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Research Article Compliance of the Morphometric Characteristics of Bees in Benin with Those of *Apis mellifera* Adansonii

^{1,2}Armand Paraïso, ^{1,2}Grégoire Paraïso, ^{1,2}Valère K. Salako, ^{1,2}Waliou Abiola, ^{1,2}Aude Kelomey, ³Romain Glele Kakaï,
 ⁴Patrick A. Edorh, ⁵Lamine Baba-Moussa, ⁶Ambaliou Sanni and ⁷Adole Isabelle Glitho

¹Département des Sciences et Techniques de Production Végétale, Faculté d'Agronomie, Université de Parakou, Benin ²Laboratoire de Protection des Végétaux, de Pathologie et de Parasitologie des abeilles (LAPPAB), Parakou, Benin

³Département des Sciences et Techniques de Production Végétale, Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, Benin ⁴Laboratoire de Recherche en Biochimie et en Toxicologie de l'Environnement, Faculté des Sciences et Techniques,

Université d'Abomey-Calavi, 03 BP 0994 Cotonou, Benin

⁵Laboratoire de Biologie et de Typage Moléculaire en Microbiologie, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, 05 BP 1604 Cotonou, Benin

⁶Département de Biologie moléculaire, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, 01 BP 526 Abomey-Calavi, République du Benin

⁷Laboratoire d'Entomologie Appliquée, Faculté des Sciences, Université de Lomé, BP 1515 Lomé, Togo

Abstract

Background: Only one subspecies of bee, Apis mellifera adansonii was described for all West and Central Africa. Preliminary studies carried out in Benin suggested the presence of other subspecies. **Objective:** The objective of this study was to evaluate the conformity of the morphometric characteristics of bees in Benin with those of A. madansonii described before. Materials and Methods: To this end, 100-300 bees were collected by locality and by hive. The samples were collected from 30 localities (colonies) belonging to 8 phytodistricts and to the three climate zones of Benin. In each locality, 100 bees were measured (except the site of Yarakeou where 60 bees were measured). Thus, 14 morphometric characteristics were measured per bee with a microscope equipped with graduated eyepieces. But three characteristics including the length of the proboscis, the width of the tomentum and the cubital index were used for the tests. Results: The ascending hierarchical classification and the t-tests of student to a sample at 5% threshold were done. The condition of application of the test, that is the normality of the data was previously verified. These analyses were done using the software SAA v.9.2. The ascending hierarchical classification of 2960 bees measured on the basis of the 14 morphometric characteristics allowed us to identify 10 statistically different morphotypes. The t-tests of student showed that in general, bees in Benin had the cubital index, the length of the proboscis and the width of the tomentum significantly different (p<0.001) from those of A. mellifera adansonii. Conclusion: Bees in Benin are characterized by a cubital index, a length of the proboscis and a width of the tomentum that are statistically different from those of the subspecies Apis mellifera adansonii. However, these three characteristics may not be sufficient alone to deduct that bees in Benin are systematically different from Apis mellifera adansonii. Some of the studied morphometric characteristics of bees in Benin are statistically different from those of the subspecies Apis mellifera adansonii. We suggest that molecular studies be carried out in order to determine the genotype of the different morphotypes listed.

Key words: Apis mellifera adansonii, conformity, morphotypes, morphometric characteristics

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Corresponding Author: Armand Paraïso, Département des Sciences et Techniques de Production Végétale, Faculté d'Agronomie, Université de Parakou, Benin Tél: (00) 229 9719 0503

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Benin has a great apiarian potential¹. Domestic bees are not spared by the regression of pollinating insects² and several species and subspecies could disappear without being identified. Honey hunting, a practice which consists in burning the swarm of wild bees in order to harvest its honey is developed in North-West Benin. It is practiced due to the lack of training in beekeeping and the number of farm assets in the households³. It is also an adaptation strategy of some beekeepers to the effects of climate changes⁴. This practice is a threat to biodiversity because it increases pressure on bee's diversity. A better knowledge of autochthonous bees is an indispensable step to develop beekeeping. The distinction between the different species, subspecies and ecotypes of bees is mainly oriented to the morphology called morphometrics. In general, all bees from tropical Africa were called "Adansonii", name originally given to a bee in Senegal⁵. Latreille⁶ described a single subspecies, Apis mellifera adansonii, for the entire Central and West Africa. The existence of a single subspecies is unlikely in such a vast region with contrasted climates. According to Ruttner⁵, among the samples collected in West Africa, none of them is from Benin. Morphometric studies carried out in the North-East of Benin revealed the existence of three statistically distinct groups of bees^{1,7}. The same studies suggested the presence of other subspecies in addition to A. mellifera adansonii. The objective of this study is to assess the compliance of the morphometric characteristics of honeybees of Benin with those of A. mellifera adansonii. Due to the great variations induced by the climate⁸⁻¹¹ and the vegetation^{12,13} on the morphometric characteristics of bees, it is hypothesized that certain morphometric characteristics of bees in Benin do not comply with those of A. mellifera adansonii.

MATERIALS AND METHODS

Study zone: The samples of bees were collected from several beekeepers located in 30 localities belonging to height phytodistricts in the three climate zones of the Republic of Benin (Fig. 1). The Republic of Benin is limited in the North by the Republics of Niger and Burkina Faso, in the South by the Atlantic Ocean, in the West by the Republic of Togo and in the East by the Republic of Nigeria¹⁴. It is located between the parallels 6°15' and 12°25' of latitude North and 0°40' and 3°45' of longitude East and covers a surface area¹⁵ of 112,622 km. The country has ten phytodistricts¹⁶⁻¹⁸ and a

varied range of climates characterized by three climate zones stretched from the South to the North: The Guinea-Congo zone, the Sudan-Guinea zone and the Sudanian zone¹⁹.

The Guinea-Congo zone has four seasons and stretches from the coast (6°25 N) to 7°30 latitude north. It has a medium rainfall of 1200 mm year⁻¹ with on average 250 rainy days. The average daily temperature varies from 25-29°C. Air moisture varies between 69 and 97%. It is subdivided into four phytogeographical districts: Coast, Pobe, Valley of Oueme and Plateau.

The Sudan-Guinea zone is located between 7°30' North and 9°45' North. The rainfall regime in the Sudan-Guinea zone is unimodal (May-October) and the average annual rainfall varies between 900 and 1110 mm often distributed over 113 days on average. Relative moisture varies from 31-98% in this zone. Average sunshine is 2305 h year⁻¹. Temperatures vary between 25 and 29°C in this zone. It is subdivided into three phytogeographical districts: Bassila, Zou and South Borgou.

The Sudanian zone is located between 9°45 N and 12°25 N. Rainfall in this zone varies from 900-1100 mm year⁻¹, spread on average on 145 days. Air moisture varies from 18% during harmattan (December-February) to 99% in August during the rainy season. Average annual temperature varies from 24-31°C in this zone. The total number of sunshine hours is 2862 h year⁻¹. It is subdivided into three phytogeographical districts: North Borgou, Chain of Atacora and Mekrou Pendjari.

Sampling of bees: The samples of bees were collected in 30 localities located in 8 phytodistricts and the three climate zones of Benin. Depending on the availability of the biological material and the willingness of the beekeepers, the 100-300 samples of bees were collected per locality and per hive. The laths containing the broods on which young bees hang² were removed and shook into a plastic pot covered automatically with fine grid of 0.05 mm diameter. Bees collected were killed using ether and conserved in ethanol at 70%.

Data collection: The 100 honeybees of each locality were counted and measured following 14 morphometric characteristics, except the locality of Yarakeou were only 60 honey bees were measured because of the non-availability of the animal material. Measures were taken on 2960 workers²⁰ because haploid males are not representative in a bee population^{19,21-23}. These measures were taken using a microscope equipped with graduated eyepiece^{22,23}. Measures taken on each honeybee were included.



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Fig. 1: Collection sites of bee's samples

The length of the honeybee, the length and the width of fore and hind wings, the length of the tongue, the width of the tomentum on the fourth abdominal tergite, the width of the hairy zone of the 5th abdominal tergite, cubital ribs A and B which ratio A/B gives the cubital index, the length of the radial cell, the distance between the radial cell and the

discoidal point, the distance between the radial cell and the left end of rib A, the discoidal transgression and the angle of lag of the discoidal point, which were measured as described by Cornuet *et al.*²³. All measures are in mm except the cubital index which is dimensionless and the angle of lag of the discoidal point which is in degree (°).

Statistical analyses: The Ascending Hierarchical Classification (AHC) of bees was done at the level of individual bees. The number of representative morphotypes was determined based on the value of the coefficient of determination (R²). The considered threshold²⁴ is 50%. Student's t-test with one sample were done at the threshold of 5%. The test application condition, that is the normality of data was previously checked. When condition was not checked, a natural logarithm transformation was done before doing the test. The statistical analysis of data was done using the software SAS v.9.2.

RESULTS

The ascending hierarchical classification of 2960 bees measured on the basis of the 14 morphometric characteristics identified ten statistically different morphotypes with a coefficient of determination R² of 59.8%. Although, each morphotype contains bees that are morphologically similar, all the morphotypes are nevertheless a mixture of bees of several localities except the morphotypes C4 (100% of Yarakeou) and C10 (94.19% of Adjiro and 5.81% of Yarakeou). Three groups of bees were recorded in the hives of the North-East of Benin. Group I comprises 79.2% of the bees sampled on all the study sites, group II comprises 20.4% of all the bees sampled and group III comprises 0.4% of all the bees sampled. The three groups are characterized by the size of the bee, the length of the fore wing, the length of the proboscis and the cubital index. The comparison between the 10 morphotypes obtained and these three groups (compliance test in Table 1) showed that, at the threshold of 5%, bees of the morphotype 9 had a size that statistically complies (p = 0.664) with bees of group I recorded in North-East Benin. Bees of the morphotypes 3 (p = 0.562) and 4 (p = 0.377) had a size that complies with the size of bees of group II identified in the North-East of Benin. Bees of all the ten morphotypes have a size that was statistically different (p<0.001) from that of the bees of group III recorded in the Nort-East of Benin. Moreover, bees of the phytodistrict of Bassila had a size that statistically complies (p = 0.091) with that of bees of group II found in the North-East of Benin.

Bees of all the morphotypes had the length of the proboscis statistically different (p<0.001) from that of the bees of group I, II and III recorded in the North-East of Benin (Table 1).

At the threshold of 5%, the cubital index of the bees of the morphotype 9 (p = 0.276) and of the phytodistrict of Plateau (p = 0.584) statistically complied with that of the bees of group I studied in the North-East of Benin. The cubital index of the bees of the morphotype 10 (p = 0.971) and of the phytodistrict of the Coast (p = 0.133) statistically complied with that of the bees of group III collected in the North East of Benin. No morphotype has bees with a cubital index that statistically complies with that of the bees of group II recorded in the North-East of Benin (Table 1).

The length of the fore wing of the bees of the morphotype 4 (p = 0.087) and of the Sudanian climate zone (p = 0.513) statistically complied with that of the bees of group I found in the Nort-East of Benin. The length of the bees of the morphotypes 6 (p = 0.791), 9 (p = 0.129), 10 (p = 0.602) and of the phytodistricts of the Chain of Atacora (p = 0.089) and of Mekrou Pendjari (p = 0.474) statistically complied with that of the bees of group II collected in the North-East of Benin. Bees of all morphotypes had the length of the fore wing statistically different (p<0.001) from that of the bees of group III recorded in the North-East of Benin.

At the threshold of 5%, bees of the morphotype 1 had a proboscis length that statistically complied (p = 0.780) with that of *A. mellifera* adansonii described in tropical Africa. Bees of the nine other morphotypes had a proboscis length statistically different (p<0.001). At the same threshold, bees of all the morphotypes had a proboscis length different (p<0.001) from that found in Tanzania in Chad and in West Africa. Bees of the phytodistrict of Bassila had a proboscis length that statistically complies (p = 0.919) with that indicated in tropical Africa (Table 2).

At the threshold of 5%, bees of all the ten morphotypes had a length of the tomentum statistically different (p<0.001) from that of *A. mellifera* adansonii of Chad. With regard to cubital index, bees of the morphotype 6 had a cubital index that complies (p = 0.097) with that of *A. mellifera* adansonii of Chad and bees of the morphotype 7, had a cubital index that statistically complies (p = 0.335) with that of *A. mellifera* adansonii of tropical Africa. Bees of the phytodistricts of North Borgou (p = 0.203), South Borgou (p = 0.737) and Mekrou Pendjari (p = 0.061) had a cubital index that statistically complies with that of *A. mellifera* adansonii of Tchad. Bees of the phytodistricts of North Borgou (p = 0.394), South Borgou (p = 0.112), Mekrou Pendjari (p = 0.603) and Zou (p = 0.348)

	Group I				Group II	(Group III			
	Size	L fore w	Proboscis	a/b	Size	L fore w	Proboscis	a/b	Size	L fore w	Proboscis	a/b
Characteristics (mm)*	$\mu = 11.35$	$\mu = 8.62$	$\mu = 2.93$	$\mu = 2.28$	$\mu = 12.02$	$\mu = 8.71$	$\mu = 2.65$	μ = 1.96	μ = 14.80	$\mu = 11.14$	$\mu = 1.20$	μ = 2.09
Global	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Morphotypes level												
Morphotype 1	0.000	0.000	0,000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029
Morphotype 3	0.000	0.000	0.000	0.000	0.562	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 4	0.004	0.087	0.000	0.000	0.377	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 5	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 6	0.000	0.000	0.000	0.029	0.000	0.791	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 9	0.664	0.000	0.000	0.276	0.000	0.129	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 10	0.000	0.002	0.000	0.000	0.128	0.602	0.000	0.000	0.000	0.000	0.000	0.971
Phytodistricts level												
Bassila	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000
North Borgou	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
South Borgou	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Chain of Atacora	0.000	0.000	0.000	0.000	0.000	0.089	0.000	0.000	0.000	0.000	0.000	0.000
Coast	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.133
Mekrou Pendjari	0.000	0.000	0.000	0.000	0.000	0.474	0.000	0.000	0.000	0.000	0.000	0.000
Plateau	0.000	0.000	0.000	0.584	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Zou	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Climate zones level												
Guinea-Congo	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sudan-Guinea	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sudanian	0.000	0.513	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Size: Length of the bee, L fo	ore w: Length of	the fore wing,	a/b: Cubital inde>	K, *Except the re	elationship a/b w	vhich is dimens	ionless					

Table 1: Compliance of the characteristics of the bees studied with the characteristics described for Apis mellifera by Viniwanou⁷ and Paraiso¹ in Benin

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i	Length of the proboscis			Tomentum		Cubital index		
Characteristics (mm)* References	$\mu = 5.45$ Gadbin ²⁵	µ = 5.69 Ruttner⁵	$\mu = 5.86$ Radloff ²⁷	$\mu = 6.05$ Ntenga ²⁶	$\mu = 0.97$ Gadbin ²⁵	$\mu = 2.35$ Gadbin ²⁵	μ = 2.39 Ruttner⁵	μ = 1.94 Amakpe ²⁸
Global	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000
Morphotypes level								
Morphotype 1	0.000	0.780	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 3	0.009	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 5	0.000	0.000	0.000	0.000	0.000	0.012	0.005	0.000
Morphotype 6	0.000	0.000	0.000	0.000	0.000	0.097	0.000	0.000
Morphotype 7	0.000	0.000	0.000	0.000	0.000	0.000	0.335	0.000
Morphotype 8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Morphotype 10	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000
Phytodistricts level								
Bassila	0.000	0.919	0.001	0.000	0.000	0.000	0.000	0.000
North Borgou	0.000	0.000	0.000	0.000	0.000	0.203	0.394	0.000
South Borgou	0.000	0.000	0.000	0.000	0.000	0.737	0.112	0.000
Chaine of Atacora	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Coast	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mekrou Pendjari	0.000	0.000	0.000	0.000	0.000	0.061	0.603	0.000
Plateau	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Zou	0.000	0.000	0.000	0.000	0.000	0.028	0.348	0.000
Climate zones level								
Guinea-Congo	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sudan-Guinea	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sudanian	0.000	0.000	0.000	0.000	0.000	0.278	0.000	0.000

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Table 2: Compliance of the characteristics of the bees studied with the characteristics described for Apis mellifera adansonii in the literature

*Except the cubital index which is dimensionless

had a cubital index that statistically complies with that of *A. mellifer*a adansonii of tropical Africa. Bees of the Sudanian climate zone (p = 0.278) had a cubital index that statistically complies with that of *A. mellifer*a adansonii of Chad. In other respects, bees of all the morphotypes had a cubital index statistically different (p<0.001) from the average of that of *A. mellifer*a adansonii found in the commune of Djidja (South Benin) (Table 2).

DISCUSSION

This study on the morphometric characteristic of bees is among the first of the kind in Benin. Samples of bees of the apiaries in the North-Eastern zone were not collected as previously studies have been already undertaken in that region. With its short pilosity, the race *A. mellifera* adansonii is similar to *A. mellifera* lamarckii, *A. mellifera* syriaca and *A. mellifera* sahariensis²⁵. It is indicated that *A. mellifera* Adansonii is yellow and small⁵. At the threshold of 5%, bees of all the morphotypes had a proboscsis length statistically different (p<0.001) from that of the bees of the three groups recorded in the North-East of Benin^{1,7}. Bees of the morphotype 1 (p = 0.780) and of the phytodistrict of Bassila (p = 0.919) had a proboscis length which statistically complies with that of *A. mellifera* adansonii described in tropical Africa⁵. Bees of all the morphotypes had a proboscis length different (p<0.001) from that of *A. mellifera* adansonii studied in Tanzania²⁶, in Chad²⁵ and in West Africa²⁷.

With regard to cubital index which is specific to each race or ecotype of European bees^{2,20}, at the threshold of 5%, the cubital index of the bees of the morphotype 9 (p = 0.276) and of the phytodistrict of Plateau (p = 0.584) statistically complied with that of the bees of group I recorded in the North-East of Benin^{1,7}. The cubital index of the bees of the morphotype 10 (p = 0.971) and of the Coast (p = 0.133) statistically complied with that of the bees of group III identified in the North-East of Benin^{1,7}. Bees of all the morphotypes had a cubital index statistically different (p<0.001) from that of the bees studied in the commune of Djidja (South Benin)²⁸. Bees of the morphotype 6, of the phytodistricts of North Borgou (p = 0.203), South Borgou (p = 0.737) and Mekrou Pendjari (p = 0.061) and of the Sudanian climate zone (p = 0.278) had a cubital index that complies (p = 0.097) with that of A. mellifera adansonii recorded in Chad²⁵. Bees of the morphotype 7 and of the phytodistricts of North Borgou (p = 0.394), South Borgou (p = 0.112), Mekrou Pendjari (p = 0.603) and Zou (p = 0.348)had a cubital index which statistically complies (p = 0.335) with that of A. mellifera adansonii identified in tropical Africa⁵.

The insects of the 10 morphotypes had a tomentum width statistically different (p<0.001) from that of *A. mellifera* adansonii in Chad²⁵.

At the threshold of 5%, bees of the morphotype 9 had a size which statistically complies (p = 0.664) with that of the bees of group I studied in the North-East of Benin^{1,7}. The honey bees of the morphotypes 3 (p = 0.562) and 4 (p = 0.377) and of the phytodistrict of Bassila (p = 0.091) had a size which complies with that of the bees of group II recorded in the North-East of Benin^{1,7}. The insects of the 10 morphotypes have a size statistically different (p < 0.001) from that of the bees of group III found in North-East of Benin^{1,7}. The comparison of the morphometric characteristics of the bees in the North-Eastern region of the country showed that, bees in Benin are not only from the sub-species *A. mellifera* adansonii, but constituted a mixture of more than one sub-species as observed by Kelomey²⁹ in a recent work.

With regard to the length of the forewing, at the threshold of 5%, it can be deducted that, bees of the morphotype 4 (p = 0.087) and those of the Sudanian climate zone (p = 0.513) statistically complied with the length of the forewing of the bees of group I found in the North-East of Benin^{1,7}. The length of the forewing of the bees of the morphotypes 6 (p = 0.791), 9 (p = 0.129), 10 (p = 0.602) and of the phytodistricts of the Chain of Atacora (p = 0.089) and Mekrou Pendjari (p = 0.474) statistically complied with that of the bees of group II identified in the North-East of Benin^{1,7}. These results indicated again that, bees in Benin are made of different sub-species.

By comparing the proboscsis of A. mellifera adansonii of the different researchers on the one hand and the cubital index of A. mellifera adansonii of these authors on the other hand, it can be observed that the means are significantly different from one researcher to another. As all the above mentioned researchers have worked on the same race. A. mellifera adansonii, the means should be statistically identical. In other words, for the same characteristics, when a morphotype complies with the mean of an researcher, it should be the same for the means of the other researchers. According to our comparison results, this is not the case. Bees of the morphotype 1 had a proboscis length which statistically complies with the mean indicated by Ruttner⁵, while they were showing a proboscis length statistically different from the mean of the other researchers. The same is true for the cubital index. Even if Fresnaye²⁰, considers that the variations of biometric measures are less important between the means obtained by each researcher, it should be recognized that the methodological differences can lead to a significant variation

of measures²⁵. In morphometry, results obtained depend on the sampling methods and the choice of the study zones³⁰. A uniform sampling throughout the study zone may prevent from better discriminating the populations of bees, contrary to other sampling methods³¹. The number of samples is more limited when the object of the study is to show a morphological differentiation between bees of various origins than when the objective is the characterization of each population studied³². Moreover, it has to be added that it is rare that two operators find exactly the same results on the same bees^{20,33}.

CONCLUSION

Some morphotypes of bees recorded in Benin are characterized by a cubital index, a length of the proboscis and a width of the tomentum that are statistically different from those of the subspecies *Apis mellifera* adansonii. However, these three characteristics may not be sufficient alone to deduct that bees in Benin are systematically different from *Apis mellifera* adansonii. Howether, the comparison of the morphometric characteristics of bees of different region of the country showed that, bees in Benin are not only from the sub-species *A. mellifera* adansonii, but constituted a mixture of more than one sub-species. It is suggested that molecular studies be carried out in order to determine the genotype of the different morphotypes listed.

SIGNIFICANCE STATEMENTS

Up to recently, only one subspecies of bee, Apis mellifera adansonii, was described for all West and Central Africa. The presence of other subspecies is recorded in preliminary studies carried out in the North Western part of Benin. The ascending hierarchical classification of 2960 bees measured on the basis of the 14 morphometric characteristics allowed us to identify 10 statistically different morphotypes. This study discusses the conformity of the morphometric characteristics of bees in Benin with those of A. mellifera adansonii described before. Results showed that some morphotype of bees in Benin are characterized by a cubital index, a length of the proboscis and a width of the tomentum statistically different from those of the subspecies Apis mellifera adansonii. However, these three characteristics may not be sufficient alone to deduct that bees in Benin are systematically really different from Apis mellifera adansonii. Molecular studies should be carried out in order to determine the genotype of the different morphotypes listed.

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