

## **Morphometrical Studies of Honeybees in Dinder Biosphere Reserve, Sudan**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author SKN designed the study, wrote the first draft of the manuscript and discuss the conclusion. Author LMA managed the literature searches, analyses of the study and performed the structural equation modeling. Author IMH wrote the protocol. All authors read and approved the final manuscript.*

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**Short Communication**

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### **ABSTRACT**

This study was conducted in Dinder Biosphere Reserve during the dry season of 2009 (February - May) and February 2010. The objective of this research is to conduct morphometrical study of honeybees in DBR in order to know if there are more than one species and their distribution in the reserve. Samples of honeybee workers were collected from wild honeybee colonies nesting in tree cavities and branches. They were selected randomly from 20 locations from the three ecosystems: Maya, Riverine and Dehra. Furthermore, 25 workerbees were selected randomly from each sample, dissected and were mounted on glass slides for the morphometric study. Fourteen traits

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were measured using a micrometer with the help of stereo-binocular microscope for each worker bee. The obtained data was subjected to one way ANOVA with the three ecosystems as main source of variation. In general, the results obtained showed that, all the samples are for bees belong to one species which is *Apis mellifera*. Moreover, the statistical analysis of the results indicated significant differences ( $P < 0.05$ ) among the bees in the three ecosystems with respect to forewing length and width, percentage of yellow coloration, first wax mirror width and the distance between wax mirrors. However, the cubital index was highly significantly different ( $P < 0.01$ ). It could be concluded that there in the Dinder Biosphere Reserve there is only one species with more than one subspecies and they are associate with Riverine, Maya and Dehra ecosystems.

**Keywords:** *Apis mellifera*; Dahara; tree cavity; Maya; riverine.

## 1. INTRODUCTION

The Sudanese honey bee was classified and named as *Apis mellifera nubica* based on samples originating from the semi desert zone [1]. Its small size and short appendices were remarkable and accordingly it was placed as a separate race [2]. Later on, two independent subspecies of honeybees were suggested to be in the Sudan: *A. m. sudanensis* that was distributed all over Sudan between latitudes 3°N and 16°N; and *A. m. nubica*, mixed bees that were distributed along Sudan-Ethiopian-Ugandan international boundaries [3]. However, the name "*nubica*" was withdrawn in favor of *yemenitica*, a name that included bees from Saudi Arabia, Yemen, Oman, Somalia, and Chad [4]. The dwarf honeybee *A. florea* was discovered in Khartoum gardens at the vicinity of the International Airport in 1985 [5]. Within seven years of its discovery, *A. florea* has advanced 150 Kilometers southeast along the Blue Nile River [6]. A survey conducted along the Nile indicated that *A. florea* crossed the border between Sudan and Ethiopia at Al Daim border station on the Blue Nile and also was found in Yarenja refugees camp near Mankush town in Ethiopia [7]. Therefore, the objective of this research is to conduct morphometrical study of honeybees in DBR in order to know if there are more than one species and their distribution in the reserve.

## 2. MATERIALS AND METHODS

This study was conducted in Dinder Biosphere Reserve (Fig. 1) which lies at the southeastern portion of Sudan against Ethiopia, approximately 400 kilometers from Khartoum. It was conducted during the dry season of 2009 and 2010. Samples of honeybee workers were collected from honeybee colonies nesting in tree cavities and on tree branches at 20 locations. They were

selected randomly from three ecosystems: the Maya, the Riverine and Dehra. Furthermore, 25 worker bees, randomly selected from each sample, were killed in boiling water to ensure full extension of their proboscis. They were then preserved in a solution of ethanol, glycerin and distilled water (1:1:1 by Volume) [8]. The samples were then washed 3 to 4 times in distilled water. The wings were removed before maceration, and the bees were then heated in 10% potassium hydroxide solution (KOH) for 20 to 30 minutes to ensure complete maceration. The worker bees were then cooled and washed with distilled water. The following parts were dissected from each individual worker: the proboscis, right antenna, hind leg, third sternite and the third and fourth abdominal tergites. The dissected parts were dehydrated in a series of alcohol solutions (25, 50, 75, 90 and 100%) for about 5 minutes in each concentration. After that, the dissected parts were mounted on glass slides using DPX. The following measurements were taken using a micrometer with the help of stereo-binocular microscope:

1. Length of proboscis.
2. Length of antenna (10 apical segments).
3. Length and width of the right forewing.
4. Cubical index.
5. Number of hamuli on the right hind wing.
6. Length and width of basitarsus III and the number of hair-rows on the inner surface of the same basitarsus.
7. Percentage of yellow coloration on the third abdominal tergite.
8. Mathematical summation of the length of third and fourth abdominal tergites.
9. Length and width of the wax mirror in sternite III and the distance between wax mirrors of the same sternite.

The obtained data were then subjected to one way ANOVA with the three ecosystems as main source of variation.

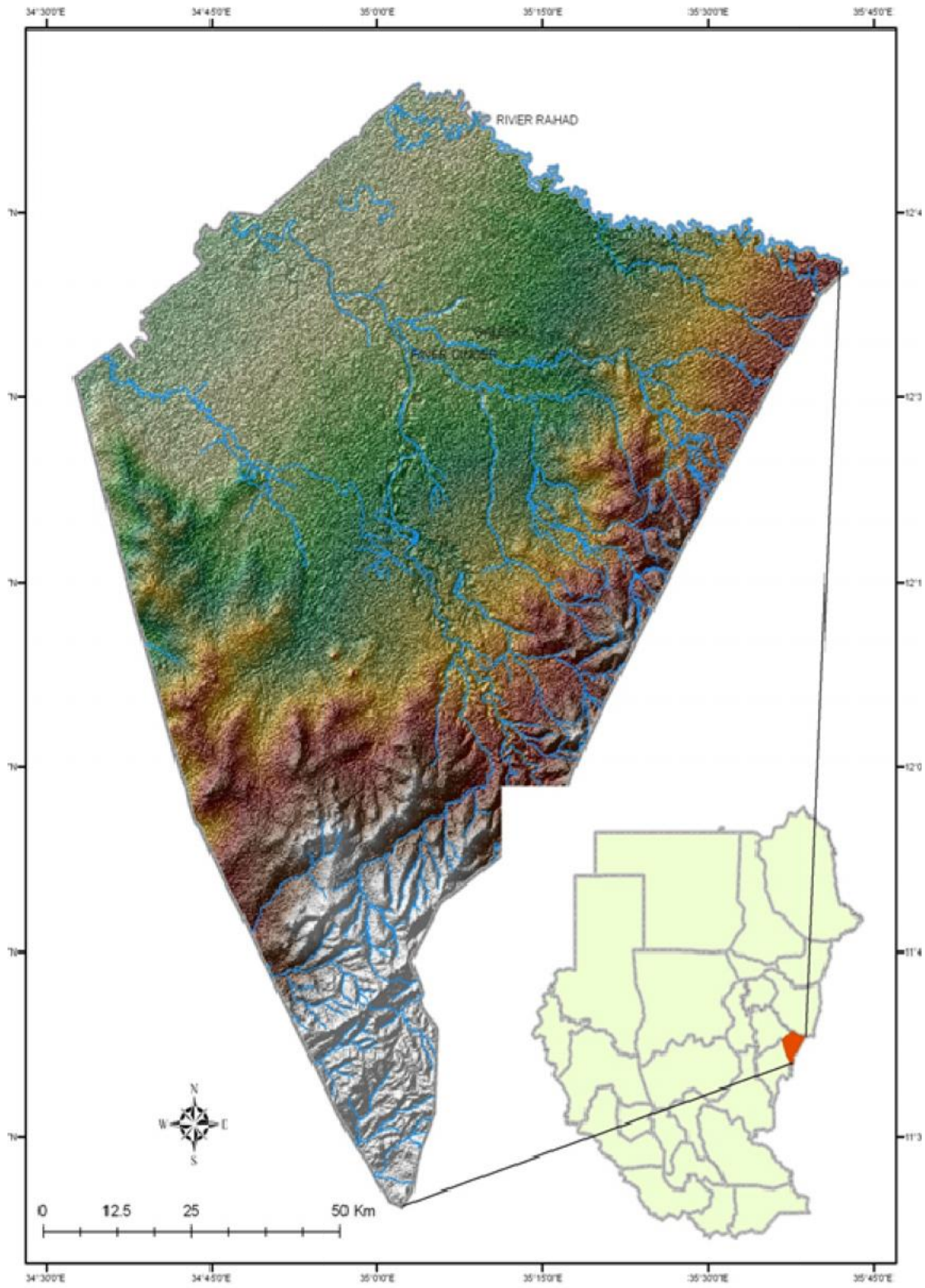


Fig. 1. Location of Dinder biosphere reserve

### 3. RESULTS

The mean measurements (Table 1) showed considerable variations among the bees in the three ecosystems. Measurements that were relatively high in the Maya ecosystem were the antennal length, proboscis length, and the wax mirror width. On the other hand, the means of: number of hooks, number of hair rows and length of tergites 3+4 were slightly higher in Dehra ecosystem. Measurements that were relatively high in the Riverine ecosystem were the forewing length and width, cubital index, basitarsus III length and width, the percentage of yellow coloration and the wax mirror length. The distance between wax mirrors was equal in the Dehra and Riverine ecosystems, but relatively higher in the Maya ecosystem.

Table 1 also indicates that the forewing length and width, the percentage of yellow coloration in the third abdominal tergite, the first wax mirror width and the distance between the wax mirrors of the same sternite were significantly different ( $P < 0.05$ ) among the bees in the three ecosystems whereas; the cubital index was highly significantly different ( $P < 0.01$ ).

### 4. DISCUSSION

In general, the measurements obtained in this study show that, all the samples are for bees belong to the species *Apis mellifera*. However, the analysis revealed variations in the fourteen

characters measured. The variation in the morphometric might be considered an indication that the honeybee samples do not belong to one subspecies and even may be obtained as a result of hybridization between the different subspecies. These results agree with those of many researchers who found highly significant differences for the morphometrical traits they studied in the Sudanese honeybees and concluded that the samples they studied did not belong to one race [9-11]. Similarly high degree of regional variations was reported in the Sudanese honeybees [12,13,3,14,15]. Similar variations were also observed for the neighboring Ethiopian honeybees [16,17,4,18,20] and Kenyan honeybees [21].

The finding of this study can also be supported by the findings of the genetical study (mitochondrial discrimination) of the Sudanese honeybees where it was concluded that, "Sudanese honeybee populations are surrounded by a suite of various subspecies with different mitochondrial haplotypes, including the O-lineage in the north (Egypt), the Y-lineage in the east (Ethiopia) and the A-lineage in the south and west. Honeybees of the wet savannah and forest ecosystems showed the A-lineage, identical to *A. m. adansonii* and *A.m. scutellata*. The honeybees in the desert, semi desert, and dry savannah of Sudan have the O-lineage, similar to *A. m. lamarkii* and *A.m. syriaca* haplotype. Moreover,  $C_2$  was found in apiaries with imported stock (*A. m. carnica*). This

**Table 1. The mean values of measurements of the honey bee workers in the Dinder Biosphere Reserve**

Parameter	Mean $\pm$ SE			Significance
	Maya N = (7)	Dehara N = (8)	Riverine N = (5)	
Antennal L. (mm)	2.706 $\pm$ 0.223	2.606 $\pm$ 0.168	2.576 $\pm$ 0.236	NS
Proboscis L. (mm)	5.353 $\pm$ 0.588	5.301 $\pm$ 0.503	5.143 $\pm$ 0.399	NS
Forewing L. (mm)	7.986 $\pm$ 0.188	7.891 $\pm$ 0.099	8.025 $\pm$ 0.250	*
Forewing W. (mm)	2.833 $\pm$ 0.091	2.843 $\pm$ 0.055	2.926 $\pm$ 0.170	*
Cubital index	2.018 $\pm$ 0.162	2.108 $\pm$ 0.114	2.204 $\pm$ 0.129	**
No. of hooks	20.790 $\pm$ 0.379	20.931 $\pm$ 0.893	20.878 $\pm$ 0.864	NS
Basitarsus I/II L. (mm)	1.891 $\pm$ 0.055	1.893 $\pm$ 0.050	1.919 $\pm$ 0.053	NS
Basitarsus III W.( mm)	1.015 $\pm$ 0.021	1.016 $\pm$ 0.028	1.016 $\pm$ 0.30	NS
No. of hair-rows	8.857 $\pm$ 0.254	9.250 $\pm$ 1.103	8.920 $\pm$ 0.829	NS
Yellow coloration (%)	66.609 $\pm$ 2.364	66.408 $\pm$ 1.333	67.693 $\pm$ 1.356	*
Tergites (3+4) L.(mm)	3.784 $\pm$ 0.333	3.919 $\pm$ 0.196	3.884 $\pm$ 0.383	NS
Wax mirror L.(mm)	1.209 $\pm$ 0.086	1.195 $\pm$ 0.127	1.262 $\pm$ 0.227	NS
Waxmirror W.(mm)	2.756 $\pm$ 0.184	2.510 $\pm$ 0.246	2.615 $\pm$ 0.365	*
Distance between Wax mirrors (m m)	1.348 $\pm$ 0.092	1.451 $\pm$ 0.231	1.451 $\pm$ 0.090	*

N = Number of colonies studied. L = length; W = width.

\*\*  $P < 0.01$ ; \*  $P < 0.05$ . ; NS= not significant

reclassification of the honeybees from Sudan has consequences for the interpretation of the biogeography of *A. mellifera* in the Maghreb and Mashriq regions [22].

Although it was previously reported that the bees of the Blue Nile State possessed the largest measurements among the studied Sudanese honeybees [3,14], but when comparing the means of the measured characters obtained in this study with those obtained in the previous studies, it was observed that the measurements obtained in this study are very small. Moreover, they were found to be very close to those of the honeybees present in the White Nile and North Kordofan provinces [23] and also to those of the bees collected from the semi-desert zone [14]. However, this could be attributed to that; bees from the semi-desert zone might migrate; due to the drought conditions during the past twenty years; to the park since it is a naturally reserved zone and hence act as an attractive site. This came in accordance with the researchers who reported that the migration of honeybee colonies in the tropics is considered as an evolutionary adaptation to escape harsh period and also as means of exploiting resource available in different ecological habitats at different times [24,25,20].

The classification of the honeybees' native to Sudan is seem to be controversial. Although subspecies naming was initially generous including *A. m. nubica* [17], *A. m. sudanensis* [15] and *A. m. bandasii* [14], today the subspecies has been classified to *A. m. jemenitica* [4]. Mitochondrial DNA (mtDNA) has also been used to classify honeybee subspecies of Sudan. Recently mitochondrial DNA analyses of 75 sampled colonies throughout Sudan revealed that native honeybees of Sudan are much more diverse and are composed of six different haplotypes (A1, A4, A8, A13, O1', O1) corresponding to two different lineages, A and O instead of only one expected lineage (Y) [26,27]. However, studies in this field are still patchy and much more information is still needed particularly in Biosphere Reserve areas in Sudan.

## 5. CONCLUSION AND RECOMMENDATION

In conclusion, the predominant honeybee in Dinder Biosphere Reserve is *Apis mellifera*. There are indications that subspecies are associate with Riverine, Maya and Dehra ecosystems. However, taxonomic study through

molecular genetics and strains should be conducted, especially for the bee in the southern region of the biosphere reserve which is not covered by this study.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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