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Morpho-Classification of Morphoclusters of *Apis mellifera* L. in the Savannah Vegetation of Nigeria

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ABSTRACT: Morphometric features were used to discriminate *Apis mellifera* L. species in the Savannah Vegetation of Nigeria into morphoclusters. Data obtained were analysed with parametric statistical tools of mean, standard deviation and standard error. Means of morphometric features of the honeybee morphoclusters were presented in centroids and the confidence intervals (95%) were expressed. Taxonomy of savannah vegetation honeybee samples based on morphometric features produced two distinct morphoclusters of honeybees. Morphocluster 2 had the highest (56.7%) percentage of honeybee in the region. The within cluster percentage of state of *Apis mellifera* in the savannah zone of Nigeria based on variations of the morphometric features showed that all honeybee samples obtained from apiaries sited in Abuja (100%) and Kaduna (100%) were of morphocluster 1 while majority of morphocluster 2 honeybee were present in Kebbi (100%) and Kwara (100%) states. Though, Adamawa State had some honeybee of morphocluster 1(16%), the larger number of bees from this state was of morphocluster 2 (84%).

Keywords: Savannah vegetation, morphometric features, *Apis mellifera*, morphoclusters, taxonomy.

INTRODUCTION

The honeybees have ability to adapt to a wide range of ecological conditions that give them the chance of existing in virtually all parts of the world. Nigeria has wide range of climates and habitats based on geographic variation and the presence of honeybees with different morphological, physiological and behavioural aspects. The two main vegetation zones in Nigeria are the forest and savannah. The two vegetations cut across the southern and northern regions of the country and are endowed with adequate flora and edaphic factors ideal for bee foraging and sustainability (Ande *et al.*, 2008 a&b; Oyerinde and Ande, 2006; Oyerinde *et al.*, 2012).

Taxonomy studies of honeybees have used morphometry, geometric morphometry, biochemical and molecular techniques. Based on the use of morphometric features, the European honeybees *Apis mellifera* L. were classified into four main groups: West Mediterranean and Northeast Europe (M branches), Central and Southeast Europe (C branches), Near east (0 branches) and Tropic Africa (A branches) (Kauhaussen-Keller *et al.*, 1997; Ruttner, 1988). The molecular techniques also confirmed this morphometric classification (Arias and Sheppard, 1996; Franck *et al.*, 2000).

The need to determine the morphoclusters of honeybee in northern Nigeria is germane in order to establish the morphoclusters/subspecies of honeybees that are kept in beekeeping practice in the savannah vegetation zone of the country and to document the influence of the variations of

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morphometric features of honey bees in the area. This study identifies morphoclusters of honeybee in the savannah vegetation zone of Nigeria with the use of variations in the morphometric features of the morphoclusters and also established the level of association between the morphometric differences in morphoclusters of honeybee from different states in the zone.

MATERIALS AND METHODS

Study Site and Collection of Sample

A total of 7,500 samples of honeybee workers were collected from 250 colonies situated in the savannah vegetation zone of Nigeria. Samples of 30 honeybee workers were collected from 50 colonized hives sited in established honeybee farms in Ilorin (Kwara State), Abuja (FCT), Katari (Kaduna State), Mayo Belwa (Adamawa State) and Zuru (Kebbi State) in the savannah vegetation zone of Nigeria. All samples were taken from colonies with captured swarms and unmanaged for queen replacement. The samples were stored separately in 70% ethanol in a small labelled plastic container according to the state of collections. These were used for further morphometric analysis in the laboratory in order to determine the morphoclusters of honeybees collected from colonies in the different states in the vegetation zone.

Morphometric Studies

Multivariate morphometric analyses were performed on ten randomly selected samples of honeybee workers obtained from 250 colonies representing the five randomly selected states in the savannah vegetation of Nigeria. This was done by adopting the use of morphometric analyses of *Apis florea* (Hepburn *et al.*, 2005; Michener, 2007) and *A. mellifera* (Andere *et al.*, 2008; Michener, 2007). All measurements were made with the aid of a calibrated hand held digitalized MiScope microscope with magnification range of 40-140x in millimetre (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 largest part of the body (abdomen) at the widest point, the length and width of the forewing and hind wing. Each morphometric feature measurement was carefully recorded per hive and all the data collected were statistically analysed.

Data Analyses

Data obtained from the morphometric measurements were analysed with SPSS version 17 software. The analysis involved parametric statistics tools of mean, standard deviation and standard error. The distribution and relation between them were subjected to two-step cluster analysis. Morphoclusters means were presented in centroids and also the simultaneous confidence intervals (95%) of means were calculated.

RESULTS

Taxonomy of honeybees collected from apiaries sited in the savannah vegetation zone of Nigeria based on variations of morphometric features revealed that only two distinct morphoclusters of *Apis mellifera* exist in this region of the country (Table 1). Morphocluster 2 had the highest (56.7%) percentage of honeybee in the region. The within cluster percentage of state of *Apis mellifera* in the savannah zone of Nigeria based on variations of the morphometric features (Table 2) showed that all honeybee samples obtained from apiaries sited in Abuja (100%) and Kaduna (100%) were of morphocluster 1 while majority of morphocluster 2 honeybee were present in Kebbi (100%) and Kwara (100%) states.

Table 1: **Frequency of honeybee samples from the savannah vegetation zone in different morphoclusters identified on basis of morphometric features**

Morphocluster	No	% of Total Honeybee samples
1	324	43.3%
2	426	56.7%
Combined	750	100.0%
Total	750	

Table 2: **Distribution by states in the savannah vegetation zone of two morphoclusters of *Apis mellifera* identified on the basis of morphometric features**

Morphocluster	1	2	Combined
Abuja FCT			
Frequency	150	0.0	150
Percentage (%)	100	0.0	100
Adamawa State			
Frequency	24	126	150
Percentage (%)	16	84.0	100
Kaduna State			
Frequency	150	0.0	150
Percentage (%)	100	0.0	100
Kebbi State			
Frequency	0	150	150
Percentage (%)	0.0	100.0	100
Kwara State			
Frequency	0	150	150
Percentage (%)	0.0	100.0	100.0

Though, Adamawa State had some honeybee of morphocluster 1(16%) but, the larger number of bees from this State were of morphocluster 2 (84%).

Centroid of the two honeybee morphoclusters from savannah vegetation zone of Nigeria based on variations of morphometric features (Table 3) indicated that morphocluster 1 had the highest mean values of the TBL (5.67 mm), HLP (2.74 mm), WFW (1.18 mm) and LRC (2.38 mm), while morphocluster 2 recorded higher mean values in the other morphometric features i.e., WLP (1.85 mm), AL (1.64 mm), PL (1.83 mm), LF (0.99 mm), LT (0.86 mm), LM (0.73 mm), LPB (1.22 mm), WPB (0.44 mm), LHW (2.44 mm) WHW (0.66 mm), LFW (3.60 mm) and WRC (0.19 mm) compared to the means of morphocluster 1, as well as the combined means of the variables in the region.

The within cluster variation simultaneous 95% confidence intervals of means of morphometric features (Fig. 1) in the savannah vegetation zone of Nigeria showed that the interval of means of TBL (Fig. 1a), HLP (Fig. 1b) and WFW (Fig. 1k) in morphocluster 2 were above the overall means for the respective morphometric variables. In addition, morphocluster 1 had mean intervals higher than the overall means in WLP (Fig. 1c), AL (Fig. 1d), PL (Fig. 1e), LPB (Fig. 1f), WPB (Fig. 1g), LHW (Fig. 1h), WHW (Fig. 1i), LFW (Fig. 1j), LF (Fig. 1l), LT (Fig. 1m), LM (Fig. 1n), LRC (Fig. 1o) and WRC (Fig. 1p) while five landmarks were noticed on the radial cell of the two morphoclusters.

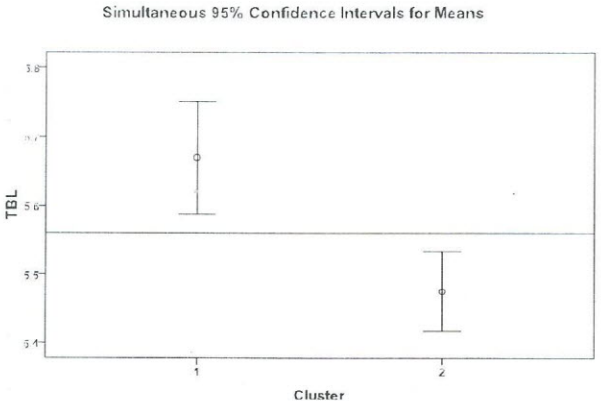
Table 3: **Centroids of ecotype *Apis mellifera* based on morphometric features in the savannah vegetation zone of Nigeria**

Morphocluster	TBL		HLP		WLP		AL		PL		LF		LT		LM		LPB		WPB	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	5.67	0.65	2.74	0.49	1.70	0.12	1.53	0.10	1.53	0.33	0.93	0.07	0.81	0.06	0.65	0.06	1.19	0.07	0.39	0.05
2	5.48	0.54	2.53	0.52	1.85	0.21	1.64	0.15	1.83	0.38	0.99	0.08	0.86	0.06	0.73	0.08	1.22	0.07	0.44	0.05
Combined	5.56	0.60	2.62	0.52	1.79	0.19	1.59	0.14	1.70	0.39	0.97	0.08	0.84	0.06	0.70	0.08	1.21	0.07	0.42	0.05
+SEM	0.02		0.02		0.01		0.01		0.01		0.00		0.00		0.00		0.00		0.00	

Morphocluster	LHW		WHW		LFW		WFW		LRC		WRC	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	2.33	0.21	0.64	0.07	3.43	0.16	1.18	0.08	2.38	11.48	0.18	0.01
2	2.44	0.13	0.66	0.07	3.60	0.09	1.17	0.07	1.30	0.06	0.19	0.02
Combined	2.39	0.18	0.65	0.07	3.53	0.15	1.17	0.08	1.77	7.57	0.19	0.02
+SEM	0.01		0.00		0.01		0.00		0.00		0.00	

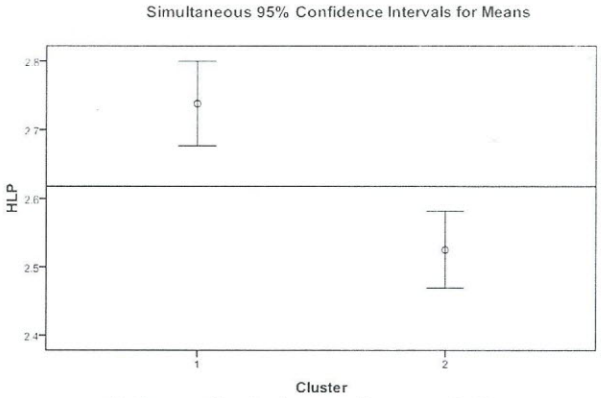
Key: Total Body Length (**TBL**), Height at Largest Part (**HLP**), Width at Largest Point (**WLP**), Antennae Length (**AL**), Proboscis Length (**PL**), Length of Femur (**LF**), Length of Tibia (**LT**), Length of Tarsi (**LM**), Length of Pollen Basket (**LPB**), Width of Pollen Basket (**WPB**), Length of Hindwing (**LHW**), Width of Hindwing (**WHW**), Length of Forewing (**LFW**), Width of Forewing (**WFW**), Length of Radial Cell (**LRC**), Width of Radial Cell (**WRC**)

Figure 1: **Simultaneous 95% confidence intervals of means of morphoclusters based on morphometric features in the savannah vegetation zone of Nigeria**



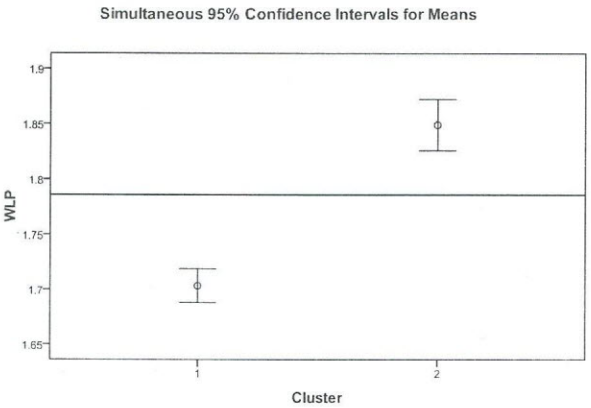
Reference line is the overall mean = 5.56

Figure 1a: **Within cluster variation of means of Total Body Length (TBL)**



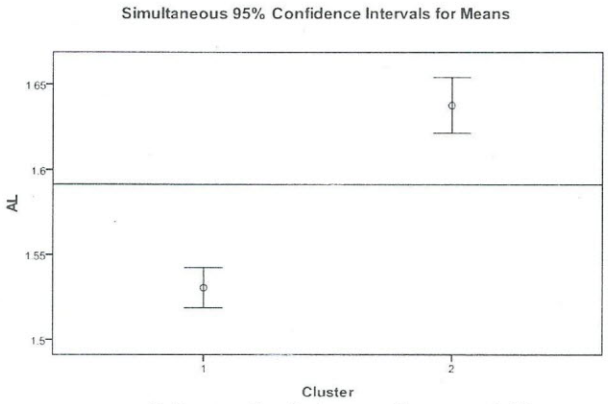
Reference line is the overall mean = 2.62

Figure 1b: **Within cluster variation of means of Height at Largest Part (HLP)**



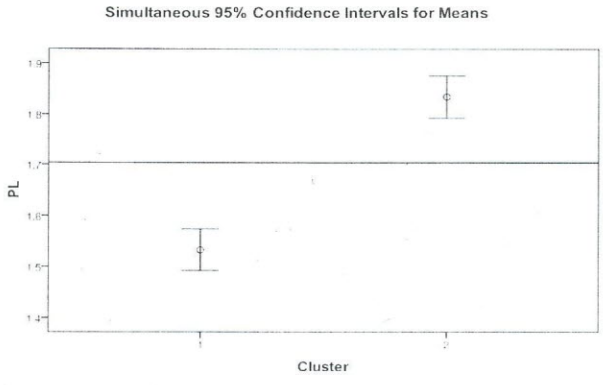
Reference line is the overall mean = 1.79

Figure 1c: **Within cluster variation of means of Width at Largest Part (WLP)**



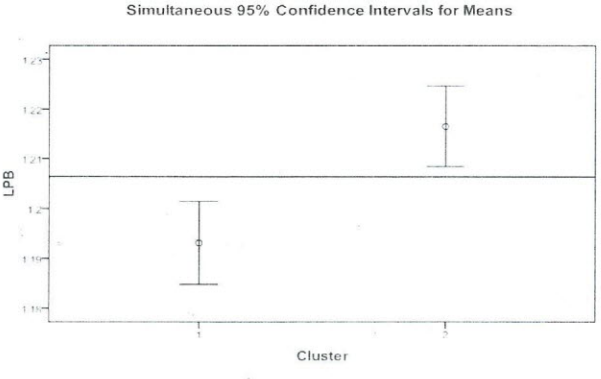
Reference line is the overall mean = 1.59

Figure 1d: **Within cluster variation of means of Antennae Length (AL)**



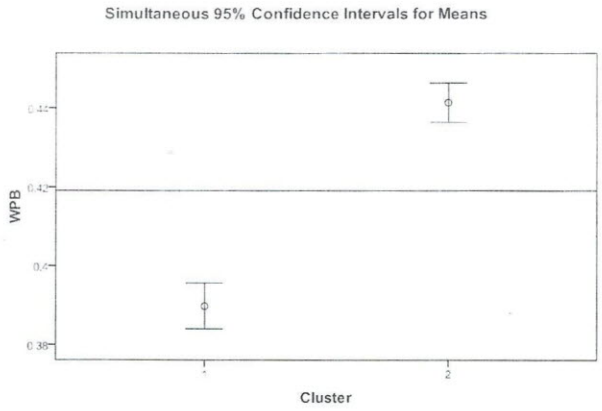
Reference line is the overall mean = 1.70

Figure 1e: **Within cluster variation of means of Proboscis Length (PL)**



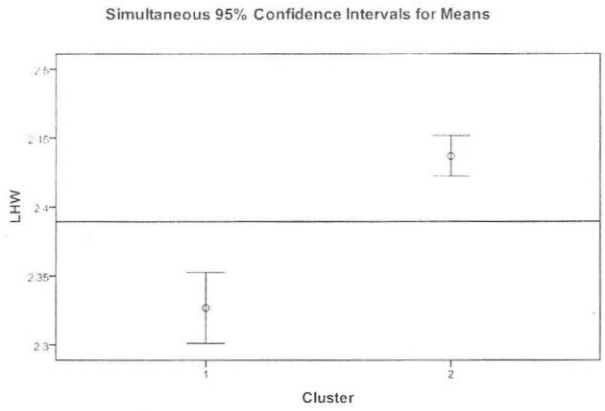
Reference line is the overall mean = 1.21

Figure 1f: **Within cluster variation of means of Length Pollen Basket (LPB)**



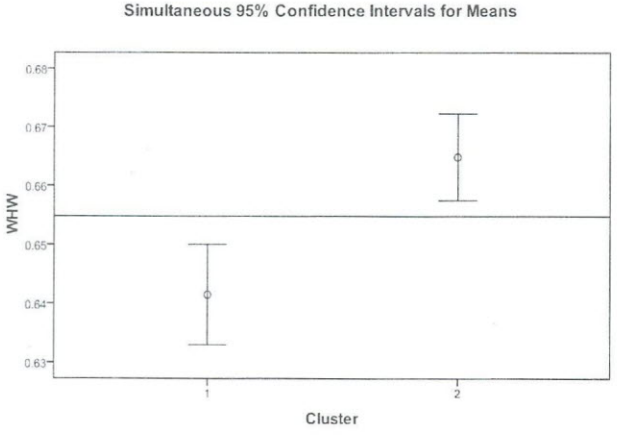
Reference line is the overall mean = 0.42

Figure 1g: **Within cluster variation of means of Width Pollen Basket (WPB)**



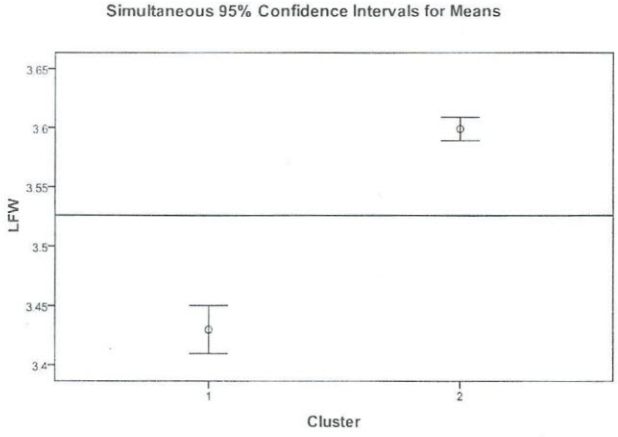
Reference line is the overall mean = 2.39

Figure 1h: **Within cluster variation of means of Length Hindwing (LHW)**



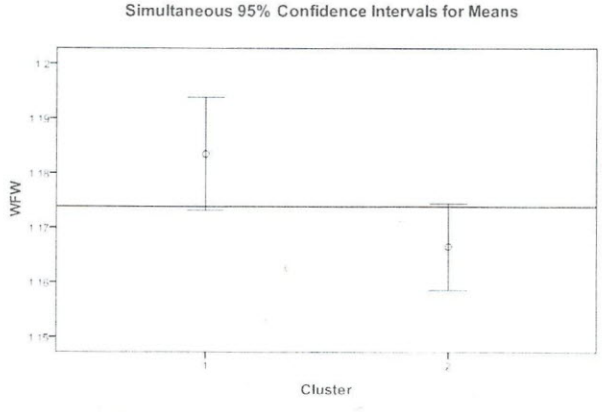
Reference line is the overall mean = 0.65

Figure 1i: **Within cluster variation of means of Width Hindwing (WHW)**



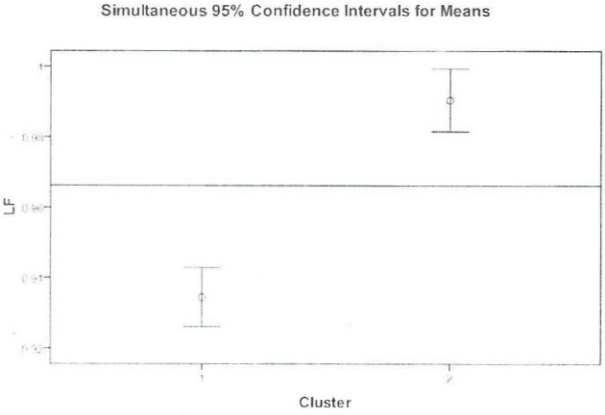
Reference line is the overall mean = 3.53

Figure 1j: **Within cluster variation of means of Length Forewing (LFW)**



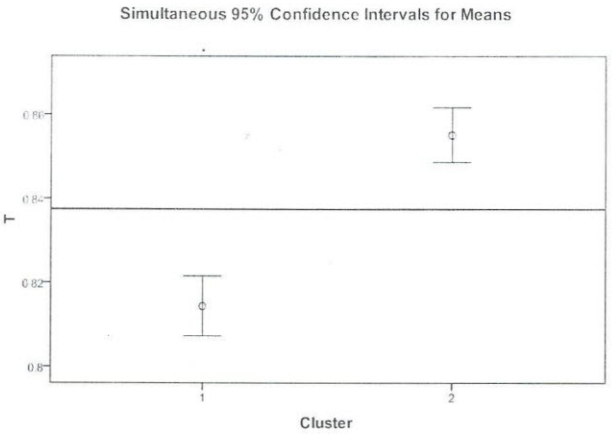
Reference line is the overall mean = 1.17

Figure 1k: **Within cluster variation of means of Width Forewing (WFW)**



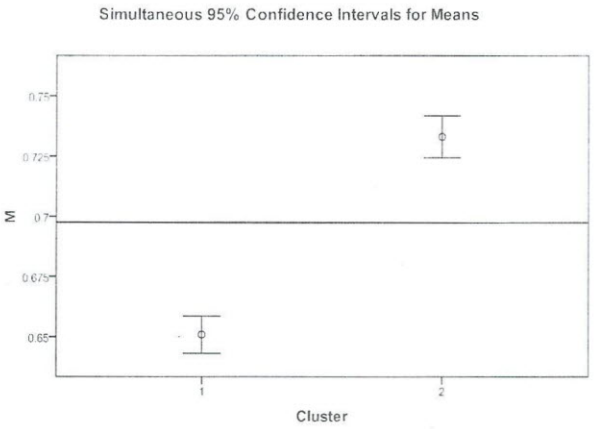
Reference line is the overall mean = 0.97

Figure 1l: **Within cluster variation of means of Length Femur (LF)**



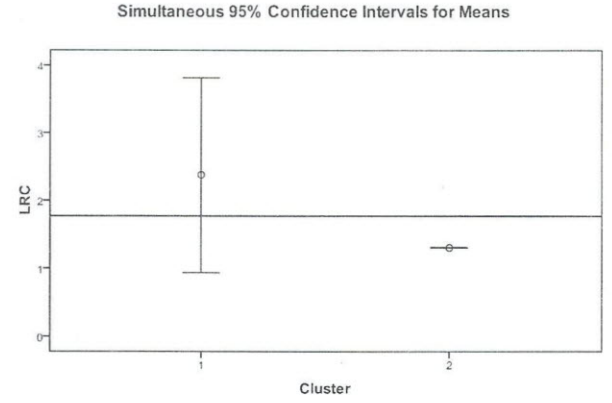
Reference line is the overall mean = 0.84

Figure 1m: **Within cluster variation of means of Length Tibia (T)**



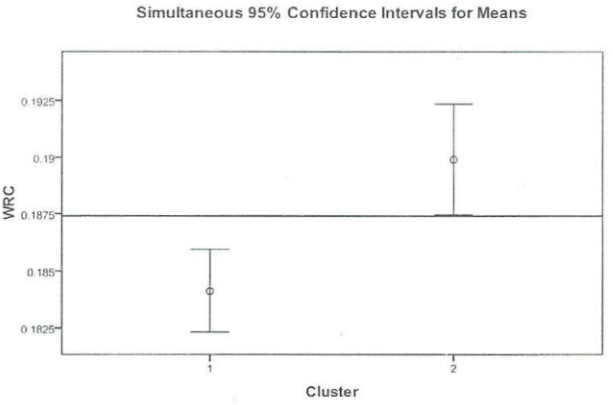
Reference line is the overall mean = 0.70

Figure 1n: **Within cluster variation of means of Length Tarsi (M)**



Reference line is the overall mean = 1.29

Figure 1o: **Within cluster variation of means of Length of Radial Cell (LRC)**



Reference line is the overall mean = 0.19

Figure 1p: **Within cluster variation of means of Width of Radial Cell (WRC)**

DISCUSSION

Results obtained from this study have revealed that honeybee samples collected from states of the savannah vegetation zone formed different morphoclusters. Also, differences existed between the morphometric measurements of the various anatomic features of the morphoclusters of honeybees in the savannah vegetation zones of Nigeria. This is due to the wide disparity between the ecological factors of the states within the zone, which conformed to Ruttner (1988) and Shepperd *et al.*, (1997) earlier report on the impact of vegetation variation on the distribution of honeybee's species in the tropics.

The relativity of the morphometric features of morphoclusters of honeybees obtained from the different state in the belt could be deduced to similarity of biology of honeybee species in the same geographical zone (Winston *et al.*, 1983). The two morphoclusters obtained in the five savannah vegetation states indicated that honeybee in the vegetation zone had narrower morphometric variations. This confirmed the influence of geographical location on the distribution of honeybee subspecies (Ojeleye, 1999; Oyerinde *et al.*, 2012a&b; Sheppard *et al.*, 1997). Also, the low number of morphoclusters established in the savannah vegetation states based on variations in the morphometric features of the morphoclusters showed wide distribution of the two morphoclusters over a wide range of states in the belt. This is in agreement with Amorin and Ribeiro (2001), which stated that honeybee species could migrate over wider vegetation belt in search of

food (nectar and pollen), water and appropriate nesting site during swarming season or in period of adverse environmental condition.

The efficacy of use of anatomical parts morphometric features variations is an indication that this technique can be adopted for taxonomy of honeybee from the savannah vegetation zones of Nigeria into their respective morphoclusters. This is in line with the report of the successful use of morphometric of *A. mellifera* by Andere *et al.* (2008).

CONCLUSION

This study entails morphometric classification of honeybee workers sampled from honeybee colonies sited in the savannah vegetation zone of Nigeria. The use of multivariate morphometric features in classification of the honeybee reared in the savannah vegetation of Nigeria implied that any of the techniques can be adopted by taxonomists for classifying the honeybee into morphoclusters in the zone.

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