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Morphometric variability of wild honey bees (*Apis mellifera adansonnii L.*) in different agro-ecosystems in coastal Côte d'Ivoire, West Africa

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ABSTRACT

Objectives: The aim of this work is to study the morphometric diversity of wild bees of the *Apis mellifera* (Linné, 1758) species in the Gboklé region, with a view to the possibility of beekeeping, which is still non-existent in the area.

Methodology and Results: For this purpose, worker bees were randomly collected over three months from wild nests in localities of Lélédou, Kpata cacao, Kpata Jachère and Dassioko Plage representing different agro-ecological zones. Sixteen morphometrical descriptors were recorded on dissected parts of each specimen. Mean morphological characters were analysed by the Kruskal-Walli's test. Separately, these groups of describers were submitted to the Principal Component Analysis and to the Hierarchical Ascending Classification. The results showed a significant variation between hives and the cubital index, allowed the bees to be classified into one variety.

Conclusions and application of findings: In the whole, there is a great morphometric diversity in bees, resulting from a probable high underlying genetic variability together with diversity of available plants and sustainable agriculture practice. It is therefore possible to practice perennial beekeeping in the area.

Keywords: morphometric diversity, cubital index, beekeeping, Gboklé, Côte d'Ivoire.

INTRODUCTION

Bees are insects belonging to the order Hymenoptera and the Apoidea superfamily, forming the largest and most specialized group of pollinators (Michener, 2001; Le Conte and Navajas, 2008). Among them, the species *Apis mellifera* (Linné, 1758), has long been domesticated by humans for food, medical and economic interests (Michener, 2001; Crane, 1990). To date, there are around 29 subspecies of *Apis mellifera* (Sheppard and Meixner, 2003; Shaibi *et al.*, 2009), including *Apis mellifera adansonii* (Latreille, 1804) or "African bee" present throughout West and Central Africa. Due to the very wide distribution of *A. mellifera*, a multitude of races and ecotypes adapted to different habitats are now reported across the world (Paraïso *et al*., 2011). The ecological interest of bees in maintaining plant diversity through their pollination services is no longer to be demonstrated (Winston, 1987; Breeze *et al.*, 2011). It is certain that the bee is the most influential insect for humankind, both in nature and in myriad of benefits to human well-being. Unfortunately, the present-day animal-mediated pollination populations and bees particularly are declining as a result of habitat loss, habitat degradation and other factors including pesticides, pathogens, parasites and climate change (Winfree *et al*., 2009). Their loss and the negative impact on natural habitat support, plant productivity, food webs, and ultimately in human well-being justify calls for stronger governance around this pollinator conservation (FAO, 2021). Obviously, one of the straightforward approaches which spark increased interest for pollinator conservation could be beekeeping. It terms the science and arts of rearing honey bees. Therefore, it allows the care and management of honey bees for hive products, food, medicine and income. Above all, this practice of maintaining honey bee colonies helps preserve bee populations. Although this occupation tends to be perceived as a hobby or as a side-line activity worldwide, valuable not only in pollination services and environmental purposes but also in farming sector. However, the most important prerequisites to ensure the long-term future of beekeeping activities in a given area are sustainable agricultural practices, floral diversity and nutritional landscapes (Pfiffner and Müller, 2016). Particularly, in view of the coevolution based on mutualism between bees and plants (Crepet, 1984), bee diversity could be a proxy of the above conditions which are linked each other. In fact, fruit set and seed set of crop plants appear to improve in tandem with increased diversity of flower-visiting bee species. In turn, flower diversity significantly impacts on species diversity of bees (Pfiffner and Müller, 2016). Here the study subject is morphometric diversity of wild bees in Gboklé locality, in coastal Côte d'Ivoire, where beekeeping activities are not yet present. In this area where access to income is limited to agricultural resources, small-scale beekeeping and related trades can therefore contribute significantly to livelihood security. Furthermore, morphometrical approach is, indeed, commonly used to distinguish between bee groups (Meixner *et al*., 2007) and offer, on the whole, interesting perspectives for the study of biodiversity patterns. Our aim with this paper is to study the morphometric diversity of wild bees of the *Apis mellifera* species with a view to the possibility of beekeeping.

MATERIALS AND METHODS

The study Area: The study was conducted in four different locations in different agroecological zones in the Gboklé region (4° 57′ 04″ North latitude and 6° 05′ 19″) in coastal Côte d'Ivoire (Fig.1). Located in the southwest of Côte d'Ivoire, the region of

Gboklè is limited to the southeast by the region of Grands-Ponts, to the southwest by the region of San-Pedro, to the north and northwest by the Nawa region, and to the northeast by the Lôh-Djiboua region. To the south, it is bounded by the Atlantic Ocean. The region is characterized by the existence of four (04) seasons (December-April), the dry season; (April-July), great rainy season; (August-September), short dry season; (October-November), small rainy season. The annual average temperature is 27 ° C with a humidity of 80%. The annual rainfall, it is estimated to 2200 mm (Dibié and Rondeau, 2008). The capital of the region is Sassandra and the population of the region is 400,798 according to the general population and housing census (RGPH, 2014). The climate of the region is characterized by a wet and dry season: The wet season includes a long (April to July) and short (October-November) rainy season, while the dry season includes a long (December - March) and short (August-September) dry season. Agriculture is the main source of income for the populations in the area. The region's economic activity is mainly focused on agriculture, with cocoa, coffee, palm oil and rubber trees as the main crops (Yao, 2014). Food crops such as cassava, plantains and maize are also produced in the area. The present study was conducted in different habitats (primary forest, secondary forest, oil palm plantation, cocoa plantation, cassava plantation, fallow land, etc.) located in 3 villages. These localities are Dassioko, Lélédou and Kpata. They are at least about 4 to 15 kilometres apart and located between the municipalities of Sassandra and Fresco. Two sampling sites were chosen in kpata village, named Kpata Cacao and Kpata Jachère (figure 1). The former is a cocoa plantation whereas the latter is a fallow land.

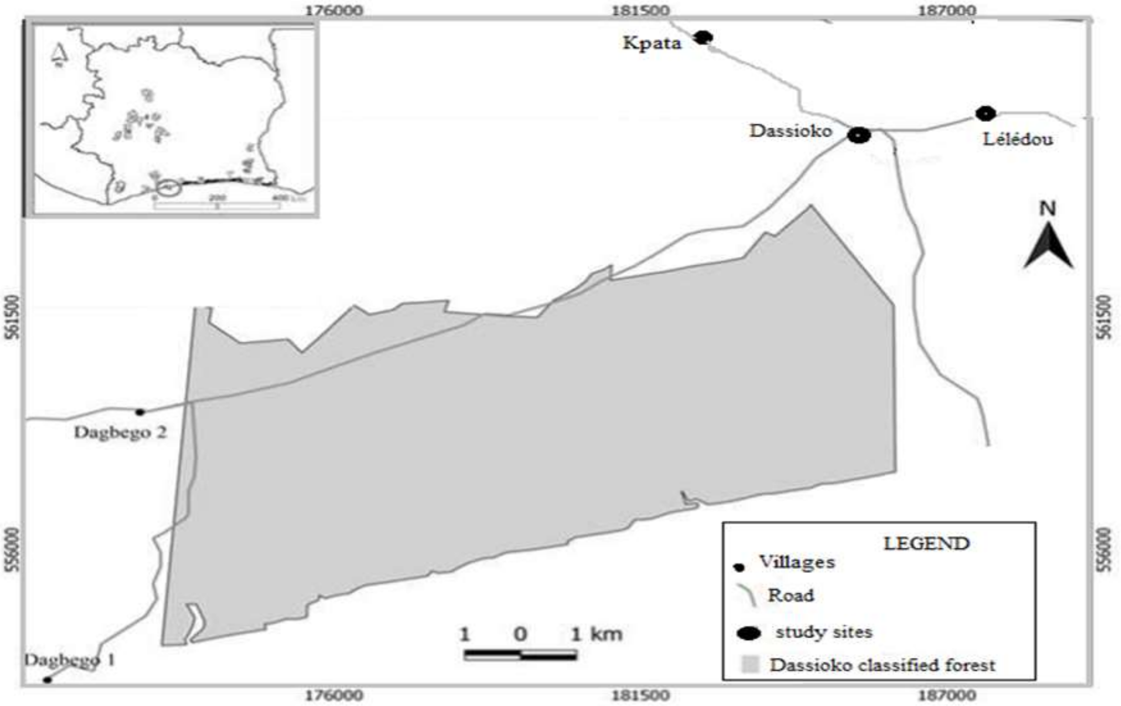

Figure 1: Location of the study area (Kouao *et al.*, 2020)

Insect collection and dissection: From September to November 2018, worker bees were randomly collected from four colonies in different agro-ecological zones in three localities (Lélédou, Dassioko Plage and Kpata) in the Gboklé region. Bees were collected, from wild nests. For each nest, 40 individuals were collected and preserved in 70 % ethanol on the field and transported at the Laboratory of Zoology at University Félix Houphouët Boigny in Abidjan, Côte d'Ivoire. Samples were dissected for morphometry following standard procedure (Meixner *et al*., 2007).The worker honeybee samples were dissected using dissecting kit to separate body parts (tongue, right fore wing, right hind wing, and right hind leg) into the different characters to be studied (Table 1). The dissected parts were further mounted on microscope slides for studying and viewing while characters were measured under electronic stereo-microscope fitted with an ocular scale at a magnification of 40 x. Morphometric analyses were performed on ten purposively selected samples of honeybee workers from each of the study areas. Fifteen morphological characters of honeybees presented below were measured and compared statistically according to the methods of (Sheppard and Meixner, 2003; Meixner *et al*., 2007). Morphometric measurements involved 16 characters, rely on great level of morphological discrimination and honey production (Meixner *et al*., 2007). These are: body length (Lc); thorax length (Lth); abdomen length (La); hind leg length (Lpa); tibia length (Lti); femur length (Lf); metatarsal length (Lome); metatarsal width (Lame); forewing length (Laa1); forewing width (Laa2); hindwing length (Lap1); hindwing width (Lap2); antenna length (Lant), cubital vein A (VcuA); cubital vein B (VcuB); cubital index (Icu) which was calculated as VcuA / VcuB.

Data analysis: Firstly, arithmetic mean, standard deviation, minimum (Xmin), maximum (Xmax) and amplitude were determined to reveal the morphological characteristics of the collected bees communities. Pairwise comparison of the means of each of the 16 biometric traits was also performed. In the absence of normality of the studied characters, the Kruskal-Wallis test was applied to determine the morphometric variations existing between these bee communities at the 5 % threshold and to classify the individuals into homogeneous groups for each character. In addition, the intensity of the relationship between the characters taken two by two was measured by calculating the Bravais-Pearson linear correlation coefficient. Moreover, a Principal Component Analysis (PCA) was carried out to search for a possible morphological differentiation between the bee colonies. Finally, a hierarchical ascending classification (HAC), based on Euclidian distances, was used to both classify the sites and analyse the degrees of separation between them. All analyses were performed using the XLSTAT version 2018 software.

RESULTS

Coefficient of variation of biometric parameters: The 16 biometric traits measured on all the bees collected showed generally low to moderate variations (Table 1). Only the length of the hind leg (Lpa) shows a very high homogeneity between the sampled bees, with a coefficient of variation (CV) of 1.53%. Concerning the other biometric traits, a low homogeneity is observed with a CV varying from 2.24 % to 12.92 %. None of the biometric traits analysed showed heterogeneity between the sampled bees.

Morphological variability of bees: The pairwise comparative study performed showed a very highly significant difference ($p < 0.001$) between sites for thorax length (Lth), hind leg length (Lpa), tibia length (Lti), femur length (Lf). This difference was observed for metatarsal length (Lome), forewing length (Le1), forewing width (Le2), vein length A (VcuA) and B (VcuB). This is the same for the cubital index (Icu), hindwing length (Lap1), hindwing width (Lap2) and antenna length (Lant). On the other hand, it shows a highly significant difference (p = 0.002) for the character width of the metatarsus (Lame) and a just significant difference for the character body length (Lc) (p = 0.018). For the abdomen length (La), the difference was not significant (p = 0.097).

Table 1: Statistical tests of 16 external morphological characters measured in 4 sites.

	Lélédou	**Kpata jachère**	**Kpata cacao**	**Dassioko plage**	**GM**	**CV %**	**p-value**
Lc	12.58^{b}	12.27^{ab}	12.07^{a}	12.52^{ab}	12.36 ± 0.80	6.47	0,018
Lth	3.63^{b}	3.40^{a}	3.48^{a}	3.50^{a}	3.57 ± 0.24	6.86	<0,001
La	7.12^{a}	6.80^{a}	6.96^{a}	6.72^{a}	6.90 ± 0.83	12.03	0,097
Lpa	10.94^{a}	11.18^{c}	11.16^{c}	11.05^{b}	11.08 ± 0.17	1.53	<0,001
Lti	2.95 ^{a}	3.02^{b}	3.00^{b}	2.94^{a}	2,98 ± 0.08	2.63	<0,001
Lf	2.25^{a}	2.35^{b}	2.37^{b}	2.37^{b}	2.34 ± 0.1	4.27	<0,001
Lome	2.13^{ab}	2.19^{c}	2.17^{bc}	2.13^{a}	2.16 ± 0.06	2.70	<0,001
Lame	1.14^{b}	1.12^{a}	1.14^{b}	1.14^{b}	1.14 ± 0.04	3.51	0,002
Laa1	8.86^{a}	8.98^{b}	9.00^{b}	8.81^{a}	8.91 ± 0.2	2.24	<0,001
Laa2	2.95^{a}	3.08^{c}	2.97^{c}	3.00^{b}	3.00 ± 0.07	2.30	<0,001
VcuA	0.47^{a}	0.50^{bc}	0.47^{ab}	0.50^{c}	0.48 ± 0.04	8.30	<0,001
VcuB	$0,24^{b}$	$0,24^{b}$	$0,20^{a}$	$0,26^{c}$	0.24 ± 0.03	12.50	<0,001
Icu	1.97^{ab}	2.09^{b}	2.35^{c}	1.93^{a}	2.09 ± 0.27	12.92	<0,001
Lap1	6.18^{b}	6.17^{b}	6.25^{c}	6.09^{a}	6.17 ± 0.13	2.11	<0,001
Lap2	1.73^{b}	1.77^{c}	1.71^{a}	6.09^{a}	1.75 ± 0.05	2.86	<0,001
Lant	$4,22^{ab}$	$4,26^{b}$	$4,22^{b}$	$4,14^{a}$	4,21 ± 0.13	3.09	<0,001

In each table line, values sharing a same superscript letter are not significantly different (P> 0.05).

GM: great mean, CV: coefficient of variation.

Body length (Lc); thorax length (Lth); abdomen length (La); hind leg length (Lpa); tibia length (Lti); femur length (Lf); metatarsal length (Lome); metatarsal width (Lame); forewing length (Laa1); forewing width (Laa2); hindwing length (Lap1); hindwing width (Lap2); antenna length (Lant), cubital vein A (VcuA); cubital vein B (VcuB); cubital index (Icu) which was calculated as VcuA / VcuB.

Determination of interdependent variables: The matrix of correlation (Table 2) shows a positive and significant correlation for the forewing length (Laa1) and forewing width (Laa2) (r = 0.173; p = 0.028). Highly significant positive correlations were observed between hindwing length (Lap1) and forewing length (Laa1) (r = 0.370; p <0.001), between hindwing width (Lap2) and forewing width (Laa2) (r = 0.644; p <0.001), and between body length and abdominal length (r = 0.563; p <0.001). The cubital index (Icu) is positively correlated with the length of vein A (Vcua) (r = 0.426; p <0.001), and negatively correlated with the length of vein B (Vcub) (r = -0.790; p <0.001).

Table 2: Linear correlation matrix of 16 morphometric traits external morphological characters measured in 4 sites.

Variables	LC	LTh	LA	Lpa	Lti	LF	Lome	Lame	Laa1	Laa2	Vcua	Vcub	Icu	Lap1	Lap2	Lant
LC																
LTh	0.062 (0.435)															
LA	**0.563** (0.000)	0.113 (0.153)														
Lpa	-0.118 (0.137)	-0.09 (0.243)	0.034 (0.666)													
Lti	**-0.159** (0.044)	-0.095 (0.232)	-0.008 (0.923)	**0.601** (0.000)												
LF	-0.090 (0.259)	**-0.159** (0.045)	0.009 (0.906)	**0.737** (0.000)	**0.161** (0.042)											
Lome	-0.017 (0.832)	0.025 (0.752)	0.059 (0.456)	**0.661** (0.000)	**0.414** (0.000)	**0.282** (0.000)										
Lame	-0.021 (0.794)	**0.168** (0.034)	-0.022 (0.783)	0.004 (0.957)	0.102 (0.200)	-0.023 (0.773)	0.004 (0.964)									
Laa1	0.025 (0.752)	-0.098 (0.219)	0.125 (0.116)	**0.324** (0.000)	**0.314** (0.000)	**0.158** (0.047)	**0.288** (0.000)	0.038 (0.632)								
Laa2	0.034 (0.670)	-0.030 (0.707)	-0.058 (0.466)	**0.379** (0.000)	**0.273** (0.000)	**0.197** (0.013)	**0.300** (0.000)	**-0.174** (0.028)	**0.173** (0.029)							
Vcua	0.045 (0.573)	-0.035 (0.657)	-0.044 (0.580)	0.121 (0.129)	-0.065 (0.412)	**0.203** (0.010)	0.035 (0.664)	-0.098 (0.216)	0.053 (0.510)	**0.227** (0.004)						
Vcub	0.134 (0.092)	0,119 (0,135)	-0,064 (0,423)	-0,118 (0,138)	**-0,234** (0,003)	-0,049 (0,537)	-0,080 (0,317)	-0,030 (0,710)	**-0,308** (0,000)	**0,185** (0,019)	**0,199** (0,012)					
Icu	-0,091 (0,253)	-0.132 (0.095)	0.028 (0.724)	**0.201** (0.011)	**0.164** (0.038)	**0.192** (0.015)	0.112 (0.159)	-0.028 (0.723)	**0.326** (0.000)	-0.037 (0.641)	**0.426** (0.000)	**-0.790** (0.000)				
Lap1	-0.051 (0.520)	0.062 (0.436)	**0.167** (0.034)	**0.161** (0.042)	**0.275** (0.000)	-0.029 (0.712)	**0.203** (0.010)	0.119 (0.134)	**0.370** (0.000)	0.134 (0.091)	**-0.161** (0.041)	**-0.352** (0.000)	**0.235** (0.003)			
Lap2	0.141 (0.075)	0.083 (0.296)	-0.113 (0.154)	**0.155** (0.050)	-0.012 (0.882)	**0.174** (0.028)	0.079 (0.321)	-0.060 (0.452)	-0.016 (0.845)	**0.644** (0.000)	**0.343** (0.000)	**0.553** (0.000)	**-0.299** (0.000)	-0.086 (0.277)		
Lant	-0.024 (0.759)	0.026 (0.742)	0.061 (0.447)	**0.256** (0.001)	0.147 (0.064)	0.078 (0.324)	**0.331** (0.000)	0.023 (0.776)	**0.218** (0.006)	**0.204** (0.010)	-0.010 (0.902)	-0.123 (0.121)	0.104 (0.191)	**0.197** (0.013)	-0,016 (0,844)	

Values in bold correspond to Pearson correlation coefficients significantly different from zero. Values in brackets correspond to the probability of significance (risk α=5). Body length (Lc); thorax length (Lth); abdomen length (La); hind leg length (Lpa); tibia length (Lti); femur length (Lf); metatarsal length (Lome); metatarsal width (Lame); forewing length (Laa1); forewing width (Laa2); hindwing length (Lap1); hindwing width (Lap2); antenna length (Lant), cubital vein A (VcuA); cubital vein B (VcuB); cubital index (Icu) which was calculated as VcuA / VcuB.

Structuring the morphological diversity of bees

Principal Components Analysis: PCA analyses revealed four clusters of bees according to the factorial plan formed by axis 1 (48.95 %) and axis 2 (38.14 %) both accounting for 87.09 % of the total variability (Figure 2). Axis 1 (F1) contrasts two types of individuals: on the left, individuals in group I, consisting of Dassioko plage and group IV, consisting of Lélédou, characterized by a large size and a long B vein, with individuals in group II, consisting of Kpata cacao and group III, consisting of Kpata Jachère, located on the right side of the axis, which have large anterior and posterior wings, long tibia, a long metatarsus and a high cubital index. Axis 2 (F2) opposes groups I and II represented respectively by the sites of Dassioko plage and Kpata jachère which are characterized by a large anterior and posterior wing and a long A vein to those of groups III and IV constituted successively by the sites of Kpata cacao and Lélédou characterized by a thorax and a rather long abdomen with a large metatarsus.

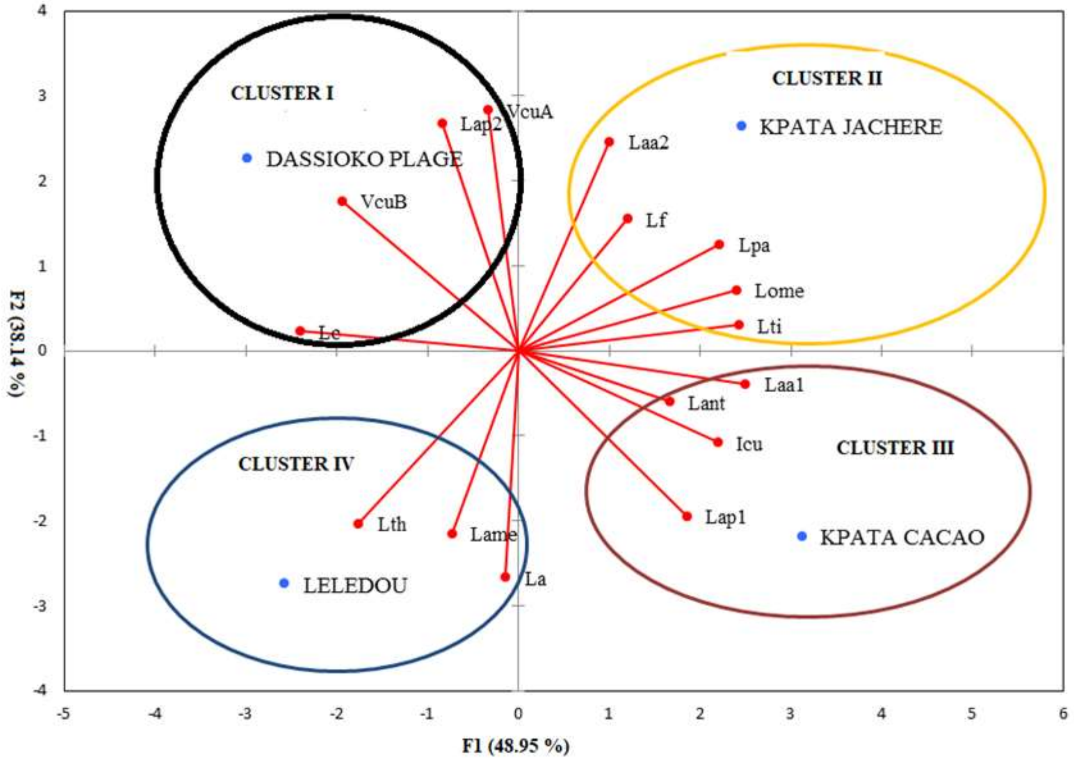

Figure 2: Biplot representation of first two axis from the principal component analysis.
Body length (Lc); thorax length (Lth); abdomen length (La); hind leg length (Lpa); tibia length (Lti); femur length (Lf); metatarsal length (Lome); metatarsal width (Lame); forewing length (Laa1); forewing width (Laa2); hindwing length (Lap1); hindwing width (Lap2); antenna length (Lant), cubital vein A (VcuA); cubital vein B (VcuB); cubital index (Icu) .

Hierarchical classification: Concerning the hierarchical ascending classification, the dendrogram shows three distinct clusters at truncation point 12 (Figure 3). Cluster 1, represented by the site of Lélédou, cluster 2 constituted by the site of Kpata Jachère and Kpata Cacao, cluster 3 is represented by the site Dassioko Plage. Moreover, for a truncation point lower than 12, the dendrogram shows four clusters which correspond to None of the biometric traits analyzed 4 samples sites.

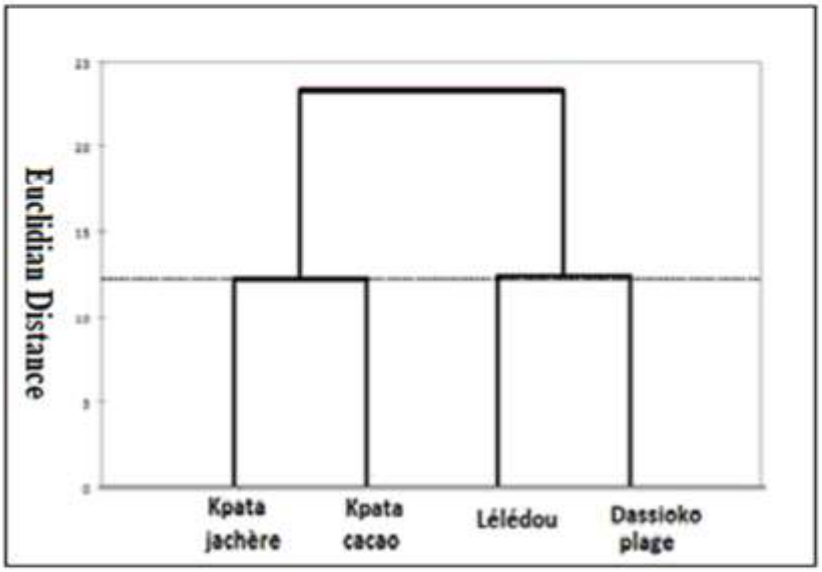

Figure 3: Dendrogram derived from hierarchical cluster analysis using Euclidian distance.

DISCUSSION

A great morphological variability was observed between colonies. These results corroborate those of (Brou *et al.*, 2019) and (Kouonon *et al.*, 2020) who showed a high morphological variability within the subspecies *Apis mellifera adansonii* respectively in central and southwestern Côte d'Ivoire. Other studies, especially these of (Ruttner *et al.*, 1978) have highlighted morphological variability within *Apis mellifera adansonii*. These morphological variations could be attributed to several reasons. Firstly, the great capacity of dispersion of *Apis mellifera*, and its great level of panmixia allow among other things, the queen, during the nuptial flight, to be inseminated by several males not belonging necessarily to the same colony (Chevalet and Cornuet, 1982). This great heterogeneity observed between colonies may therefore be the result of the mixing of genes of various genotypes (Eckholm *et al.*, 2015). It seems to be no decline in diversity of these wild bees. We believe also that this result is a consequence of maintaining bees' food sources and nest sites as well as the sites rich in floral resources and habitat features. Apart from these anthropogenic factors, other factors adversely affecting diversity of bees such as agricultural inputs with herbicides and pesticides uses seem to be reduced in sampling area. At fact, sustainable cropping system geared towards agroecology demonstrably contribute to maintaining bee populations (Pfiffner and Müller, 2016). From all these, it seems to be a straightforward relation between diversity of bees and sustainable agriculture. It is thus reasonable to consider that cropping system applied in Gbôklé area is yet sustainable. In other hand, this result could suggest the presence of different bee races in this region or the adaptation of these bees to different ecotypes. Indeed, the cubital index, which is one of the most accurate indicators of differentiation of bees into ecotypes or races (Fresnaye, 1965; Ruttner, 1988), revealed three differentiated groups in the present study. In West Africa. Amakpe (2010) and Mendes *et al*. (2007) who worked successively on honeybees in the South and North-East of Benin, showed a differentiation of the populations of the studied areas based on the cubital index. These authors revealed the existence of two subspecies of *A. mellifera* in particular, *A. m. adansonii*, *A. m. jemenitica*, and a third subspecies with an intermediate character (hybrid). These results seem to corroborate ours. However, the results of the principal component analysis and those of the hierarchical ascending classification, showed 4 clusters honeybee individuals relate to the geographical location of the colonies, which are Lélédou, Dassioko Plage, Kpata Cacao and Kpata Jachère. Moreover, since beekeeping activity is still absent in this area, it is certain that this zone is out of from certain beekeeping practices, such as the importation of queens and the transhumance of colonies, seen to be efficient and likely to belong to other subspecies. In the same way, recent phylogeny studies on the mitochondrial DNA of honeybees from 33 colonies in the 4 agro-ecological zones of Côte d'Ivoire, have shown the existence of a single mitochondrial haplotype (Coulibaly *et al*., 2019). In addition, the high homogeneity of the coefficient of variation of various morphological traits notices surely the idea of a single and same group of honeybees in this locality. It is therefore possible that the distribution pattern of honeybees in this study area is more a differentiation into ecotypes than into different subspecies. In general way, biotic factors such as competition for food and parasitism effects, especially ectoparasites, affect the phenotype of bees (Radlof *et al*., 2003). In addition, the climatic conditions of a given area and the type of plants visited by the bees, together with the morphological characteristics of the foraged flowers, strongly affect their shape and size (Eckholm *et al*., 2015; Szabo and Lefkovich, 1988). At climate, level, this seems to be true considering the size of bees in the present study and in the study of Paraïso *et al*. (2019) in Northern Benin. In the present study, the bees appear significantly larger (12.36 mm average size) compared to those in northern Benin (11.28 mm average size). A temperature difference of 4 to 9°C between the Gboklé region (variable temperature of 21°C to 35°C) and the North-East of Benin (temperature between 30°C and 39°C) could explain the difference in size observed between these two studies, as postulated by Bergman who stated that individuals from colder climates tend to be larger than those of the same species from warmer climates (Ruttner, 1988). In the present study, the variation between colonies cannot be attributed to eco-climatic factors, since the bees live in the same biotope. In total, specific characteristics of the bee nesting sites, including the action of possible biotic factors, brood size, and vegetation type could have played a key role in the different patterns observed. In the whole, the difference between biometric parameters generates the morphological structuring observed with PCA and hierarchical clustering. This structuring is linked to the sampling site. It shows that bees seem to be better adapted to their respective zones. Each sampling area thus has what it takes to ensure the bees' biological cycle.

CONCLUSION AND APPLICATION OF RESULTS

The Gbokle region can be considered a favourable area for beekeeping. There is a good reservoir of variability in wild bee genetic resources, thanks to agricultural practices that are still sustainable and the existence of acceptable floral diversity.

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