

ZIBELINE INTERNATIONAL™  
PUBLISHING

ISSN: 2521-5051 (Print)

ISSN: 2521-506X (Online)

CODEN: ASMCCQ



## RESEARCH ARTICLE

**MORPHOMETRIC CHARACTERIZATION OF THREE DIFFERENT HONEYBEES (HYMENOPTERA: APIDAE) SPECIES FROM BANGLADESH**Tanzum Islam Nabila<sup>a</sup>, Md Mamunur Rahman<sup>a\*</sup>, Mst Nura Jahan<sup>a</sup>, Md Ahsanul Haque<sup>a</sup>, Pratik Raj Karki<sup>b</sup> and Mohammad Mehruz Hassan Saikat<sup>c</sup><sup>a</sup>Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh<sup>b</sup>Faculty of Agriculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh<sup>c</sup>Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh\*Corresponding Author Email: [mamun@bsmrau.edu.bd](mailto:mamun@bsmrau.edu.bd)

This is an open access article distributed under the Creative Commons Attribution License CC BY 4.0, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ARTICLE DETAILS

## Article History:

Received 24 May 2023

Revised 28 June 2023

Accepted 01 August 2023

Published 10 August 2023

## ABSTRACT

The honeybee is an eusocial species that plays an important role in conservation, pollination, and environmental protection. In Bangladesh, apiculture has become a profitable venture for the last few decades. Taking into account the potential of different honeybee species in pollinating and producing bee products, knowledge of different species can help maximize the production of bee products and increase pollination. To identify these species using their morphometric characteristics, three samples of honey species, namely *Apis mellifera* L., *Apis cerana* F., and *Apis dorsata* F. were collected to analyze their morphometric characteristics. Approximately 172 worker honeybee samples were collected from 24 colonies in 7 districts of Bangladesh. For each of the collected samples, 22 (22) morphometric characters were measured in the entomology laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University. Analysis of variance was performed to understand the variability of the morphometric characters between honeybee species, which indicated that the morphometric characters are suitable for identifying honeybee species. The principal component analysis delineated three morphometric characters (along with their morphometric characters in parentheses): The distance of the cubular vein a (19), longitudinal length of the forewing (17), and the angle of venation of the forewing B4 (22), which contributed the most to the variance. Classification of honeybee species using multilayer perceptron considering only these three morphometric characters achieved an accuracy of 100% while significantly reducing the overall computation required. The findings of this study can be helpful in identifying the discriminating morphometric characteristics of the honeybee for improving the apiculture sector in Bangladesh.

## KEYWORDS

Apiculture, genetic diversity, morphometry, taxonomy, honeybees

## 1. INTRODUCTION

Honey bees are eusocial insects that play a crucial role in food security, sustainable farming, biodiversity, and environmental protection. They are one of the most efficient and numerous pollinator species in the world. Around 80% of the world's flowering plants are pollinated by honey bees (Randall, 2021). Through pollination, honey bees increase the amount of production in agriculture and improve the quality and resistance of crop plants.

Honey bees belong to the Apidae family consisting of around 20,000 species. Among them, honey-producing bees come from two subfamilies: Apinae (honey bees) and Meliponinae (stingless bees). Among honey bees, there are four well-recognized species in the world, namely *Apis cerana* F. and *Apis dorsata* F. *Apis florea* F. and *Apis mellifera* L. *Apis mellifera* L., also known as the European honey bee, is the most domesticated and widely distributed species in the world. The name *Apis* comes from the Latin word for 'bee' and *mellifera* comes from the Latin word for 'honey bearing'. It originates from Asia and spreads through Africa, the Middle East, and Europe naturally, and through North America, South America, Australia, New Zealand, and eastern Asia through human help (Han et al., 2012). Along with selective breeding for honey production, synchronized colony cycle with the local flowering pattern, winter clustering during cold seasons, migratory swarming, and enhanced foraging of *Apis mellifera* L.

have made them highly adaptable to different ecological conditions of the world, resulting in 33 different subspecies (Ilyasovetal, 2020).

The Indian or Asiatic or Eastern honey bee, *Apis cerana* F. are small honeybees of southern and southeast Asia, such as China, India, Japan, Malaysia, Nepal, Bangladesh, and Papua-Neuve Guinea (Abrol, 2009). *A. cerana* F. constructs parallel combs in darkened closures such as caves, rock cavities, and hollow tree trunks. The species is widely domesticated, stores sealed ripe honey and is susceptible to wax moths. The "small body size" of *A. cerana* F. was alleged by many authors to be the major discriminant factor. However, this assumption favored misidentifications, e.g., the allocation of "*Apis cerana* F." races to Senegal and Cameroun (Buttel-Reepen, 1906; Enderlein, 1906; Maa, 1953).

*Apis dorsata* F., the giant honey bee, is a honey bee of South and Southeast Asia, found mainly in forested areas such as the Terai of Nepal. These social bees are known for their aggressive defense strategies and vicious behavior when disturbed. Although it was not domesticated, indigenous peoples have traditionally used this species as a source of honey and beeswax, a practice known as honey hunting. The greatest populations of *Apis dorsata* F. are found in China, Malaysia, Indonesia, India, Pakistan, and Sri Lanka. In the Philippines, which used to have one of the greatest populations of *Apis dorsata* F., the populations have now become relatively rare due to deforestation and people's "mindsets" towards the bees. The

## Quick Response Code



## Access this article online

## Website:

[www.actascientificmalaysia.com](http://www.actascientificmalaysia.com)

## DOI:

10.26480/asm.02.2023.37.49

giant honeybee is certainly the most spectacular of the three honeybee species; it is an individual bee of the length of a hornet, living in the open in huge colonies.

A global analysis of the modern diversity of *A. mellifera* L., *A. cerana* F., and *A. dorsata* F. using genetic approaches characterized Bangladesh as one of the geological zones due to the effects of the Last Glacial Maximum (LGM) (Eimanifar et al., 2018). Bangladesh, located in the southern part of Asia between 20°34' to 26°38' North latitude and 88°01' to 92°41' East longitude, has an area of 1.47 million square kilometers. It borders India to the west, north and east, Myanmar to the southeast, and the Bay of Bengal to the south. Located in the Indo-Asiatic region, Bangladesh lies within moist deciduous forests of the Lower Gangetic Plains, Mizoram-Manipur-Kachin rain forests freshwater swamp forests, and mangroves of the Sundarbans mangroves (Dinerstein et al., 2017). With diversified geographical characteristics, variable climatic conditions, and a variety of natural and cultivated vegetation, the country is suitable for the existence of different subspecies of honey bees. According to the record of Pleistocene glaciation, topography, and climatic variation in this continent, Bangladesh could be a potential zone of the divergent gene pool of honey bee population. For this reason, the interspecific characteristics of honey bees must be analyzed to design and implement strategies to conserve local bees and facilitate profitable beekeeping.

In Bangladesh, beekeeping is one of the oldest traditions, which started more than 400 years ago (Sivaram et al., 2012). Three species *Apis dorsata* F., *Apis cerana* F., and *Apis florea* F. occurred naturally in Bangladesh, and in 1992, *Apis mellifera* L. was introduced due to its higher honey yield potential (Sivaram et al., 2012). With the help of government and non-government organizations, beekeeping has become a profitable venture over the last few decades. Considering the agricultural dependence of Bangladesh and the pivotal role of honey bees in cross-pollination, improvement of seed and fruit quality, forest conservation and the maintenance of biodiversity the need for beekeeping is paramount (Minja and Nku-milwa, 2016). Despite the need for beekeeping, the amount of research focused on honey bees is limited. Beekeepers often employ a traditional system of differentiating bees by size (small, medium, large) and color (black, yellow, orange). To make things worse, the system lacks consistency, as it can sometimes be challenging to find uniform color within a colony, and even then it might vary from region to region. This lack of information on honey bee species contributes to the lack of understanding of the behavior, biology, distribution, and productivity of existing honey bees. Since different species of honey bees have different potentials for pollination and the production of honey, beeswax, bee nectar, pollen and other bee products, knowledge about different species can help maximize the production of bee products and increase pollination. Additionally, improved management techniques for each honey bee in a particular ecology are essential to maximize the output of beekeeping. The selection and use of these honeybee species according to potential is not possible without basic morphometric information about these honeybees. Morphometric characterization of honey bee species in Bangladesh can generate appropriate honey bee management systems best suited for different agroecology, help select the best performing and productive honey bee species to breed and disseminate to beekeepers, and develop policies for the conservation of local species. With all these goals in mind, the objective of this study is to identify three different species of honeybees in Bangladesh and to describe their morphometric

characteristics to differentiate the species of *A. mellifera* L., *A. cerana* F. and *A. dorsata* F. allowing Bangladeshi beekeepers to adopt the superior species of honeybee for uplifting their production.

## 2. MATERIALS AND METHODS

### 2.1 Sampling Sites

The sampling sites are located in the northern, central, southeastern and south-western parts of Bangladesh between 22°21'43.9"N to 24°51'06.1"N latitude and 88°45'08.1"E to 91°48'42.0"E longitude. Samples were collected from Rajshahi, Satkhira, Jhenaidah, Chattogram, Gazipur, Tangail, and Bogura districts. These include localities such as Charchat, Kalaroa, Shyamnagar, Atulia, Kotchandpur, Khulsi, Sreepur, Mirzapur, Bogura Sadar, and Dhunut. These localities have prominent beekeeping practices due to their flowering season, flowering pattern, and geographical areas favorable for honey bees.

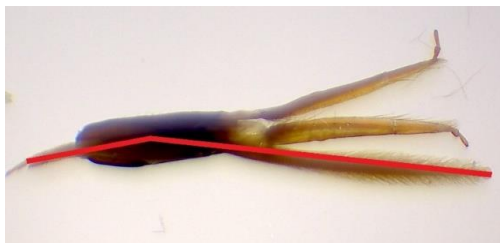
### 2.2 Sample Collection

From October 2020 to March 2021, 172 worker honey bees from 24 colonies were sampled from 10 localities. These samples were collected from bee hives and instantly preserved in ethanol. Samples of each colony were stored in a single vial. Each vial was given a unique identification number. The location, date, and collector information for each vial was marked with the vial number. These vials were then transferred to the entomology laboratory at Bangabandhu Sheikh Mujibur Rahman Agricultural University for morphometric measurements. The samples were then stored in a preservative solution prepared using 30 parts distilled water, 15 parts 95% ethanol, 7 parts 38% to 40% formaldehyde, and 2 parts acetic acid to prevent sample deformation.

### 2.3 Morphometric Characteristics of Honeybees

Morphometric measurements were performed in the entomology laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, from June 2021 to August 2021. A total of 22 morphometric characters (in parentheses) representing body size were extracted: including Proboscis length (4), Femur length (5), Tibia length (6), Metatarsus length (7), Metatarsus width (8), Tergite 3 longitudinal (9), and Tergite 4 longitudinal (10) (Figure 1); forewing: including Forewing longitudinal (17), Forewing transversal (18), Cubital vein distance a (19) and Cubital index distance b (20), Wing venation angle A4 (21), B4 (22), D7 (23), E9 (24), G18 (25), I10 (26), I16 (27), K19 (28), L13 (29), N23 (30) and O26 (31). (Figure 2).

To take these measurements of the morphometric characteristics of honey bee samples, individual body parts were dissected and measured using the Euromex Stereo Blue microscope equipped with CMEX-5f DC.5000f. The 2.5-inch CMOS sensor with a rolling shutter can take RGB images of 1272 × 952 pixels with a pixel size of 2.2 μm × 2.2 μm. The sensor is accompanied by ImageFocus Plus v2.2.0 software to facilitate the correct measurement. First, a micrometer scale was used to calibrate the microscope. The dissected body part was placed on the stage plate and an image was captured using the software. Finally, the measurements were taken on the calibrated scale and stored together with the original image for future reference.



(a) Proboscis length (4)



(b) Femur length (5)

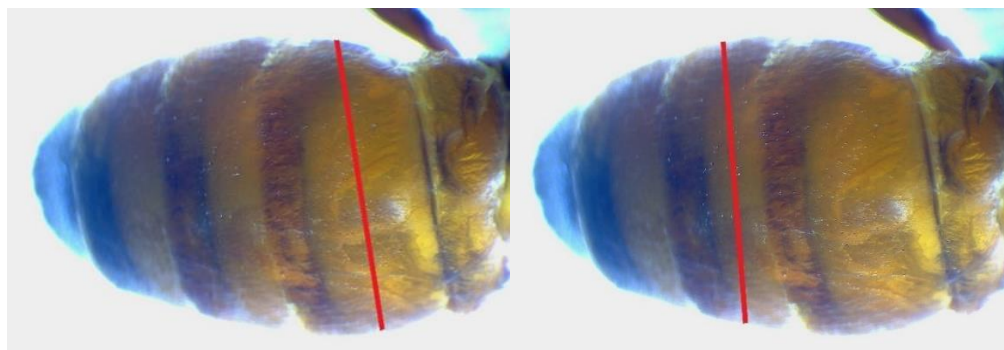


(c) Tibia length (6)



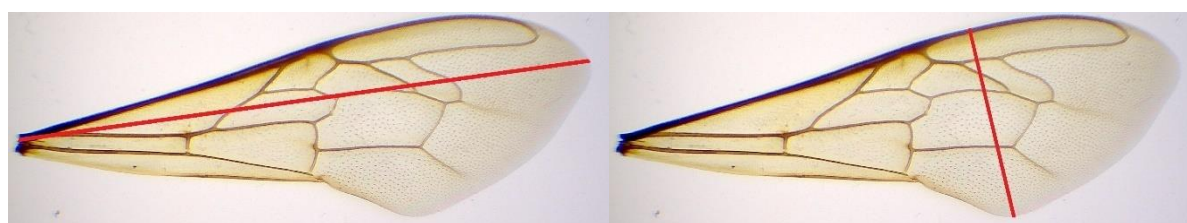
(d) Metatarsus length (7)

(e) Metatarsus width (8)



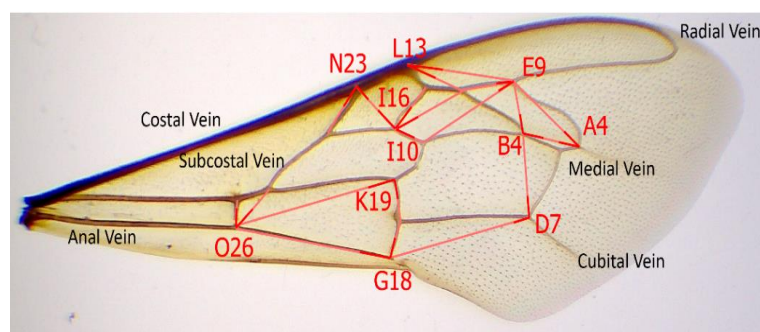
(f) Tergite 3 longitudinal (9)

(g) Tergite 4 longitudinal (10)

**Figure 1:** Measured morphometric characters related to honey bee body size (a-g)

(c) Cubital vein distance a (19)

(d) Cubital index distance b (20)

**Figure 2:** Measured morphometric characters related to honey bee forewing

(e) Wing venation angles A4 (21) between medial and radio-medial vein 2, B4 (22) between medial and radio-medial vein 1, D7 (23) between cubital and medio-cubital vein 2, E9 (24) between radial sector and radio-medial vein 2, G18 (25) between anal and cubito-anal vein 2, I10 (26) radial sector and medial vein, I16 (27) radial sector and radio-medial vein, K19 (28) between cubital and cubito-anal vein 2, L13 (29) between radial sector and radial vein, N23 (30) between radial sector and costal vein, and O26 (31) between anal and cubito-anal vein 1. Angle names are taken from (Ruttner, 1988).

## 2.4 Statistical Analysis

### 2.4.1 Mean and Standard Deviation

The mean and standard deviation were computed using the following formula. For the morphometric character  $c$ ,

$$\mu_c = \frac{\sum_{i=1}^n x_i}{n}$$

Here,

$x_i$  = Measurement of morphometric character of the  $i$ -th sample

$n$  = Total number of samples

The standard deviation were measured through

$$\sigma_c = \sqrt{\frac{\sum_{i=1}^n (x_i - \mu_c)^2}{n}}$$

Here,

$x_i$  = Measurement of morphometric character  $c$  of the  $i$ <sup>th</sup> sample

$\mu_c$  = Mean of the  $c$ <sup>th</sup> sample

$n$  = Total number of samples

### 2.4.2 Pearson's Correlation Coefficient

To quantify the relationship among the morphometric characteristics of honey bee species, the Pearson correlation coefficient was calculated.

Given a pair of random variables  $(X, Y)$  representing two morphometric characters of the honey bee:

$$\rho_{X,Y} = \frac{E[XY] - E[X]E[Y]}{\sqrt{(E[X^2] - (E[X])^2)(E[Y^2] - (E[Y])^2)}}$$

Here,

$E$  = The expectation

The correlation coefficient ranges from (-1) to 1. An absolute value of exactly 1 implies that a linear equation perfectly describes the relationship between X and Y, with all data points lying on a line. The correlation sign is determined by the regression slope: A value of (+1) implies that all data points lie on a line for which Y increases as X increases, and vice versa for (-1). A value of 0 implies that there is no linear dependency between the variables.

### 2.4.3 Analysis of Variance (ANOVA)

Analysis of variance (ANOVA) were performed using the following formula

$$H_0: \mu_1 = \mu_2 = \dots = \mu_k \text{ (all } k \text{ species means are equal)}$$

$$H_1: \text{(At least one of the } k \text{ species means is not equal to the others)}$$

Here,

$$\mu_i = \text{Population mean of the } i^{\text{th}} \text{ species } (i = 1, 2, \dots, k)$$

### 2.4.4 Principal Component Analysis (PCA) and Multilayer Perceptron

To determine the number of principal components to be extracted, a scree plot is generated. Based on the inflection point in the scree plot, the number of components to be extracted can be determined. In addition to that, the number of components with eigenvalues greater than 1 is extracted.

A multilayer perceptron (MLP) is a class of feed-for-word artificial neural networks (ANN). An MLP consists of at least three layers of nodes: an input layer, a hidden layer, and an output layer. Except for the input nodes, each node is a neuron that uses a non-linear activation function. To determine whether the set of morphometric characters, found by performing PCA, contributes to the discrimination capability or not, honey bee samples can be classified using MLP. A simple MLP can consist of a single hidden layer. The number of nodes in the input layer is equal to the number of characters considered for classification, and the number of nodes in the output layer is equal to the number of subspecies found. Typically, 70% of the samples are used to test the MLP and 30% of the samples are used to

test accuracy.

## 3. RESULTS AND DISCUSSIONS

### 3.1 Morphometric Characteristics of Honeybee Species in Bangladesh

The morphometric data retrieved from the different honeybee samples were analysed to enumerate the following results.

#### 3.1.1 Morphometry Study of *Apis Mellifera* L.

To discriminate between the species of *Apis mellifera* L., 22 morphometric characters were measured. These characters can be grouped into three main categories (with their Ruttner numbers in brackets): (i) body size including Proboscis length (4), Femur length (5), Tibia length (6), Metatarsus length (7), Metatarsus width (8), Tergite 3 longitudinal (9) and Tergite 4 longitudinal (10); (ii) forewing characters such as Fore wing longitudinal (17), Fore wing transversal (18), Cubital vein distance a (19) and Cubital index distance b (20), wing venation angles A4 (21), B4 (22), D7 (23), E9 (24), G18 (25), I10 (26), I16 (27), K19 (28), L13 (29), N23 (30) and O26 (31).

The measurements of these characters were made on 172 worker honey bees, collected from 10 localities in Bangladesh. The means, ranges, and standard deviations of each morphometric character of the honey bees are indicated in Table 1. The statistical results show that the proboscis length varied from 4.46 mm to 5.82 mm with an average of  $5.10 \pm 0.43$  mm. The length of the femur and tibia varied from 2.35 mm to 2.62 mm and 2.50 mm to 3.07 mm with an average of  $2.50 \pm 0.10$  mm and  $2.90 \pm 0.17$ , respectively. The metatarsus length and width ranged from 1.67 to 2.03 mm and 0.79 mm to 1.13 mm, with an average of  $1.86 \pm 0.14$  mm and  $1.02 \pm 0.11$  mm, respectively. On average, tergite 4 is shorter than tergite 3 in the longitudinal direction. The longitudinal length of tergite 3 and tergite 4 ranged from 3.81 mm to 4.43 mm and 3.37 mm to 4.15 mm with an average of  $4.10 \pm 0.26$  mm and  $3.71 \pm 0.28$  mm in that order. The transverse longitudinal length of the forewing varied from 8.67 to 9.08 mm and from 2.86 mm to 3.04 mm with an average of  $8.83 \pm 0.13$  mm and  $2.96 \pm 0.07$  mm, respectively. The distance of the cubital vein is arranged from 0.48 mm to 0.55 mm with an average of  $0.52 \pm 0.02$  mm. The cubital index distance b varies from 0.20 mm to 0.36 mm with an average of  $0.24 \pm 0.05$  mm. The findings were found with the similar findings from Amssal (2003).

**Table 1:** Ranges, Means, and Standard Deviations of Samples of Samples of Morphometric Characters of *Apis mellifera* L. Samples from Bangladesh

Ruttner No.	Morphometric Characters	Range		Mean	Std. Deviation
		Min.	Max.		
4	Proboscis Length	4.46	5.82	5.10	0.43
5	Femur Length	2.35	2.62	2.50	0.10
6	Tibia Length	2.50	3.07	2.90	0.17
7	Metatarsus Length	1.67	2.03	1.86	0.14
8	Metatarsus Width	0.79	1.13	1.02	0.11
9	Tergite 3 Longitudinal	3.81	4.43	4.10	0.26
10	Tergite 4 Longitudinal	3.37	4.15	3.71	0.28
17	Forewing Longitudinal	8.67	9.08	8.83	0.13
18	Forewing Transversal	2.86	3.04	2.96	0.07
19	Cubital Vein Distance a	0.48	0.55	0.52	0.02
20	Cubital Index Distance b	0.20	0.36	0.24	0.05
21	Forewing Venation Angle A4	26.62	29.97	28.28	1.10
22	Forewing Venation Angle B4	109.50	120.57	114.06	3.82
23	Forewing Venation Angle D7	95.80	103.36	98.32	2.50
24	Forewing Venation Angle E9	92.55	105.65	98.02	4.85
25	Forewing Venation Angle G18	89.79	102.19	94.42	3.95
26	Forewing Venation Angle I10	47.76	55.19	50.69	2.77
27	Forewing Venation Angle I16	87.75	94.52	90.60	2.68
28	Forewing Venation Angle K19	74.92	81.80	79.72	2.38
29	Forewing Venation Angle L13	12.20	33.80	17.83	8.04
30	Forewing Venation Angle N23	84.53	90.40	87.10	2.08
31	Forewing Venation Angle O26	29.44	42.44	37.29	4.30

Angles A4, B4 and D7 varied from  $26.62^\circ$  to  $29.97^\circ$ ,  $109.50^\circ$  to  $120.57^\circ$  and  $95.80^\circ$  to  $103.36^\circ$  with an average of  $28.28 \pm 1.10^\circ$ ,  $114.06 \pm 3.82^\circ$ , and  $98.32 \pm 2.50^\circ$  in that order. The forewing venation angles E9 and G18 ranged from  $92.55^\circ$  to  $105.65^\circ$  and  $89.79^\circ$  to  $102.19^\circ$  with an average of

$98.02 \pm 4.85^\circ$  and  $94.42 \pm 3.95^\circ$  respectively. Again, angles I10 and I16 varied from  $47.76^\circ$  to  $55.19^\circ$  and  $87.75^\circ$  to  $94.52^\circ$  with an average of  $50.69 \pm 2.77^\circ$  and  $90.60 \pm 2.68^\circ$ , respectively. Finally, the wing venation angles K19, L13, N23, and O26 ranged from  $74.92^\circ$  to  $81.80^\circ$ ,  $12.20^\circ$  to

33.80°, 84.53° to 90.40°, and 29.44° to 42.44° with an average of 79.72 ± 2.38°, 17.83 ± 8.04°, 87.10 ± 2.08°, and 37.29 ± 4.30° in that order.

### 3.1.2 Morphometry of *A. Cerana* F

To discriminate between *Apis cerana* F. 22 morphometric characters were measured. These characters can be grouped into three main categories (with their Ruttner numbers in brackets): (i) body size including Proboscis length (4), Femur length (5), Tibial length (6), Metatarsus length (7), Metatarsus width (8), Tergite 3 longitudinal (9) and Tergite 4 longitudinal (10); (ii) forewing characters such as Fore wing longitudinal (17), Fore wing transversal (18), Cubital vein distance a (19) and Cubital index distance b (20), wing venation angles A4 (21), B4 (22), D7 (23), E9 (24), G18 (25), I10 (26), I16 (27), K19 (28), L13 (29), N23 (30), and O26 (31).

The measurements of these characters were made on 172 worker honey bees, collected from 10 localities in Bangladesh. The means, ranges, and standard deviations for each morphometric character of the honey bees are indicated in Table 2. The statistical results show that the proboscis length ranged from 3.22 mm to 4.01 mm with an average of 3.61 ± 0.21 mm. The length of the femur and tibia varied from 1.74 to 2.17 mm and from 2.47 mm to 2.68 mm with an average of 1.98 ± 0.12 mm and 2.57 ±

0.06, respectively. The length and width ranged from 1.68 to 1.72 mm and 0.67 mm to 0.80 mm with an average of 1.70 ± 0.01 mm and 0.72 ± 0.04 mm, respectively. On average, tergite 4 is shorter than tergite 3 in the longitudinal direction. The longitudinal lengths of tergite 3 and tergite 4 ranged from 3.24 mm to 3.79 mm and 2.62 mm to 3.28 mm with an average of 3.43 ± 0.17 mm and 2.93 ± 0.18 mm in this order. The longitudinal and transverse lengths of the forewing varied from 7.54 to 7.55 mm and 2.54 mm to 2.59 mm with an average of 7.55 ± 0.00 mm and 2.56 ± 0.01 mm, respectively. The cubital vein distance ranges from 0.43 to 0.54 mm with an average of 0.49 ± 0.03 mm. The cubital index distance b varies from 0.14 mm to 0.17 mm with an average of 0.16 ± 0.01 mm.

Angles A4, B4 and D7 varied from 28.11° to 34.11°, 94.22° to 105.88°, and 85.21° to 92.66° with an average of 31.22 ± 1.62°, 101.64 ± 3.46°, and 88.43 ± 2.06° in that order. The forewing venation angles E9 and G18 ranged from 79.35° to 89.70° and 94.65° to 96.44° with an average of 85.31 ± 2.90° and 95.38 ± 0.50° respectively. Again, angles I10 and I16 varied from 40.19° to 48.29° and 97.90° to 108.62° with an average of 44.86 ± 2.25° and 102.79 ± 2.92°, respectively. Finally, the wing venation angles K19, L13, N23, and O26 ranged from 74.80° to 82.71°, 14.05° to 33.92°, 81.58° to 85.27°, and 32.29° to 39.45° with an average of 78.20 ± 2.18°, 20.87 ± 6.10°, 82.79 ± 1.14°, and 34.92 ± 2.10° in that order.

**Table 2:** Ranges, Means, and Standard Deviations of Samples of Morphometric Characters of *Apis Cerana* F. Samples From Bangladesh

Ruttner No.	Morphometric Characters	Range		Mean	Std. Deviation
		Min.	Max.		
4	Proboscis Length	3.22	4.01	3.61	0.21
5	Femur Length	1.74	2.17	1.98	0.12
6	Tibia Length	2.47	2.68	2.57	0.06
7	Metatarsus Length	1.68	1.72	1.70	0.01
8	Metatarsus Width	0.67	0.80	0.72	0.04
9	Tergite 3 Longitudinal	3.24	3.79	3.43	0.17
10	Tergite 4 Longitudinal	2.62	3.28	2.93	0.18
17	Forewing Longitudinal	7.54	7.55	7.55	0.00
18	Forewing Transversal	2.54	2.59	2.56	0.01
19	Cubital Vein Distance a	0.43	0.54	0.49	0.03
20	Cubital Index Distance b	0.14	0.17	0.16	0.01
21	Forewing Venation Angle A4	28.11	34.11	31.22	1.62
22	Forewing Venation Angle B4	94.22	105.88	101.64	3.46
23	Forewing Venation Angle D7	85.21	92.66	88.43	2.06
24	Forewing Venation Angle E9	79.35	89.70	85.31	2.90
25	Forewing Venation Angle G18	94.65	96.44	95.38	0.50
26	Forewing Venation Angle I10	40.19	48.29	44.86	2.25
27	Forewing Venation Angle I16	97.90	108.62	102.79	2.92
28	Forewing Venation Angle K19	74.80	82.71	78.20	2.18
29	Forewing Venation Angle L13	14.05	33.92	20.87	6.10
30	Forewing Venation Angle N23	81.58	85.27	82.79	1.14
31	Forewing Venation Angle O26	32.29	39.45	34.92	2.10

### 3.1.3 Morphometry Study of *Apis Dorsata* F

To discriminate the species of *Apis dorsata* F. 22 morphometric characters were measured. These characters can be grouped into three main categories (with their Ruttner numbers in brackets): (i) body size including Proboscis length (4), Femur length (5), Tibia length (6), Meta tarsus length (7), Meta tarsus width (8), Tergite 3 longitudinal (9), and Tergite 4 longitudinal (10); (ii) forewing characters such as Fore wing longitudinal (17), Fore wing transversal (18), Cubital vein distance a (19) and Cubital index distance b (20), wing venation angles A4 (21), B4 (22), D7 (23), E9 (24), G18 (25), I10 (26), I16 (27), K19 (28), L13 (29), N23 (30) and O26 (31).

The measurements of these characters were made on 172 worker honey bees, collected from 10 localities in Bangladesh. The means, ranges, and standard deviations for each morphometric character of the honey bees

are indicated in Table 3. The statistical results show that the proboscis length ranged from 4.19 mm to 4.51 mm with an average of 4.40 ± 0.10 mm. The length of the femur and tibia varied from 2.92 to 3.18 mm and from 3.48 mm to 4.12 mm with an average of 3.08 ± 0.07 mm and 3.87 ± 0.18, respectively. The length and width ranged from 2.77 to 2.94 mm and 1.18 mm to 1.28 mm with an average of 2.85 ± 0.05 mm and 1.22 ± 0.03 mm, respectively. On average, tergite 4 is shorter than tergite 3 in the longitudinal direction. The longitudinal lengths of tergite 3 and tergite 4 ranged from 4.10 mm to 4.90 mm and 3.67 mm to 4.90 mm with an average of 4.52 ± 0.22 mm and 4.10 ± 0.37 mm in this order. The longitudinal and transverse lengths of the forewing varied from 12.52 to 12.91 mm and 3.94 to 4.19 mm with an average of 12.72 ± 0.10 mm and 4.06 ± 0.07 mm, respectively. The cubital vein distance ranges from 1.04 to 1.12 mm with an average of 1.08 ± 0.02 mm. The cubital index distance b varies from 0.13 mm to 0.16 mm with an average of 0.15 ± 0.01 mm.

**Table 3:** Ranges, Means, and Standard Deviations of Samples of Samples of Morphometric Characters of *Apis Dorsata* F. Samples from Bangladesh

Ruttner No.	Morphometric Character	Range		Mean	Std. Deviation
		Min.	Max.		
4	Proboscis Length	4.19	4.51	4.40	0.10
5	Femur Length	2.92	3.18	3.08	0.07
6	Tibia Length	3.48	4.12	3.87	0.18
7	Metatarsus Length	2.77	2.94	2.85	0.05
8	Metatarsus Width	1.18	1.28	1.22	0.03
9	Tergite3 Longitudinal	4.10	4.90	4.52	0.22
10	Tergite 4 Longitudinal	3.67	4.90	4.10	0.37
17	Forewing Longitudinal	12.52	12.91	12.72	0.10
18	Forewing Transversal	3.94	4.19	4.06	0.07
19	Cubital Vein Distance a	1.04	1.12	1.08	0.02
20	Cubital Index Distance b	0.13	0.16	0.15	0.01
21	Forewing Venation Angle A4	36.63	39.41	37.71	0.80
22	Forewing Venation Angle B4	72.20	81.28	76.23	2.48
23	Forewing Venation Angle D7	82.74	89.70	86.62	1.90
24	Forewing Venation Angle E9	57.73	66.15	61.34	2.32
25	Forewing Venation Angle G18	97.70	101.10	99.77	0.97
26	Forewing Venation Angle I10	33.60	35.95	34.94	0.65
27	Forewing Venation Angle I16	81.61	90.43	84.72	2.66
28	Forewing Venation Angle K19	67.43	74.96	71.68	2.07
29	Forewing Venation Angle L13	11.01	14.46	13.17	1.01
30	Forewing Venation Angle N23	67.93	72.99	70.00	1.42
31	Forewing Venation Angle O26	31.44	40.21	36.27	2.39

Angles A4, B4 and D7 varied from 36.63° to 39.41°, 72.20° to 81.28° and 82.74° to 89.70° with an average of 37.71 ± 0.80°, 76.23 ± 2.48°, and 86.62 ± 1.90° in that order. The forewing venation angles E9 and G18 ranged from 57.73° to 66.15° and 97.70° to 101.10° with an average of 61.34 ± 2.32° and 99.77 ± 0.97° respectively. Again, angles I10 and I16 varied from 33.60° to 35.95° and 81.61° to 90.43° with an average of 34.94 ± 0.65° and 84.72 ± 2.66°, respectively. Finally, the wing venation angles K19, L13, N23, and O26 ranged from 67.43° to 74.96°, 11.01° to 14.46°, 67.93° to 72.99° and 31.44° to 40.21° with an average of 71.68 ± 2.07°, 13.17 ± 1.01°, 70.00 ± 1.42°, and 36.27 ± 2.39° in that order. Taking into account the mean and standard deviations of the morphometric characteristics of *A. mellifera* L., *Apis cerana* F., and *Apis dorsata* F. of Bangladesh, the angles of the forewing venation exhibit the highest variability. Specifically, the difference between the minimum and maximum value of the venation angles B4, E9, L13, and G18 is greater than 10. Even the other forewing venation angles have greater variability than morphometric characters related to body size or forewing distances. This indicates their higher discriminatory capacity to differentiate *A. mellifera* L., *A. cerana* F., and *A. dorsata* F. species.

This supports the results derived by (Hepburn and Radloff, 1998; Radloff and Hepburn, 1997b). There are different opinions as to why such variability is observed. This could reflect distinct species or geographical races an adaptation of ecotypes derived from adjacent populations (Kerr, 1992), and/or the product of asynchronous gene fluctuations within an essentially contiguous metapopulation that might not be considered as separate subspecies (Radloff and Hepburn, 1997 a; Hepburn and Crewe, 1991; Ruttner, 1988/1992).

One of the interesting results of this study was that, unlike the previous study of Amssalu et al. (2004), significant differences were observed in the morphometric characters related to body size. Among these morphometric characters, proboscis length, the longitudinal distance of forewing, tergite 3, and tergite 4 show variability, supporting the result derived by (Kassaye, 1990). However, further analysis is required considering the species label to understand their contribution to the discrimination of the *A. mellifera* L., *A. cerana* F., and *A. dorsata* F. species.

### 3.2 Classification of Honeybee Samples in Bangladesh

The species of colonies collected from different localities in Bangladesh are shown in Table 4. According to the table, these colonies were spread throughout the sampling regions in Rajshahi, Satkhira, Jhenaidah, Chattogram, Gazipur, Tangail, and Bogura. *Apis mellifera* L. was found in eight colonies in Rajshahi, Gazipur, Tangail, and Satkhira. *Apis cerana* F. was found in eight colonies from Satkhira, Jhenaidah, and Chattogram. And *Apis dorsata* F. was found in eight colonies from Satkhira and Bogura.

Among the four species found in Bangladesh, *A. mellifera* L. was the most abundant species for its higher honey yield. Samples of this species were found in all localities from which the samples were collected. *A. mellifera* L. originates from the central and northeastern Mediterranean. The mild, humid climate and rich flora of Bangladesh, similar to that of the Mediterranean region (Ruttner, 1988), favors the needs of honey bees. In addition to that, this species can defend itself successfully against insect pests, while also being extremely gentle in its behavior toward beekeepers (Lane, 2019b). During the dearth period, *A. mellifera* L. is particularly adapted by adapting the worker population to nectar availability (Canada Agriculture and Food Museum, 2015). It relies on these rapid adjustments of population levels to rapidly expand worker bee populations after nectar becomes available in the spring and again to rapidly cut off brood production when nectar ceases to be available in quantity. It meets periods of large nectar with large worker populations and, consequently, stores large quantities of honey and pollen during those periods. It is resistant to some diseases and parasites that can debilitate hives of other subspecies. It is long-distance foragers with a very smooth frequency curve of waggle runs (Ruttner, 1988). All these characteristics made them popular among beekeepers in Bangladesh.

*Apis cerana* F., the "Eastern Honeybee" is the exact equivalent, in the eastern part of the Old World, of its occidental sister species, *A. mellifera* L. (Butler, 1954). It has an equally wide area of distribution with a similar capacity for a broad spectrum of adaptations. *A. cerana* F. is a species of honey bee native to South, Southeast and East Asia. *A. cerana* F. shows the same ability of adaptation to a very dry, semi-desert-like environment in the mountains or plains. *A. cerana* F. encompasses a wide range of climatic zones, including moist tropical rainforests, wet-dry tropical savannas, mid-latitude steppes, dry mid-latitude grasslands, moist continental deciduous forests, and taigas (Radloff et al., 2010).

### 3.3 Correlation Among the Morphometric Characters

#### 3.3.1 Correlation Among the Morphometric Characters of *A. Mellifera* L. In Bangladesh

A statistically significant correlation ( $p < 0.01$ ) can be found between proboscis length and the femur length, tibial length and the width of the metatarsus, and forewing venation angles B4 and E9.

A highly positive correlation can be observed between the forewing venation angle B4 and width of the E9 (0.98), the length and metatarsus (0.90) and the length of the proboscis of the length and femur (0.85). This correlation indicates that an increase in one character size can result in an increase in the other character and vice versa.

A highly negative correlation can be observed between the length of the

tibia and the longitudinal length of the forewing (-0.83) and metatarsus width and the longitudinal length of the forewing (-0.83). This correlation

indicates that an increase in one character size can decrease the other character size, and vice versa.

**Table 4: Classification of Honeybee Species of Bangladesh**

Colony ID	Locality	Species
M1	Charghat, Rajshahi	<i>Apis mellifera</i> L.
M2	Charghat, Rajshahi	<i>Apis mellifera</i> L.
M3	Sreepur, Gazipur	<i>Apis mellifera</i> L.
M4	Horinogor, Satkhira	<i>Apis mellifera</i> L.
M5	Kalaroa, Satkhira	<i>Apis mellifera</i> L.
M6	Shyamnagar, Satkhira	<i>Apis mellifera</i> L.
M7	Shyamnagar, Satkhira	<i>Apis mellifera</i> L.
M8	Mirzapur, Tangail	<i>Apis mellifera</i> L.
C1	Atulia, Satkhira	<i>Apis cerana</i> F.
C2	Atulia, Satkhira	<i>Apis cerana</i> F.
C3	Kotchandpur, Jhenaidah	<i>Apis cerana</i> F.
C4	Kotchandpur, Jhenaidah	<i>Apis cerana</i> F.
C5	Kotchandpur, Jhenaidah	<i>Apis cerana</i> F.
C6	Khulsi, Chattogram	<i>Apis cerana</i> F.
C7	Khulsi, Chattogram	<i>Apis cerana</i> F.
C8	Khulsi, Chattogram	<i>Apis cerana</i> F.
D1	Atulia, Satkhira	<i>Apis dorsata</i> F.
D2	Atulia, Satkhira	<i>Apis dorsata</i> F.
D3	BoguraSadar, Bogura	<i>Apis dorsata</i> F.
D4	BoguraSadar, Bogura	<i>Apis dorsata</i> F.
D5	BoguraSadar, Bogura	<i>Apis dorsata</i> F.
D6	Dhunut, Bogura	<i>Apis dorsata</i> F.
D7	Dhunut, Bogura	<i>Apis dorsata</i> F.
D8	Dhunut, Bogura	<i>Apis dorsata</i> F.

### 3.3.2 Correlation Among The Morphometric Characters of *A. Cerana* F. In Bangladesh

A statistically significant correlation ( $p < 0.01$ ) can be found between many morphometric characters. The length is significantly correlated with the angles of venation of the forewing A4, B4, E9, L13 and N23. The length is significantly correlated with the length of the tibia, the length of the metatarsus, the longitudinal length of tergite 3, the longitudinal length of the forewing, the transverse length of the forewing, cubital index distance b, the venation angles B4, E18, I10, I16, K19, and O26. The length is significantly correlated with the transverse length of the forewing, cubital index distance b, the forewing venation angles B4, G18, I10, I16, K19, L13 and N23. The length is significantly correlated with the longitudinal length of tergite 3, the longitudinal length of tergite 4, the longitudinal length of the forewing, the transverse length of the forewing, cubital vein of the distance a, the cubital index distance b, the angles of venation D7 and O26. The width is significantly correlated with the longitudinal length of the tergite 4, the cubital vein distance a, and the angle of venation of the forewing D7. The longitudinal length of tergite 3 is significantly correlated with the longitudinal length of tergite 4, the longitudinal length of the forewing, the distance of the cubital vein distance a, the cubital index distance b, the venation angles of the forewing D7 and O26. The longitudinal length of tergite 4 is significantly correlated with the longitudinal length of the forewing, cubital vein distance a, and forewing venation angles D7 and O26. The longitudinal length of the forewing is significantly correlated with the transverse length of the forewing, the cubital vein distance a, the cubital distance index b, the forewing venation angles D7 and O26. The transverse length of the forewing is significantly correlated with the cubital index distance b, the forewing venation angles E18, I10, I16, K19, and O26. The cubital vein distance a is significantly correlated with the forewing venation angles D7 and O26. The distance of the cubital index b is significantly correlated with the venation angles D7, I10, I16, K19 and O26. The angle of venation of the forewing A4 is significantly correlated with forewing B4 and E9. The angle of the venation B4 is significantly correlated with forewing venation of the forearm E9, E18, I10, I16, K19, L13, and N23. The angle of venation of the forewing D7 is significantly correlated with the angle of venation of the forewing O26. The angle of venation of the forewing E9 is significantly correlated with forewing venation angles E18, I10, L13 and N23. The angle of venation angle E18 is significantly correlated with the venation of the forearm I10, I16, K19, L13, and N23. The angle of forewing venation I10 is significantly correlated with forewing venation angles I16, K19, L13, and N23. The

angle of venation of the forewing I16 is significantly correlated with forewing venation angles K19, L13 and N23. The angle of venation of the forewing K19 is significantly correlated with the angle of venation of the forewing L13 and N23. The angle of venation of the forewing L13 is significantly correlated with the angle of venation of the forewing N23.

Many highly positive correlations can be seen among the morphometric characteristics of *A. cerana* F. The length is positively correlated with the forewing venation angles B4 (0.90) and E9 (0.96). The length of the femur is positively correlated with the length of the tibia (0.92), the longitudinal length of the forewing (17), the forewing venation angles E18 (0.89), I16 (0.93) and K19 (0.91). The length of the tibia is positively correlated with forewing venation angles E18 (0.99), I16 (1.00), K19 (0.99), L13 (0.93), and N23 (0.94). The length of the metatarsus is positively correlated with the longitudinal length of tergite 3 (0.99), the longitudinal length of tergite 4 (0.96), the transverse length of the forewing (0.90), the cubital index distance b (0.95), the venation angles D7 (0.98) and O26 (0.99). Metatarsus width is positively correlated with cubital vein distance a (0.91). The longitudinal length of tergite 3 is positively correlated with the longitudinal length of tergite 4 (0.94), the transverse length of the forewing (0.93), the cubital index distance b (0.97), the forewing venation angles D7 (0.97) and O26 (0.99). The longitudinal length of tergite 4 is positively correlated with the cubital index distance b (0.83), the angle of venation of the forewing D7 (0.99) and O26 (0.96). The longitudinal length of the forewing is positively correlated with the cubital vein distance a (0.96). The transverse length of the forewing is positively correlated with cubital index distance b (0.99), forewing venation angle D7 (0.82), I10 (0.89) and O26 (0.91). The cubital index distance b is positively correlated with the venation angle D7 (0.89), I10 (0.84) and O26 (0.91). The angle of the A4 venation of the forewing of venation is positively correlated with the angle of venation of the forewing L13 (0.83) and N23 (0.82). The of the forewing of venation B4 is positively correlated with the angle of venation of the forewing E9 (0.99) and I10 (0.94). The forewings of the venation angle D7 is positively correlated with the angle of venation of the forewings of venation angle O26 (0.98). The angle of forewing venation E9 is positively correlated with the angle of forewing venation I10 (0.87). The of the forewing of G18 is positively correlated with the angle of venation of the forewing I16 (0.99), K19 (0.99), L13 (0.96) and N23 (0.97). The of the forewing I16 is positively correlated with the angle of venation of the forewing K19 (0.99), L13 (0.93) and N23 (0.93). The of the K19 the forewings of venation angle is positively correlated with the of the forewings of the L13 (0.94) and N23 (0.95). The of the forewing L13 is

positively correlated with the angle of venation of the forewing N23 (0.99). These highly positive correlations indicate that an increase in one character size can increase the other character, and vice versa.

Some highly negative correlations can be seen among the morphometric characters of *A. cerana* F. The length is negatively correlated with the forewing venation angles A4 (-0.97), L13 (-0.85), and N23 (-0.84). The length of the femur is negative correlated with metatarsus (-0.89), longitudinal length of tergite 3 (-0.92), transverse length of forewing (-0.97), cubital index distance b (-0.97), angle of venation of the forewing (-0.97), the angle of venation of the forewing venation angles D7 (-0.80) and I10 (-0.87). The length of the tibia is negatively correlated with the transverse length of the forewing (-0.91), the cubital index distance b (-0.85), the angle of venation of the forewing D7 (-0.88), E9 (-0.81), and I10 (-0.97). The length of the metatarsus is negatively correlated with the longitudinal length of the forewing (-0.96) and the cubital vein a (-0.94). The width of the metatarsus is negatively correlated with the longitudinal length of tergite 4 (-0.90) and the forewing venation angle D7 (-0.86). The longitudinal length of tergite 3 is negatively correlated with the longitudinal length of the forewing (-0.97) and the distance from the cubital vein a (-0.92). The longitudinal length of tergite 4 is negatively correlated with the longitudinal length of the forewing (-0.91) and the cubital vein distance a (-0.97). The longitudinal length of the forewing is negatively correlated with the transversal length of the forewing (-0.90), the cubital index distance b (-0.94) and the forewing venation angle D7 (-0.94). The transverse length of the forewing is negatively correlated with the venation angle E18 (-0.87), I16 (-0.92), and K19 (-0.89). The distance of the cubital vein a is negatively correlated with the cubital index distance b (-0.83), the angle of venation of the forewing D7 (-0.97), and O26 (-0.95). The radius of the cubic index b is negatively correlated with the forewing venation angles G18 (-0.81), I16 (-0.86) and K19 (-0.84). The angle of venation of the forewing of venation A4 is negatively correlated with the forewing B4 (-0.84), and E9 (-0.91). Forewing venation angle B4 is negatively correlated with forewing venation angles E18 (-0.92), I16 (-0.87), K19 (-0.89), L13 (-0.97), and N23 (-0.97). The angle of forewing venation E9 is negatively correlated with forewing venation angles E18 (-0.85), K19 (-0.83), L13 (-0.95) and N23 (-0.94). The forewings of the venation angle E18 is negatively correlated with the of the forewings of venation angle I10 (-0.98). The of the forewing I10 is negatively correlated with the angle of venation of the forewing I16 (-0.97), K19 (-0.98), L13 (-0.93) and N23 (-0.94). These highly negative correlations indicate that an increase in one character size can decrease the other character, and vice versa.

### 3.3.3 Correlation Among the Morphometric Characters of *A. dorsata* F. in Bangladesh

A statistically significant correlation ( $p < 0.01$ ) can be found between many morphometric characters. The length of the femur is significantly correlated with the length of the tibia, the length, the metatarsus length, metatarsus width, the longitudinal length of the forewing, the transverse length of the forewing, the angle of venation of the forewing B4, D7, E9, I16, K19 and N23. The length is significantly correlated with the length of the metatarsus, the width of the metatarsus, the longitudinal length of the forewing, the angle of the venation of the the transverse length of the forewing, forewing B4, D7, E9, I16, K19 and N23. The length is significantly correlated with the width of the metatarsus, the longitudinal length of the forewing, the transversal length of the forewing venation angles B4, D7, E9, and I16. The width is significantly correlated with the longitudinal length of the forewing, transversal length of the forewing, forewing venation angle of the forewing B4, D7, E9 and I16. The longitudinal length of tergite 3 is significantly correlated with the forewing venation angles A4, E18, I10, L13, and O26. The longitudinal length of tergite 4 is significantly correlated with the distance between the cubital veins between the distance a, cubital index b, forewing venation angle A4, E18, and O26. The longitudinal length of the forewing is significantly correlated with the transverse length of the forewing venation angle B4, D7, E9, and I16. The transverse length of the forewing is significantly correlated with the venation angles B4, D7, E9 and I16. The distance of the cubital vein a is significantly correlated with the distance of the cubital index b, forewing venation angle I16, K19, and N23. Cubital index distance b is significantly correlated with venation angle A4, E18, K19, and O26. The angle of venation of the forewing of venation A4 is significantly correlated with the angle of venation of the forewing E18 and O26. The forewing of venation B4 is significantly correlated with the angle of venation of the forewing D7, E9, I16, and N23. The of the forewing E9 is significantly correlated with the angle of venation of the forewing E9 and L13. The angle of the venation E18 is significantly correlated with forewing venation of the forearm I16 and N23. The of the forewing I10 is significantly correlated with the angle of venation of the forewing O26. The of the forewing I16 is significantly correlated with the angle of venation of the forewing L13 and O26. The of

the forewing of venation K19 is significantly correlated with the angle of venation of the forewing K19 and N23. The of the forewings of venation angle K19 is significantly correlated with the of the forewings of venation angle N23.

Highly positive correlations can be seen between the morphometric characters of *A. dorsata* F. Femur length is positively correlated with tibia length (0.99), longitudinal length of the forewing (0.97) and forewing venation angle K19 (0.87). The length is positively correlated with the longitudinal length of the forewing (0.96) and the a cubital vein (0.80). The length is positively correlated with the width of the metatarsus (1.00), the transverse length of the forewing (0.99), the forewing venation angles of the forewing B4 (0.99), D7 (0.96), E9 (0.99), I16 (0.93) and N23 (0.80). The width is positively correlated with the transverse length of (0.99), forewing venation angles of the forewing B4 (0.99), D7 (0.95), E9 (0.99), I16 (0.94) and N23 (0.82). Tergite 3 longitudinal length is positively correlated with the tergite 4 longitudinal length of tergite 4 (0.8), forewing venation angles A4 (0.90), I10 (0.99) and L13 (0.95). The longitudinal length of tergite 4 is positively correlated with the distance from the cubital vein a (0.85) and the angle of forewing vein A4 (0.98). The transverse length of the forewing is positively correlated with the venation angles B4 (0.99), D7 (0.98) E9 (0.98), and I16 (0.90). The distance of the cuboidal vein a is positively correlated with the angle of venation of the forewing K19 (0.99). The cubital index distance b is positively correlated with the forewing venation angle E18 (0.92), N23 (0.82), and O26 (0.84). The angle of forewing venation A4 is positively correlated with the angle of forewing venation I10 (0.83). The angle of venation of the forewing of venation B4 is positively correlated with the angle of venation of the forewing D7 (0.93), E9 (0.99), I16 (0.96) and N23 (0.86). The of the forewing of venation D7 is positively correlated with the angle of venation of the forewing E9 (0.91), I10 (0.81) and L13 (0.90). The of the forewing is positively correlated with the angle of venation of the forewing I16 (0.97) and N23 (0.88). The angle of forewing venation E18 is positively correlated with the angle of forewing venation O26 (0.98). The of the forewing I10 is positively correlated with the angle of venation of the forewing L13 (0.99). The of the forewing I16 is positively correlated with the angle of venation of the forewing N23 (0.97). These highly positive correlations indicate that an increase in one character size can increase the other character, and vice versa.

There are a few negative correlations among the morphometric characteristics of *A. dorsata* F. Femur length is negatively correlated with metatarsus length (-0.97), metatarsus width (-0.97), transverse length of the forewing (-0.95), forewing venation angle B4 (-0.98), D7 (-0.87), E9 (-0.99), I16 (-0.97), and N23 (-0.90). The length is negatively correlated with the length of the forewing (-0.94), the angle of venation of the forewing B4 (-0.98), D7 (-0.85), E9 (-0.98) I16 (-0.98), and N23 (-0.91). The length is negatively correlated with the longitudinal length of the forewing (-0.97). The width is negatively correlated with the longitudinal length of the forewing (-0.98). Tergite 3 longitudinal length is negatively correlated with the forewing venation angles E18 (-0.89) and O26 (-0.95). The longitudinal length of tergite 4 is negatively correlated with the distance of the cubital index distance b (-0.97), the of the flange of venation angle E18 (-0.97) and O26 (-0.95). The longitudinal length of the forewing is negatively correlated with the transverse length of the forewing (-0.99), forewing B4 (-0.98), D7 (-0.96), E9 (-0.97), and I16 (-0.90). The distance of the cubital vein a is negatively correlated with the cubital index b (-0.93), the angle of the venation forewing I16 (-0.87) and N23 (-0.96). The cubital index distance b is negatively correlated with the forewing venation angle A4 (-0.92) and K19 (-0.86). The angle of forewing venation A4 is negatively correlated with the angle of forewing venation E18 (-0.99) and O26 (-0.97). The angle of venation of the forewing of E9 is negatively correlated with the angle of venation of the forewing K19 (-0.82). The of the forewing E18 is negatively correlated with the angle of venation of the forewing I10 (-0.92). The of the forewing of venation angle I10 is negatively correlated with the angle of venation of the angle of venation of the forewing venation O26 (-0.91). The of the the forewing of venation I16 is negatively correlated with the forewing K19 (-0.93). The of the forewing of K19 is negatively correlated with the angle of venation of the forewing N23 (-0.98). The of the forewing L13 is negatively correlated with the forewing O26 (-0.83). These highly negative correlations indicate that an increase in one character size can decrease the other character, and vice versa.

### 3.4 Analysis of Variance Among the Morphometric Characteristics of Honey Bee Species In Bangladesh

The result of the analysis of variance for each of the morphometric characters used for the discrimination of honey bee species is shown in Table 5. The result reveals a highly significant variation among all the morphometric characters, as denoted by the F and the tabulated F value.



Analysis of variance indicated that the collected samples were not from the same species. This means that there are more than one species in Bangladesh. The variability between species also indicates that morphometric characters are suitable tools for species identification.

However, ANOVA only indicates the high variance among the morphometric characters. Supplemental analyzes that use these characters are necessary to understand how their contributions discriminate between honeybee species in Bangladesh

**Table 5: Analysis of Variance of The Morphometric Characters of Honey Bee Samples in Bangladesh**

Category	Morphometric Characters	Sum of Squares	df	Mean Square	F Value	Tab. F	
Body Size	Proboscis Length	Between Groups	8.932	2	4.466	56.153	0
		Within Groups	1.67	21	0.08	-	-
		Total	10.603	23	-	-	-
	Femur Length	Between Groups	4.81	2	2.405	251.019	0
		Within Groups	0.201	21	0.01	-	-
		Total	5.012	23	-	-	-
	Tibia Length	Between Groups	7.324	2	3.662	166.471	0
		Within Groups	0.462	21	0.022	-	-
		Total	7.786	23	-	-	-
	Metatarsus Length	Between Groups	6.185	2	3.093	410.558	0
		Within Groups	0.158	21	0.008	-	-
		Total	6.343	23	-	-	-
	Metatarsus Width	Between Groups	1.008	2	0.504	112.759	0
		Within Groups	0.094	21	0.004	-	-
		Total	1.102	23	-	-	-
	Tergite 3 Longitudinal	Between Groups	4.841	2	2.42	50.335	0
		Within Groups	1.01	21	0.048	-	-
		Total	5.851	23	-	-	-
Tergite 4 Longitudinal	Between Groups	5.635	2	2.818	34.126	0	
	Within Groups	1.734	21	0.083	-	-	
	Total	7.369	23	-	-	-	
Forewing Distance	Forewing Longitudinal	Between Groups	116.208	2	58.104	6474.005	0
		Within Groups	0.188	21	0.009	-	-
		Total	116.396	23	-	-	-
	Forewing Transversal	Between Groups	9.711	2	4.855	1598.373	0
		Within Groups	0.064	21	0.003	-	-
		Total	9.775	23	-	-	-
	Cubital Vein Distance a	Between Groups	1.771	2	0.886	1441.893	0
		Within Groups	0.013	21	0.001	-	-
		Total	1.784	23	-	-	-
	Cubital Index Distance b	Between Groups	0.041	2	0.02	23.577	0
		Within Groups	0.018	21	0.001	-	-
		Total	0.059	23	-	-	-
Category	Morphometric Characters	Sum of Squares	df	Mean Square	F Value	Tab. F	
Forewing Angles	Wing Venation Angle A4	Between Groups	372.805	2	186.402	125.008	0
		Within Groups	31.314	21	1.491	-	-
		Total	404.118	23	-	-	-
	Wing Venation Angle B4	Between Groups	5950.39	2	2975.195	272.939	0
		Within Groups	228.913	21	10.901	-	-
		Total	6179.302	23	-	-	-
	Wing Venation Angle D7	Between Groups	634.187	2	317.093	67.458	0
		Within Groups	98.712	21	4.701	-	-
		Total	732.899	23	-	-	-
	Wing Venation Angle E9	Between Groups	5548.55	2	2774.275	222.926	0
		Within Groups	261.341	21	12.445	-	-
		Total	5809.892	23	-	-	-
	Wing Venation Angle G18	Between Groups	130.227	2	65.114	11.638	0
		Within Groups	117.489	21	5.595	-	-
		Total	247.716	23	-	-	-
	Wing Venation Angle I10	Between Groups	1014.297	2	507.149	115.586	0
		Within Groups	92.14	21	4.388	-	-
		Total	1106.437	23	-	-	-
	Wing Venation Angle I16	Between Groups	1360.233	2	680.116	89.538	0
		Within Groups	159.512	21	7.596	-	-
		Total	1519.744	23	-	-	-
	Wing Venation Angle K19	Between Groups	291.428	2	145.714	29.761	0
		Within Groups	102.819	21	4.896	-	-
		Total	394.248	23	-	-	-
	Wing Venation Angle L13	Between Groups	240.332	2	120.166	3.505	0.049
		Within Groups	719.956	21	34.284	-	-
		Total	960.287	23	-	-	-
Wing Venation Angle N23	Between Groups	1265.216	2	632.608	247.328	0	
	Within Groups	53.713	21	2.558	-	-	
	Total	1318.929	23	-	-	-	
Wing Venation Angle O26	Between Groups	22.537	2	11.268	1.184	0.326	
	Within Groups	199.896	21	9.519	-	-	
	Total	222.433	23	-	-	-	

### 3.5 Analysis on The Morphometric Characteristics Of Honey Bees In Bangladesh

To determine whether the principal component analysis is suitable for our data or not, we performed the Bartlett sphericity test. The results are shown in Table 6.

Table 6: Bartlett's Test of Sphericity	
Approx. Chi-Square	2012.269
Degrees of Freedom (df)	231
Significance	0.0001

According to the table, the significance value of  $p < 0.0001$  denotes that the

analysis of the principal components gives us a significant result in terms of dimensional reduction. This means that the extracted components can be used to classify the honey bee species.

Table 7 shows the eigenvalues generated for each component. Here, component 1 contributes about 63% variance shown in the collected samples. Component 2 captures around 20% of the variance. To capture around 90% of the variance, 3 components are required. These 3 components have an eigenvalue greater than 1. Components 16 and onward have almost insignificant eigenvalues, denoting their inadequate contribution to the variance.

To determine the inflection point using which we can further confirm the number of components to be extracted, a scree plot was generated using the eigenvalues. The generated scree plot can be seen in Figure 3.

Table 7: Generated Eigenvalues for Each Component and Their Contribution to the Variance			
Component	Generated Eigenvalues		
	Eigenvalue	% of Variance	Cumulative %
1	13.842	62.918	62.918
2	4.492	20.417	83.334
3	1.39	6.316	89.651
4	0.886	4.026	93.677
5	0.558	2.538	96.214
6	0.301	1.368	97.582
7	0.195	0.887	98.469
8	0.15	0.68	99.149
9	0.074	0.336	99.486
10	0.05	0.227	99.713
11	0.032	0.146	99.859
12	0.018	0.084	99.942
13	0.008	0.038	99.981
14	0.003	0.014	99.995
15	0.001	0.005	99.999
16	0.0	0.001	100
17	5.13E-05	0.0	100
18	4.17E-07	1.90E-06	100
19	9.82E-08	4.46E-07	100
20	2.45E-08	1.11E-07	100
21	1.67E-08	7.57E-08	100
22	5.44E-09	2.47E-08	100

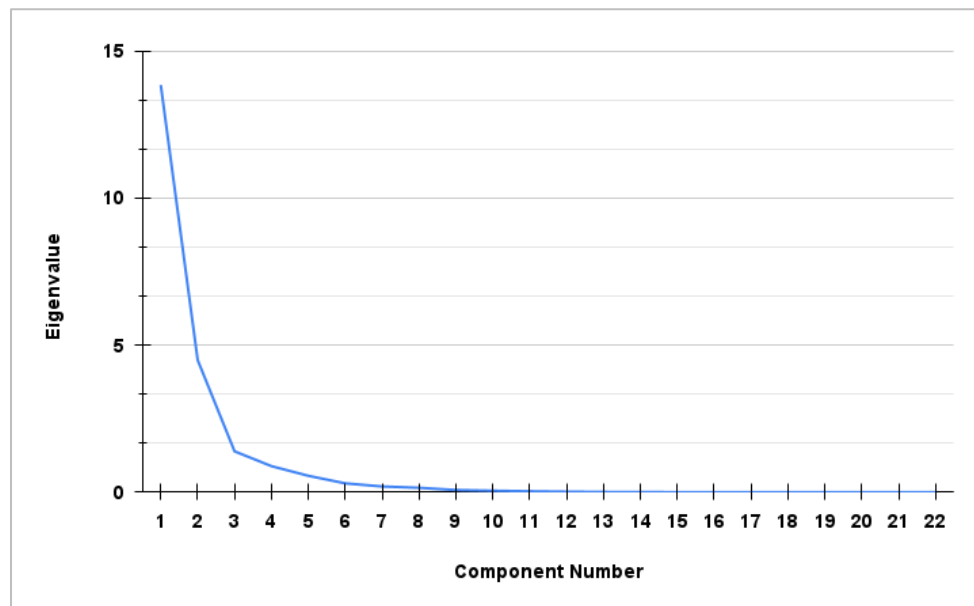


Figure 3: Scree plot showing the eigenvalues of each principal component

According to the scree plot, the inflection point is shown in component 3. This means that at least 3 components should be extracted.

The axis was rotated using nonorthogonal direct oblimin rotation, considering the high correlation among the morphometric characters. The loading of each morphometric character is shown in Table 8.

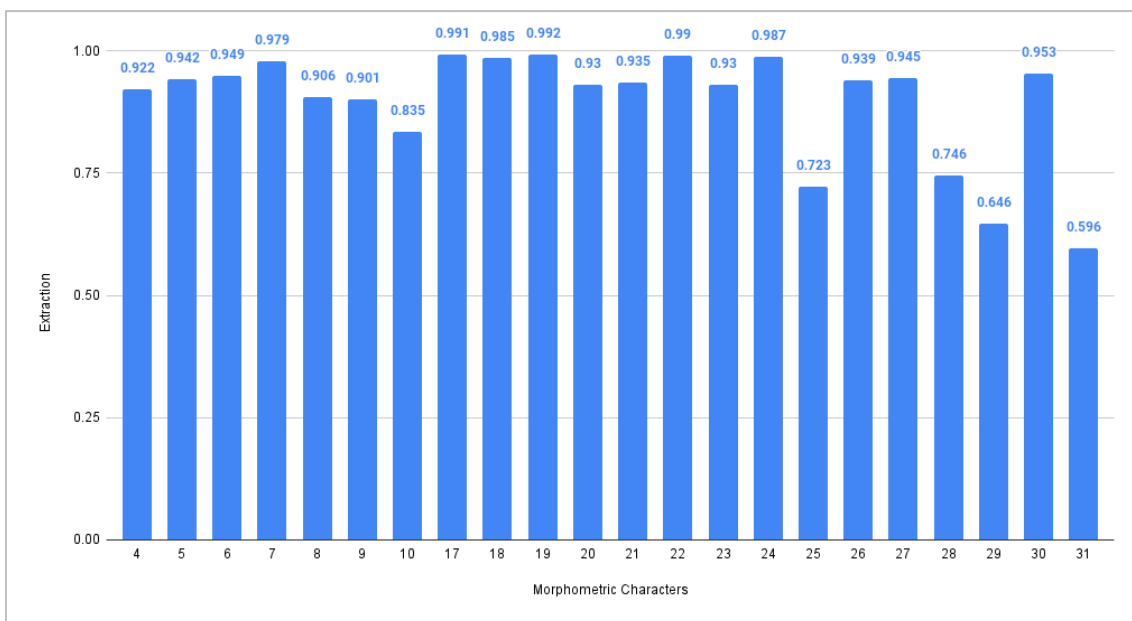
Among morphometric characters: femur length, tibia length, metatarsus length, metatarsus width, tergite 3 and 4, longitudinal and transverse length of the forewing, cubital vein distance a, cubital index distance b, forewing venation angle A4, B4, D7, E9, G18, I10, I16, K19, N23, and L13 were associated with component 1. The length of the the prastic, the length of the femur, the width of the metatarsus, the longitudinal length of tergite

3 and 4, the distance of the cubital index b, forewing venation angle A4, B4, D7, E9, I10 and I16 were associated with component 2. Finally, the cubital

index distance b, the forewing venation angle G18, L13, and O26 were associated with component 3.

**Table 8:** Rotation Component Matrix Generated Using Direct Oblimin Rotation Method

Morphometric Characters	Component		
	1	2	3
Proboscis Length	-	0.95	-
Femur Length	0.896	0.373	-
Tibia Length	0.96	-	-
Metatarsus Length	0.986	-	-
Metatarsus Width	0.811	0.498	-
Tergite 3 Longitudinal	0.775	0.544	-
Tergite 4 Longitudinal	0.72	0.54	-
Forewing Longitudinal	0.984	-	-
Forewing Transversal	0.978	-	-
Cubital Vein Distance a	0.995	-	-
Cubital Index Distance b	-0.41	0.725	0.486
Wing Venation Angle A4	0.894	-0.369	-
Wing Venation Angle B4	-0.904	0.408	-
Wing Venation Angle D7	-0.517	0.813	-
Wing Venation Angle E9	-0.893	0.423	-
Wing Venation Angle G18	0.708	-	0.419
Wing Venation Angle I10	-0.851	0.462	-
Wing Venation Angle I16	-0.766	-0.598	-
Wing Venation Angle K19	-0.822	-	--
Wing Venation Angle N23	-0.936	-	-
Wing Venation Angle L13	-0.477	-	0.602
Wing Venation Angle O26	-	-	0.711



**Figure 4:** Communalities of the Morphometric Characteristics of Honey Bee Samples

Based on the extraction values of the morphometric characters (Figure 4), the cubital vein distance a, the longitudinal length of the forewing, and the forewing venation angle B4 contribute the most to the variance of the morphometric characters analyzed. Therefore, these characters can be considered to discriminate between the honey bee species in Bangladesh.

### 3.6 Classification Of Honey Bee Species Using The Selected Morphometric Characters

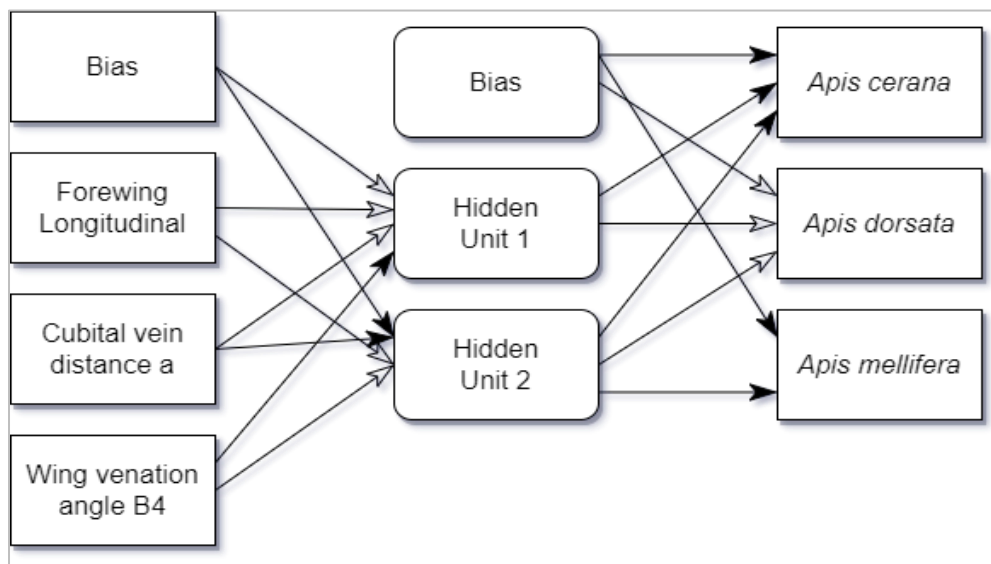
The observed samples were classified using a multilayer perceptron with a single hidden layer to determine whether the 3 selected morphometric characters contribute to the discrimination capability. The number of nodes in the input layer was equal to the number of characters considered for classification and bias. The number of nodes in the output layer was equal to the number of species. The number of nodes in the hidden layer

was 3. 75% of the samples were used to train the perceptron and 25% of the samples were used to test the accuracy of the multilayer perceptron. Figure 5 shows the multilayer perceptron.

The overall accuracy of the system considering the 3 morphometric characters was 100%. This shows that the three-selected morphometric characters can accurately classify the honey bee species, reducing the overall computation required to discriminate among the species.

### 3.7 Morphometric Characteristics In Honey Bee Species Of Bangladesh

Three species of honeybee were found in Bangladesh. The means and standard deviations of the morphometric characters used in the discriminant functions of these species are shown in Table 9.



**Figure 5:** The architecture of the multilayer perceptron. Here, the hollowed arrow denotes a negative synaptic weight, whereas the filled arrow denotes a positive synaptic weight.

**Table 9:** Comparison of the Means and Standard Deviations of The Morphometric Characteristics of Each Honey Bee Species (Measurements in Mm, Angles in Degree)

Ruttner No.	<i>Apis mellifera</i> L.		<i>Apis cerana</i> F.		<i>Apis dorsata</i> F.	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
17	8.83	0.13	7.55	0.00	12.72	0.10
19	0.52	0.02	0.49	0.03	1.08	0.02
22	114.06	3.82	101.64	3.46	76.23	2.48

For *A. mellifera* L. samples, the longitudinal length of the forewing ranges from 8.67 to 9.08mm with an average of  $8.83 \pm 0.13$ mm. The cubital vein distance a ranges from 0.43 mm to 0.54 mm with an average of  $0.49 \pm 0.03$  mm. The angle of forewing venation B4 ranges from  $109.5^\circ$  to  $120.57^\circ$  with an average of  $114.06 \pm 3.82^\circ$ . Among the three species considered, *A. mellifera* L. has the largest forewing venation angle B4.

For *A. cerana* F. samples, the longitudinal length of the forewing ranges from 7.54 mm to 7.55 mm with an average of 7.55 mm. The cubital vein distance a ranges from 0.43 mm to 0.54 mm with an average of  $0.49 \pm 0.03$  mm. The angle of forewing venation B4 ranges from  $94.22^\circ$  to  $105.88^\circ$  with an average of  $101.64 \pm 3.46^\circ$ . Among the three species considered, *A. cerana* F. has the shortest longitudinal length of the forewing and cubital vein distance a.

For *A. dorsata* F. samples, the longitudinal length of the forewing ranges from 12.52 mm to 12.91 mm with an average of  $12.72 \pm 0.10$  mm. The cubital vein distance a ranges from 1.04 mm to 1.12 mm with an average of  $1.08 \pm 0.02$  mm. The angle of forewing venation B4 ranges from  $72.20^\circ$  to  $81.28^\circ$  with an average of  $76.23 \pm 2.48^\circ$ . Among the three species considered, *A. dorsata* F. has the longest longitudinal length of the forewing and the cubital vein distance a. However, it has the smallest forewing venation angle B4 than the other species.

#### 4. CONCLUSION

This is the first study in Bangladesh on the availability of *A. mellifera* L, *A. cerana* F., and *A. dorsata* F. species on a morphometric basis. This study identifies and differentiates three species of honeybee found in Bangladesh, providing beneficial information on honeybee species and their taxonomy. A strong correlation was observed among the angles of the sizes of the venation of the forewing and body parts. ANOVA showed significant variance among the morphometric characters. These results justify the use of morphometric characteristics for the classification of honey bee species. Multivariate analysis was performed to determine the important morphometric characteristics for the discrimination of honeybee species. Among the 22 measured morphometric characters, only three can be used to successfully discriminate the *A. mellifera* L., *A. cerana* F., and *A. dorsata* F. species in Bangladesh. This study also underscored the importance of more research in this field focused on collecting and analyzing more samples from more location, incorporating additional morphometric characters, such as pigmentation. In addition, molecular analysis methods can be performed for further confirmation of the species. At first glance, this study represents a notable advancement in

understanding the diversity of honeybee species in Bangladesh and offers valuable insights to improve the apiculture sector in Bangladesh.

#### ACKNOWLEDGEMENT

The authors acknowledged the Ministry of Science and Technology, Peoples Republic of Bangladesh for partial funding of this research.

#### DATA AVAILABILITY

Data are available from the corresponding author on reasonable request.

#### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to publish this article.

#### REFERENCES

- Abrol, D. P., 2009. Distinguishing Characters of the Cavity Nesting Honeybees. In *Bees and Beekeeping in India*. Kalyani Publishers, Ludhiana. Pp 80-81.
- Amssalu, A.B., 2003. Multivariate morphometric analysis and behaviour of honeybees (*Apis mellifera* L.) in the southern regions of Ethiopia. Ph D Thesis. Rhodes University, Faculty of Science, Zoology and Entomology. Bottom of Form
- Amssalu, B., Nuru, A., Radloff, S. E., Hepburn, H. R., 2004. Multivariate morphometric analysis of honeybees (*Apis mellifera*) in the ethiopian region. *Apidologie*, 35(1):Pp. 71-81.
- Butler C. G., 1954. *The world of the honeybee*. Collins London.
- Buttel-Reepen, H., 1906. *Apistica. Beitrage zur Systematik, Biologie, sowie zur geschichtlichen und geographischen Verbreitung der Honigbiene (Apismellifera L.), ihrer Varietaten und der iibrigen Apis-Arten*. Veroff Zool Mus Berlin Pp. 118-120.
- Canada Agriculture and Food Museum, 2015. What is a bee? Retrieved October 14, 2021, from <https://bees.techno-science.ca/english/bees/what-is-a-bee/carniolan.php>.
- Dinerstein, E., Olson, D., Joshi, A., Vynne, C., Burgess, N. D., Wikramanayake, E., Saleem, M., 2017. An ecoregion-based approach to protecting

- half the terrestrial realm. *Bio Science*, 67(6), Pp. 534-545.
- Eimanifar, A., Kimball, R. T., Braun, E. L., Ellis, J. D., 2018. Mitochondrial genome diversity and population structure of two western honey bee subspecies in the republic of south africa. *Scientific reports*, 8(1):Pp. 1-11.
- Enderlein, G. (1906). Neue Honigbienen Und Beitrage Zur Kenntnis Der Verbreitung Der Gattung Apis. *StettinerEntomol Zeitg*, 67, Pp. 331-344.
- Han, F., Wallberg, A., Webster, M. T., 2012. From where did the Western honeybee (*Apis mellifera*) originate?. *Ecology and evolution*, 2(8), Pp. 1949-1957.
- Hepburn, H. R., Crewe, R. M., 1991. Portrait of the cape honeybee, *Apis mellifera capensis*. *Apidologie*, 22(6):Pp. 567-580.
- Hepburn, H. R., Radloff, S. E., 1998. *Honeybees of Africa*. Springer Berlin Heidelberg, Berlin, Heidelberg.
- Ilyasov, R. A., Lee, M. L., Takahashi, J. I., Kwon, H. W., Nikolenko, A. G., 2020. A revision of subspecies structure of western honey bee *Apis mellifera*. *Saudi Journal of Biological Sciences*, 27(12), Pp. 3615-3621.
- Kassaye, A., 1990. The honeybees (*Apis mellifera*) of Ethiopia - a morphometric study.
- Kerr, W. E., 1992. Abejas Africanas su introduccion y expansion en el continente Americano. *Subespecies y ecotipos Africanos*. *Ind. Apic*, 13, Pp. 12-21.
- Lane, R., 2019b. The common races of Honey Bees. Retrieved October 15, 2021, from <https://www.perfectbee.com/learn-about-bees/the-science-of-bees/common-races-of-honey-bee>.
- Maa, T. C., 1953. An inquiry into the systematics of the tribus Apidini or honeybees (Hym.). *Archipel. Treubia*, 21, Pp. 525-640
- Minja, G. S., Nkumilwa, T. J., 2016. The role of beekeeping on forest conservation and poverty alleviation in Moshi Rural District, Tanzania. *European Scientific Journal*, 12(23).
- PhD thesis, Agricultural University of Norway, Norway.
- Radloff, S. E., Hepburn, C., Hepburn, H. R., Fuchs, S., Hadisoelilo, S., Tan, K., Kuznetsov, V., 2010. Population structure and classification of *Apis cerana*. *Apidologie*, 41(6), Pp. 589-601.
- Radloff, S. E., Hepburn, H. R., 1997a. Multivariate analysis of honeybee populations, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), from western central Africa: Morphometrics and pheromones. *African Entomology*, 5(2).
- Radloff, S. E., Hepburn, H. R., 1997b. Multi variate analysis of honey bees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), of the Horn of Africa. *African Entomology*, 5(1), Pp. 57-64.
- Randall, B., 2021. The Value of Birds and Bees. Retrieved October 15, 2021, from <https://www.farmers.gov/blog/conservation/value-birds-and-bees>.
- Ruttner, F., 1988. *Biogeography and Taxonomy of Honeybees*. Springer Berlin Heidelberg, Berlin, Heidelberg.
- Ruttner, F., 1992. *Naturgeschichte der Honigbienen*. Franckh-Kosmos Verlag, Stuttgart, Germany.
- Sivaram, V., 2012. Status, prospects and strategies for development of organic beekeeping in the South Asian Countries. Division of Apiculture and Biodiversity, Department of Botany, Bangalore University.
- Snodgrass, R. E., 2018. *Anatomy of the honey bee*. Cornell University Press.

