

Morphometrical Studies of Honeybees in Dinder Biosphere Reserve, Sudan

Lubna Mohammed Abdalla¹, Siham Kamil Nagi^{2**} and Ibrahim Mohammed Hashim³

¹ Wildlife Research Centre, Animal Resources Research Corporation, Ministry of Animal Resources and Fisheries, Khartoum, Sudan . ^{2**} the National Centre for Research, Apiculture Research Department , P.O.Box 6096 Khartoum, Sudan (Academy of Sciences, Biological Science, New Technologies and Environment Sector).. ³ Sudanese Wildlife Society, Sudan.

Abstract—This study was conducted in Dinder Biosphere Reserve during the dry season (February - May) 2009 and February 2010. Samples of honeybee workers were collected from wild honeybee colonies nesting in tree cavities and branches at 20 locations that were selected randomly from three ecosystems: the Maya, the Riverine and Dehra. Furthermore, 25 workerbees were selected randomly from each location, dissected and the measurements of 14 traits were taken by a micrometer with the help of stereo-binocular microscope. The data was subjected to one way ANOVA with the three ecosystems as main source of variation. In general, the measurements obtained show that, all the samples are for bees belong only to the species *Apis mellifera*. However, the analysis of the results indicated that the forewing length and width, the percentage of yellow coloration , the first wax mirror width and the distance between the same wax mirrors were significantly different ($P < 0.05$) among the bees in the three ecosystems whereas; the cubital index was highly significantly different ($P < 0.01$). In other words there are indications that subspecies associate with Riverine, Maya and Dehra ecosystems.

Key words: *Apis mellifera*; Dahara, tree cavity; Maya, Riverine.

I. INTRODUCTION

The Sudanese honey bee was classified and named as *Apis mellifera nubica* [1] based on samples originating from the semi desert zone. In [2] noted that the small size and the short appendices of Sudanese bee were remarkable. Accordingly he placed the bee in a separate race. [3] suggested that there were two independent subspecies of honeybees 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. However, [4] withdrew the name "*nubica*" in favor of *yemenitica*, a name that included bees from Saudi Arabia, Yemen, Oman, Somalia, and Chad. The dwarf honeybee *A. florea* was discovered in Khartoum gardens at the vicinity of the International Airport in 1985 [5]. [6] were reported that, within seven years of its discovery *A. florea* has advanced 150 Kilometers southeast along the Blue Nile River by February, 1992. 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 found in Yarenja refugees camp near Mankush town in Ethiopia [7]. However 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.

II. 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. Established in 1935, during the dry season in 2009 and 2010. Samples of honeybee workers were collected from honeybee colonies nesting in tree cavities and on tree branches at 20 locations that 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) as mentioned by [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, the

right antenna, the hind leg, the 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. Measurements of the following organs were taken by 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 the 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 data were then subjected to one way ANOVA with three ecosystems as main source of variation.

III. 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 number of hooks, the number of hair rows and the length of tergite 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, the 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 (2) indicated 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 same wax mirrors were significantly different ($P < 0.05$) among the bees in the three ecosystems whereas; the cubital index was highly significantly different ($P < 0.01$).

Table 1: The mean values of measurements taken for the different organs of honey bee workers in the Dinder Biosphere Reserve

Organ	Mean $\pm$ SD		
	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
Proboscis L ,mm	5.353 $\pm$ 0.588	5.301 $\pm$ 0.503	5.143 $\pm$ 0.399
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. hooks	20.790 $\pm$ 0.379	20.931 $\pm$ 0.893	20.878 $\pm$ 0.864
Basitarsus I II L ,mm	1.891 $\pm$ 0.055	1.893 $\pm$ 0.050	1.919 $\pm$ 0.053
Basitarsus III W, mm	1.015 $\pm$ 0.021	1.016 $\pm$ 0.028	1.016 $\pm$ 0.30
No. Hair-rows	8.857 $\pm$ 0.254	9.250 $\pm$ 1.103	8.920 $\pm$ 0.829
Yellow coloration (%)	66.609 $\pm$ 2.364	66.408 $\pm$ 1.333	67.693 $\pm$ 1.356

Tergite (3+4) L, mm	3.784 ± 0.333	3.919 ± 0.196	3.884 ± 0.383
Wax mirror L, mm	1.209 ± 0.086	1.195 ± 0.127	1.262 ± 0.227
Waxmirror W, mm	2.756 ± 0.184	2.510 ± 0.246	2.615 ± 0.365
Distance between Wax mirror, m m	1.348 ± 0.092	1.451 ± 0.231	1.451 ± 0.090

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

Table 2: ANOVA of honeybee workers' measurements in Dinder Biosphere Reserve

Organs	Source of variation	Degree of freedom	Sum of Squire	Mean Squire	F Calculated	Significance
Antenna length, mm	Ecosystem	2	0.059	0.02954	0.700	NS
	Error	17	0.717	0.04219		
	Total	19	0.776			
Proboscis length, mm	Ecosystem	2	0.134	0.06718	0.255	NS
	Error	17	4.480	0.2640		
	Total	19	4.615			
Forewing L, mm	Ecosystem	2	0.064	0.03222	1.030	*
	Error	17	0.532	0.03128		
	Total	19	0.596			

Forewing width, mm	Ecosystem	2	0.029	0.01452	1.316	*
	Error	17	0.188	0.0104		
	Total	19	0.217			
Cubital index	Ecosystem	2	0.101	0.05072	2.742	**
	Error	17	0.314	0.01849		
	Total	19	0.416			
No. hooks	Ecosystem	2	0.085	0.04244	0.036	NS
	Error	17	19.989	1.176		
	Total	19	20.073			
Basitarsus III length mm	Ecosystem	2	0.003	0.001453	0.491	NS
	Error	17	0.050	0.002961		
	Total	19	0.050			
Basitarsus III width, mm	Ecosystem	2	0.001	0.000326	0.472	NS
	Error	17	0.010	0.000691		
	Total	19	0.010			
No Hair-rows	Ecosystem	2	0.657	0.329	0.479	NS
	Error	17	11.653	0.685		
	Total	19	12.310			
Yellow	Ecosystem	2	5.472	2.736	0.872	*

colouration (%)	Error	17	53.312	3.136		
	Total	19	58.784			
Tergite(3+4)L, mm	Ecosystem	2	0.071	0.0354	0.679	NS
	Error	17	1.518	0.0893		
	Total	19	1.589			
Wax mirror L, mm	Ecosystem	2	0.015	0.007259	0.340	NS
	Error	17	0.363	0.02134		
	Total	19	0.377			
Wax mirror width ,mm	Ecosystem	2	0.227	0.113	1.660	*
	Error	17	1.160	0.06821		
	Total	19	1.386			
Distance between Wax mirror,mm	Ecosystem	2	0.049	0.0243	0.901	*
	Error	17	0.459	0.02697		
	Total	19	0.507			

** P < 0.01; * P < 0.05. ; NS= not significant ; L = length; W =width.

IV. DISCUSSION

In general, the measurements obtained in this study show that, all the samples are for bees belong only 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 agrees with [9],[10] and [11] who found highly significant differences for all twelve traits they studied and concluded that the samples they studied did not belong to one race. Similarly [12], [13], [3], [14] and [15] were reported a high degree of regional variation of the Sudanese honeybees.

Similar variation was also observed for the neighboring Ethiopian honeybees [16],[17],[4], [18], [19], [20] and Kenyan honeybees [21].

The finding of this study can also be supported by the finding of the genetically studied Sudanese honeybees. [22] studied the mitochondrial discrimination of honeybees (*Apis mellifera*) of Sudan. They 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; C₂ was found in apiaries with imported stock (*A.m.carnica*). This reclassification of the honeybees from Sudan has consequences for the interpretation of the biogeography of *A .mellifera* in the Maghreb and Mashriq regions"

Although Rashad et al [3] and [14] reported that the bees of the Blue Nile State possessed the largest measurements among the studied Sudanese honeybees, but when comparing the means of the measured characters obtained in this study with those obtained in the previous studies we will observe that, they were very smaller than the Sudanese bees obtained by the different previously mentioned researchers. Moreover, the means of the measured characters obtained in the present study were found to be very close to those obtained by [23] who studied the honeybees in the White Nile and North Kordofan provinces and also to the ones obtained by [14] for the bees collected from the semi-desert zone. 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 agreed with [24], [25] and [20] 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. The classification of the honeybees' native to Sudan seems 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 particular in Biosphere Reserve areas in Sudan

CONCLUSION AND RECOMMENDATION

In conclusion, the predominant honeybee in Dinder Biosphere Reserve is *Apis mellifera*. There are indications that subspecies 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.

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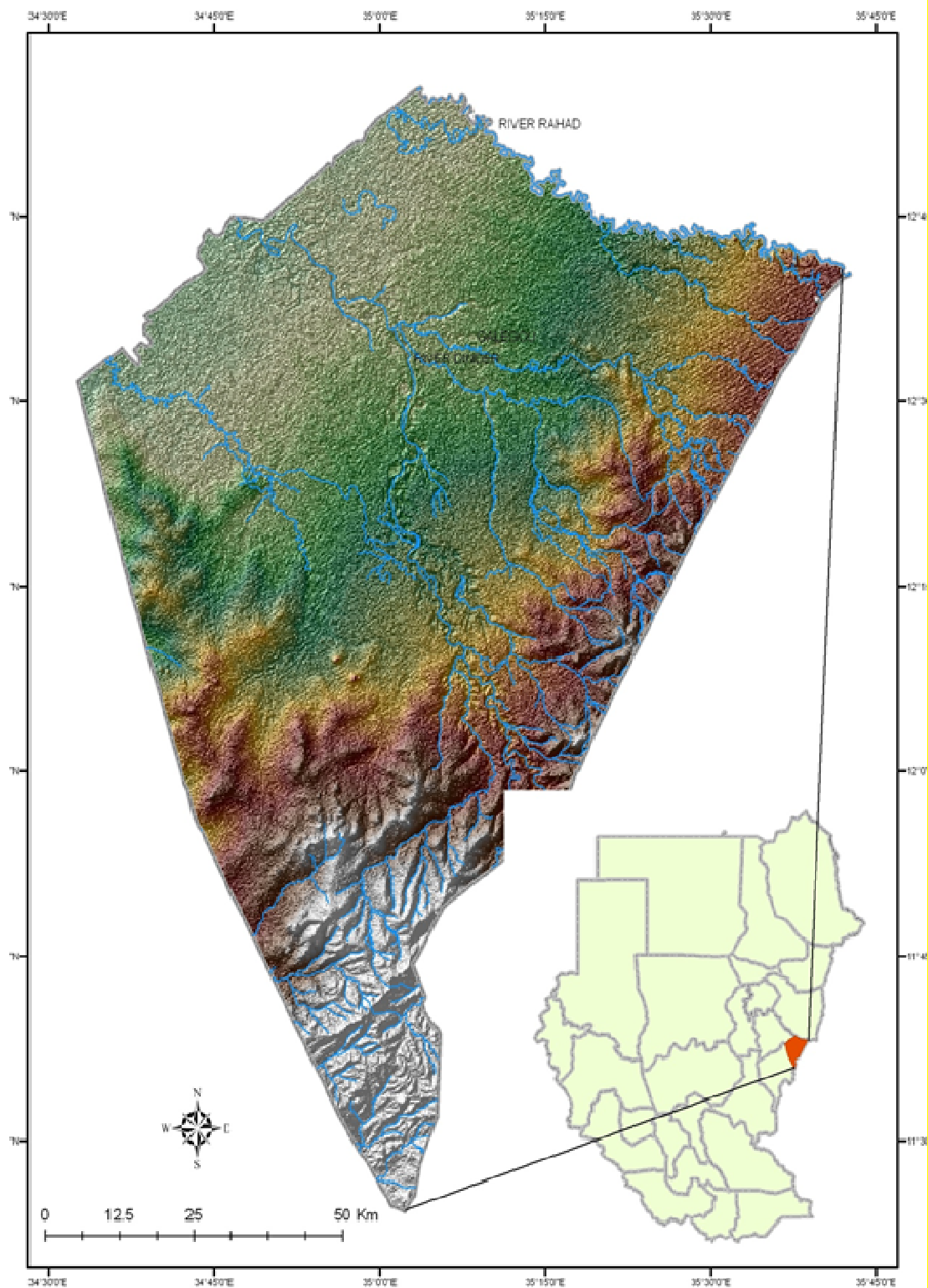


Fig. 1. Location of Dinder Biosphere Reserve