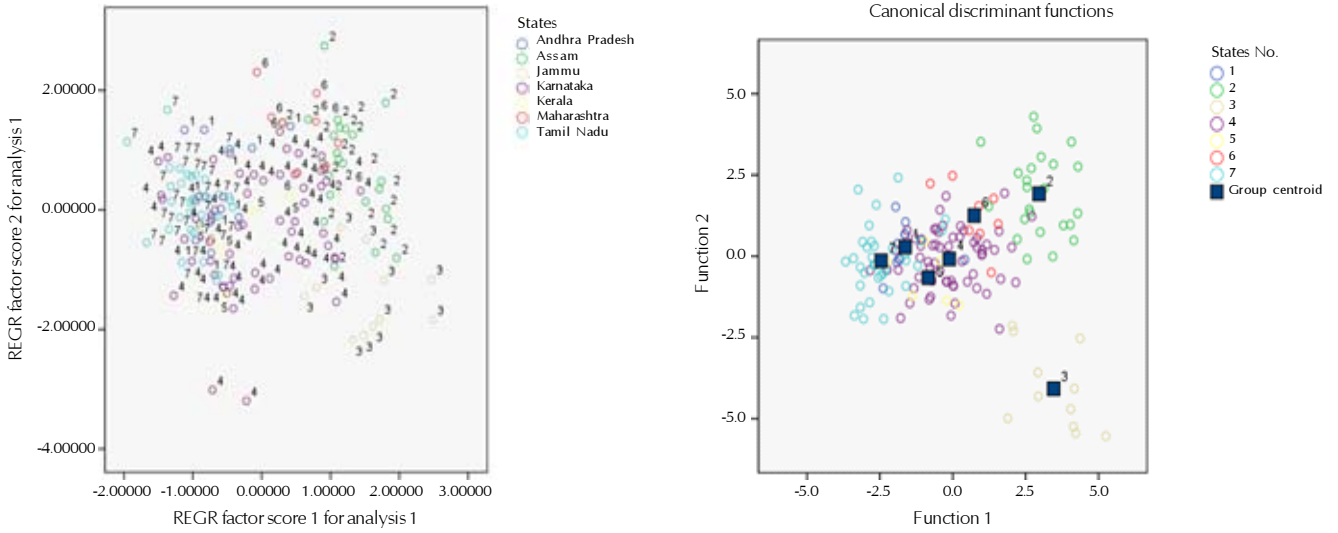


Figure 1: Clustering of *Apis cerana* drones based on Principal Component and Canonical Discriminant analysis

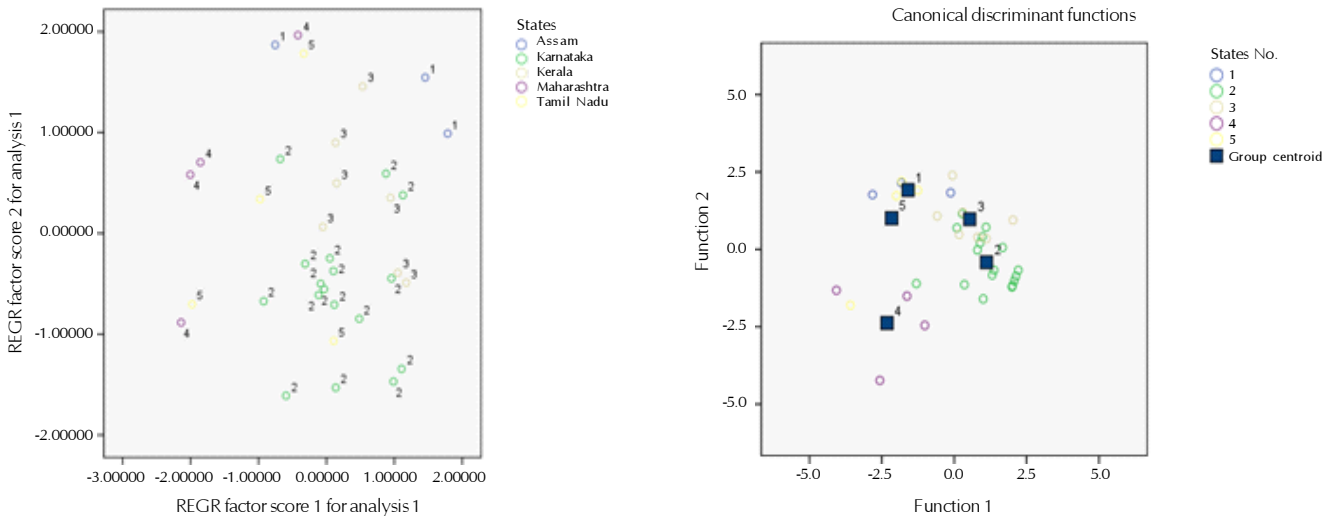


Figure 2: Clustering of *Apis dorsata* drones based on Principal Component and Canonical Discriminant analysis

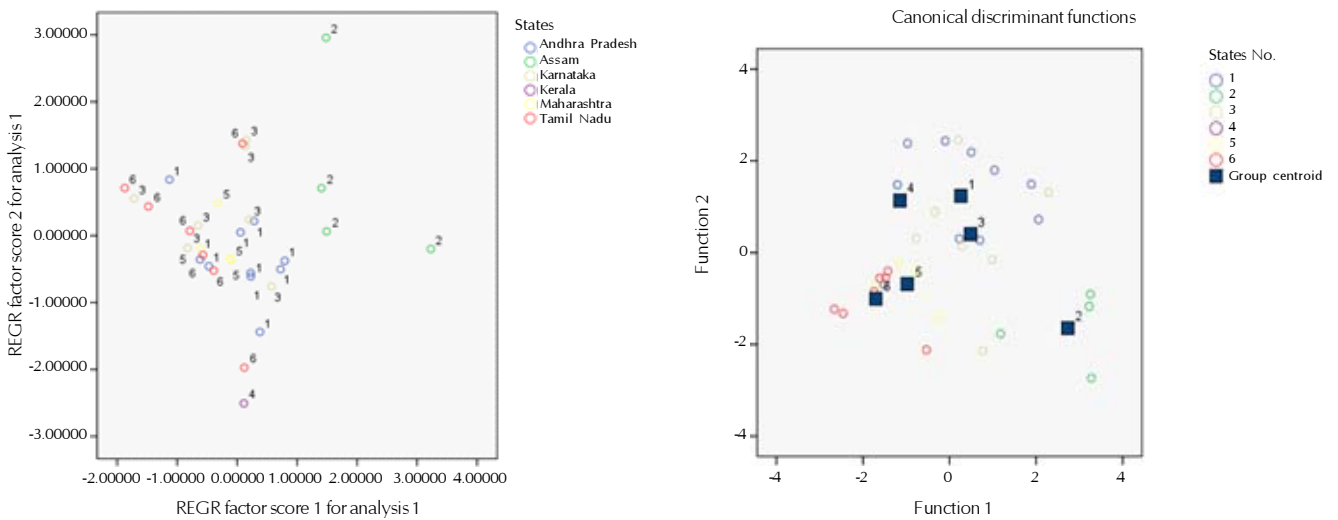


Figure 3: Clustering of *Apis florea* drones based on Principal Component and Canonical Discriminant analysis

Table 14: Rotated component matrix^a

	Component				
	1	2	3	4	5
HW	0.858	0.005	0.073	0.065	-0.061
4 th TL	0.855	0.091	-0.013	0.071	0.122
HFL	0.468	-0.175	0.382	-0.414	0.421
HAM	0.418	-0.227	-0.114	-0.278	-0.247
DOOD	-0.052	0.758	0.147	-0.171	0.090
HTL	0.108	-0.739	0.383	-0.112	0.005
DBO	0.382	0.645	0.254	0.045	-0.288
TBPLW	-0.189	-0.044	0.790	0.354	0.005
FWLW	0.143	0.086	0.669	-0.047	-0.099
BL	0.367	0.028	0.013	0.756	-0.183
CI	-0.257	-0.245	0.301	0.635	0.255
MLW	0.008	0.000	-0.121	-0.006	0.869

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. a. Rotation converged in 13 iterations.

Table 15: Canonical discriminant functions of drones of *Apis florea* from seven states of India

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
HW	2.135 ^a	48.5	48.5	0.825
BL	1.390 ^a	31.6	80.1	0.763
CI	0.624 ^a	14.2	94.2	0.620
HTL	0.254 ^a	5.8	100.0	0.450

a. First 4 canonical discriminant functions were used in the analysis.

cerana and *A. florea* but longer than these species. On the ventral side it had small 8-9 hairy patches. The fimbriate lobe showed variations. It had three distinct lobes with no fringes (drones from Assam). Drones from Karnataka, Kerala and Tamil Nadu had lobe with three lobes and the middle lobe distinctly longer than the lateral lobes and bifurcated near the apex. However, the drones from Maharashtra had distinctly cleft four lobes. The bulb was smaller than that of *A. cerana* and often filled with hardened mucous as in *A. cerana*.

In *A. florea* ventral cornuae were strongly curved and hook like terminally (Plate 3). However, dorsal cornuae were with indistinct bulges as reported by Koeniger *et al* (1991). The vestibulum had hairy patch. The cervix was straight with a longitudinal band of hairy patch on the dorsal side. But the ventral patches were indistinct. The fimbriate lobe with three bulges of which the first one was broad and 'U' shaped. The bulb was the smallest compared to *A. cerana* and *A. dorsata*, tube like and indistinct from the ejaculatory duct.

Present morphometric studies revealed that the drones of *A. cerana* and *A. dorsata* from seven states formed overlapping clusters indicating close relationship among honey bee populations. It also strongly indicates an extensive migration and interbreeding with exchange of gene pool among the populations of each species. However, *A. florea* drones of Assam formed a distinct cluster while the drones from the southern states formed overlapping clusters. The genitalia of drone of each species of honey bee were distinct but the genitalial structures within the species varied.

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