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Morphometric variations of species of tsetse fly (Diptera: Glossinidae) and their prevalence in federal capital territory (FCT) Abuja, Nigeria

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Abstract
Total sample of four hundred and eighty (480) tsetse flies (Glossina) were collected from three randomly selected Area Councils of Federal Capital Territory (FCT), Abuja namely: Abaji, Kwali and Gwagwalada. The multivariate morphometric analysis was made with MiScope microscope (Mag. 40-140x). The morphometric variables measured include: the length of body, proboscis, antennae, femur, tibia, tarsi, largest part of the body (abdomen) at the widest point and length and width of the forewing. Data were analyzed with parametric statistic tools of mean and standard deviation. The distribution and relation between them were subjected to two-step and hierarchical cluster analysis. Also, the dendrogram plot was used to show the phylogenetic relationship of the species. Results show the presence of two morphometrically distinct Glossina species in the FCT. *Glossina tachinoides* recorded 67.5% while *G. morsitans* was 32.5 % of collections. The distribution of species per Area Council showed majority of *G. tachinoides* i.e. 73.4%, 51.6% and 81.9% in Gwagwalada, Abaji and Kwali respectively while the remaining flies were *G. morsitans*. The correlation of morphometric features with sampling sites i.e. Area Councils gave insignificant negative correlations while highly significant correlations ($p<0.01$) were established between the morphometric variables of the tsetse flies. The Dendogram plot based on variations in morphometric features revealed linkages between the different samples collected. This gives an indication that the samples were of the same descent and shows that the morphometric based features can be engaged in classifying tsetse fly into species in FCT, Abuja.

Keywords: Tsetse fly, morphometric features, area council, classification, species

Introduction
Tsetse (Diptera: Glossinidae) also known as tik-tik flies, are large biting flies that inhabit much of Tropical Africa. Tsetse flies include all the species in the genus Glossina, which are placed in the family, Glossinidae. The tsetse flies are obligate parasites that live by feeding on the blood of vertebrate animals. They are vectors of trypanosomes that cause sleeping sickness (Trypanosomosis) in humans and nagana in livestock across sub-Saharan Africa. The insect has major negative impact on agriculture, especially on livestock economies [1, 2].

Morphometric analyses have become interesting tools for the study of population genetics. It measures the micro-evolutionary continuous changes in an insect and represents a low-cost tool adapted to the study of phenotypic evolution. This tool has shown considerable epidemiological significance for medically important insects such as the vectors of leishmaniasis and Chagas disease in Latin America [2]. In addition, the use of geometric morphometrics was established for comparisons of many samples with homologous landmarks, e.g. the vein-patterns on wings [3, 4].

Also, the variation of morphometric features was used to determine how close or distant tsetse fly populations with factors such as size and rotation of the wings from the different populations [5]. This technique allows a more sensitive appraisal of the relationship between groups than genetic techniques involving microsatellites. The technique had significantly revealed the structure of vector populations and provides means to isolate geographical distribution of vectors to prevent casual mixing with other populations and enhance future vector control efforts [6].

Tsetse flies are classified based on a combination of distributional, behavioural, molecular and morphological characteristics to the Genus Glossina. It is grouped as the sole member of the family Glossinidae that is essentially one of the four families of blood-feeding obligate

parasites [4]. They are in the order Diptera, the true flies. Up to 34 species and subspecies of tsetse flies were recognized, depending on the particular classification methods used by taxonomists [7].

The objective of initiative to eliminate tsetse flies and trypanosomosis in Africa currently employed control strategies which include the use of screens, tsetse traps and area-wide techniques to exterminate pockets of tsetse infestations urgently needs improvement. The success of these control strategies requires proper identification of species of isolated tsetse populations where elimination can be undertaken. The proper identification of tsetse populations is critical and will direct the choice between elimination and suppression of tsetse flies in a specific area.

This study was done at Abuja the Federal Capital Territory of Nigeria to characterize the species of tsetse flies that were infesting livestock in the Area Councils of the territory. The research engaged the comparison of established variations in the morphological differences and prevalence of species of Glossina (Tsetse flies) in different Area Councils to group them into their respective species. This is germane to the provision of appropriate control of the colonies of tsetse flies in the area to combat the possible outbreak of tsetse related diseases.

Materials and Methods

A total sample of four hundred and eighty (480) tsetse flies was collected from three randomly selected Area Councils of FCT, Abuja namely: Abaji, Kwali and Gwagwalada. All samples were trapped in nomadic herdsmen settlements in the Area Councils with the use of biconical traps for six months (i.e. April- October, 2017). Samples collected were stored separately in 70% ethanol in a small labeled plastic vial according to the location.

The multivariate morphometric analysis was made with the aid of a calibrated hand held digitalized MiScope microscope with magnification range of 40-140x in millimeter at the Insect Museum of Department of Crop Protection, Ahmadu Bello University, Zaria-Nigeria. The morphometric variables measured include: the length of the body, proboscis, antennae, femur, tibia, tarsi, the largest part of the body (abdomen) at the widest point, length and width of the forewing. This technique was used to establish the species of tsetse flies collected from different locations and also to confirm the possible correlations in the morphometric features of tsetse flies encountered in the Area Councils. The variables measured were carefully recorded per location and all the data collected were statistically analyzed.

Data were analyzed with SPSS software Version 17. The analysis involved parametric statistic tools of mean and standard deviation. The distribution and relation between them were subjected to two-step and hierarchical cluster analysis. Species means were presented in centroids and the simultaneous confidence intervals (95%) of means were calculated. In addition, the dendrogram plot was used to show the phylogenetic relationship of the samples from the different Area Councils of the FCT. This allows classification of the tsetse flies collected into species. Attempt was made at correlating the morphometric features distinguishing the identified species to ascertain the morphometric features that have relationships. The Pearson's correlation method was used for this analysis.

Results

The morphometric analysis of unknown species of tsetse fly was done on collections from three selected Area Councils of FCT, Abuja; Namely Abaji, Kwali and Gwagwalada. The result showed the presence of two morphometrically distinct Glossina species in the regions. *Glossina tachinoides* recorded 67.5% while *G. morsitans* was 32.5 % of the number of samples collected from the three Area Councils (Table 1).

Table 1: Frequency of Tsetse fly samples from selected Area Councils in FCT, Abuja on the basis of morphometric features

Species	No	% of Total Tsetse fly samples
Glossina tachinoides	324	67.5%
Glossina morsitans	156	32.5%
Combined	480	100.0%
Total	480	

The distribution of the tsetse fly species per Area Council based on morphometric features variation (Table 2) showed that, Gwagwalada, Abaji and Kwali had majorly i.e. 73.4%, 51.6% and 81.9% *G. tachinoides* respectively while the remaining flies were *G. morsitans*.

Table 2: Distribution by Area Councils of two species of Tsetse fly identified on the basis of morphometric features

Species	Gwagwalada		Abaji		Kwali	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
Glossina tachinoides	105	73.4%	97	51.6%	122	81.9%
Glossina morsitans	38	26.6%	91	48.4%	27	18.1%
Combined	143	100.0%	188	100.0%	149	100.0%

A total of 10 different morphometric features measured on the tsetse flies samples caught at different Area Councils in FCT Abuja, gave variations drawn into centroid (Table 3). The mean value of Head / Eyes (HE) of *G. morsitans* (10.47 mm) was above combined mean of (9.61mm) while the least HE was recorded in *G. tachinoides* (8.75 mm). Also the Body Length (BL) gave a similar mean value as HE. The mean value of the BL recorded in *G. morsitans* (75.96 mm) and *G. tachinoides* (55.46 mm) portrayed *G. morsitans* having the longest mean than the combined mean (65.71). The means of Antenna (AN) ranged between 8.08 mm in *G. morsitans* and 9.17 mm in *G. tachinoides*. The mean length of femur (FE) recorded were 23.93 mm and 18.93 mm in *G. tachinoides* and *G. morsitans* respectively. The combined mean (21.43 mm) measured was less than the values of *G. tachinoides*.

The Tibia length (TI) recorded mean value of 13.39 mm in *G. tachinoides* and 17.43 mm in *G. morsitans* while the mean value of Tarsal (TA) was 18.53 mm in *G. tachinoides* and 19.92 mm in *G. morsitans*. In addition, the mean of length of the Abdomen (AB) at the widest point in *G. tachinoides* was 18.79 mm while *G. morsitans* had 28.94 mm. Also, the mean value of the Wing Length (WL) was 42.74 mm in *G. tachinoides* and 57.37 mm in *G. morsitans* while the mean of the Thorax (TH) was 27.95 mm in *G. tachinoides* and 36.27 mm in *G. morsitans*.

Table 3: Centroids of Species of Tsetse fly Based on Morphometric Features in Area Councils of the FCT, Abuja

Species	HE		BL		AN		FE		TI		TA		AB		WL		TH	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Glossina tachinoides	8.75	0.42	55.46	2.67	8.08	0.71	18.93	0.68	13.39	2.06	18.53	0.40	18.79	1.99	42.74	4.85	27.95	0.43
Glosssina mortisan	10.47	0.16	75.96	2.33	9.17	0.04	23.93	1.82	17.43	0.96	19.92	0.06	28.94	0.49	57.37	3.29	36.27	1.53
Combined	9.61	0.92	65.71	10.65	8.62	0.74	21.43	2.87	15.41	2.59	28.22	0.76	23.86	5.32	50.06	8.46	32.11	4.35

The within cluster variation simultaneous 95% confidence intervals of means of morphometric features (Fig. 1) in the Area Councils of the HE in FCT showed that the means of HE in *G. morsitans* was above the overall means of 9.61 mm while the HE of *G. tachinoides* fell below the overall mean while the BL value of *G. morsitans* was above the overall means and higher than the value of *G. tachinoides*. Also, the results of AN, FE, TI, TA, AB, WL and TH (9.17 mm, 23.93 mm, 17.43 mm, 28.22 mm, 28.94 mm, 57.37 mm and 36.27 mm) recorded in *G. morsitans* were above their respective overall means.

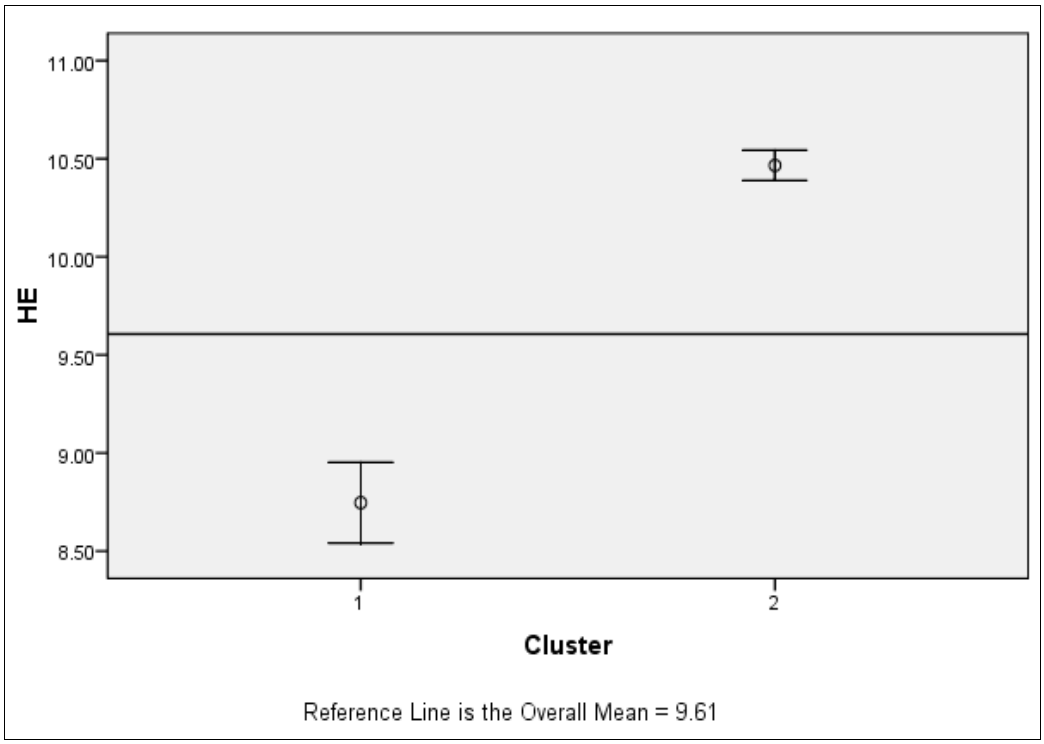


Fig 1a: Within Cluster Variation of Means of Head/ Eye (HE)

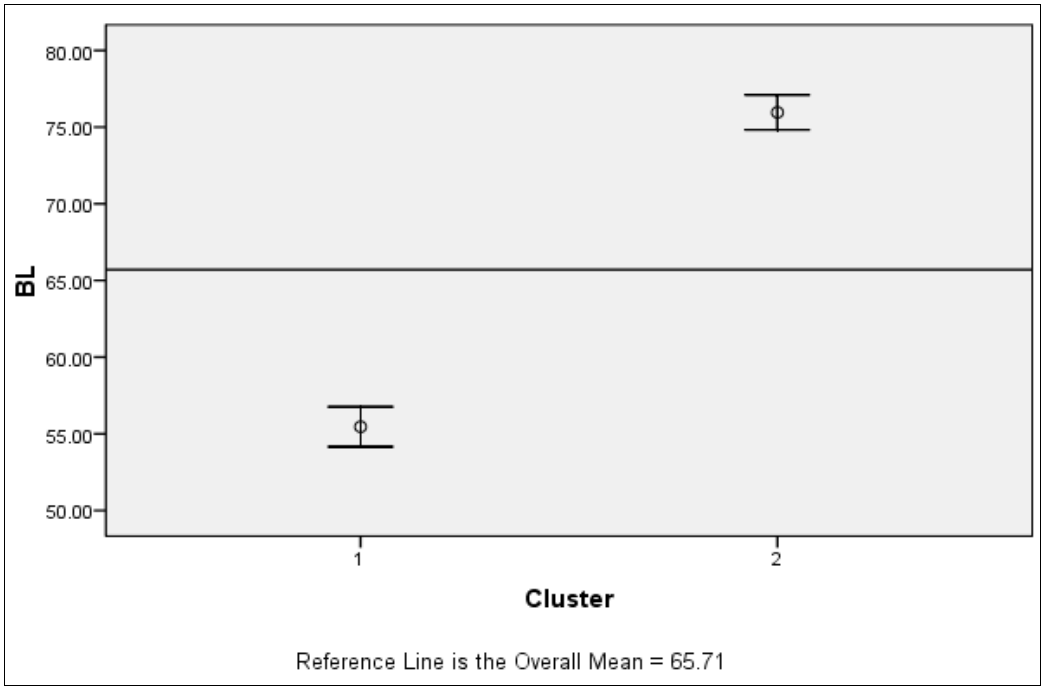


Fig 1b: Within Cluster Variation of Means of Body Length (BL)

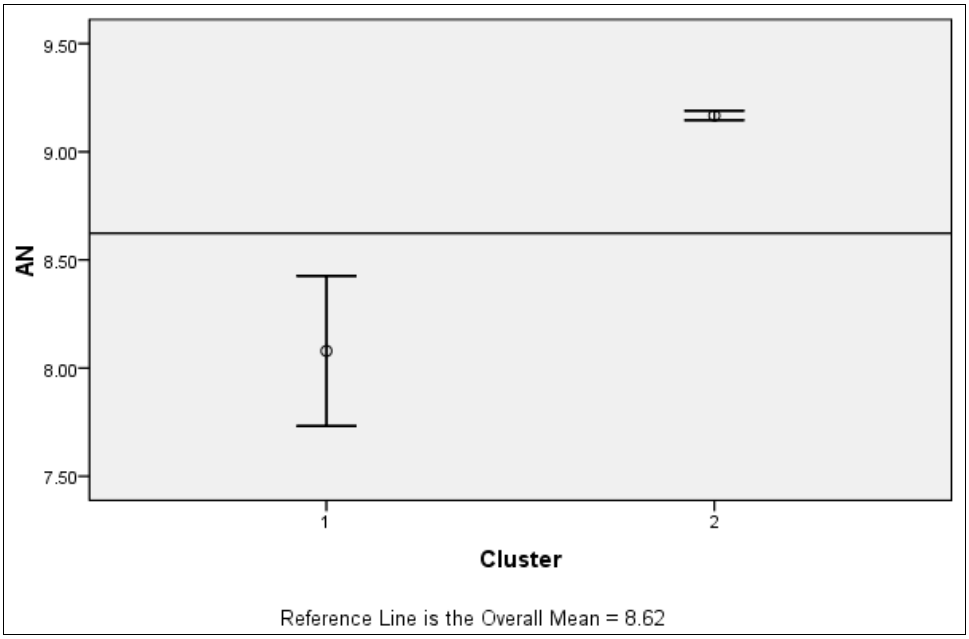


Fig 1c: Within Cluster Variation of Means of Antenna (AN)

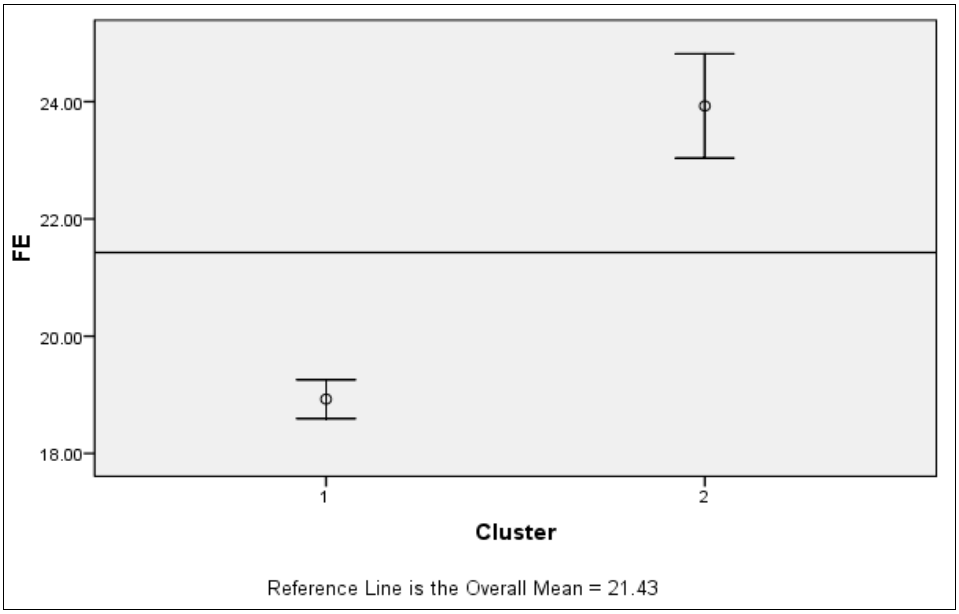


Fig 1d: Within Cluster Variation of Means of Femur (FE)

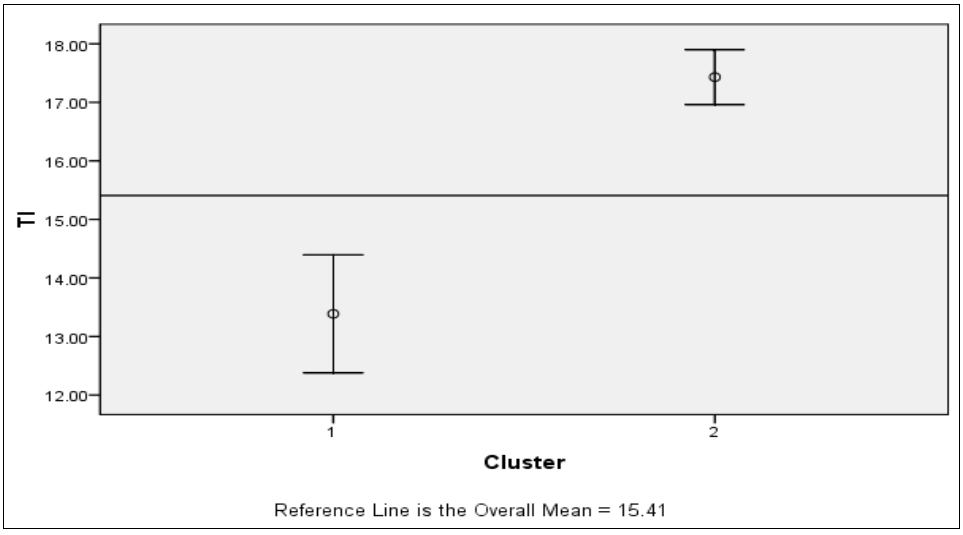
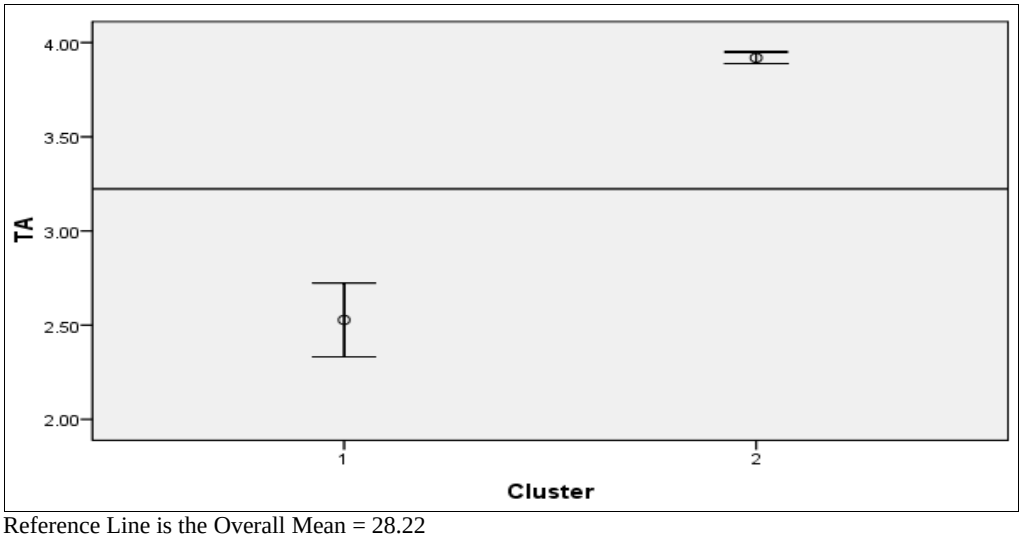


Fig 1e: Within Cluster Variation of Means of Tibia (TI)



Reference Line is the Overall Mean = 28.22

Fig 1f: Within Cluster Variation of Means of Tarsi (TA)

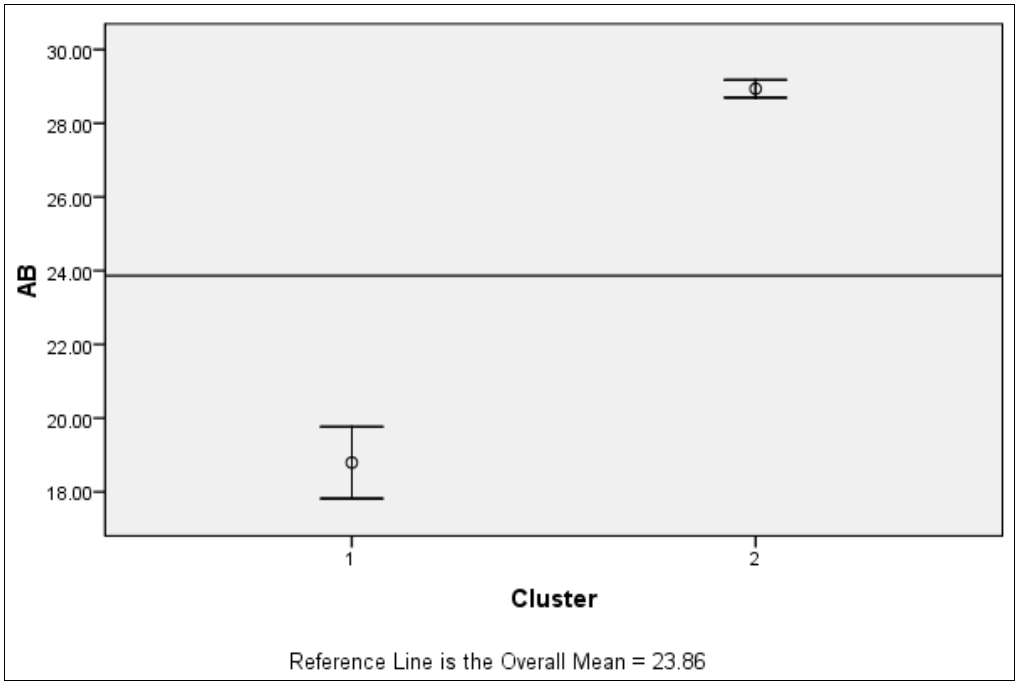


Fig 1g: Within Cluster Variation of Means of Abdomen (AB)

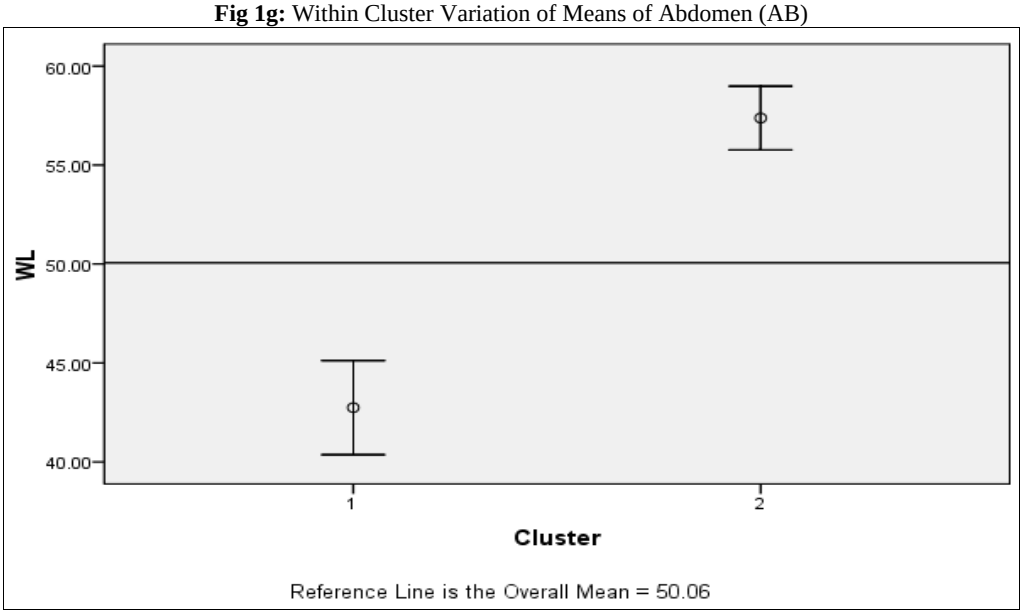


Fig 1h: Within Cluster Variation of Means of Wing Length (WL)

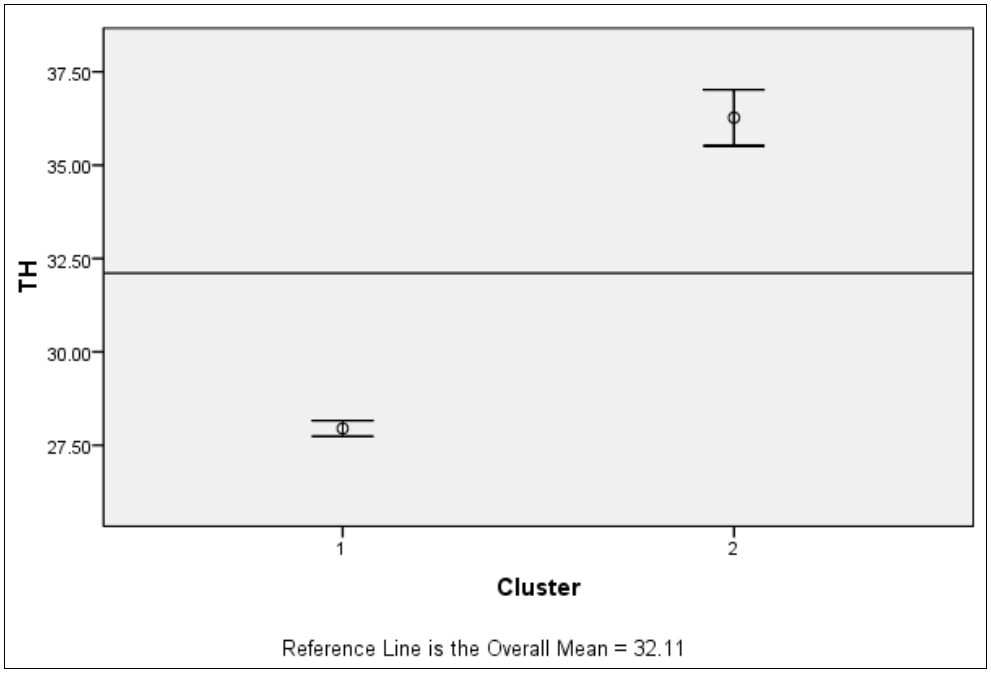


Fig 1i: Within Cluster Variation of Means of Thorax (TH)

Table 4 showed that the correlation coefficient of the morphometric features of tsetse fly samples with the variations in the sampling site i.e. Area Councils gave insignificant negative correlations while highly significant positive correlations ($p<0.01$) were established between the morphometric features of the tsetse flies encountered in the study.

Table 4: Correlations of tsetse fly morphometric features with the sampling site in FCT, Abuja

	HE	BL	AN	FE	TI	TA	AB	WL	LM	TH
HE	1									
BL	.981**	1								
AN	.832**	.837**	1							
FE	.848**	.909**	.683**	1						
TI	.613**	.753**	.519**	.852**	1					
TA	.978**	.962**	.902**	.800**	.588**	1				
AB	.986**	.992**	.878**	.865**	.691**	.988**	1			
WL	.813**	.906**	.809**	.909**	.916**	.812**	.877**	1		
LM	.925**	.942**	.812**	.860**	.709**	.908**	.932**	.866**	1	
TH	.945**	.986**	.771**	.953**	.830**	.910**	.960**	.930**	.940**	1
LOC	-.043	-.058	-.020	-.083	-.092	-.027	-.044	-.079	.000	-.071

**. Correlation is significant at the 0.01 level (2-tailed).

Key: Head / Eyes (HE), Body Length (BL), Antenna (AN), Length of Femur (FE), Tibia length TI, Tarsal TA, Abdomen (AB), Wing Length (WL), Thorax (TH), Sampling site (LOC).

The Dendogram plot of hierarchical cluster analysis of the two identified species of tsetse flies (Fig. 2) revealed linkages between the different samples collected from the three Area Councils. This gives an indication that the samples were of the same descent based on morphometric features and portray that the variations in morphometric features can be engaged in classifying tsetse fly into species in FCT, Abuja.

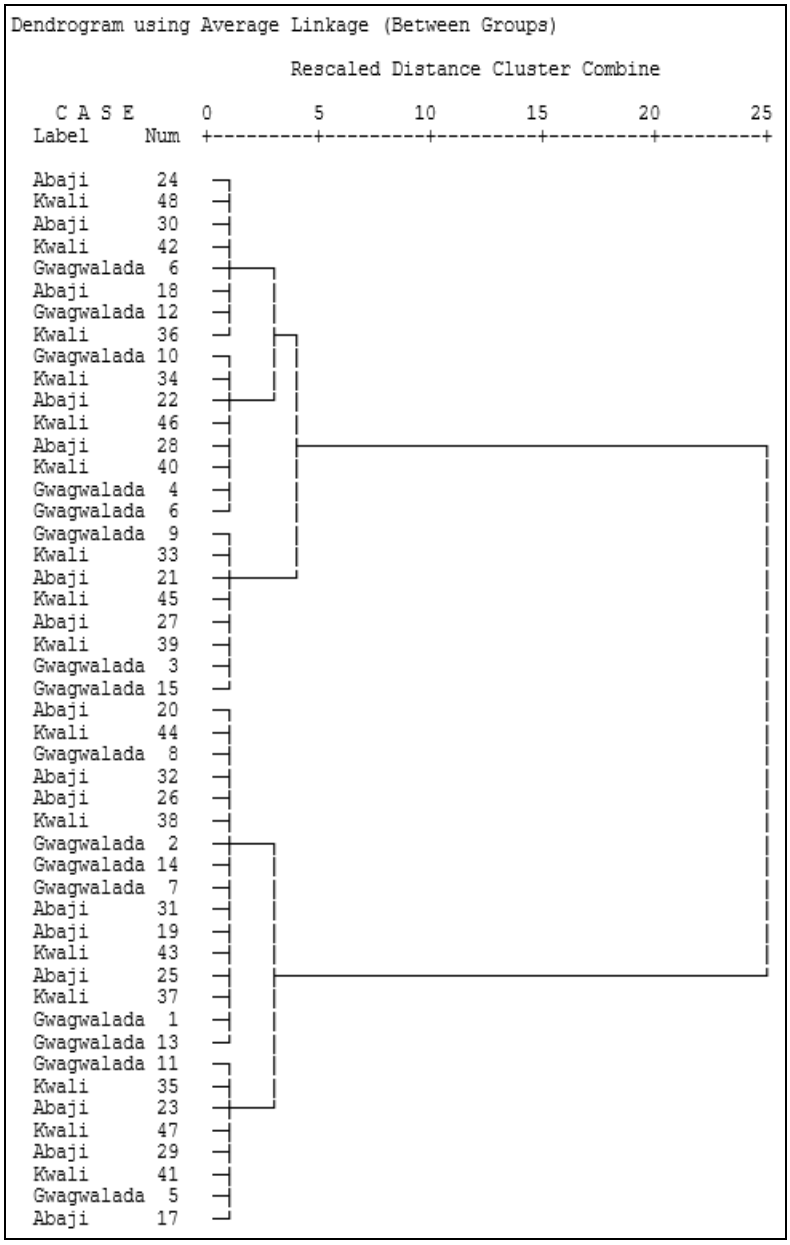


Fig 2: Morphometric Features Based Hierarchical Cluster Analysis Dendrogram of Tsetse Fly in FCT, Abuja

Discussion

Taxonomy of tsetse fly in the sub Saharan Africa is limited due to the non-availability of appropriate equipment and the scarcity of insect taxonomist in the continent. The successful classification of tsetse flies into two distinct species i.e. *G. tachinoides* and *G. morsitans* reflects the abundance of the two species in the savanna vegetation zone of Nigeria. This result conforms to earlier findings on the occurrence of the two species in the savannah belt of Nigeria [6, 8, 9, 10].

The prevalence of *G. tachinoides* over *G. morsitans* in the study area can be ascribed to the environmental condition that favour the breeding of *G. tachinoides* in the study area and were in line with earlier reports which identified colonies of the two species in Nigeria and Burkina- Faso [11, 12] but, contradicts the assertion that describes *G. m. submortisan* as the most common species of tsetse fly in Africa [13].

Higher number of tsetse flies encountered in Gwagwalada and Kwali Area Councils can be attributed to the proximity of the two Councils to locations with high population of herder settlements near villages in the Area Councils. This can also be as a result of availability of green area that facilitates the availability of pasture for the animal and presence of flowing rivers that can sustain the water requirement of the cattle colonies all year round. On the other hand the relatively close distribution of the two species in Abaji Area Council can be as a result of suitable environmental condition for the two species in the Area.

The successful engagement of variation in morphometric features to discern the tsetse collections from the three Area Councils of the FCT portrayed that the technique can be adopted to characterize tsetse flies in FCT, Abuja. This is in line with the use of morphometric features in taxonomy of insects [5, 14, 15, 16, 17].

The use of within cluster variations of the morphometric features that portrayed *G. mortisan* as having higher value compared with the overall means than the *G. tachinoides* conform to the use to establish confidence interval in morphometric measurement and corroborate the possibility on the use of the tools as reported in taxonomy of honeybees and maize weevils in Nigeria [16, 17].

The negative correlation established between tsetse flies in the Area Councils are indication of no link in the samples in the different areas while the positivity of the correlation values established in the morphometric features of the two species

identified in this study coupled with the linkages on the dendrogram plots revealed the closeness of the morphometric features of the two species and conform to the earlier report on the relationship of morphometric features within tsetse fly sub genera [14, 15].

The prevalence of the two species in the Area Councils of FCT, Nigeria is an indication that the duo has favourable environment that support breeding. This shows the urgency in undergoing further research that will provide efficient control for the species in the territory. Furthermore, it will deter the outbreak of diseases that are related to the species of tsetse flies in human and livestock in the area.

Conclusion

Based on the findings from this study, it can be concluded that the species of tsetse fly that affects livestock in Abuja have related morphometric features. Thus, it is appropriate that livestock farmers in the Area Councils of FCT, Abuja can always interact to share practical ideas that can encourage their pest management practices. Since, all morphometric features were positively correlated; it is possible to use the type and size of the morphometric features of tsetse fly to identify the species of tsetse fly infesting livestock in the area to foster the development appropriate control or management methods. This will improve productivity of the animals and in turn boost the livestock farmers' returns on investment.

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