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Morphometric and wing landmarks analysis of races of *Apis mellifera adansonii* L. in Nigeria

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Abstract

Morphometrics is the measurement and analysis of morphological characters and it is widely applied to study insect life history, physiology and systematics. Morphometric analysis has been used as a useful tool to assess subspecies limits in *Apis mellifera* and to study of genetic variability of honeybees. The identification of the species of honeybee in any beekeeping practice is germane to productive apicultural practice in any part of the World. This study entails the use of variations in morphometric and wing landmarks features to classify the samples *A. mellifera* collected from established apiaries in the forest and savannah vegetation zones of Nigeria. The result showed that honeybee samples collected from the two vegetation zones of the country produced five distinct morphoclusters. Morphoclusters 3 and 1 had the lowest (1.2%) and highest (26.4%) number of bee respectively. 20 landmarks was observed on the forewings of morphoclusters 1, 2, 4 and 5 while, morphoclusters 3 had 19 landmarks. Also, a similar trend of landmarks occurred on the hindwings. Morphoclusters 1, 2, 4 and 5 had six landmarks while, morphoclusters 3 had five respectively. The distinctive morphometry and wing landmark features of the identified morphoclusters of *A. mellifera* encountered in beekeeping practice in Nigeria portrayed differences in the sizes of the morphometric features of the morphoclusters. The uniqueness of the morphometric and wing landmark features of the morphoclusters was conveniently used to group the honeybees as distinct races of *A. mellifera adansonii* in the country.

Keywords: Honeybees, morphometrics, wing Landmarks, races, Nigeria

1. Introduction

Beekeeping otherwise known as apiculture is a unique primary industry. It depends on floral resources (i.e. nectar and pollen) about 80% of which are produced from native flora (Gibbs and Muirhead, 1998) ^[9]. Honeybees are kept for their highly coveted products which include: venom, propolis, beeswax, honey, pollen, royal jelly and pollination services (Oyerinde and Ande, 2006) ^[15]. Apiculture also provides people that take beekeeping as secondary occupation with extra source of income and nutrition and as well generates foreign exchange earnings, thus it is seen lately and valued as a sustainable form of agriculture (Oyerinde and Ande, 2006) ^[15]. More so, the practice is not detrimental to the environment, since it helps in the regeneration of forest resource, reclamation of eroded land and pasture improvement (Ojeleye, 1999) ^[14].

Morphometrics is the measurement and analysis of morphological characters and it is widely applied to study insect life history, physiology and systematics. Morphometrics also measures both genotypic and phenotypic characteristics. These include three categories: pigmentation, body size, and venation of forewing angle, which represent 82.1% of the total variation found in *Apis mellifera* (Kekeçoğlu *et al.*, 2007) ^[12]. Morphometric analysis has been used as a useful tool to assess subspecies limits in *A. mellifera* (Ruttner, 1988) ^[18], and to study of genetic variability of honeybees (Kekeçoğlu *et al.*, 2007) ^[12].

The domestic honeybee, *A. mellifera* is a species of bee that was first described by Carolus Linnaeus in 1758. Africa is the largest area where *A. mellifera* originally lived; but the borderlines between the different races are not well known (DBAR, 2008) ^[5]. Of note is the fact that ever since Linnaeus classification, several attempts have been made to classify species of *A. mellifera*, leading to identifying many subspecies originating from Europe, Middle East and Africa (Winston *et al.*, 1983) ^[20] but, in Nigeria where beekeeping is presently gaining ground, no effort has been made to identify the races of honeybee in the country. This study was conducted to identify the races of honeybee in Nigeria.

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Materials and Methods

Study Site and Collection of Sample

A random sample of 12,000 honeybee workers was collected from 400 colonies situated in the two major vegetation zones of Nigeria i.e. forest and savannah zones. Samples of 30 honeybee workers were collected from 50 colonized hives in established honeybee farms at Oyo, Osun and Ebonyi States in the forest ecological zone, as well as, Kwara, Kaduna, Adamawa, Kebbi States and Abuja (FCT) in the savannah vegetation zone. All samples were taken from colonies located in apiaries initiated with captured swarms and unmanaged for queen replacement. The samples of the honeybee workers collected were stored separately in 70% ethanol in small plastic containers labeled according to the vegetation zone of collection in Nigeria. These were used for morphometric analysis in the laboratory in order to determine the races of honeybee present in the colonies at different locations in Nigeria.

Morphometric Studies

The morphometric analyses were performed on ten randomly selected samples of honeybee workers by adopting the methods used in the morphometric analyses of *Apis florea* (Hepburn *et al.*, 2005; Michener, 2007) ^[10, 13] and *A. mellifera* (Michener, 2007; Andere *et al.*, 2008) ^[1, 13] as well as the use of a number of wing landmarks, which entailed manual or computer program evaluation of the wing revealing points of correspondences between and within the wing morphology of populations (Bookstein, 1991) ^[3] as was done for bumble bees by Aytekin *et al.* (2007) ^[2], and a number of landmarks on the radial cell of the forewing of Africanized honeybee (Francoy *et al.*, 2006; 2008) ^[7, 8] were adopted. All measurements were made with the aid of a calibrated hand held digitalized MiScope microscope with magnification range of 40-140x in millimeter (mm).

The following variables were measured on the honeybee samples: the length of the total body, proboscis, antennae, femur, tibia and tarsi, as well as, the length and width of the pollen basket and the abdomen at the widest point. The length, width at the widest points, and the number of landmarks on the forewing, hindwing and radial cell of the forewing were also measured (Plate I-XVI). Each measurement was carefully recorded per hive and all the data collected were statistically analysed.



Plate II: Maximum Width of Abdomen (MWA)



Plate III: Antenna Length (AL)



Plate I: Median Length of Abdomen (MLA)



Plate IV: Proboscis Length (PL)



Plate V: Length of Femur (LF)

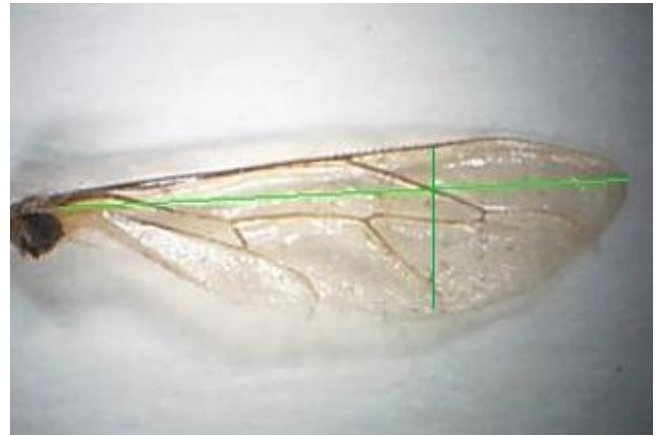


Plate IX: Length and Width of Hindwing (LHW & WHW)

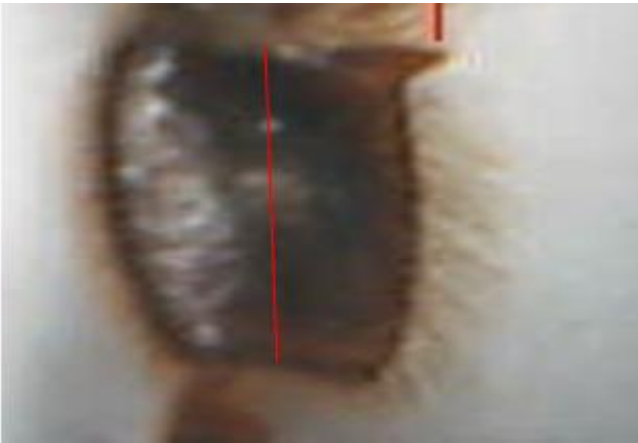


Plate VI: Length of Tibia (LT)



Plate X: Width of Forewing (WFW)



Plate VII: Length of Tarsus (LM)



Plate XI: Length and width of Radial Cell of Forewing (LRC & WRC)

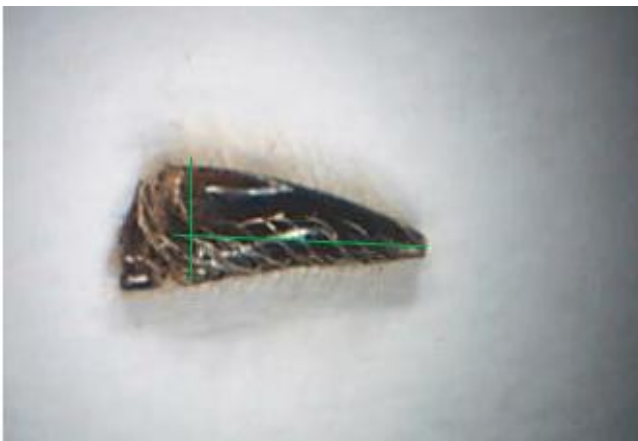


Plate VIII: Length and width of Pollen Basket (LPB & WPB)



Plate XII: Twenty Landmarks on Forewing



Plate XIII: Nineteen Landmarks on Forewing



Plate XIV: Six Landmarks on Hindwing



Plate XV: Five Landmarks on Hindwing



Plate XVI: Five Landmarks on Radial Cell of Forewing

Data Analysis

Data obtained from the preceding measurements were analyzed with SPSS version 18 software. The analysis involved the use of parametric statistical tools of mean, standard deviation and standard error. These were subjected to two-step and hierarchical cluster analysis to show their distribution and relationships. Means of clusters were presented in centroids and also the dendrogram plot, which is a branching diagram that represents the relationships of similarity among a group of entities (Dryden and Mardia, 1998) [6], was computed to illustrate the hierarchical clustering of morphometric and wing landmark features of the honeybee samples. The results of the dendrogram plots of hierarchical cluster analysis were used to show the phylogenetic relationship of the various honeybee samples obtained from the different vegetation zones of the country. This allowed classification of the honeybee collected into races. In addition, a utilitarian key was constructed for the identification of races of *Apis mellifera adansonii* based on the variations in the morphometric and wing landmark features of the respective honeybees encountered in this study.

Results

Morphometric and wing landmark features of honeybee samples collected from the two major vegetation zones of Nigeria i.e. Forest and Savannah produced five distinct morphoclusters (Table 1), Morphoclusters 3 and 1 had the lowest (1.2%) and highest (26.4%) number of bees respectively. The distribution by States in Nigeria of *Apis mellifera* samples identified based on morphometric and landmark feature variations (Table 2) showed that Kaduna (100%), Kebbi (100%) and Oyo (100%) States had their entire honeybees in a single morphoclusters, which respectively were morphoclusters 1, 5 and 4. On the contrary, the other five States recorded honeybee from two different morphoclusters. Majority of honeybee in Abuja (FCT), Adamawa, Ebonyi, Kwara, and Osun States were of morphoclusters 1(98.0%), 4(86.7%), 2(98.7%), 2(86.0%) and 5(94.0%) respectively.

Table 3 shows the centroids of combined morphometric features of different honeybee morphoclusters identified in the two main vegetation zones of Nigeria. The mean value of total body length (TBL) ranged from 5.19 ± 0.02 mm to 5.68 ± 0.01 mm in morphoclusters 4 and 1. Also, Median length of abdomen (MLA) produced similar range of mean value as TBL. The highest and lowest MLA values were obtained in morphoclusters 1 (2.75 ± 0.01 mm) and 4 (2.25 ± 0.01 mm), whereas, highest and lowest mean values of maximum width of abdomen (MWA) were recorded in morphoclusters 2 (1.86 ± 0.01 mm) and 1 (1.71 ± 0.01 mm) respectively.

The antenna length (AL) means ranged from 1.53 ± 0.01 mm to 1.69 ± 0.01 mm in morphoclusters 1 and 2 while the proboscis (PL) mean values were between 1.54 ± 0.00 mm and 1.90 ± 0.00 mm in morphoclusters 1 and 4 respectively. Morphometric analysis means of the leg features showed that length of femur (LF), length of tibia (LT) and length of tarsus (LM) were highest in morphoclusters 2 (1.00 ± 0.00 mm), 2 & 4 (0.87 ± 0.00 mm) and 2 (0.77 ± 0.00 mm) respectively. On the other hand, morphoclusters 3 and 4 had the highest values of length of pollen basket (LPB) and width of pollen basket (WPB) i.e. 1.56 ± 0.00 mm and 0.46 ± 0.00 mm.

Variations of mean value of honeybee fore and hindwings morphometric features in Nigeria followed a similar trend

with the means recorded in LF and LT. Morphoclusters 2 had the highest means value of 2.51 ± 0.01 mm, 3.67 ± 0.01 mm and 0.63 ± 0.00 mm for length of hindwing (LHW), length of forewing (LFW) and length of radial cell of forewing (LRC) while highest width of hindwing (WHW) (0.66 ± 0.00 mm) and width of forewing (WFW) (1.19 ± 0.00 mm) occurred in both morphoclusters 2 and 4. The width of radial cell of forewing (WRC) means values were between 0.18 ± 0.00 mm and 0.19 ± 0.00 mm.

Numbers of landmarks observed on the hind and forewings of honeybee samples from the two main vegetations in Nigeria slightly varied (Table 3). On the forewings morphoclusters 1, 2, 4 and 5 had 20 landmarks while, morphoclusters 3 had 19 landmarks. Also, a similar trend of landmarks occurred on the hindwings. Morphoclusters 1, 2, 4 and 5 had six landmarks while, morphoclusters 3 had five respectively. On the other hand, numbers of landmarks i.e. five recorded on the radial cells of all the five morphoclusters were the same.

The distinctive morphometry of the identified morphoclusters of *A. mellifera* encountered in beekeeping practice in Nigeria portrayed differences in the sizes of the morphometric features of the morphoclusters. This affirmed that the morphoclusters can be conveniently grouped as distinct races of *A. mellifera adansonii* in the country (Table 4). Recognition of the respective races can be achieved by observing the variations in their morphometric features and wing landmarks. *A. mellifera adansonii* belonging to race 1 group had TBL and median MLA that measured 5.7 mm and 2.8 mm respectively while race 2 recorded 0.8 mm LM. Also, race 3 can be identified with the presence of 19 landmarks on forewing and five landmarks on hindwing while race 4 had a PL of 1.9 mm and also *A. mellifera adansonii* of race 5 have LM less than 0.8 mm or LFW that measured 3.5 mm.

Dendrogram plot of hierarchical cluster analysis of the five races identified based on morphometric features of honeybees in Nigeria (Figure 1) revealed linkages between the various samples collected from apiaries sited in the eight different States, as well as, inter and intra morphoclusters relationship in the country. This gave an indication that the honeybee races reared in beekeeping practice in Nigeria had the same descent and in turn could be traced to share morphometric features.

Discussion

Combined analysis of morphometric features and wing landmarks data obtained from *A. mellifera* found in the two vegetation zones of Nigeria established five different races instead of the six morphoclusters (i.e. four morphoclusters in forest and two morphoclusters in savannah). This finding contradicts the earlier report of six morphoclusters (i.e. four and two in the forest and savannah vegetation respectively) (Oyerinde *et al.*, 2012a; Oyerinde *et al.*, 2012b) [16, 17]. This was because the identified morphoclusters 2 in the forest vegetation zone was eliminated in the pooled analysis. Thus, indicating that there were relationships between the morphometric features and wing landmarks of the established honeybee morphoclusters from the two vegetation zones. Also, the result was in line with the earlier report on the distribution and diversity of *A. mellifera* in Nigeria (Hussein, 2000) [11] while the distribution of two races in Abuja (FCT) as well as Adamawa, Kwara, Ebonyi and Osun States portrayed high diversity of *A. mellifera* in the various States

in their respective vegetation zones. This complied with ecotype variations of *A. mellifera* in Africa (Winston, 1980) [19].

Despite variations that were established with the number of morphoclusters of honeybee found in the forest (four) and the savannah (two) vegetation zones of Nigeria which later produced five distinct races in the pooled analysis; it is worthy of note that the different races of honeybee were with varying and interrelated morphometric features and wing landmarks. This was probably due to the variation in the ecology of the two zones as well as the ability of the races to swarm over kilometers of land in search of appropriate nesting place.

The uniqueness of the respective morphometric features that classified the honeybee's collection into morphoclusters could be associated with the likeliness of variations of honeybees in Nigeria. Consequently, all the morphoclusters had forewings that fell within the range for *A. mellifera adansonii* (i.e. below 8mm) (David, 2007) [4]. This revealed that samples of honeybees encountered in the study can be classified as races of *A. mellifera adansonii*. Furthermore, beekeepers in the forest and savannah vegetation zones of Nigeria can use the utilitarian keys to the identification of the races of honeybee drawn in this study to identify and as well, develop honeybee of races 3 and 4 as well as race 2 respectively for productive beekeeping practice in their regions.

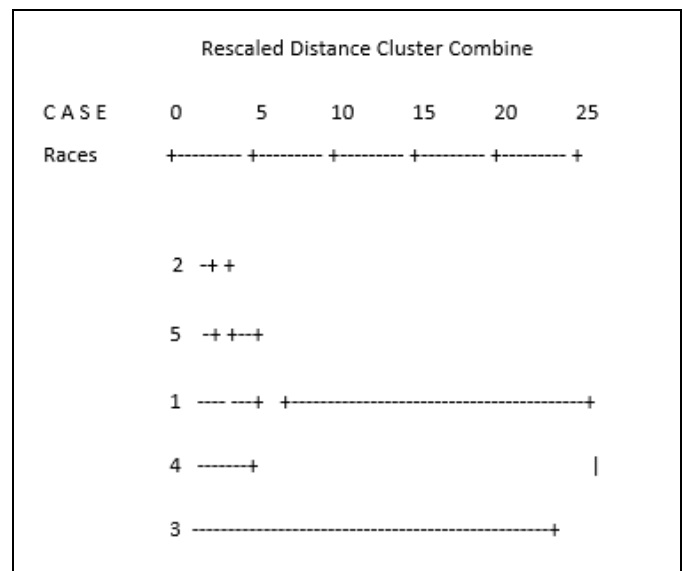


Fig 1: Morphometric Features Based Hierarchical Cluster Analysis Dendrogram of Races of *Apis mellifera* in Nigeria

Table 1: Pooled Frequency of honeybee samples in different morphoclusters in both the forest and savanna zones of Nigeria identified basis of morphometric and landmark features

Morphoclusters	No of Bees	% of Total Honeybee samples
1	317	26.4
2	277	23.1
3	14	1.2
4	301	25.1
5	291	24.3
Combined	1200	100.0
Total	1200	

Table 2: Distribution by States of five morphoclusters of *Apis mellifera* identified on the basis of combined morphometric features of *Apis mellifera* in Nigeria

Morphoclusters	Abuja (FCT)		Adamawa State		Ebonyi State		Kaduna State		Kebbi State		Kwara State		Osun State		Oyo State	
	Freq	Percentage	Freq	Percentage	Freq	Percentage	Freq	Percentage	Freq	Percentage	Freq	Percentage	Freq	Percentage	Freq	Percentage
1	147	98.0	20	13.3	0	0.0	150	100.0	0	0.0	0	0.0	0	0.0	0	0.0
2	0	0.0	0	0.0	148	98.7	0	0.0	0	0.0	129	86.0	0	0.0	0	0.0
3	3	2.0	0	0.0	2	1.3	0	0.0	0	0.0	0	0.0	9	6.0	0	0.0
4	0	0.0	130	86.7	0	0.0	0	0.0	0	0.0	21	14.0	0	0.0	150	100.0
5	0	0.0	0	0.0	0	0.0	0	0.0	150	100.0	0	0.0	141	94.0	0	0.0
Combined	150	100.0	150	100.0	150	100.0	150	100.0	150	100.0	150	100.0	150	100.0	150	100.0

Table 3: Combined centroids (in mm) of morphoclusters of *Apis mellifera* in Nigeria identified on basis of morphometric and landmarks features variations

Morphoclusters	TBL (mm)		MLA (mm)		MWA (mm)		AL (mm)		PL (mm)		LF (mm)		LT (mm)		LM (mm)		LPB (mm)		WPB (mm)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	5.68	0.64	2.75	0.49	1.71	0.12	1.53	0.09	1.54	0.33	0.94	0.07	0.81	0.06	0.65	0.06	1.19	0.07	0.39	0.05
2	5.61	0.47	2.68	0.48	1.86	0.23	1.69	0.17	1.81	0.41	1.00	0.08	0.87	0.07	0.77	0.07	1.23	0.08	0.44	0.06
3	5.62	0.26	2.29	0.21	1.72	0.09	1.57	0.10	1.68	0.43	0.89	0.10	0.81	0.08	0.70	0.06	1.56	0.41	0.39	0.04
4	5.19	0.42	2.25	0.36	1.77	0.15	1.63	0.13	1.90	0.34	0.97	0.07	0.87	0.05	0.72	0.09	1.25	0.09	0.46	0.05
5	5.55	0.68	2.52	0.51	1.80	0.17	1.65	0.07	1.84	0.30	0.96	0.06	0.84	0.06	0.70	0.05	1.19	0.06	0.42	0.05
Combined	5.51	0.60	2.55	0.50	1.78	0.18	1.62	0.14	1.76	0.38	0.97	0.08	0.85	0.07	0.71	0.08	1.22	0.10	0.42	0.06
±SEM	0.02		0.01		0.01		0.00		0.01		0.00		0.00		0.00		0.00		0.00	

Morphoclusters	LHW (mm)		WHW (mm)		LFW (mm)		WFW (mm)		NLF		NLH		NLR		LRC (mm)		WRC (mm)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	2.35	0.09	0.64	0.07	3.43	0.16	1.18	0.08	20.00	0.40	6.00	0.44	5.00	0.00	1.27	0.06	0.18	0.01
2	2.51	0.12	0.66	0.08	3.67	0.11	1.19	0.10	20.00	0.40	6.00	0.47	5.00	0.00	1.33	0.06	0.19	0.02
3	1.79	0.77	0.57	0.06	3.26	0.65	1.17	0.07	19.00	0.71	5.00	0.90	4.79	0.80	1.28	0.03	0.19	0.01
4	2.42	0.12	0.66	0.06	3.64	0.12	1.19	0.05	20.00	0.06	6.00	0.50	5.00	0.00	1.30	0.04	0.18	0.02
5	2.39	0.08	0.65	0.05	3.52	0.10	1.16	0.07	20.00	0.22	6.00	0.48	5.00	0.00	1.28	0.06	0.19	0.02
Combined	2.40	0.16	0.65	0.07	3.56	0.18	1.18	0.08	19.87	0.61	5.63	0.49	5.00	0.09	1.29	0.06	0.19	0.02
±SEM	0.01		0.00		0.01		0.00		0.02		0.01		0.00		0.00		0.00	

Key: Total Body Length (TBL), Median Length of Abdomen (MLA), Maximum Width of Abdomen (MWA), Antennae Length (AL), Proboscis Length (PL), Length of Femur (LF), Length of Tibia (LT), Length of Tarsi (LM), Length of Pollen Basket (LPB), Width of Pollen Basket (WPB), Length of Hindwing (LHW), Width of Hindwing (WHW), Length of Forewing (LFW), Width of Forewing (WFW), No of Landmarks on Forewing (NLF), No of Landmarks on Hindwing (NLH), No of Landmarks on Radial Cell (NLR), Length of Radial Cell (LRC), Width of Radial Cell (WRC)

Table 4: Morphometric and Wing Landmarks for Identification of Races of *Apis mellifera adansonii* in Nigeria

Race	TBL	MLA	MWA	AL	PL	LF	LT	LM	LPB	WPB	LHW	WHW	LFW	WFW	NLF	NLH	LRC	WRC
1	5.68	2.75	1.71	1.53	1.54	0.94	0.81	0.65	1.19	0.39	2.35	0.64	3.43	1.18	20.00	6.00	1.27	0.18
2	5.61	2.68	1.86	1.69	1.81	1.00	0.87	0.77	1.23	0.44	2.51	0.66	3.67	1.19	20.00	6.00	1.33	0.19
3	5.62	2.29	1.72	1.57	1.68	0.89	0.81	0.70	1.56	0.39	1.79	0.57	3.26	1.17	19.00	5.00	1.28	0.19
4	5.19	2.25	1.77	1.63	1.90	0.97	0.87	0.72	1.25	0.46	2.42	0.66	3.64	1.19	20.00	6.00	1.30	0.18
5	5.55	2.52	1.80	1.65	1.84	0.96	0.84	0.70	1.19	0.42	2.39	0.65	3.52	1.16	20.00	6.00	1.28	0.19

Key: Total Body Length (TBL), Median Length of Abdomen (MLA), Maximum Width of Abdomen (MWA), Antennae Length (AL), Proboscis Length (PL), Length of Femur (LF), Length of Tibia (LT), Length of Tarsi (LM), Length of Pollen Basket (LPB), Width of Pollen Basket (WPB), Length of Hindwing (LHW), Width of Hindwing (WHW), Length of Forewing (LFW), Width of Forewing (WFW), No of Landmarks on Forewing (NLF), No of Landmarks on Hindwing (NLH), No of Landmarks on Radial Cell (NLR), Length of Radial Cell (LRC), Width of Radial Cell (WRC).

Conclusion

Based on the findings from this study, it can be concluded that the races of honeybee that were engaged in beekeeping in Nigeria have related morphometric features and wing landmarks. Thus, it can be appropriately recommended that beekeepers in both forest and savannah vegetation zones of the country can always interact to share practical ideas that can encourage their apicultural practices and as well possible for taxonomist or beekeepers interested in characterizing honeybees kept on their farms with the use of the utilitarian key to races of honeybee given below:

Key to Races of Honeybee in Nigeria

1. Wings present, the front pair transparent or membranous, similar in texture to the hind pair; forewings generally with obvious veins; wings sometimes covered with scales or hairs ----- (APOCRITA). 2
 - Body clearly divided into mesosoma and metasoma. Forewing usually with closed anal cell. Length greater than 3mm ----- (SYMPHYTA)
2. Pubescence simple, with hairs not plumose or branched; hind basitarsi not broadened. Pubescence on face often silvery ----- APOIDEA Sphecidae
 - Pubescence at least in part (e.g. below tegulae) plumose or branched. Hind basitarsi usually rather broader frequently densely hairy long, stout hair ----- APOIDEA Apidae 3
3. Large broad body size with short limbs, short proboscis and few dark hairs on worker ----- *Apis mellifera mellifera*
 - Medium or small slim body size, with long limbs -----4
4. Grey or lead grey coloured worker, with very long proboscis and long tomentum width with much hair ----- *Apis mellifera caucasica*
 - Yellow coloured worker with 1, 2 or 3 yellow scutellum, with long proboscis ----- 5
5. Forewing length average between 8 mm-9.7 mm with worker cell ranged between 4.7 mm-4.9 mm ----- *Apis mellifera scutellata*
 - Forewings average size below 8 mm ----- *Apis mellifera adansonii* 6
6. Nineteen landmarks on forewing and five landmarks on hindwing ----- Race 3
 - Twenty landmarks on forewing and six landmarks on hindwing -----7
7. Total body length and height at the largest part about 5.7 mm and 2.8 mm respectively ----- Race 1
 - Total body length less than 5.7 mm and width hindwing 0.7 mm ----- 8
8. Proboscis length 1.9 mm ----- Race 4
 - Proboscis length less than 1.9 mm and antennae length 1.7 mm ----- 9
9. Length of tarsus 0.8 mm ----- Race 2
 - Length of tarsus less than 0.8 mm or length of forewing 3.5 mm ----- Race 5

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