

Morphological Variation and Distribution of Honeybees *Apis Mellifera* (L) Across Altitudinal Gradient in Selected Areas of Tanzania

Nicephor Lesio^{1*} Emmanuel Mmassy²

1. Tanzania Forestry Research Institute, P. O. Box 1854, Morogoro, Tanzania

2. Tanzania Wildlife Research Institute, P. O. Box 661, Arusha, Tanzania

* E-mail of the corresponding author: lesio.np@gmail.com

Abstract

The study aimed at determining morphological variations and distribution of honeybee populations along altitudinal gradient of Tanzania. Samples of the honeybees were collected from 93 feral colonies from sea level to 3000m above sea level. Twenty characters from right hind legs and forewings were measured for morphometric analysis. The descriptive analysis of the same characters by both Principal Component Analysis (PCA) and Discriminant Factor Analysis (DFA) showed weak correlation with altitude. On the other hand, Analysis of Variance (ANOVA) of the 20 characters indicated that only 45% of the characters had significant variations. Among the measured parameters, only wing distance L4 and fibula length (FL) varied in all populations. Characters L2, D7, E9 and O26 varied significantly ($P > 0.05$) only between populations 1 and 2; and character L3 between populations 2 and 3. Besides, there was high overlap in both scattergram and visualization space analyses, suggesting increased intermixing among the populations. However, a further study by using other analytical tools such as molecular analysis was required.

Keywords: Morphological Variation, Distribution, Honeybees

DOI: 10.7176/JBAH/12-12-04

Publication date: June 30th 2022

1. Introduction

The honeybee species *Apis mellifera* (L) is naturally distributed throughout Africa, Europe, and Middle East (Galindo-Cardona *et al.* 2013). However, a long time of international transportation by humans has resulted to its cosmopolitan distribution (Hung *et al.*, 2018) while its importance to human life make it one of the most-studied invertebrate globally (Arias *et al.* 2008, Hung *et al.*, 2018). Honeybees have become a subject for scientific research due to their high value to nature and livelihood of both rural and urban populations (Pirk *et al.* 2013). *A. mellifera* produces honey, beeswax, royal jelly and bee venom; and also collects pollen and propolis apart from being the most economically valuable pollinator of agricultural crops and wild plants worldwide (Costanza *et al.*, 1997; Le Conte and Navajas, 2008, Hung *et al.*, 2018). Therefore characterization of the *A. mellifera* and understanding of its population dynamics is critically important.

Efforts for description of the honeybees (*Apis mellifera* L.) started as early as the beginning of 19th century, although it has a lot of confusion due to lack of definition on their taxonomic positions (Ruttner *et al.*, 1978). This was followed by use of exact morphological character measurements to understand variation and races of the honey bees (Alpanov 1929). DuPraw (1964) introduced the interpretation of honeybee morphometric results using multivariate statistical methods.

The first comprehensive investigation of the honeybees by quantitative analysis of forms (morphometric) gave an insight of the available races of honeybees (Ruttner *et al.* 1978) and the biogeography in the world (Ruttner 1988). Thirty six morphometric characters, including significant parts of the body such as the abdomen (hairbands, tergite pigmentation and wax plate sizes), thorax (wings and legs) and head (proboscis and scutellum) were used to describe worldwide honeybee *A. Mellifera*, revealed presence of 24 subspecies (Ruttner 1988) including three described earlier in Tanzania (Smith 1961). Although these honeybee were categorized based on measurements of 36 characters, fewer characters could be used in the morphometric analysis of the African honeybees (Ruttner 1988). Thus, a quantitative description of shape variation is adequately done using morphometric techniques (Rohlf 1990); in honeybees, it uses measurements of anatomical features for classification to subspecies level (Adams *et al.* 2004).

The Tanzanian subspecies which were reported to occupy three different ecological zones along elevational diversity gradient (Smith, 1961; Ruttner, 1988). The Indian Ocean Coastal area subspecies from sea level to 500m elevation with a hot and humid climate harboured *A. m. litorea*. Others were the savannah subspecies *A. m. scutellata* whose ecological zone ranged from 500 to 2000 meters above sea level and mean annual temperature between 16°C and 23°C, and *A. m. monticola* occurring in the mountain forest ecological zone (specifically surrounding Mt. Meru and Mt. Kilimanjaro), above 2000m elevation with mean annual temperature of 11.2°C. A follow-up study on the ecological distribution of *A. m. monticola* and *A. m. scutellata* around Mt. Kilimanjaro

and Meru, Tanzania (Meixner *et al.* 1989) was congruent to earlier studies (Smith 1961). The elapsed time since last study report and the associated ecological changes that resulted from the impact of climate change and anthropogenic activities necessitated a comparative study to understand the current distribution of the honeybee taxa across altitudinal gradients in Tanzania through analysis of their morphological characters. The study hypothesizes that morphology and distribution of subspecies of honeybees in Tanzania do not differ across altitudinal gradients.

2. Materials and Methods

2.1 Study Area

Tanzania is a tropical climate country that can be divided into (i) the hot humid coastal plain; (ii) the semi-arid central plateau; (iii) the high rainfall lake regions; and (iv) the temperate highlands (Makoi, 2008). The country is located between latitudes 29°27'S and 40°20'S, and longitudes 01°07'E and 11°51'E with the size of 937,062 sq. km. Kenya and Uganda border it to the north, Burundi, Rwanda, the Democratic Republic of Congo (DRC) and Zambia to the west, Malawi and Mozambique to the south and the Indian Ocean to the east.

Tanzania has a few remarkably elevated features which include Mt Kilimanjaro with its two peaks i.e. Kibo (5,895m) and Mawenzi (– 5,148), Mt Meru (4,562.13m), Mt. Ol Doinyo Lengai whose volcano is still active (2,962m) and the Eastern Arc Mountains with the highest peak at Uluguru (2,630m). As the altitude decreases, Tanzania is endowed with a vast Savannah area that occupies most of the country, with waterbodies and a varied distribution of vegetation types. Further low is the vast litoral zone of the Western Indian Ocean laying between Tanzania-Kenya and Tanzania-Mozambique borders. In addition, Tanzania has a few islands in the Indian Ocean, the largest being Unguja, Pemba and Mafia.

2.2 Study of Subspecies Populations and Sites

Three honeybee populations namely *A. m. litorea* in the Coast, (population 1), *A. m. scutellata* in the Savannah (population 2) and *A. m. monticola* in Mountains (population 3) (Smith, 1961; Ruttner, 1988) were investigated. The selected study sites considered only areas where the ecological habitats for the three populations were in relative proximity. Twelve districts of eight regions investigated are presented in Table 1

Table 9: Districts and regions visited for sample and data collection

Habitat	District	Region
Coastal (below 500m asl)	Lindi	Lindi
	Mafia	Pwani
	Rufiji	Pwani
	Mkuranga	Pwani
	Kisarawe	Pwani
	Pangani	Tanga
	Muheza	Tanga
	Morogoro	Morogoro
	Kyela (Matema Beach)	Mbeya
Savannah (500m-2000m asl)	Lindi	Lindi
	Muheza	Tanga
	Morogoro	Morogoro
	Same	Kilimanjaro
	Manyoni	Singida
	Arusha City	Arusha
	Arumeru	Arusha
Mountainous (above 2000m asl)	Arumeru	Arusha

In this study, honeybee colonies from Kyela District, Mbeya Region are considered in population 1 because they were collected from Matema Beach, which is part of Lake Nyasa Basin, below 500m asl.

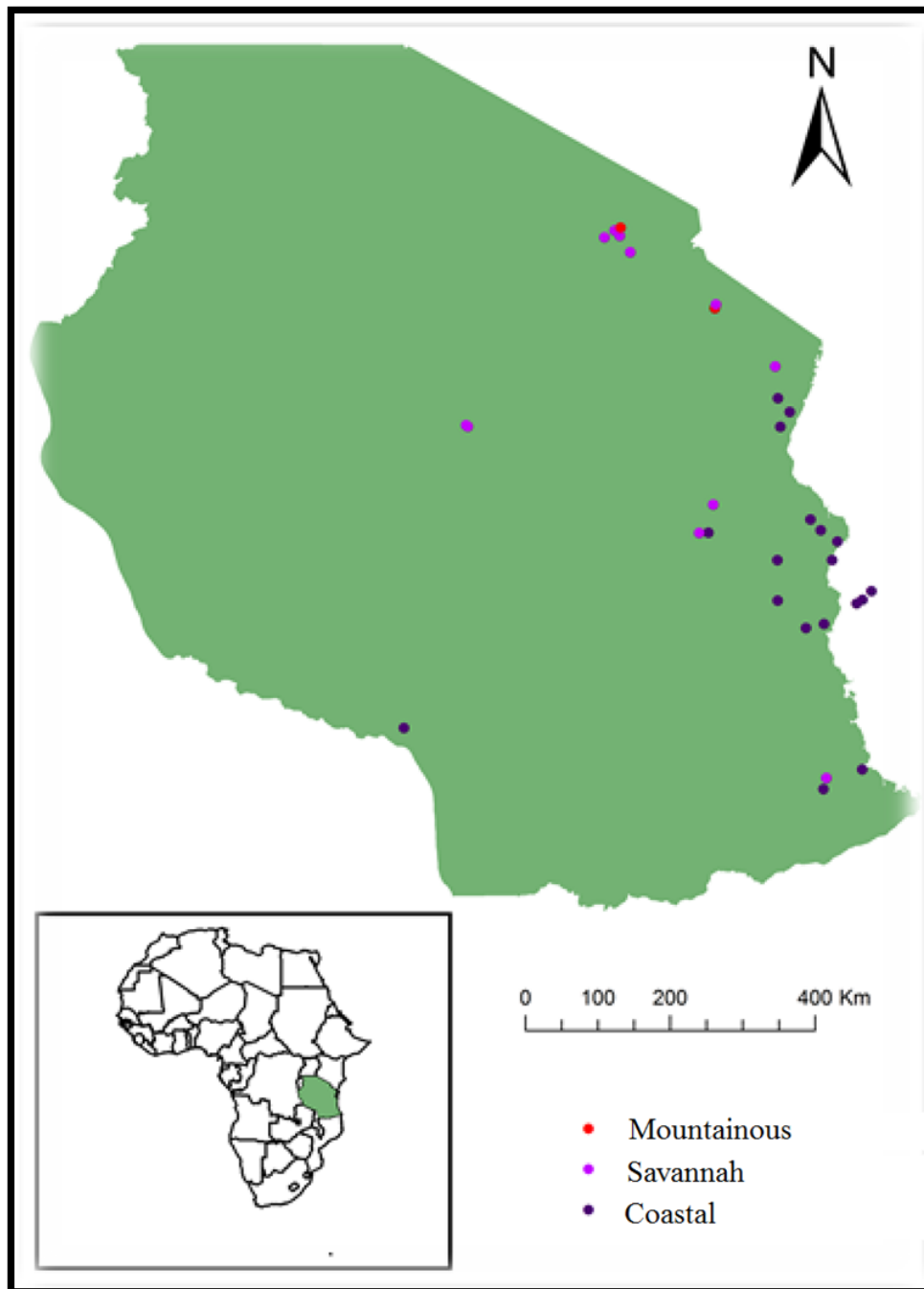


Figure 1: Map of Tanzania showing areas where data for this study were collected.

2.3 Collection of Honeybee Samples

Ten honeybee samples per honeybee nest/colony were collected from 93 bee colonies between 2011 and 2013. Collection of bee samples from nest combs was intended to avoid the collection of drifting or robber bees (Quezada-Euán *et al.* 2003) that might come from other populations. All collected bee samples were preserved in absolute alcohol (95% ethanol) and all points of collection georeferenced using GPS MAP 60CSx (Garmin International, Inc, USA) (Table 2).

Table 10: Georeferences for honeybee samples (each code represents a sample from one honeybee colony)

Code	Latitude	Longitude	Elevation	District	Region
KSJ	7.17513	39.40601	4	Mkuranga	Pwani
KSJ	7.407	39.33699	11	Mkuranga	Pwani
MKR	7.03556	39.19665	41	Mkuranga	Pwani
KSR	6.90083	39.07152	210	Kisarawe	Pwani
CHL	7.40762	38.65799	331	Kisarawe	Pwani
MTB	7.06625	37.80344	362	Morogoro	Morogoro
MSN	6.71853	37.86136	515	Morogoro	Morogoro
MTB	7.06878	37.6893	1002	Morogoro	Morogoro
MAF	7.94568	39.64158	-4	Mafia	Pwani
MAF	7.79284	39.82644	9	Mafia	Pwani
MAF	7.89814	39.71627	14	Mafia	Pwani
MKN	5.7311	34.79496	625	Same	Kilimanjaro
MNY	5.74747	34.81488	1253	Manyoni	Singida
MNY	5.7311	34.79496	1311	Manyoni	Singida
MTM	9.49197	34.02435	490	Kyela	Mbeya
MER	3.58297	36.83287	923	Arusha	Arusha
MER	3.40128	36.51212	1260	Arusha	Arusha
MER	3.31314	36.63892	1545	Arusha	Arusha
MER	3.28092	36.713	2277	Arusha	Arusha
MER	3.37913	36.70244	1272	Arusha	Arusha
HLE	5.39778	38.66568	34	Muheza	Tanga
AMN	5.00277	38.63078	1198	Muheza	Tanga
PGN	5.75277	38.69643	57	Pangani	Tanga
PGN	5.5662	38.81278	83	Pangani	Tanga
RFJ	8.19901	39.23917	-2	Rufiji	Pwani
RFJ	8.25005	39.01335	57	Rufiji	Pwani
RFJ	7.90764	38.66288	83	Rufiji	Pwani
LND	10.00551	39.71337	7	Lindi	Lindi
LND	10.24957	39.23074	358	Lindi	Lindi
LND	10.11519	39.26623	744	Lindi	Lindi
NDL	4.23257	37.89617	1587	Same	Kilimanjaro

2.4 Honeybees' Morphological Character Measurements

Morphometrics is used as a tool to quantitatively describe, analyse and interpret shape variation at specific and infraspecific levels in honeybees (Rohlf 1990). It uses measurements of anatomical features of the honeybees for classification to subspecies level (Adams *et al.* 2004). In this study right fore wing and a hind leg for each honeybee, the sample was used for measurement and analysis.

Right hind legs and fore wings from 10 honeybee samples per colony as suggested by Ruttner (1988) were amputated with the visual aid of dissecting microscope. Canada balsam was used for mounting the amputated organ on glass slides following Gramacho *et al.*, (2003) and Dolati (2013). The slides were observed through sufficient resolution images taken with Omax 3.7 digital Camera fixed on a dissecting microscope at magnification X40. Morphometric characters were measured from the images by using OmaxToupView computer software (version 3.7). Twenty characters including cubital vein (a and b) distances, two other vein distances and eleven angles of the right forewing, as well as four hind leg distances were measured from each honeybee sample (Ruttner *et al.* 1978; Ruttner 1988) modified by Nazzi (1992); and Dedej *et al.* (1996) and modified in the present study to add the measurement of distance L5 (Fig 2B) for reason that it cannot be distorted during amputation or through the natural wearing of the wing.

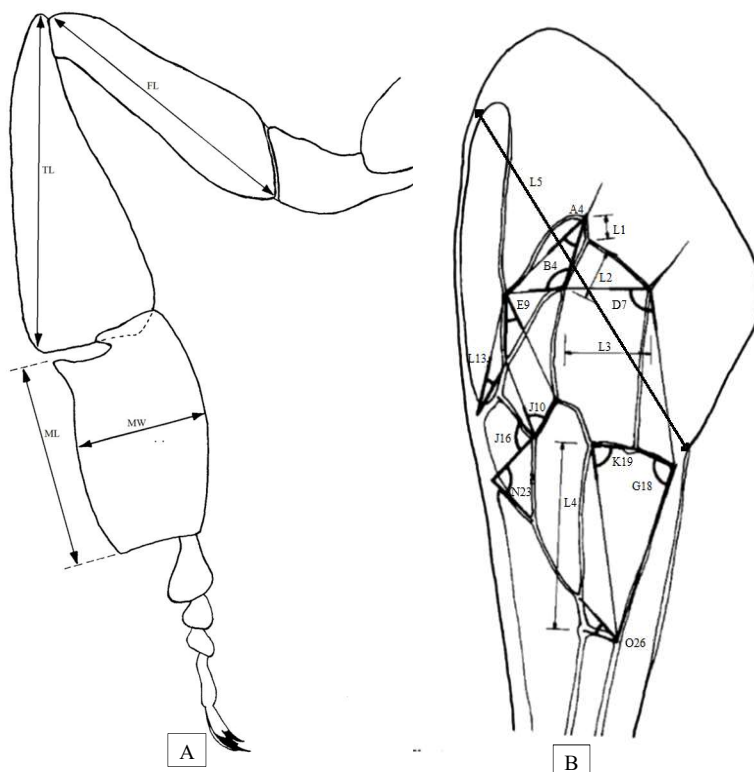


Figure 2: Organs whose characters were measured for morphometric analysis.

Key: **A:** Hind leg of a worker honeybee FL= femur length; TL= Tibia length; MW metatarsus width; ML metatarsus length. **B:** Forewing of a honeybee L1 = Cubital vein distance L1; L2 = cubital vein distance L2; L3 = wing distance L3, L4= wing distance L4; L5 = wing distance L5, 11 wing angles A4; B4; D7; E9; L13; J10; J16; N23; G18; K19; O26 (Adapted from (Ruttner 1988, Nazzi 1992) with slight modification.

2.5 Data Analysis

Statistical Package for Social Scientists (SPSS version 20, IBM Statistics) was used for univariate and multivariate analyses to test the statistical significance of morphometric variations among colonies of the three studied populations (DuPraw 1964). Normality tests, including Shapiro-Wilk test, Anderson-Darling and Jarque-Bera JB were implemented in PAST before Analysis of Variance (ANOVA). An ordination of the colonies was produced by analysing principal component analysis (PCA) using PAST package (Version 3.05; developed by Natural History Museum, University of Oslo). The principal components analysis (PCA), based on the covariance matrix, was carried out to determine allometric coefficients. The first eight values of the PCA denote a general-size factor, resulting from the within-group variance-covariance matrix. Each character was regressed independently on the size factor (PC1). The ellipses were imposed at a concentration of 95%. Also, neighbour-joining clustering of the same dataset used in PCA was performed to determine the affinity between and within colonies of different populations by using Mahalanobis distances. The multi-dimensional image of the populations was produced by analysing discriminant function analysis (DFA) by using the IBM SPSS (Version 20) package (SPSS Inc., Chicago, Illinois, USA).

The stepwise discriminant method was used to partition data for verification of data obtained in descriptive statistics within the range (Table 3). F values were entered at a maximum probability of 0.05. Features with lowest overall Wilk's lambda were entered at each step. Individual characters were assigned to the samples using canonical functions, and percentage of correct group assignment added as a measure of differentiation among samples. Percentage of correctly classified individuals was determined to give a measure of morphological variability of the samples. The number of misclassified individuals indicates the degree of overlap between the colonies. Linear discriminant function (D) was calculated according to Tabachnick and Fidell (1996), which is $D = 0.439L4 + 0.126L5 - 0.448B4 + 0.306E9 + 0.183L13 + 0.396J16 + 0.293K19 + 0.315O26 + 0.225MW - 0.452FL$.

3. Results

Fifty colonies from Population 1, forty one from Population 2 and two from Population 3 were sampled. Ten honeybee samples per colony were involved in morphological character measurements, yielding 930 honeybee samples. Some of the colonies studied across populations had a mixture of black and yellow individual honeybees (examples from Mafia, Manyoni and Mt. Meru Forest). The descriptive analysis of the 20 characters indicated that 50% (L2, L3, L4, L5, B4, N23, ML, MW, TL, FL) increased with altitude for all populations, 25% (E9, L13, J10, G18, K19) increased for populations 1 and 2 but decreased with increasing altitude in population 3, and 15% (L1, A4 and D7) decreased with increasing altitude in all populations. No particular trend was shown by J16 and O26 (10% of all characters). Overall, the increase in characters' size was, to some extent, positively correlated to altitude. Univariate analysis (ANOVA) of the 20 characters indicated that only 45% had significant variations across the elevational gradient.

The follow-up *post hoc* analysis (Table 3) showed that seven wing and leg distance-related (L2, L3, L4, L5, ML, MW and FL) and four wing vein angle-related (D7, J10 and O26) characters varied significantly in all populations, showing 55% of total variation. Some of the significantly varying characters further indicated differences between and among population (Table 4). While L2 and L5 differed between populations 1 and Population 2, L3 differed between population 1 and Population 3. On the other hand L4, D7 and O26 differed among population 1 and population 2 and population 1 and population 3. Only character FL differed significantly among all the populations under study.

Table 3: Morphometric characters of the elevation populations between groups delineation significance (\pm Standard Deviation).

Character	Individual mean and standard deviation			F-value	Probability	Significance level
	Population 1	Population 2	Population 3			
L1	36.45 \pm 4.0	36.42 \pm 3.83	35.23 \pm 3.37	0.9452	0.389	NS
L2	68.31 \pm 8.46	72.87 \pm 7.89	73.2395 \pm 6.42	36.1	0.000	S
L3	102.71 \pm 6.52	106.12 \pm 6.53	109.64 \pm 6.90	37.39	0.000	S
L4	247.47 \pm 9.36	253.82 \pm 7.85	262.68 \pm 7.95	79.85	0.000	S
L5	518.53 \pm 15.91	534.33 \pm 13.92	551.24 \pm 12.61	153.4	0.000	S
A4	31.05 \pm 2.52	30.93 \pm 2.23	30.93 \pm 1.98	0.2929	0.7461	NS
B4	102.37 \pm 7.64	102.40 \pm 7.21	102.6 \pm 6.99	0.0106	0.9897	NS
D7	97.47 \pm 4.48	96.44 \pm 4.17	95.32 \pm 4.64	7.816	0.000	S
E9	16.58 \pm 1.78	16.76 \pm 1.87	16.241 \pm 1.87	1.606	0.201	NS
L13	15.64 \pm 2.17	15.82 \pm 1.94	15.25 \pm 1.59	1.378	0.253	NS
J10	45.44 \pm 5.44	46.30 \pm 5.89	42.13 \pm 4.76	6.839	0.001	S
J16	90.24 \pm 6.73	91.25 \pm 6.26	90.75 \pm 6.47	2.679	0.069	NS
N23	86.30 \pm 6.74	86.96 \pm 6.54	87.68 \pm 8.29	1.334	0.264	NS
G18	95.33 \pm 5.32	95.41 \pm 5.07	91.79 \pm 4.03	4.661	0.010	S
K19	80.57 \pm 4.40	80.89 \pm 4.24	79.06 \pm 5.57	1.998	0.1362	NS
O26	39.95 \pm 3.16	38.43 \pm 3.54	39.55 \pm 2.84	23.51	0.000	S
ML	237.86 \pm 9.90	239.97 \pm 11.24	248.25 \pm 7.96	12.51	0.000	S
MW	120.95 \pm 7.53	123.21 \pm 8.97	131.02 \pm 7.37	20.56	0.000	S
TL	359.73 \pm 12.88	363.74 \pm 14.22	375.08 \pm 12.86	19.83	0.090	NS
FL	299.66 \pm 8.45	305.3 \pm 10.09	308.70 \pm 5.12	47.67	0.000	S

Key;

S = significant, NS = not significant

Table 4: Means comparisons of characters for the elevation populations based on Tukey's Honestly significant difference test.

Character	Population comparison	Mean difference	Standard Error	P - value
L2	1vs2	-3.40834*	.53383	0.000
	1vs3	-6.33802	1.89021	0.992
	2vs3	-2.92968	1.89999	0.272
L3	1vs2	-5.8684	.45079	0.395
	1vs3	-4.84814*	1.53853	0.005
	2vs3	-4.26130	1.54649	0.018
L4	1vs2	-4.12795*	.60988	0.000
	1vs3	-9.26553*	2.08149	0.000
	2vs3	-5.12758	2.09227	0.016
L5	1vs2	-5.48569*	1.14058	0.000
	1vs3	-8.14422	3.89276	0.092
	2vs3	-2.65852	3.91290	0.776
D7	1vs2	2.0882*	.28343	0.000
	1vs3	3.75267*	.96735	0.000
	2vs3	1.66385	.97235	0.202
O26	1vs2	1.02017*	.22439	0.000
	1vs3	2.62399*	.76584	0.002
	2vs3	1.60382	.76981	0.094
FL	1vs2	2.34017*	.63198	0.001
	1vs3	-7.68943*	2.15692	0.001
	2vs3	-10.02960*	2.16808	0.000

Population Clusters

Scattergram produced from PCA (Fig 3), showed an intensive overlap, with its high intensity pooled towards zero at the centre of axes. Despite the overlaps, the scattergram indicated a weak, increasing trend of character sizes with increasing altitude. There were a few colonies from populations 1 and 2 that did not fall into overlap (Fig. 3). While two colonies from population 1 lay in the positions outside the population boundary to the upper left quadrant, three colonies from population 2 lay in different positions (Fig. 3). The outliers of population 1 were collected from Mafia Island while those of population 2 were from Manyoni district, Amani Nature Reserve in Muheza district and Makanya in Same district. Generally, the colonies from population 3 did not separate well with those of populations 1 and 2.

Discriminant function (DF) assessment of morphological measurements of honeybees from the different populations under study did not show population separation (Fig. 4). Discriminant function 1 (DF1) axis, which is 'size-dependent' showed 85.6% of the total variation. In contrast, the DF2 axis, which is a shape-dependent component had less than 14.4% eigenvalues percentage of variance, indicating a weak differentiation of the three populations on the shape basis. Even though overall correct classification from predicted group membership to actual group membership was 70%, individual group membership was variable. Population 1 colonies clustered with Populations 2 and 3 by 23.7% and 25%, respectively. Also, Population 2 colonies clustered with those of Population 3 by 65%, thus making the total clustering of population 3 with others to be 90%.

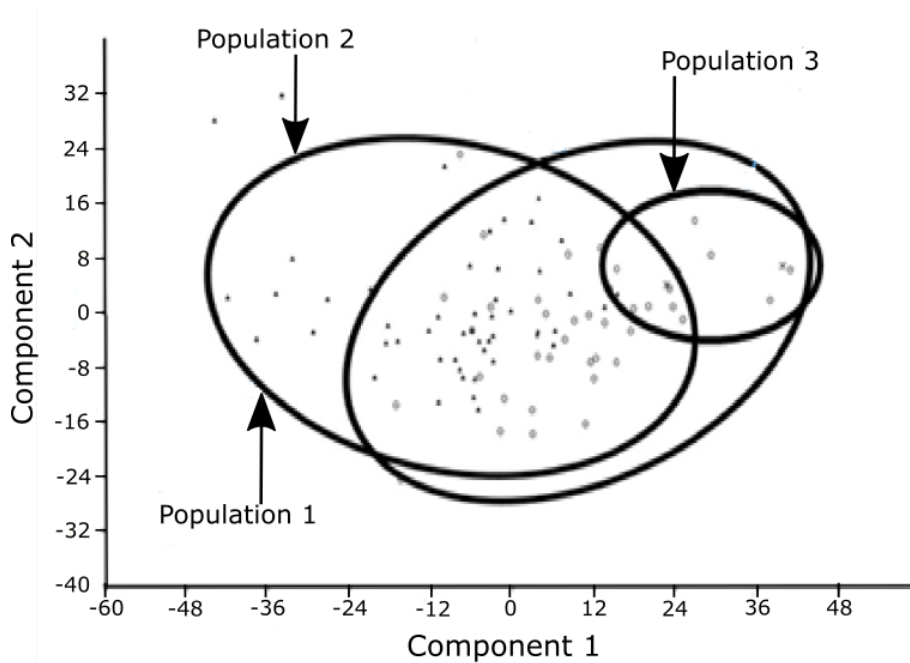


Figure 3: Principal component analysis (Components 1 and 2) of the morphometrics characters of colonies from the populations 1, 2 and 3 (Ellipses indicate 95% confidence limit).

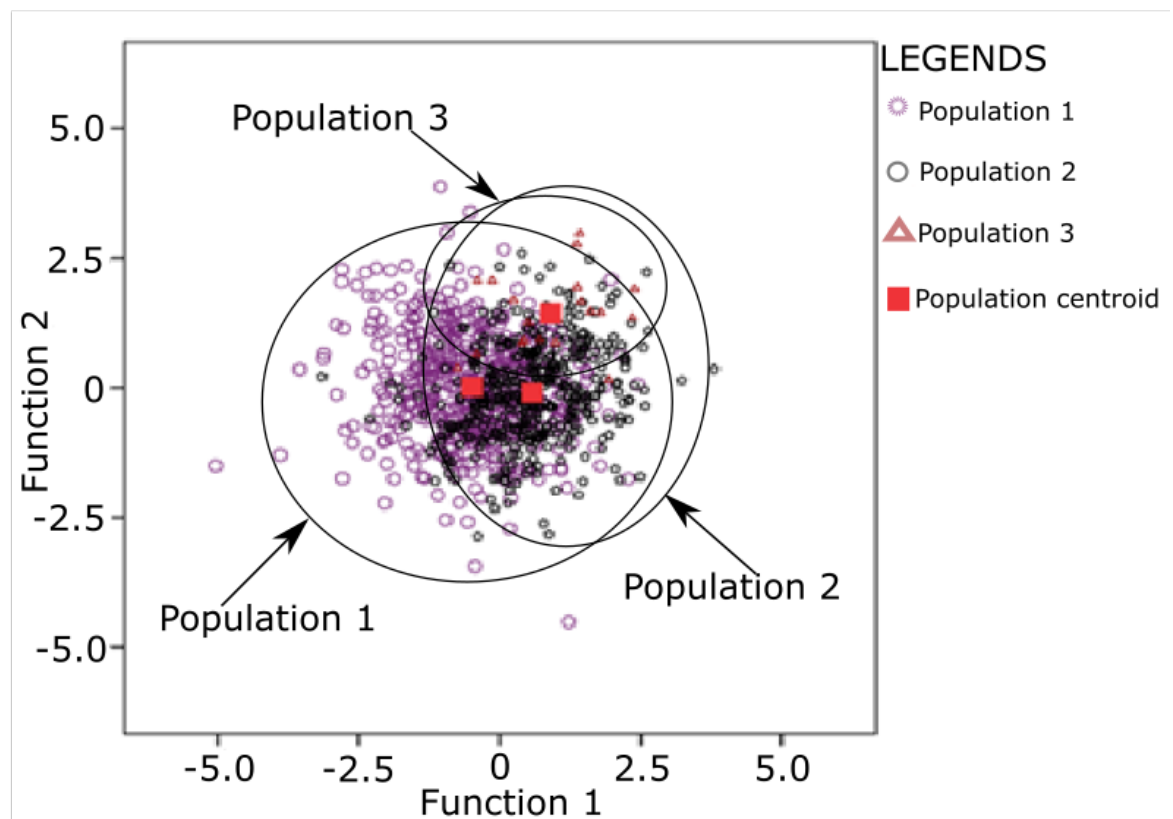


Figure 4: Visualization space for discriminant function 1 (DF1) and DF2 of morphometrics of different populations (the means of characters are represented by centroids).

4. Discussion

Using the morphometric technique analysis, the present study showed weak morphological variations of honeybee subspecies along the altitudinal gradient from the lowest to the highest elevation. This trivial variation is contrary to (Smith 1961, Ruttner *et al.*, 1978, Ruttner 1988 and Meixner *et al.*, 1989) whose morphological studies on honeybee subspecies in Tanzania showed distinct morphological variation among them in the ecological altitudinal range. The observed weak differences in the size of morphological characters in this study might indicate mixing and interbreeding of the subspecies as a result of disturbance of ecological boundaries, through increased anthropogenic activities and climate change leading to lack of apparent variations and distinction among subspecies (Smith 1961 and Ruttner 1988). According to Smith (1961) and verification by Ruttner (1988) the population 1 subspecies, *A. m. litorea*, consisted of individual honeybees that were comparatively smallest in size with bright yellow colour, *A. m. scutellata* were medium sized brown, while *A. m. monticola* were relatively big and black, the results of which are contradicting to the finding of this study.

Contradicting results among these studies (Smith 1961; Ruttner 1988; Meixner 1989) and the present might be attributed to several biological and environmental factors, including hybridization among subspecies, expansion of the habitat range of *A. m. scutellata* at the expense of *A. m. littorea* and *A. m. monticola* (Le Conte and Navajas, 2008). The biophysical environmental changes include temperature and land use land cover change. For instance, annual average temperatures for Tanzania range between 17°C and 27°C, depending on location (Agrawala *et al.* 2003, McSweeney *et al.*, 2009), whereas *A. m. monticola* subspecies requirement is 11.2°C (Smith, 1961; Ruttner 1988). This implies, for instance, that for *A. m. monticola* colonies to survive they ought to adapt higher temperatures than the original, probably by changing anatomical, physiological and / or behavioural aspects.

The effect of honeybees' subspecies hybridization on population structures may lead to genetic depression. In Africa, Ethiopia and Kenya (Ngong Hills) for instance, natural hybridization between different honeybee subspecies has been observed, causing low variation among morphoclusters (Amssalu *et al.* 2004, Meixner *et al.* 2000, Hepburn and Radloff 1998). This can be explained as a result of panmixia of the neighbouring subspecies caused by their high migratory behavior (Franck *et al.*, 2001; Gruber *et al.*, 2013). Based on this study, hybridization of honeybees may exist in Tanzania as individual honeybees from same colonies were seen to have different colours, which may be caused by multiple mating of the honeybee queen with drones from colonies of the same and different subspecies in a common congregation area (Gruber *et al.* 2013). The perceived hybridization may be impacted by climate change, urbanization and the associated anthropogenic activities that increase reduction of ecological barriers between the honeybee subspecies zones and thus changing ecological characteristics favoured by the subspecies. Fragmentation of the coastal forests and mangroves, for instance, have changed ecological characteristics of savannah honeybee *A. m. scutellata*, to expand its habitat range to the coast. This is congruent to postulation that anthropogenic activities can lead to change in ecological characteristics responsible for diversification and isolation of *A. mellifera* populations in Tanzania (Ruttner and Kauhausen 1984). This study has observed influence of environmental changes to the expansion of *A. m. scutellata* habitat beyond the known distribution boundaries, and reduction of the ecological habitats of the other populations (Meixner *et al.* 2000; Le Conte and Navajas 2008, Buescu *et al.*, 2018).

5. Conclusion

Findings of this study, whose samples were collected from traditionally known ecological zones of the three honeybee populations, indicated inconsistent and insignificant morphological differences. The results further indicate that honeybee subspecies in Tanzania are mixing and share ecological habitats as the study did not show clear cut existence based on morphological variations along altitudinal gradients. The insignificant and inconsistent morphological differences could be attributed by hybridization that is brought about by diminishing of the ecological boundaries due to anthropogenic activities and climate change.

Studies are required to identify different variants of honeybees within an area, which this study could not do. The required study should be followed by determining bee space for each variant, as the theory of having only one bee race leading to a standard bee space in the hives has been defeated.

REFERENCES

- Adams, D. C., Rohlf, F. J. and Slice, D. E. (2004) Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*, 71(1): 5-16.
- Agrawala, S., Moehner, A., Andreas Hemp, A., Maarten Van- Aalst, M., Hitz, S., Smith, J., Meena, H., Mwakifwamba, S. M., Hyera, T and Obeth U. Mwaipopo, O. U (2013) Development and climate change in Tanzania: Focus on Mount Kilimanjaro. Organisation for Economic Co-operation and Development-OECD 2 rue André Pascal, 75775 Paris, Cedex 16, France.
- Alpatov, W. (1929) Biometrical studies on variation and races of the honey bee (*Apis mellifera* L.). *The Quarterly Review of Biology*, 4(1): 1-58.

- Amssalu, B., Nuru, A., Radloff, S. and Hepburn, H. R. (2004) Multivariate morphometric analysis of honeybees (*Apis mellifera*) in the Ethiopian region. *Apidologie*, 35(1): 71-81.
- Arias, M. C., Silvestre, D., de Oliveira-Francisco, F., Weinlich, R. and Sheppard, W. S. (2008) An oligonucleotide primer set for PCR amplification of the complete honey bee mitochondrial genome. *Apidologie*, 39(4): 475-480 Elena
- Buescu, E., Gurau, M. R. and Danes, D. (2018) Identification of the Honeybee Subspecies from Some Romanian Counties Using a Semiautomatic System for Analyzing Wings. CBU International Conference on Innovations in Science and Education. March 21-23, 2018, Prague, Czech Republic. www.cbuni.cz, www.journals.cz
- Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, R. G., Sutton, P. and van den Belt, M (1997). *Nature* 387: 253-260
- Dedej, S., Biasiolo, A. and Piva, R. (1996) Morphometric and Alloenzymatic characterisation in the Albanian honeybee population *Apis mellifera* L. *Apidologie*, 27: 121-132.
- Dolati, L., Rafie, J. N. and Khalesro, H. (2013) Landmark-based morphometric study in the fore and hind wings of an Iranian race of European honeybee (*Apis mellifera*). *Journal of Apicultural Science* 57 (2) 187-197
- DuPraw, E. (1964) Non-linnean taxonomy. *Nature*, 202: 849-852.
- Franck, P., L. Garnery, A. Loiseau, B. P. Oldroyd, H. R. Hepburn, M. Solignac, et al. (2001). Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data. *Heredity* 86:420-430.
- Galindo-Cardona, A., Acevedo-Gonzalez, J. P., Rivera-Marchand, B. and Giray, T. (2013) Genetic structure of the gentle Africanized honey bee population (gAHB) in Puerto Rico. *BMC genetics*, 14(1): 1.
- Gramacho, K. P., Goncalves, L. S., Stort, A. C. and Noronha, A. B. (2013) Is the number of antennal plate organs (sensilaplacea) greater in hygienic than in non-hygienic Africanized honeybees? *Genetics and Molecular Research* 2 (3) 309- 316
- Gruber, K., Schöning, C., Otte, M., Kinuthia, W. and Hasselmann, M. (2013) Distinct subspecies or phenotypic plasticity? Genetic and morphological differentiation of mountain honey bees in East Africa. *Ecology and evolution*, 3(10): 3204-3218. doi: 10.1002/ece3.711
- Hepburn, H. R. and Radloff, S. E. (1998). *Honeybees of Africa*. Berlin Heidelberg, New York, Springer-Verlag
- Le Conte, Y. and Navajas, M. (2008) Climate change: impact on honey bee populations and diseases. *Revue Scientifique Technique-Office International des Epizooties*, 27(2): 499-510.
- Makoi, J. (2018) Description of cropping systems, climate, and soils in Tanzania. *The Global Yield Gap Atlas*. <https://www.yieldgap.org/tanzania> (accessed April 15, 2020)
- McSweeney, C., New, M., Lizcano, G. and Lu, X. (2020) Improving the Accessibility of Observed and Projected Climate Information for Studies of Climate Change in Developing Countries. *The UNDP Climate Change Country Profiles*. http://journals.ametsoc.org/bams/article-pdf/91/2/157/3737654/2009bams2826_1.pdf (Accessed 14 August 2020)
- Meixner, M., Ruttner, F., Koeniger, N. and Koeniger, G. (1989) The mountain bees of the Kilimanjaro region and their relation to neighbouring bee populations. *Apidologie*, 20(2): 165-174.
- Meixner, M., Arias, M. C. and Sheppard, W. (2000) Mitochondrial DNA polymorphisms in honey bee subspecies from Kenya. *Apidologie*, 31(2): 181-190.
- Nazzi, F. (1992) Morphometric analysis of honey bees from an area of racial hybridization in northeastern Italy. *Apidologie*, 23(2): 89-96.
- Pirk, C. W. W., de Miranda, J. R., Kramer, M., Murray, T. E., Nazzi, F., Shutler, D., van der Steen, J. J. M. and van Dooremalen, C. (2013). Statistical guidelines for *Apis mellifera* research. *Journal of Apicultural Research*, 52:4, 1-24, DOI: 10.3896/IBRA.1.52.4.13
- Quezada-Euán, J. J. G., Pérez-Castro, E. E. and May-Itzá, W. D. J. (2003) Hybridization between European and African-derived honeybee populations (*Apis mellifera*) at different altitudes in Perú. *Apidologie*, 34: 217-225.
- Hung, K-L. J., Kingston, J. M., Albrecht, M., Holway, D. A., Kohn, J. R. (2018). The worldwide importance of honey bees as pollinators in natural habitats. *Proc. R. Soc. B* 285: 2017 - 2140. <http://dx.doi.org/10.1098/rspb.2017.2140>
- Rohlf, F. J. (1990) Morphometrics. *Annual Review of ecology and Systematics*, 299-316.
- Ruttner, F. (1988) *Biogeography and Taxonomy of Honeybees*. Berlin Heidelberg New York, Springer-Verlag.
- Ruttner, F. and Kauhausen, D. (1984). Honeybees of tropical Africa: ecological diversification and isolation. In: Third International Conference on Apiculture in Tropical Climate, Nairobi, Kenya. *International Bee Research Association*
- Ruttner, F., Tassencourt, L. and Louveaux, J. (1978) Biometrical-statistical analysis of the geographic variability of *Apis mellifera* L. *Apidologie*, 9(4): 363-381.
- Smith, F. G. (1961) The races of honeybees in Africa. *Bee World*, 42(10): 255-260.