

Morphometric Analysis of Giant Honeybee, *Apis Dorsata* Worker Bees of Different Areas of Mysore District, Karnataka, India

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Abstract: The morphological characters of *A.dorsata* worker bees were analyzed after collecting moribund worker bees from different areas in Mysore District that experiences climate of semi-malnad type in southern dry zone part of Karnataka. Around 32 morphological characters were measured with the help of computer aided microscope Axion Vision ref. 4.8 software. The thorax, abdomen and overall body length showed significant variation between the workers bees. However, head length didn't show significant variation ($F=1.058$; $P>0.05$) between the worker bees which belong to different areas. Interestingly, the head breadth, tongue length, mandible and antenna length showed significant variation ($F= 6.599$; $P>0.05$) between the worker bees collected from different areas of Mysore District. Further, length of thorax, length & breadth of fore wing, hind wing and hamuli numbers also showed significant difference ($F= 18.23$; $P>0.05$) between *A.dorsata* worker bees except the thorax breadth. Furthermore, fore leg and mid leg length showed significant difference ($F=5.849$; $P>0.05$) between worker bees of different areas excepting the hind leg. Pearson's correlation studies revealed that there is a considerable relationship existed between few morphological traits and the body length of *A. dorsata* worker bees collected from different areas of Mysore District. Thus, it can be concluded that morphometric variations perhaps discriminate intraspecific groups of honeybee colonies as different ecotypes. Although it is simple, computer program-assisted morphometric analysis of the wing and body parts might provide useful information for honeybee biodiversity studies.

Keywords: *Apis dorsata*, worker bees, morphometric traits, Mysore District. * Corresponding Author.

I. Introduction

Honeybee morphological characteristics are measured for different reasons. The major use is to identify and characterized honeybee races and individuals (Ruttner, 1988 and Meixner *et al.*, 2007). Hence, morphological analysis is the key aspect to discriminate the honeybee races, colonies and species (Moradi and Kandemir, 2004; Raina and Kimbu, 2005; Farhoud and Kence, 2005; Shaibi *et al.*, 2009; Rattanawanee *et al.*, 2010; Nedić *et al.*, 2011). Further, morphological traits are genetically determined by polygenes and are highly constant (Collins, 1986), preserved even when the honeybees move to an entirely new environment (Alpatov, 1929). Recent years, morphometric traits analysis has become one of the most widely used authoritative tool for identifying honeybee races, because of its high practicability and low cost (Francoy *et al.*, 2008). It is also used for the discrimination between honeybee subspecies also (Toilski 2004; Abou-Shaara and Al-Ghamdi 2012). The wing length and width, tongue length were used to differentiate the honeybee subspecies (Buco *et al.*, 1987; Rinderer *et al.*, 1993; Crewe *et al.*, 1994; Ftayeh *et al.*, 1994; Diniz-Filho and Malaspina, 1995; Szymula *et al.* 2010). Tongue length was found to be an indicator of geographical variation of honeybees (Marghitas *et al.*, 2008; Morimoto, 1968 and Souza *et al.*, 2002). Szymula *et al.* (2010) has identified the difference with proboscis length between *Apis mellifera mellifera*, *A.m.carnica* and *A.m.caucasica*. Waddington (1989) has identified the correlation existed between body size and colony productivity. Mostajeran *et al.* (2002) have reported that honey production was related to tongue length, fore wing length and width, hind wing length, leg length, femur length, tibia length and metatarsus width. Edriss *et al.* (2002) have indicated that honey production has positive correlation with fore wing width. All these studies were attempted to create evidence that honeybee morphological characters are very important and have positive correlation with the colony productivity.

Makhmoor and Ahmad (1998) have studied 16 morphological characters by analyzing 10 honeybees belong to *A. florea*, *A. mellifera*, *A. cerana* and *A. dorsata* from Jammu region of India. Honeybees from the higher altitude were larger and darker compared to those from low altitudes (Atsalek *et al.*, 2012). *A. cerana indica* from Tamil Nadu showed a higher degree of intercolonial variation in association with major geographical features. The tongue of open nesting honeybee's namely *A. dorsata* and *A. florea* showed considerable variation. The scanning electron microscopic studies revealed that the shape of flagellum is oval to *A. dorsata* and triangular for *A. florea* which indicated the diversity of honeybee (Neelama *et al.*, 2015). Thus, certain morphometric characters are most widely used as quantitative traits for identification of honeybee races

and it has proved to be a useful tool for detecting both intraspecific and interspecific variation among *Apis* species. *A. dorsata* is one of the major pollinators, plays a major pollination service to innumerable number of tropical plant species and other crops (Wongsiri *et al.*, 2001; Corlett, 2011 and Partap, 2011). Interestingly, around 70 to 80 % honey produced in Nepal, India and other south-east Asian countries are coming from *A. dorsata* colonies alone (Woyke *et al.*, 2008). The hive products harvested from *A. dorsata* colonies provides an additional source of income for people who live in the vicinity of forest areas and at diversified agro-ecosystems (Basavarajappa *et al.*, 2009). As *A. dorsata* live at diversified ecosystems, reports on its morphological characters are fragmentary. Its ferocious nature hindered the economic parity with domesticated honeybees such as *A. mellifera* and *A. cerana* and resulted less attention (Ruttner, 1988) on its existence. There are few reports available on morphometrics of *A. dorsata* from central Karnataka (Basavarajappa, 1998). However, reports on morphological characters of *A. dorsata* in southern Karnataka are poor. This has necessitated conducting morphometric analysis of *A. dorsata* in this part of the State. Since, it is one of the premier multifloral honey producer's in this region (Raghunandan, 2014) information on different geographical variants/ecotypes of *A. dorsata* is fragmentary. Moreover, there are no published reports on morphometrics of *A. dorsata* population and its subspecies in southern Karnataka. Hence, the present study was conducted.

II. Material and Methods

Study area: To study the morphological characters, *A. dorsata* worker bees were collected from different areas in Mysore, Manasagangotri (12.31 ° N latitude and 76.62 ° E longitude) and Devaraj flower market (12.30 ° N latitude and 76.64 ° E longitude), T. Narasipura (12° 12' 36" N longitude and 76° 54' 23" E latitudes) areas lies in semi-malnad type of climate in southern dry zone of Karnataka. Moreover, this region experience dry climate most of the time. The region lies at 770 meters above msl, and experience an annual rainfall 804.2 mm (Kamath, 2001).

Methodology: The moribund *A. dorsata* worker bees were collected from their natural hives and preserved in 70% alcohol as per Adl *et al.* (2007). The body parts *viz.*, head, antenna, tongue, thorax, abdomen, fore wing, hind wing, foreleg, mid leg and hind leg were dissected under the Lieca EZ4 Stereozoom microscope with the help of surgical needles. The body parts were separately measured in millimeter with the help of computer aided microscope Axion Vision ref: 4.8software as per Ruma *et al.* (2013). Moreover, body colour, number of segments in abdomen number of hamuli in hind wing, and first tarsus segment length and breadth were measured. Collected information was compare by following standard methods as per Saha (2002).

III. Results

Morphological characters of *A. dorsata* worker bees collected from different areas of Mysore District revealed interesting results. Around 32 characters *viz.*, head length and breadth, thorax length and breadth, abdomen length and breadth, total body length, total antenna length, segment of antenna (scape, pedicel and flagellum length), mouth parts (tongue length, mandible length, sub mentum length, mentum length and width, galea, maxilla, lorum and labial palp length), fore wing length and breadth, hind wing length and breadth, hamuli number, fore leg, mid leg and hind leg length and breadth and leg parts (coxa, trochanter, femur, tibia and tarsal segments) measurements are shown in Tables 1, 2 and 3. The data from the table indicated that there is a considerable variation existed among different body parts of *A. dorsata* worker bees. The head, thorax, abdomen and overall body length of *A. dorsata* worker bee is shown in Table 1. The thorax, abdomen and overall body length has shown significant variation between the worker bees collected from different places of Mysore District. However, the head length didn't show significant difference ($F=1.058$; $P>0.05$) between the workers. Interestingly, the head breadth, tongue length, mandible and antenna length showed significant difference ($F= 6.599$; $P>0.05$) between the worker bee collected from different parts of Mysore District (Table 2). Further, length of thorax, length and breadth of fore wing, hind wing and hamuli numbers showed significant difference ($F= 18.23$; $P>0.05$) between *A. dorsata* worker bees except the thorax breadth (Table 3). Furthermore, the fore leg and mid leg length showed significant difference ($F=5.849$; $P>0.05$) between worker bees except the hind leg (Table 5).

The correlation studies revealed that there is a considerable relationship existed among morphological traits of *A. dorsata* worker bees collected from Manasagangotri, Periyapatna and T. Narasipura areas (Table 5). The worker bee abdominal length showed positive correlation with Manasagangotri ($r = 0.925$), Devaraj Market ($r = 0.936$), Periyapatna ($r = 0.912$) and T. Narasipura ($r = 0.885$). Moreover, thorax length of the worker bees collected from Periyapatna showed positive correlation ($r = 0.828$). However, correlation of worker bee's body length with scape, pedicel, flagellum, total antenna length, mandible, tongue length, sub-mentum length, mentum length and width, galea, maxilla lorum and labial palp length didn't show positive correlation for Manasagangotri, Devaraj market, Periyapatna and T. Narasipura samples (Table 6). Further, correlation between worker bee's body length with forewing and hind wing length and breadth showed negative correlation for Devaraj Market, Manasagangotri, Periyapatna and T. Narasipura samples (Table 7). The correlation of the

worker bees total body length with the leg parts viz., length and breadth of coxa, trochanter, femur, tibia and tarsus didn't show correlation (Tables 8, 9 and 10).

IV. Discussion

Various studies have shown that wing morphometrics alone could be used to identify some bee species including bumble bees (Aytekin *et al.* 2007), stingless bees (Francisco *et al.* 2008; Francoy *et al.* 2009) and honeybees. Francoy *et al.* (2006) have demonstrated that a single wing cell carried enough information to discriminate three racial groups of *A. mellifera* (Africanized, Italian, and Carniolan) with a fidelity level of nearly 99% of the individuals. The information of wing morphology and its molecular analysis could confirm the discovery and classification of a new species of stingless bee in the genus *Plebeia* (Francisco *et al.*, 2008). Similarly, Amssalu *et al.* (2004), Radloff *et al.* (2005), Hepburn *et al.* (2005), Rattanawanee *et al.* (2007), Andere *et al.* (2008), Basavarajappa (1998) and Raghunandan (2014) have made on an attempts to differentiate honeybee groups based on morphological data collected from different body characteristics namely: the body size, antenna length, proboscis length, hair length, metatarsus length and width, wing angle, wing length and width. The body morphological characteristics could be used as a simple indicator for estimating fluctuations in genetic and productive characteristics of honeybee colonies. The numbers of hamuli and their linear extent to the edge of the hind wing of honeybees have high heritability values and are readily modify by genetic selection (Hepburn *et al.*, 2004). The frequency distributions of hamuli and their extent on hind wings significantly vary at the population level and exhibited latitudinal and longitudinal clines over large distance (Hepburn *et al.*, 2004). Perhaps, due to latitudinal and longitudinal variations over large distance, there is a pronounced variation in overall body length, fore leg and mid leg length, thorax and abdomen length, head breadth, tongue, mandible and antenna length among the *A. dorsata* worker bees population. Based on these observations, it can be concluded that few morphometric variations perhaps help discriminate intraspecific groups of honeybee populations as different ecotypes. Similar types of observations were made by Radloff *et al.* (2005); Hepburn *et al.* (2005), Amssalu *et al.* (2004) and Andere *et al.* (2008). Notwithstanding it, methodology used in this work although is simple, can be extended to in depth finer identifications among honeybee species with the addition of future landmarks. However, these procedures are time-consuming for the preparation and accurate measurement of the various body parts. The computer program-assisted morphometric analysis of the wing and body parts might provide useful information for honeybee biodiversity studies further.

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Table 1. Body size of *Apis dorsata* worker bee collected from different areas of Mysore District

Sl.No.	Morphometric Parameter (mm)	Manasagangotri	Devaraj Market	Periyapatna	T.Narasipura	'F' value	
1.	Head	Length	3.89 ± 0.34	3.97 ± 0.35	3.89 ± 0.27	3.87 ± 0.24	1.058 NS
		Breadth	4.31 ± 0.14	4.25 ± 0.22	4.27 ± 0.17	4.39 ± 0.11	6.599 S
2.	Thorax	Length	5.27 ± 0.34	4.93 ± 0.50	5.10 ± 0.74	5.18 ± 0.46	3.254 NS
		Breadth	4.44 ± 0.38	4.48 ± 0.82	4.5 ± 0.33	5.15 ± 0.31	18.23 S
3.	Abdomen	Length	9.73 ± 1.33	10.53 ± 1.96	8.47 ± 1.43	10.42 ± 1.62	17.40 S
		Breadth	4.75 ± 0.45	4.62 ± 0.40	4.39 ± 0.52	4.68 ± 0.23	6.269 S
4.	Overall body size	18.61 ± 1.69	19.67 ± 2.05	17.41 ± 1.93	19.67 ± 1.84	16.235S	

Note: Each value is a mean of 50 observations. NS: Values are not significant' S: Values are significant at 5% level.

Table 2. Size of head and mouth parts of *Apis dorsata* worker bee

Sl. No.	Morphometric Traits (in mm)		Different areas of Mysore District				'F' value	
			Manasagangotri	Devaraj Market	Periyapatna	T.Narasipura		
1.	Head	Length	3.89 ± 0.34	3.97 ± 0.35	3.89 ± 0.27	3.87 ± 0.24	1.461 NS	
		Breadth	4.31 ± 0.14	4.25 ± 0.22	4.27 ± 0.17	4.39 ± 0.11	6.599 S	
2.	Antenna	Scape	1.39 ± 0.06	1.36 ± 0.06	1.32 ± 0.11	1.49 ± 0.09	33.180 S	
		Pedicel	0.27 ± 0.05	0.28 ± 0.04	0.24 ± 0.04	0.24 ± 0.04	5.403 S	
		Flagellum	2.89 ± 0.43	2.73 ± 0.42	2.94 ± 0.10	2.98 ± 0.16	4.709 S	
		Length	3.16 ± 0.00	4.33 ± 0.45	4.47 ± 0.26	4.73 ± 0.02	313.0 S	
3.	Mandible size		1.58 ± 0.46	1.49 ± 0.07	1.35 ± 0.10	1.50 ± 0.15	5.842 S	
4.	Overall Tongue length		4.28 ± 0.32	4.08 ± 0.32	4.14 ± 0.22	4.19 ± 0.11	2.716 S	
5.	Tongue	Sub Mentum		0.48 ± 0.10	0.50 ± 0.12	0.48 ± 0.07	0.64 ± 0.39	6.291 S
		Mentum	Length	1.79 ± 0.34	1.81 ± 0.41	1.70 ± 0.14	1.75 ± 0.07	1.438 NS
			Width	0.64 ± 0.09	0.65 ± 0.08	0.66 ± 0.09	0.67 ± 0.01	1.678 NS
		Galea		2.56 ± 0.30	2.52 ± 0.29	2.55 ± 0.34	1.44 ± 0.18	184.64 S
6.	Maxilla		1.70 ± 0.37	1.82 ± 0.35	1.68 ± 0.35	1.66 ± 0.32	1.827 NS	
7.	Lorum		0.62 ± 0.14	0.61 ± 0.16	0.63 ± 0.15	0.66 ± 0.06	1.602 NS	
8.	Labial palp		2.61 ± 0.28	2.57 ± 0.31	2.61 ± 0.30	2.81 ± 0.25	6.751 S	

Note: Each value is a mean of 50 observations. NS: Values are not significant' S: Values are significant at 5% level.

Table 3. Size of thorax, wing and appendages of *Apis dorsata* worker bee

Sl. No.	Morphometric Traits (in mm)		Different areas of Mysore District				F - Value
			Manasagangotri	Devaraj Market	Periyapatna	T.Narasipura	
1.	Thorax	Length	5.27 ± 0.34	4.93 ± 0.50	5.10 ± 0.74	5.18 ± 0.46	3.254 NS
		Breadth	4.44 ± 0.38	4.48 ± 0.82	4.5 ± 0.33	5.15 ± 0.31	18.23 S
2.	Fore wing	Length	12.70 ± 0.24	12.67 ± 0.28	12.50 ± 0.32	12.75 ± 0.24	7.425 S
		Breadth	4.26 ± 0.19	4.27 ± 0.34	4.36 ± 0.16	4.47 ± 0.11	7.547 S
3.	Hmd wing	Length	8.56 ± 0.27	8.59 ± 0.24	8.43 ± 0.30	8.65 ± 0.27	6.376 S
		Breadth	2.48 ± 0.11	2.50 ± 0.19	2.47 ± 0.10	2.54 ± 0.10	2.758 S
4.	No. of Hamuli on	Left wing	25 ± 1.58	25 ± 2.49	25 ± 2.00	24 ± 1.45	4.790 S
		Right wing	25 ± 2.04	25 ± 2.40	25 ± 2.00	23 ± 1.35	11.55 S
5.	Fore Leg		8.98 ± 0.63	9.1 ± 0.42	8.88 ± 0.64	8.91 ± 0.40	3.66 S
6.	Mid Leg		10.59 ± 0.90	10.76 ± 1.03	10.79 ± 2.01	10.93 ± 0.61	5.849 S
7.	Hmd Leg		13.9 ± 0.21	13.98 ± 0.74	13.79 ± 1.08	14.33 ± 0.63	1.896 NS
8.	Abdomen	Length	10.53 ± 1.96	9.73 ± 1.33	8.40 ± 1.29	10.70 ± 1.52	17.40 S
		Breadth	4.75 ± 0.45	4.62 ± 0.40	4.39 ± 0.52	4.68 ± 0.23	6.269 S

Note: Each value is a mean of 50 observations. NS: Values are not significant' S: Values are significant at 5% level.

Table 4. Coefficient correlation ('r' values) between the body length and other parts

Morphometric Character (in mm)		Body parts of <i>A. dorsata</i> worker bee					
Body length	Place	Head length	Head width	Thorax length	Thorax width	Abdomen length	Abdomen width
	Manasagangotri	0.038	0.024	0.309	-0.094	0.925	0.363
	Devaraj Market	0.247	0.119	0.544	-0.180	0.936	0.615
	T. Narsipura	0.150	0.110	0.544	0.030	0.880	0.240
	Periyapatna	0.095	0.049	0.820	0.233	0.912	0.710

Note: Data is based on Table 1 & 2. - Indicates negative correlation.

Table 5. Coefficient correlation ('r' values) between the body length and mouth parts

Sl. No.	Morphometric Character versus (in mm)	body length	<i>A. dorsata</i> worker bee body length in			
			Manasagangotri	Devaraj Market	T. Narsipura	Periyapatna
1.	Antenna	Scape	0.276	-0.240	0.089	0.103
		Pedical	0.306	-0.054	0.437	0.145
		Flagellum	-0.057	0.144	-0.024	0.380
		Overall length	0.010	-0.173	0.116	-0.067
2.	Mandible	0.137	-0.198	0.076	0.054	
3.	Tongue	Mantum length	-0.243	0.013	0.002	-0.027
		Mantum width	-0.322	-0.145	-0.129	0.085
		Sub mantum length	-0.080	-0.043	-0.100	0.062
		Overall tongue length	-0.088	-0.168	-0.114	-0.117
4.	Galea	-0.092	-0.013	-0.108	0.026	
5.	Maxilla	0.014	0.078	0.014	0.211	
6.	Lorum	0.051	0.017	-0.015	-0.138	
7.	Labial palp	-0.223	0.160	0.019	0.272	

Note: Data is based on Table 1 & 2. - Indicates negative correlation.

Table 6. Coefficient correlation ('r' values) between the body length and wings

Sl. No.	Morphometric Character versus (in mm)	body length	<i>A. dorsata</i> worker bee body length in			
			Manasagangotri	Devaraj Market	T. Narsipura	Periyapatna
1.	Fore wing	Length	0.428	0.026	0.200	0.186
		Breadth	-0.020	0.100	0.026	0.167
2.	Hind wing	Length	0.256	-0.019	-0.029	-0.044
		Breadth	-0.185	0.010	-0.047	0.026
3.	No. of Hamuli on	Left wing	0.077	0.123	0.188	0.048
		Right wing	-0.111	0.068	-0.199	0.207

Note: Data is based on Table 1 & 2. - Indicates negative correlation.

Table 7. Pearson's coefficient correlation ('r' values) between the body length and appendages

Sl. No.	Appendages (in mm)			<i>A. dorsata</i> worker bee body length in			
				Manasagangotri	Devaraj Market	T. Narsipura	Periyapatna
1.	Coxa	Length	Fore leg	0.131	-0.335	-0.043	0.256
			Mid leg	-0.164	0.038	0.147	-0.330
			Hind leg	0.187	-0.283	-0.256	0.407
		Width	Fore leg	0.069	0.116	-0.067	-0.056
			Mid leg	-0.074	0.243	0.117	-0.005
			Hind leg	-0.178	0.005	-0.007	0.412
2.	Trochanter	Length	Fore leg	0.358	-0.005	-0.277	0.096
			Mid leg	-0.087	-0.414	-0.207	0.159
			Hind leg	0.075	-0.146	0.081	0.637
		Width	Fore leg	-0.005	-0.009	-0.096	-0.012
			Mid leg	-0.177	-0.208	0.215	-0.039
			Hind leg	-0.189	-0.201	0.164	0.513
3.	Femur	Length	Fore leg	0.119	-0.048	-0.020	-0.156
			Mid leg	-0.093	0.233	-0.026	0.356
			Hind leg	-0.148	0.068	-0.033	0.348
		Width	Fore leg	-0.204	-0.204	-0.112	-0.170
			Mid leg	-0.233	-0.050	-0.045	0.283
			Hind leg	-0.237	0.265	0.212	0.332
4.	Tibia	Length	Fore leg	-0.125	0.092	0.147	-0.121
			Mid leg	0.129	0.442	0.251	0.248
			Hind leg	0.282	-0.137	0.006	0.590
		Width	Fore leg	-0.065	-0.240	0.094	0.040
			Mid leg	-0.219	-0.064	-0.028	0.212
			Hind leg	-0.188	0.125	0.149	0.424
5.	Tarsus	Length	Fore leg	-0.033	-0.087	0.035	0.201
			Mid leg	0.034	0.447	0.058	0.451
			Hind leg	-0.137	-0.032	0.098	0.187
		Width	Fore leg	-0.076	-0.262	-0.146	0.026
			Mid leg	-0.064	-0.433	-0.330	0.073
			Hind leg	0.107	0.118	0.122	0.602
6.	Over length of	Fore leg	-0.092	-0.239	0.037	0.036	
		Mid leg	-0.084	0.359	0.115	0.151	
		Hind leg	0.035	0.149	0.079	0.689	

Note: Data is based on Table 1 & 2. - Indicates negative correlation.