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A preliminary evaluation of morphometric characters of honeybee (*Apis mellifera* L.) populations in Nigeria

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Abstract
Samples of workers of honeybee were collected from 49 colonies belonging to 12 localities in Nigeria and analysed using classical morphometry. In addition, 10 colonies from three localities in neighbouring Niger and Cameroon were included in the analysis. Measurements of 35 morphological characters of body size, colour and pilosity were taken from 10 workers per colony and the data subjected to various statistical analyses. Although ANOVA revealed a considerable variation of morphological characters between the sampled localities, principal component analysis indicated that this variation was not sufficient to group the colonies into geographically separable groups. However, stepwise discriminant analysis confirmed the three *a priori* clusters into which the colonies were classified by hierarchical structural analysis. One of the clusters consisted of colonies from the plateaus while the remaining two consisted of colonies from the plains. Pearson correlation analysis revealed a significant positive correlation between altitude and the discriminant functions.

Keywords: *Apis mellifera*, honeybee, African bees, morphometry, Nigeria.

Introduction
The natural range of the western honeybee, *A. mellifera*, is western Asia, Africa and Europe: From southern Scandinavia in the north to the Cape of Good Hope in the south, from Dakar in the west to the Urals, Mashhad and the coast of Oman in the east. Geographical isolation and ecological adaptations resulted in the evolution of local populations showing considerable geographical variation, resulting in adaptation to local factors of climate, vegetation, pests and pathogens [1, 2]. These adaptations may be lost due to human activities in beekeeping that affect wild honeybees in different ways: competition for floral sources, introduction of exotic genes, pests, parasites and diseases [3, 4]. Thus, an adequate knowledge of the natural diversity of local subspecies and ecotypes is essential for their management and conservation. To protect the biological diversity of local populations of honeybees in their natural habitats, these populations must, first of all, be characterised. One of the standard methods of characterising honeybees is classical morphometry [1]. This method uses numeric data resulting from exact measurements of 36 morphological characters of body size, colour and pilosity from which means of colony characters are obtained for statistical analyses [5]. Using this method Ruttner [5], classified *A. mellifera* into 24 subspecies and four evolutionary lineages. However, whereas the European subspecies have been thoroughly studied, the study of their Asian and African counterparts is still in its infancy, in many places [1]. For example, although a few studies have been carried out, recently, in Nigeria [6, 8], they have done little in improving the situation, due to their inadequacy in coverage and/or methodology. Thus, the present study attempted to improve our knowledge of the diversity of the honeybees of Africa by analysing samples of honeybees from Nigeria using classical morphometry. The main purpose of the study was to provide reference data for future studies.

Materials and Methods
Description of the Area of Study
The area of study (Figure 1) lies approximately within 3˚ to 14˚ E and 4˚ to 14˚ N and covers the whole of Nigeria. A summary of the important physical features of the area, based on Hepburn and Radloff [9], is given below:
The area consists of four climatic zones, namely, equatorial, wet tropical, dry tropical and sahelian. These correspond, approximately, to four zones of vegetation: Tropical rainforest,

Guinea savanna, Sudan savanna and Sahel. Sahel is the transitional zone between savanna and desert (Figure 1). The forest zone is characterized by a very dense vegetation, very rich in species diversity; a very heavy rainfall and a long rainy season (more than half of the year); and a mean annual temperature of about 25 ˚C which varies a little. Plants flower throughout the year.

The savannas consist of a mixture of grasslands and woody vegetation; lighter rainfall with a short rainy season (less than half of the year); and a high variation of mean annual temperature (10 to 15 ˚C). The density of vegetation, species richness, amount of rainfall and length of the rainy season decrease with the increase in latitude. Annuals flower at the end of the rainy season while trees flower during the dry season.

The Sahel is characterized by scanty rainfall; very short rainy season; very sparse vegetation of short trees and grass; and frequent droughts.

Altitude varies from sea level, through 1200 metres on the Jos Plateau, to about 1800 metres on the Mambila Plateau.

Collection of honeybees

Samples of workers of honeybee were collected from 49 colonies in 12 localities (Figure 1 and Table 1). In addition, 10 colonies from three localities in neighbouring Niger and Cameroon were included in the analysis. Bees were collected, from wild nests or unmanaged traditional or top-bar hives populated by wild swarms, and preserved in 70% ethanol. The collection of samples and the morphometric data generated therefrom were stored at the Institut für Bienenkunde in Oberursel (Polytechnische Gesellschaft), University of Frankfurt, Germany.

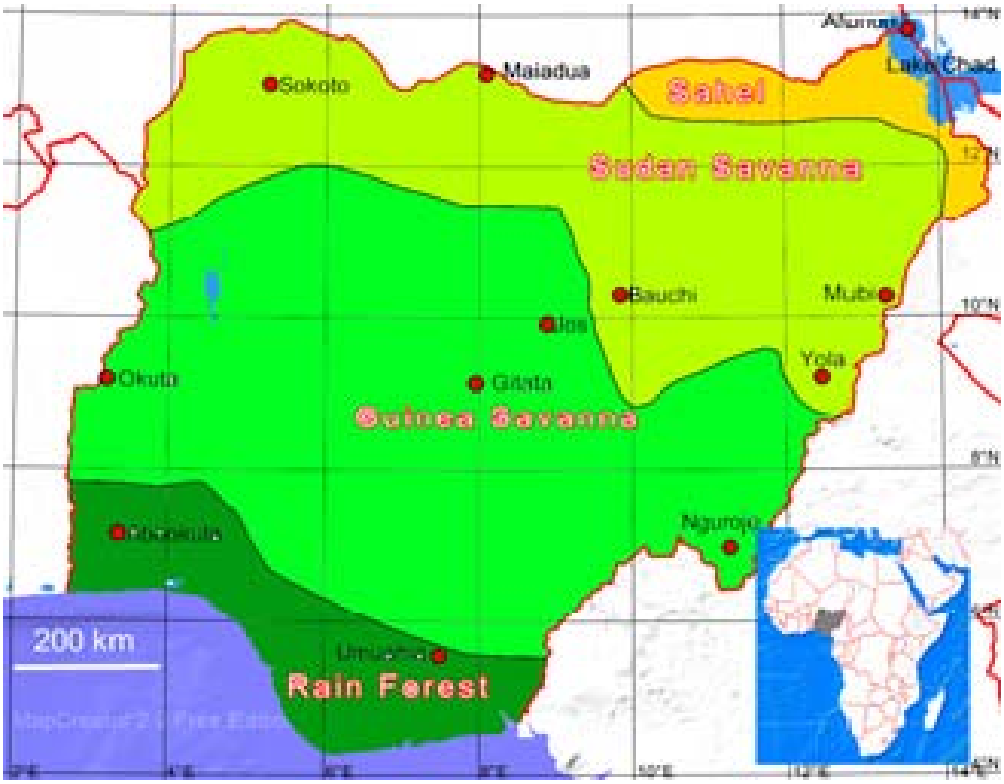

Fig 1: The area of study (Nigeria) showing sampled localities and variation in vegetation. Inset: A map of Africa showing the location of the area of study (highlighted in grey). Information on vegetation was obtained from Hoyle, Keay [10].

Table 1: Localities in Nigeria from which samples of honeybee were collected for morphometric analysis

Locality*	Latitude (°N)	Longitude (°E)	Altitude (m)	Number of Colonies
Afunori	13.70	13.33	283	5
Maiadua	13.18	8.23	469	3
Sokoto	13.05	5.23	283	5
Okuta	9.22	3.18	455	5
Gitata	9.12	7.95	444	5
Yola (Bole)	9.12	12.45	232	5
Abeokuta	7.17	3.40	131	4
Nguroje	6.95	11.12	1828	4
Umuahia (Umudike)	5.48	7.57	125	5
Bauchi	10.32	9.83	620	2
Mubi (Salmakoli)	10.02	13.10	518	4
Jos (Laminga)	9.83	8.98	1332	2
Bamenda	5.96	10.15	1241	1
Tahoua	14.90	5.27	358	4
Niamey (Gouldjo Koara)	14.90	5.27	358	5

*Bamenda is in Cameroon while Tahoua and Niamey are in Niger.

Morphometric Measurements

Morphometric measurements of 35 characters were taken from 10 bees from each colony according to Ruttner [5] and Ruttner, Tassencourt [2]. Measurements of hair and pigmentation were taken under a Leica dissecting microscope, fitted with an eyepiece graticle, at a magnification of 40 x. Measurements of wings, legs and sternites were taken with the help of a Leica CCD camera connected to a desktop computer, using the measuring program Bee Morphometric, Version 1.02 [11]. The details of the variables measured are shown in Table 2.

Table 2: List of characters measured for morphometry [1]

	Ruttner No.	Character
A. Hair		
	1	Length of cover hair on tergite 5
	2	Width of the tomentum band on the side of tergite 4
	3	Width of the dark stripe between the tomentum and the posterior rim of the tergite
B. Size		
	5	Femur, length
	6	Tibia, length
	7	Metatarsus, length
	8	Metatarsus, width
	9	Tergite 3, longitudinal diameter
	10	Tergite 4, longitudinal diameter
	11	Sternite 3, longitudinal diameter
	12	Wax plate(mirror) of sternite 3, longitudinal diameter
	13	Wax plate of sternite 3, transversal diameter
	14	Distance between wax plates of sternite 3
	15	Sternite 6, longitudinal diameter
	16	Sternite 6, transversal diameter
C. Fore-wing		
	17	Fore-wing, length
	18	Fore-wing, width
	19	Cubital vein, distance a
	20	Cubital vein, distance b
	21-31	11 angles of wing venation
		(No. 21=A4, 22=B4, 23=D7, 24=E19, 25=G18, 26=J10, 27=J16, 28=K19, 29=L13, 30=N23, 31=O26)
D. Colour		
	32	Pigmentation of tergite 2
	33	Pigmentation of tergite 3
	34	Pigmentation of tergite 4
	35	Pigmentation of scutellum
	36	Pigmentation of plates of scutellum

[1] **Sources:** [5] Ruttner F. Biogeography and taxonomy of honeybees. Berlin, Heidelberg, New York: Springer-Verlag; 1988. and [2] Ruttner F, Tassencourt L, Louveaux J. Biometrical-statistical analysis of the geographic variability of *Apis mellifera* L.: I. Material and methods. Apidologie 1978; 9:363-81.

Statistical Analyses

First, the mean and standard deviation of each of the 35 morphometric characters were calculated for every colony. Then the means for colonies were used to calculate the means and standard deviations for localities. The data were subjected to a one-way analysis of variance (ANOVA) to compare different localities. Tukey HSD tests, on the means of the 35 characters, were used to detect significant differences between the localities. The level of statistical significance chosen was p=0.05.

A PCA, using colony means of 35 morphometric characters was run in order to detect any possible clusters. The suitability of PCA was assessed, prior to the analysis, using correlation coefficients of the variables, Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity [12, 13]. Any variable that did not have a correlation with at least one other variable where $r \geq 0.3$ should be removed from the analysis. The KMO measure is used as an index of whether there are linear relationships between the variables. Its value ranges from 0 to 1, with values above 0.6 suggested as a minimum requirement for sampling adequacy. Bartlett's test of sphericity tests the null hypothesis that there are no correlations between any of the variables and, therefore, the variables cannot be reduced to a smaller number of principal components. For a PCA to be feasible the null hypothesis must be rejected: The result of Bartlett's test is used to take this decision.

Then a hierarchical cluster analysis was carried out with the assumption that samples from each locality formed a distinct population. Thus, 15 populations were assumed and the analysis was carried out using the means of the 35 morphometric characters for the 15 localities in the study.

Thereafter, a stepwise discriminant analysis (DA) was run in order to confirm the groups predicted by cluster analysis and to determine the discriminant characters. The suitability of DA was determined through log determinants and Box's M test. In DA the basic assumption is that the variance-co-variance matrices are equivalent. For this assumption to hold the log determinants should be equal and the Box's M test should not be significant. The latter tests the null hypothesis that the

covariance matrices do not differ between groups formed by the dependent variable. Wilk's lambda was used to test the discriminatory power of the discriminant functions while the significance of the distance between group centroids was tested by F-statistic.

A Pearson's product-moment correlation analysis was run to assess the relationship between altitude and the two discriminant functions. Prior to analysis, the data were assessed for linearity, normal distribution and presence of outliers [12, 13]. Interpretation of the magnitude of Pearson's correlation coefficient, *r*, was based on Cohen [14], that is: 0.1 < |*r*| <.3: small/weak correlation; 0.3 < |*r*| <.5: medium/moderate correlation; and |*r*| >.5: large/strong correlation.

All statistical analyses were carried out with IBM® SPSS® Statistics Version 20 with additional material from Burns and Burns [12] and Anonymous [13].

Results

Means of the 35 morphometric characters for the sampled localities are given in Tables 3-6. A one-way ANOVA revealed that means of 24 of the morphometric characters differed significantly ($p<0.05$) between sampled localities while those of the remaining 11 characters did not ($p>0.05$).

Table 3: Means and standard deviations of morphological characters of body hair (mm) and pigmentation of 10 worker bees each from (N) colonies from 14 localities

Locality@	N	Hair (1)***	Tom1 (2)***	Tom2(3)**	Pt2 (32)†	Pt3 (33) †	Pt4 (34)†	Scut1 (35)***	Scut2 (36)***
Afunori	5	0.14ab	0.73bcd	0.43a	9.00	8.04	4.40	7.52c	5.24d
		0.01	0.03	0.03	0.00	0.09	0.89	0.28	0.40
Maiadua	3	0.15ab	0.73bcd	0.44a	9.00	8.00	5.30	6.75abc	4.10bcd
		0.01	0.02	0.00	0.00	0.00	2.25	0.07	0.00
Sokoto	5	0.15ab	0.73bcd	0.43a	8.96	8.00	4.00	6.02a	3.04ab
		0.01	0.01	0.01	0.09	0.00	0.00	0.24	0.34
Okuta	5	0.17abc	0.63a	0.46a	9.00	8.00	4.10	6.50a	3.74abc
		0.01	0.05	0.02	0.00	0.00	0.41	0.20	0.50
Gitata	5	0.17abc	0.70abc	0.45a	8.88	7.68	4.00	6.08a	3.30ab
		0.04	0.05	0.02	0.27	0.70	0.00	0.28	0.20
Yola	5	0.13a	0.75cd	0.42a	8.96	8.00	4.02	6.62a	3.26ab
		0.00	0.01	0.01	0.09	0.00	0.04	0.20	0.09
Abeokuta	4	0.19c	0.72bcd	0.41a	9.00	8.00	4.00	6.63ab	3.03ab
		0.01	0.02	0.03	0.00	0.00	0.00	0.29	0.63
Nguroje	4	0.16abc	0.64a	0.42a	9.00	8.08	4.23	7.53c	5.23d
		0.02	0.03	0.05	0.00	0.15	0.66	0.17	0.71
Umuahia	5	0.16abc	0.76cd	0.45a	8.96	7.86	3.96	7.40bc	4.58cd
		0.02	0.02	0.01	0.09	0.31	0.09	0.37	0.36
Bauchi	2	0.20c	0.78d	0.46a	9.00	8.00	4.00	6.45a	2.65a
		0.01	0.02	0.01	0.00	0.00	0.00	0.49	1.06
Mubi	4	0.15ab	0.69abc	0.45a	9.00	7.85	4.00	6.68ab	3.28ab
		0.00	0.04	0.02	0.00	0.03	0.00	0.59	0.34
Jos	2	0.17abc	0.74bcd	0.44a	9.00	7.40	4.00	6.65ab	3.80abc
		0.01	0.00	0.00	0.00	0.00	0.00	0.21	0.28
Tahoua	4	0.16abc	0.70abc	0.47a	9.00	8.00	4.00	6.55a	3.48abc
		0.01	0.06	0.02	0.00	0.00	0.00	0.19	0.57
Niamey	5	0.15ab	0.66ab	0.45a	9.00	8.20	4.00	6.40a	3.55abc
		0.03	0.02	0.02	0.00	0.28	0.00	0.24	0.40

@Tahoua and Niamey are in Niger while the rest are in Nigeria. **Highly significant ($p < 0.01$); ***very highly Significant ($p < 0.001$); †not significant ($p > 0.05$); according to one-way ANOVA. Means within a column followed by the same letter are not significantly different at $p = 0.05$ according to Tukey HSD test. Hair: Cover hair on tergite 5; Tom1: Width of tomentum; Tom2: Width of stripe behind tomentum; Pt2,3 & 4: Pigmentation of tergites 2, 3 & 4; Scut1,2: pigmentation of scutellum and its plates. Numbers in brackets are Ruttner numbers.

Table 4: Means and standard deviations (mm) of characters of the hind leg of 10 worker bees each from (N) colonies from 14 localities

Locality†	N	Femur, length(5)***	Tibia, length(6)***	Metatarsus, length(7)***	Metatarsus, width(8)***
Afunori	5	2.37ab	2.89ab	1.82a	1.04a
		0.03	0.04	0.03	0.02
Maiadua	3	2.50def	3.05cd	1.91bc	1.13cd
		0.08	0.12	0.04	0.03
Sokoto	5	2.52ef	3.07d	1.93c	1.14d
		0.03	0.04	0.02	0.02
Okuta	5	2.45cdef	2.98bcd	1.86abc	1.09abcd
		0.03	0.04	0.01	0.02
Gitata	5	2.45cdef	2.99bcd	1.86abc	1.11bcd
		0.03	0.02	0.04	0.03
Yola	5	2.43abcd	2.94abc	1.84ab	1.07abc
		0.02	0.03	0.03	0.03

Abeokuta	4	2.35a	2.84a	1.82a	1.05ab
		0.02	0.00	0.02	0.00
Nguroje	4	2.45cdef	2.96abcd	1.87abc	1.08abcd
		0.02	0.02	0.03	0.02
Umuahia	5	2.43abcd	2.94abc	1.85abc	1.08abcd
		0.04	0.05	0.04	0.02
Bauchi	2	2.45bcde	2.98bcd	1.88abc	1.10bcd
		0.01	0.00	0.05	0.02
Mubi	4	2.41abc	2.95abc	1.85abc	1.08abcd
		0.02	0.04	0.04	0.01
Jos	2	2.48cdef	3.01cd	1.89abc	1.10bcd
		0.00	0.01	0.00	0.00
Tahoua	4	2.53f	3.05cd	1.91bc	1.11bcd
		0.03	0.07	0.05	0.02
Niamey	5	2.41abc	2.96abcd	1.86abc	1.07abc
		0.02	0.02	0.02	0.02

†Tahoua and Niamey are in Niger while the rest are in Nigeria. ***Very highly significant ($p < 0.001$), according to one-way ANOVA. Means, within a column, followed by the same letter are not significantly different at $p = 0.05$ according to Tukey HSD test. Numbers in brackets are Ruttner numbers.

Table 5: Means and standard deviations (mm) of characters of the abdomen of 10 worker bees each from (N) colonies from 14 localities

Locality@	**N**	**Lt3 (9)****	**Lt4 (10)****	**Lst3 (11)*****	**Lwm (12)*****	**Wwm (13)*****	**Dwm (14)†**	**Lst6 (15)*****	**Wst6 (16)*****
Afunori	5	1.94ab	1.87a	2.33ab	1.18ab	1.92a	0.31	2.22ab	2.64ab
		0.05	0.06	0.02	0.01	0.13	0.01	0.05	0.06
Maiadua	3	2.07b	2.01b	2.46cd	1.23bc	2.05ab	0.33	2.29bcd	2.79bcd
		0.05	0.05	0.09	0.05	0.13	0.02	0.10	0.10
Sokoto	5	2.05ab	1.99ab	2.50d	1.26c	2.07ab	0.33	2.35cd	2.90d
		0.08	0.07	0.04	0.03	0.07	0.01	0.03	0.07
Okuta	5	2.01ab	1.95ab	2.44bcd	1.21bc	2.04ab	0.30	2.22ab	2.78bcd
		0.03	0.03	0.04	0.03	0.05	0.03	0.03	0.06
Gitata	5	1.92ab	1.87a	2.45bcd	1.22bc	2.04ab	0.32	2.25abcd	2.75bcd
		0.03	0.03	0.04	0.04	0.10	0.01	0.08	0.07
Yola	5	2.01ab	1.95ab	2.36abc	1.18abc	2.03ab	0.30	2.21ab	2.71abc
		0.05	0.05	0.03	0.02	0.06	0.02	0.02	0.06
Abeokuta	4	1.95ab	1.89ab	2.28a	1.11a	1.92a	0.30	2.15a	2.58a
		0.10	0.10	0.03	0.01	0.05	0.01	0.03	0.07
Nguroje	4	2.02ab	1.97ab	2.41bcd	1.19abc	2.01ab	0.30	2.26abcd	2.75bcd
		0.03	0.03	0.05	0.05	0.06	0.03	0.06	0.04
Umuahia	5	2.01ab	1.95ab	2.42bcd	1.19abc	2.00ab	0.31	2.23abc	2.71abc
		0.07	0.07	0.05	0.04	0.04	0.04	0.04	0.05
Bauchi	2	1.99ab	1.94ab	2.41bcd	1.19abc	2.05ab	0.35	2.28bcd	2.76bcd
		0.02	0.02	0.10	0.06	0.06	0.01	0.03	0.10
Mubi	4	1.93a	1.87a	2.44bcd	1.22bc	2.05ab	0.30	2.28bcd	2.79bcd
		0.03	0.03	0.07	0.04	0.03	0.02	0.05	0.06
Jos	2	1.99ab	1.93ab	2.42bcd	1.20bc	2.06ab	0.33	2.23abc	2.77bcd
		0.03	0.03	0.04	0.01	0.05	0.01	0.03	0.01
Tahoua	4	1.98ab	1.92ab	2.53d	1.26c	2.12b	0.32	2.36d	2.90d
		0.02	0.02	0.02	0.01	0.03	0.02	0.02	0.03
Niamey	5	1.97ab	1.91ab	2.43bcd	1.22bc	2.06ab	0.30	2.29bcd	2.81cd
		0.04	0.04	0.03	0.01	0.01	0.02	0.05	0.06

@Tahoua and Niamey are in Niger while the rest are in Nigeria. **Highly significant ($p<0.01$); ***very highly significant ($p<0.001$); †not significant ($p>0.05$), according to one-way ANOVA. Means, within a column, followed by the same letter are not significantly different between each other at $p=0.05$ according to Tukey HSD test. Lt3, 4: Tergites 3, 4, longitudinal; Lst3: Sternite 3, longitudinal; Lwm: Wax mirror, longitudinal; Wwm: Wax mirror, width; Dwm: Distance between wax mirrors; Lst6: Sternite 6, longitudinal; Wst6: Sternite 6, width. Numbers in brackets are Ruttner numbers.

Table 6: Means ± standard deviations of characters of the fore-wing of 10 worker bees each from (N) colonies from 14 localities

Locality[@]	N	Fwl (17)***	Fww (18)***	Cub-a (19)†	Cub-b (20)†	A4 (21)*	B4 (22)†	D7 (23)†	E9 (24)*	G18 (25) ***	J10 (26)†	J16 (27)*	K19 (28) †	L13 (29)*	N23 (30)***	O26 (31)†
Afunori	5	8.04±0.14ab	2.77±0.06ab	48.9±4.3	21.1±1.0	32±0.7a	104±1.7	101±1.8	20±1.0a	94± 2.0ab	53±0.5	90±1.3a	79±2.0	16±0.7a	86±2.0a	37±2.4
Maiadua	3	8.50±0.18cd	2.82±0.10abc	48.4±3.2	23.0±1.3	33±1.4a	103±4.0	101±2.1	19±0.6a	96±ab	54±1.4	93±ab	78±0.5	15±0.8a	88±1.2ab	39±3.4
Sokoto	5	8.51±0.13cd	2.89±0.05bcd	47±4.3	21±0.5	33±0.3a	106±2.7	102±4.0	20±0.7a	97±1.2b	53±1.2	92±1.7ab	79±1.0	15±0.4a	87±2.6ab	38±1.1
Okuta	5	8.33±0.05bcd	2.84±0.02bcd	49.2±3.2	23±0.6	32±0.6a	104±3.5	102±1.5	19±0.7a	96±2.6ab	52±2.2	94±1.8ab	78±0.7	15±0.7a	88±1.1ab	38±2.2
Gitata	5	8.37±0.16cd	2.83±0.03abcd	50.6±3.1	21.6±2.2	32±0.9a	103±3.9	101±2.7	18±0.8a	94±0.9ab	54±2.8	93±2.9ab	80±1.7	16±1.0a	89±1.9ab	38±2.7
Yola	5	8.24±0.09abc	2.76±0.02abcd	48.0±3.1	22.7±0.8	33±1.9a	101±4.4	102±1.9	19±1.0a	98±1.7b	54±1.3	94±2.0ab	79±0.9	16±0.5a	89±1.3ab	38±0.9
Abeokuta	4	7.98±0.10a	2.71±0.04a	46.6±1.6	21.1±2.1	31±0.2a	103±1.4	101±1.1	19±0.4a	97±0.2b	53±0.8	92±1.1ab	80±0.5	16±0.5a	86±0.7a	38±0.1
Nguroje	4	8.40±0.19cd	2.86±0.04bcd	47.0±1.4	21.9±2.6	33±0.9a	105±2.6	101±1.4	19±0.1a	92±1.0a	52±2.3	93±1.7ab	80±0.6	15±1.0a	91±1.6b	37±2.2
Umuahia	5	8.20±0.16abc	2.77±0.08ab	47.1±4.9	20.7±0.7	33±1.2a	102±2.1	102±3.2	19±0.3a	97±2.0b	55±2.0	95±2.4ab	78±1.8	15±0.5a	91±1.7b	40±2.4
Bauchi	2	8.47±0.01cd	2.90±0.02cd	50.5±8.1	22.2±1.7	33±1.2a	102±2.7	99±1.5	20±0.3a	95±1.0ab	53±0.4	95±1.0b	79±0.0	15±0.9a	87±0.9ab	39±2.2
Mubi	4	8.36±0.05bcd	2.83±0.05abcd	50.1±4.1	21.7±2.9	32±0.7a	105±3.4	101±1.0	19±0.9a	97±1.3b	53±1.0	93±1.3ab	80±0.5	16±1.4a	87±2.0ab	38±1.4
Jos	2	8.56±0.03d	2.90±0.04cd	54.9±4.0	22.2±0.8	31±0.4a	104±0.2	100±1.9	19±0.7a	93±2.8ab	51±0.2	93±0.9ab	80±1.6	15±0.3a	87±0.1ab	41±3.2
Tahoua	4	8.59±0.07d	2.95±0.02d	50.2±3.1	22.6±1.6	34±0.7a	101±3.5	103±1.2	20±0.7a	94±2.6ab	54±2.7	93±2.5ab	80±1.7	15±1.0a	88±0.8ab	39±2.0
Niamey	5	8.36±0.11cd	2.82±0.04abcd	49.6±3.3	22.3±1.3	33±0.5a	102±2.1	102±0.7	18±0.8a	93±1.8ab	53±2.1	95±1.3b	79±0.5	16±0.4	89±1.9ab	39±1.1

[@]Tahoua and Niamey are in Niger while the rest are in Nigeria.*Significant ($p<0.05$); **highly significant ($p<0.01$); ***very highly Significant ($p<0.001$) and †not significant ($p>0.05$), according to one-way ANOVA. Means, within a column, followed by the same letter are not significantly different at $p = 0.05$ according to Tukey HSD test. Measurements of distance are in mm and of angles in degrees. Fwl: Fore-wing, length; Fww: Fore-wing, width; Cub-a, b: Cubital vein, distance a, b; A4-O26: 11 angles of wing veins. Numbers in brackets are Ruttner numbers.

In order to investigate the similarity of the honeybee colonies under study, a PCA, using colony means of 14 morphometric characters (21 characters were excluded from the analysis, during a preliminary PCA, due to their failure to meet some conditions) of 10 worker honeybees from each of 59 colonies at 15 localities, was run to detect possible clusters. The suitability of PCA was assessed prior to analysis. Inspection of the correlation matrix showed that all variables had the minimum requirement of at least one correlation coefficient greater than 0.3. The overall Kaiser-Meyer-Olkin (KMO) measure was 0.90 with individual KMO measures from 0.63 to 0.95, thus meeting the minimum requirement for sampling adequacy. Bartlett's test of sphericity was statistically significant ($p<.0005$), indicating that the data could be appropriately analysed using PCA [12, 13].

Two principal components, with eigenvalues greater than one were extracted. They accounted for 61.8% and 14.4%, respectively, of the total variance between the samples. A Varimax orthogonal rotation was employed to aid interpretability. There were strong loadings of characters of size on both components (Table 7).

As may be seen in Figure 2, a scatter plot of these principal components did not produce distinct clusters.

Table 7: Rotated component matrix for PCA, with Varimax rotation, of morphometric characters of *A. mellifera* colonies from Nigeria.

Character (Ruttner numbers)	Rotated Component Coefficients		Communalities
	Component 1	Component 2	
Fore-wing, length (17)	**0.916***		0.841
Sternite 6, transversal diameter (16	**0.907**		0.844
Wax plate(mirror) of sternite 3, longitudinal diameter (12)	**0.882**		0.797
Tergite 3, longitudinal diameter (9)	**0.869**		0.94
Fore-wing, width (18)	**0.859**		0.739
Femur, length (5)	**0.853**	0.377	0.869
Sternite 6, longitudinal diameter (15)	**0.840**		0.713
Wax plate of sternite 3, transversal diameter (13)	**0.836**		0.700
Tibia, length (6)	**0.825**	0.367	0.816
Metatarsus, length (7)	**0.717**	0.427	0.696
Metatarsus, width (8)	**0.665**	0.484	0.677
Angle L13 (29)	**-0.514**		0.287
Tergite 4, longitudinal diameter (10)		**0.968**	0.945
Sternite 3, longitudinal diameter (11)		**0.967**	0.796

*Major loadings for each character are shown in bold.

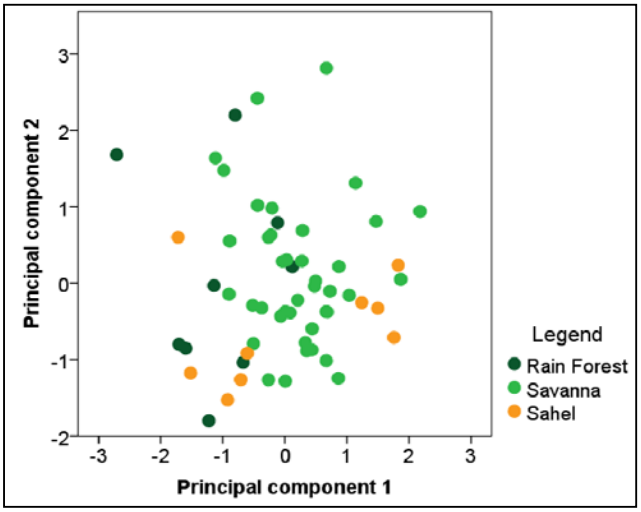

Fig 2: A scatter plot of the first two principal components of PCA, using the colony means of 14 morphological characters, of workers of *A. mellifera* from nine localities in Nigeria. Both components, which carry about 76% of the total variance, were loaded with characters of body size: The colonies are coded according to the type of vegetation of their origin.

Since PCA did not produce a clear clustering of the colonies, an alternative method, hierarchical cluster analysis, using means of 35 morphometric characters for the 15 localities under investigation, was used. Based on the output of this analysis (Figure 3A and Table 8), three tentative clusters were assumed: Cluster 1 made up of colonies from Maiadua, Yola, Umuahia, Niamey, Tahoua, Gitata, Mubi, Okuta and Bauchi; cluster 2 colonies from Abeokuta, Afunori and Sokoto; and cluster 3 colonies from Bamenda, Nguroje and Jos. A stepwise discriminant analysis (DA) was run, in order to predict the membership of the 59 colonies of *A. mellifera*, among the three *a priori* groups defined by the cluster analysis, as a means of confirming these groups. 35 morphometric characters of workers were used as predictor variables. Significant mean differences (ANOVA; $p<0.05$) were observed for 10 of the variables. While the log determinants were similar (16 and 18, respectively, for groups 1 and 2; group 3 had a few samples), Box's M indicated that the assumption of equality of covariance matrices was violated ($p<0.05$). However, given the large sample size, DA was deemed appropriate [12, 13].

Two canonical discriminant functions (with eigenvalues 1.9 and 1.1, respectively) were used in the analysis. They explained 63.8% and 36.2% of the total variance, respectively, and their Wilk's Lambda values, assessed by chi-square, were highly significant ($p<0.0005$), suggesting they had sufficient discriminatory power to group the cases. The six discriminant variables used in the analysis and their correlations with the discriminant functions are shown in Table 9. Overall, 86% of original grouped cases were correctly classified. 29 of the 37 cases in cluster 1 were correctly classified while five and three cases were misclassified into clusters 2 and 3, respectively. Of the 29 correctly classified cases, 18 were classified with a posterior probability of 95% and above and only one of the misclassified cases was classified with this probability. For clusters 2 and 3, all the cases were correctly classified. Eight out of the 14 cases in cluster 2 were classified with a posterior probability of at least 95% while all, but one, of the seven cases in cluster 3 were classified with this probability.

A pairwise comparison of the groups, using F-statistic, revealed a very highly significant difference between the groups' centroids ($p<0.0005$). A scatter plot of the two discriminant functions is shown in Figure 3B and the distribution of the members of the three groups (morphoclusters), in the area of study, in Figure 4.

Table 8: Proximity (dissimilarity) matrix of 15 populations of A. mellifera

	Squared Euclidean Distance														
Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1:Abeokuta	**0**	29	67	41	32	108	22	19	67	53	30	20	42	39	22
2:Afunori	29	**0**	63	53	31	71	31	27	46	52	30	29	36	70	51
3:Bamenda	67	63	**0**	31	31	52	36	28	70	46	21	46	55	58	49
4:Bauchi	41	53	31	**0**	20	44	20	29	66	24	27	51	26	49	28
5:Gitata	32	31	31	20	**0**	45	16	12	40	13	15	34	12	35	21
6:Jos	108	71	52	44	45	**0**	72	58	105	61	64	98	54	129	102
7:Maiadua	22	31	36	20	16	72	**0**	21	45	22	11	25	16	23	11
8:Mubi	19	27	28	29	12	58	21	**0**	54	37	15	18	30	46	28

9:Nguroje	67	46	70	66	40	105	45	54	**0**	30	40	55	52	55	60
10:Niamey	53	52	46	24	13	61	22	37	30	**0**	23	56	15	35	25
11:Okuta	30	30	21	27	15	64	11	15	40	23	**0**	17	25	38	24
12:Sokoto	20	29	46	51	34	98	25	18	55	56	17	**0**	45	49	41
13:Tahoua	42	36	55	26	12	54	16	30	52	15	25	45	**0**	43	23
14:Umuahia	39	70	58	49	35	129	23	46	55	35	38	49	43	**0**	15
15:Yola	22	51	49	28	21	102	11	28	60	25	24	41	23	15	**0**

Table 9: Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions

Variable (Ruttner Number)	Function	
	1	2
Fore-wing, length (17)	**.344†**	-0.008
Width of the dark stripe between the tomentum and the posterior rim of tergite (3)	0.181	**.464**
Pigmentation of plates of scutellum (36)	0.106	**-.376**
Wing venation angle G18 (25)	-0.239	**.286**
Wing venation angle J10 (26)	-0.050	**.271**
Sternite 6, longitudinal diameter (15)	0.030	**.084**

†The largest absolute correlation between each variable and any discriminant function is shown in bold.

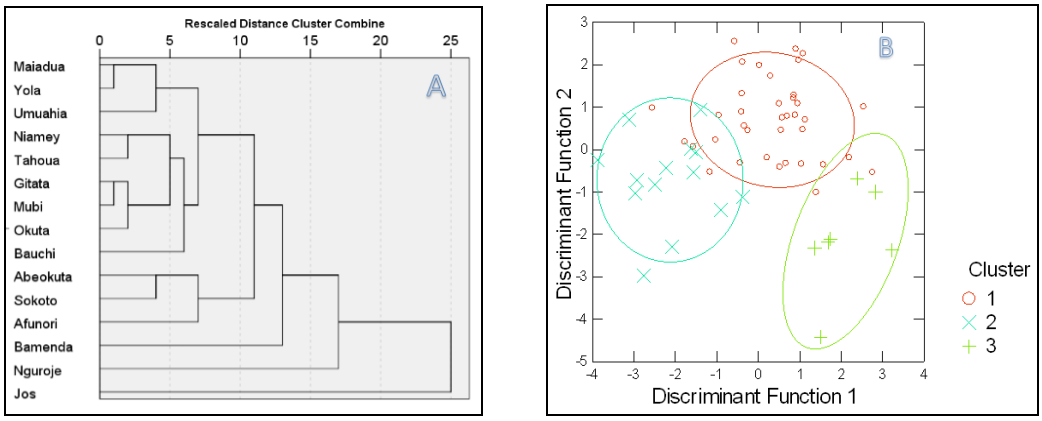

Fig 3: (A) Classification of 15 populations of *A. mellifera* from Nigeria by hierarchical cluster analysis (using average linkage (between groups)) using means of 35 morphometric characters for the 15 localities. Based on the dendrogram, three tentative groups were identified for entry into stepwise discriminant analysis. (B) Confirmation of the three groups by discriminant analysis. 86% of original grouped cases were correctly classified into their original groups and the distances between group centroids were highly significant ($p < 0.0005$) according to F-statistic. 75% confidence ellipses are shown.

Fig 4: Distribution of members of three morphoclusters of *A. mellifera* from Nigeria defined by hierarchical cluster analysis and confirmed by stepwise discriminant analysis. Only colonies assigned with a posterior probability of at least 95% (59% of total number of colonies in the study) are shown. Each circle represents one colony.

In order to test the relationship between altitude and the two discriminant functions (that is to test whether the distribution of the morphoclusters was related to altitude), a Pearson's product-moment correlation analysis was run. Preliminary analyses showed the relationship to be linear with all variables normally distributed (as assessed by visual inspection of normal Q-Q plots) and there were no outliers [13]. There was a very highly significant moderate positive relationship between discriminant function 1 and altitude (r (56) = 0.484, p<0.0005 and a very highly significant strong positive relationship between discriminant function 2 and altitude (r (56) = 0.545, p<0.0005.

Discussion

As may be seen in Table 10, the mean values of a set of the morphometric characters measured in this study are in general agreement with those reported for the subspecies of *A. mellifera* in sub-Saharan Africa [5, 8], except some values of doubtful validity reported by Ajao, Oladimeji [6] and Oyerinde, Dike [7]. This agreement is a confirmation of the correctness of the measurements taken in this study.

Although ANOVA reveals a considerable variation of morphological characters between the sampled localities, principal component analysis suggests this variation is not sufficient in grouping the colonies into geographically separable groups. As revealed by PCA, the most important morphological variation of the honeybees of this area is size of the body. As may be seen from the scatter plot of the principal components (Figure 2), the colonies form one mixed cluster. In other words, they do not segregate according to the type of vegetation of their origin. This suggests a continuous variation in size, which is not related to the present ecological variation of the area. However, grouping the colonies into three morphoclusters, by hierarchical cluster analysis in conjunction with discriminant analysis, in which all colonies from localities of altitude above 1000 m (Nguroje, Jos and Bamenda) were placed in one group, suggests a morphological variation based on altitude. This is further supported by the significant positive correlation between the discriminant functions and altitude. As may be seen in Figure 4, whereas morphocluster 3 is isolated from morphoclusters 1 and 2 by altitude, the latter two clusters lack such isolation. For example, both clusters (morphoclusters 1 and 2) are present in one locality, Mubi. Thus, it may be concluded that the colonies under investigation have been grouped into two broad groups: Highland (colonies from Nguroje, Jos and Bamenda) and lowland (colonies from other localities) groups. However, ranking these groups as ecotypes or subspecies is beyond the scope of the present study and, therefore, requires further investigation.

Table 10: Comparison of values (mean ± s.d.) of some morphometric characters of subspecies of *A. mellifera* from sub-Saharan Africa from various sources.

Character	This study	Ruttner [5]	Ajao, Oladimeji [6]	Yu, Xie [8]	Oyerinde, Dike [7]
Cover hair, length	0.13 ± 0.00 - 0.20 ± 0.01	0.20 ± 0.02 - 0.26 ± 0.04			
Fore-wing, length	7.98 ±. 0.10 - 8.59 ± 0.07	8.13 ± 0.19 - 8.95 ± 0.37	9.54 ± 0.01†	8. 42 ± 0. 66	3.43 ± 0.16†
Fore-wing, width	2.71 ± 0.04 - 2.95 ± 0.02			3. 13 ± 0. 21	1.18 ± 0.08†
Tergites 3 + 4, diameter	3.80 ± 0.03 - 4.08 ± 0.05	3.92 ± 0.10 - 4.25 ± 0.21		3. 80 ± 0. 28	
Angle J16	90.10 ± 1.30 - 95.10 ± 1.00	86.44 ± 8.37 - 96.76 ± 3.60			
Pigmentation, scutellum	6.40 ± 0.24 - 7.53 ± 0.17	0.35 ± 0.73 - 6.77 ± 0.96			

†The validity of these values is doubtful because they fall outside the range reported for all subspecies of *A. mellifera*. For example, in respect of the length of the fore-wing, the smallest value, 8.13 ± 0.19 mm, was reported for *A. m. jemenitica* and the highest value, 9.33 ± 0.11 mm, for *A. m. mellifera* [5]. Measurements of distance are in mm and of angles in degrees.

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