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Morphometric characteristics of honeybees in the cechi nature reserve in the department of agboville (south-east, Ivory Coast)

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Abstract

The study was carried out within the framework of the evaluation of the melliferous potentialities of the natural reserve of Cechi in the Department of Agboville (South-East, Ivory Coast). For this purpose, thirty worker bees were taken from each of the hives installed for the occasion, according to their coloration and shape, and then preserved in 70% ethanol. The bees are dissected and described according to the classical morphometry. Nineteen characters were selected for the study according to their apicultural importance and zoological significance. The size of the different characters is determined on graph paper with a magnifying glass. For tiny sizes the organ is photographed and projected on a computer for precise measurements. The bees have an average length of 13.23±1.11 mm, a negative discoidal transgression and an average cubital index of 1.70±0.15. These values are consistent with individuals of *Apis mellifera mellifera*. Substituting for the length of the hind legs, proboscis and wings, the honeybees studied are good foragers and honey producers.

Keywords: honeybees, morphometry, nature reserve, Ivory coast

Introduction

According to Toullec (2008) [25], the plant kingdom is made up of more than 70% of cultivated and non-cultivated flowering plants. More than 80% of them depend on the foraging activity of bees for sexual reproduction, of which the major part (76.6%) is attributed to the honey bee. Honey bees are winged insects grouped in numerous species and are diversely distributed in the world (Makhtar, 2002) [14]. In Asia one meets *Apis cerana*, *Apis corsata*, *Apis florea*. In Europe and a little everywhere in the world one meets *Apis mellifera* with more than twenty listed races and subspecies. It is the only species of honeybee present and exploited worldwide (Mackowiak, 2009) [13].

These races are adapted to specific geographical areas with variations according to the environmental requirements of the environment where they live. They are an important melliferous potential of ecological zones. Also, through their role as insect pollinators of flowering plants, these bees contribute to the conservation of plant diversity, food production and the economy (Mackowiak, 2009; Yalamoussa *et al*, 2019) [13, 27]. Indeed, honey bees are essential for human survival.

In Ivory Coast, studies on honey bees, their identification and beekeeping capacity by morphometry are those generated by Brou *et al.* (2019) [4] on colonies raised in modern hives in the Central localities and by Kouonon *et al.* (2020) [12] on colonies collected in the wild in the South-West localities; by Yalamoussa *et al.* (2019) [27], on the bee fauna in market gardening in Korhogo. On the other hand, the melliferous potential of Ivorian forest areas has been little studied. This is the work of Kouame *et al* (2020) [11], on the melliferous plants of the Yapi Daniel Nature Reserve in the Department of Agboville. The present study is a contribution to the knowledge of honeybees raised in Ivory Coast. It concerns the melliferous potentialities of the South of the country by the morphometry of the melliferous bees. The objective is to list, identify from the morphological characters the different species present and eventually consider the preservation of these species.

Material and Methods

Study Environment

The honeybees were attracted in the hives installed in the natural reserve of Cechi (Fig 1). The reserve has geographic coordinates of 6° 11' 44 02" - 6° 11' 45 3" North latitude and 4° 17' 22 45" - 4° 17' 25 34" West longitude (Kouame *et al.*, 2020) [11]. It is located in the subprefecture of Cechi in the Department of Agboville in the South-East of Ivory Coast (Fig 2). The nature reserve is limited in its northern part by the Assôko river and its tributaries, and the Seguie classified forest, which represent its natural boundaries. Its other boundaries are plantations of industrial crops (*Theobroma cacao*, *Hevea brasiliensis*); fallows and baffles developed for food crops (*Oryza saliva*).

Fig 1: Views of the collection apiary in the nature reserve

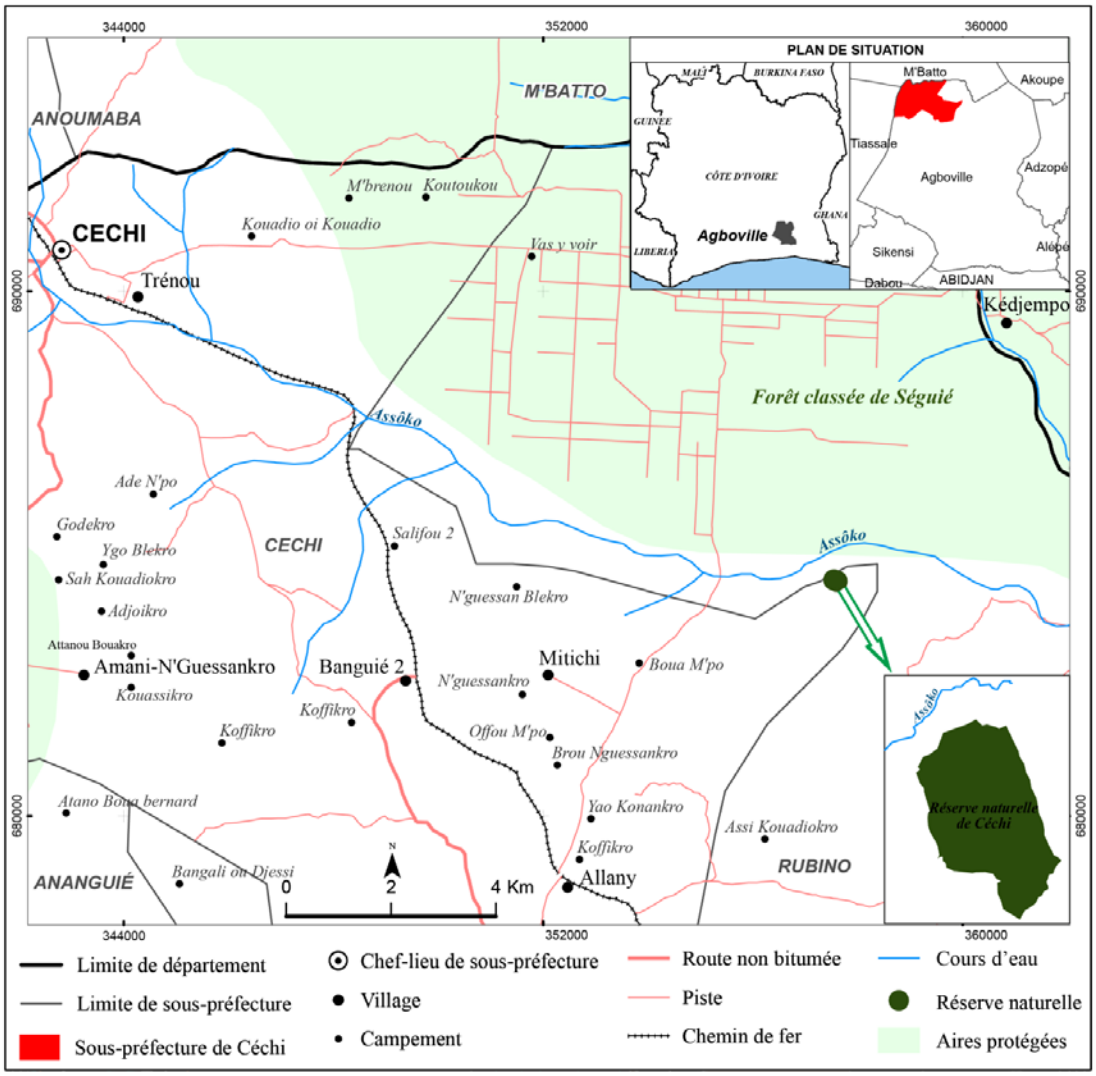


Fig 2: Geographic location of the Cechi Nature Reserve (Kouame *et al*, 2020).

Sampling

Bees were collected from the frames of the hives according to coloration and shape. Thirty (30) bees in total were collected per hive and introduced directly into jars containing 70% ethanol for preservation (Meixner *et a.*, 2011; Paraïso *et al.*, 2011) [16, 17]. According to Radloff and Hepburn (2000) [18]; Toullec (2008) [25] a total of 20 bees per hive is sufficient for the morphometric study. Measurements were performed on workers because haploid males are not representative in a bee population (Toullec, 2008; Paraïso *et al.* 2011; Brou *et al*, 2019) [5, 17, 4] in addition, the bees were provided with stingers, which is a defense organ present in females (workers).

Biometric characteristics measured

In this study 19 parameters were studied according to their

zoological significance and their apicultural importance. In addition, there are about fifty morphological characters used to study the biometry of the bee (Fresnaye, 1981) [8]. The biometric characters: the size of the bee; the length of the proboscis (Fig 3 A); the length of the antennae; the length of the hind leg; the length of the femur; the length of the tibia; the length and width of the metatarsus (Fig 3 B); the length and width of the right fore and hind wings (Fig 3 C); the coloration and width of the black and yellow bands on the 2nd abdominal tergite (Fig 3 F) concerned only yellow-black bees because black bees have an all-black abdomen; the width of the hairy area on the 5th abdominal tergite; the ulnar index (structure of the worker's forewing) were studied. The wings are divided into cells by wing veins, 3 cubital cells noted I, II and III (Toullec, 2008) [25]. Two rib segments A and B are measured on the third cubital cell (Fig 3 E). The cubital index is the ratio A/B between the lengths of the two rib portions A and B. The discoidal transgression is the measure of the discoidal angle obtained by noting the position of the discoidal point relative to the perpendicular to the major axis of the radial cell, passing through the upper corner of the third ulnar cell (Fig 3 D). When the discoidal point is toward the wing tip, the transgression is positive (+). When it is toward the point of wing attachment on the thorax, it is negative (-) and when the line passes exactly over the discoidal point, the transgression is zero (Fig 3 D) (Toullec, 2008; Paraïso *et al*, 2011; Brou *et al*, 2019) [25, 17, 4].

Method of measurement

After dissecting the bees, the organs to be studied were placed on graph paper for measurements. The parameters difficult to appreciate with the naked eye, because of their size on the forewing of the bee and other organs (segments A and B; discoidal transgression, width of the yellow and black bands), were appreciated with the help of a magnifying glass on millimeter paper, photographed and then processed on the computer for a better appreciation of the measurements.

Statistical Analyses

The means of the collected data of the honey bee types (black bees; yellow-black bee) were subjected to statistical tests with XLSAT 2014.5.03 software, to see if there is a statistical difference between the bees. The non-parametric Mann-Whitney test was performed for each parameter to test for a difference between bees. The choice of test was critical for Shapiro-Wilk normality ($p<0.05$). The means of the zoological parameters (discoidal transgression; cubital index) were significantly different between bee types ($p<0.05$). Thus, the bees are statistically different.

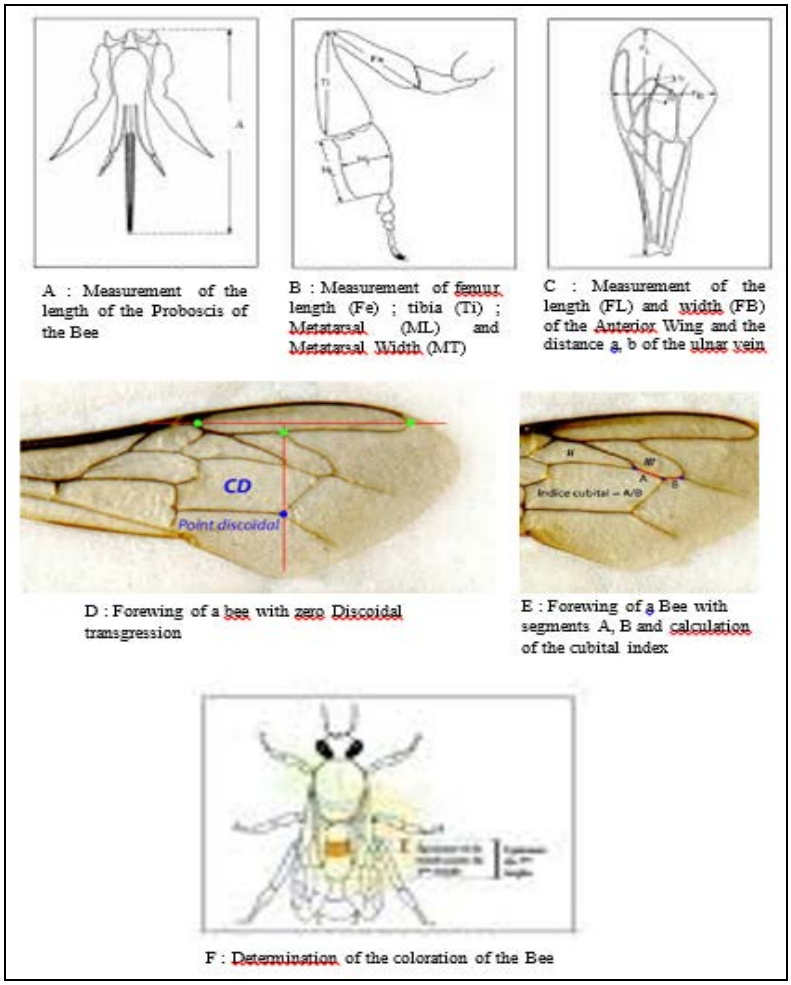


Fig 3: Taking measurements for morphometric study of bees (Raina and Kimbu, 2005; Toullec, 2008; Paraïso *et al.*, 2011; Brou *et al.*, 2019)

Results

Types of honey bees

According to the coloration of the honey bees in the study site, there are two types of honey bees: single-colored black honey bees and bicolored yellow-black honey bees. Table 1 and Fig 4 on the following page summarize the studied

morphological parameters of the bees and their measurements.

Length of honey bees

The length of the honey bees at the site varied from 10 mm to 14 mm with an average of 13.23±1.11 mm. Black bees are the longest with an average size of 13.33±0.99 mm. The yellow-black bees are smaller than the previous ones, with an average size of 13.13±1.24 mm. Statistically these bees are not different in size (p>0.05).

Length of hind legs

The length of the hind legs of the bees ranged from 9 mm to 10 mm with an average of 9.64±0.49 mm. The average length of the hind legs of black bees is 9.76±0.99 mm. The average length of the hind legs of yellow-black bees is 9.50±1.24 mm. There is no significant difference between the hind legs of the bees (p>0.05).

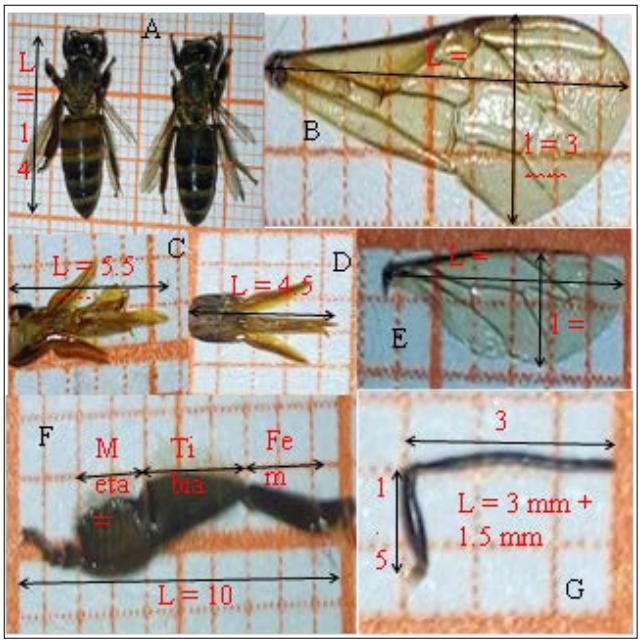
Femur, tibia, metatarsal length and widths.

Femur length, tibia length, and metatarsal length and width of honey bees were not statistically different (p>0.05). Femur length ranged from 2 mm to 3 mm, with an average of 2.5±0.13 mm. Black bees have femurs of 2.5±0.00 mm; yellow-black bees have femurs of 2.5±0.19 mm. Tibia length ranges from 2 mm to 3 mm, with an average of 2.96±0.18 mm. Black bees have tibiae of 3±0.00 mm; yellow-black bees have tibiae of 2.93±0.26 mm. Metatarsal length varies from 2 mm to 2.3 mm with an average of 2.02±0.06 mm. Black bees have a metatarsus length of 2.01±0.03 mm; yellow-black bees have a metatarsus length of 2.03±0.08 mm. The width of the metatarsus varies from 0.7 mm to 1.2 mm with an average of 1.17±0.09 mm. Black bees have a metatarsus width of 1.19±0.03 mm; yellow-black bees have a metatarsus width of 1.16±0.13 mm.

Table 1: Morphometric parameters of honey bees at the site and their measurements

Morphometric parameters	Black bee	Yellow-black bee	Average
Bee length (mm)	13.33a±0.99	13.13a±1.24	13.23±1.11
Femur length (mm)	2.5a±0.00	2.5a±0.19	2.5±0.13
Length tibia (mm)	3a±0.00	2.93a±0.26	2.96±0.18
Metatarsal length (mm)	2.01a±0.03	2.03a±0.08	2.02±0.06
Leg length (mm)	9.77a±0.37	9.50a±0.6	9.64±0.49
Metatarsal width (mm)	1.19a±0,03	1.16a±0.13	1.17±0.09
Proboscis length (mm)	5.33b±0.45	5.03c±0.3	5.18±0.4
Forewing length (mm)	8.97a±0.13	8.97a±0.15	8.97±0.14
Front wing width (mm)	3a±0.00	2.98a±0.05	2.99±0.04
Hindwing length (mm)	6.27a±0.08	6.23a±0.12	6.25± 0.10
Hindwing width (mm)	1.8a±0.04	1.79a±0.06	1.79±0.05
Black band width (mm)		0.4±0.00	
Yellow band width (mm)		1.6±0.00	
Width of hairy band (mm)	1.6a±0.00	1.6a±0.00	1.6±0.00
Antenna length (mm)	4.5a±0.00	4.5a±0.00	4.5±0.00
Discoidal transgression (°C)	-1.13b±0.71	-1.62c±0.02	-1.37±0.55
Index A (mm)	0.54b±0,02	0.49c±0.05	0.51±0.045
Index B (mm)	0.3a±0.00	0.3a±0.06	0.3±0.004
Cubital index	1.80b±0.07	1.61c±0.16	1.70±0.15

Parameters with same letter values (a) on the same line are not significantly different (p>0.05). Those with different letter values (b, c) on the same line are significantly different (p<0.05).



A: Honey bees; B: Forewing; C & D: Proboscis; E: Hind wing; F: Hind leg; G: Antennae

Fig 4: Honey bees and morphometric parts studied

Length and width of fore and hind wings

The length of the bees' forewings and hindwings ranges from 8.5 mm to 9.2 mm for the forewing with an average of 8.97±0.14 mm and 6 mm to 6.3 mm for the hindwing with an average of 6.25±0.10 mm, respectively. Wing widths range from 2.8 mm to 3 mm for the forewing with a mean of 2.99±0.04 mm and 1.70 mm to 1.90 mm for the hindwing with a mean of 1.79±0.05 mm. Black bees have forewings 8.97±0.13 mm long and 3±0.00 mm wide; yellow-black bees have forewings 8.97±0.15 mm long and 2.98±0.05 mm wide. Black bees have hind wings 6.27±0.08 mm long and 1.8±0.04 mm wide; yellow-black bees have hind wings 6.23±0.12 mm long and 1.79±0.06 mm wide. Statistically there is no significant difference between bees by wing length and width ($p>0.05$).

Length of proboscis and antennae

The proboscis length of the bees ranged from 4.5 mm to 6 mm with an average of 5.18±0.4 mm. Black bees have proboscis lengths of 5.33±0.45 mm. Yellow-black bees have proboscis lengths of 5.03±0.3 mm. Statistically by proboscis length the two types of honey bees are different ($p<0.05$). These bees have 4.5 mm long antennae.

Width of coloration bands and hairy areas of honey bees.

The width of the coloration bands on the 2 nd abdominal tergite concerned only yellow and black bees. The black bees have an all black abdomen. The yellow-black bees have yellow bands of 1.6 mm and black bands of 0.4 mm width on their second abdominal tergite. The hairy area of the 5th abdominal tergite of the bees is 1.6 mm wide.

Discoidal transgression

The discoidal transgression of honey bees varies from -1.51°C to 0°C with an average of -1.37±0.55°C. Only a few black bee individuals exhibited zero discoidal transgression (0°C). Black bees had an average discoidal transgression of -1.13±0.7°C and yellow-black bees had an average discoidal transgression of -1.62±0.02°C. Statistically the bees are different in discoidal transgression ($p<0.05$).

A-index, B-index and cubital index.

Index A and index B and cubital index are statistically different among honey bee types ($p<0.05$). The A-index varied from 0.34 mm to 0.60 mm with an average of 0.51±0.045 mm. The B index varies from 0.3 mm to 0.32 mm with a mean of 0.3±0.004 mm. The ulnar index varied from 1.13 to 2 with an average of 1.70±0.15. Black bees with average A and B indexes of 0.54±0.02 mm and 0.3±0.00 mm have an average cubital index of 1.80±0.07. Yellow-black bees with A and B indexes of 0.49±0.05 mm and 0.3±0.06 mm have a mean cubital index of 1.61±0.16.

Apicultural significance of morphometric parameters

The development of certain organs in honey bees can be a good criterion for the selection of bees for beekeeping. The length of the hind legs, the length and width of the metatarsus, are important for pollen collection and transport. Honey bees with these developed organs can collect pollen easily and in quantity to the hive. The length of the proboscis is important in nectar collection, at the corollas of flowers and at the base of some leaves. Honey bees with a developed proboscis are good nectar collectors and are able to visit several plants. The length and width of the wings make it easier for the bees to move from the food source to the hive and back. Honey bees with long, wide wings are likely to travel long distances in search of nutrients and also make rapid movements from the hive to the food source when the latter is not far away. The width of the hairy area increases the pollinating ability of the bees, by involuntary transport of pollen from visited flowers through the hairs. Bees with wider hairy bands have a lot of hairs and would be good pollinators.

Discussion

This study on the morphological description of honeybees in the study area is one of the first to assess the melliferous potential of honeybees in the south of Ivory Coast, to identify them and to conserve them. Indeed, according to Toullec (2008) [25] the identification is the first step for the safeguard, because colonies of bees belonging to the same species but occupying different habitats develop characteristics specific to the environment, called geographical subspecies or varieties or races. In addition, the development of certain organs in bees is beneficial to beekeeping and pollination (Paraiso *et al*, 2011; Brou *et al*, 2019; Kouonon *et al*, 2020) [4, 12].

Two types of honey bees were inventoried by the study according to their coloration: namely, unicolored bees with all-black abdomen and bicolored bees with yellow and black colors on the second abdominal tergite. These bees were in perfect cohabitation within the same hive. This same finding was revealed by Brou *et al.* (2019) [4], in hives in the localities of Central Ivory Coast (N'Guessankro, Soungassou, Kouassikouassikro, Lengbe-kouassikro, Yobouekro and Yamoussoukro: Institut National Polytechnique Felix Houphouët-Boigny). According to Hounkpè *et al.* (2007), it is not uncommon to observe subspecies of honey bees in the same hive.

The average length of the bees varies from 13.13±1.24 mm for the yellow-black bee to 13.33±0.99 mm for the black bee. These averages are higher than those of honey bees in north eastern Benin:10.80 mm - 11.97 mm (Paraïso *et al.*, 2011) [17]; those of bees collected from natural hives in south western Ivory Coast: 10.11mm - 12.10 mm (Kouonon *et al.*, 2020) [12] and those of *Apis mellifera* races in Kenya: 6.17 mm - 7.92 mm (Raina and Kimbu, 2005) [19]. On the other hand, the cumulative average length of both types of bees (13.23±1.11 mm) is greater than that of honey bees from the Central Ivory Coast localities of 13.10 mm (Brou *et al*, 2019) [4]. This difference between the size of the bees and those in the literature, would be related to the environment of ecological zones and the availability of nutrients. According to the latter authors, bees are larger in the South where the climate is more humid and become smaller in the North where the climate is drier. For Kouonon *et al.* (2020) [12], the variations between the sizes of the bees is related to the quantity of food available for the bees. Indeed, bees would be larger in nutrient-rich areas and smaller in less nutrient-rich areas.

Regarding the average length of the hind legs of the bees taking into account only the average length of the femurs, tibias and metatarsals, the studied bees have an average leg length of 7.48±0.27 mm. This value is close to that observed by Ruttner (1988) [20], i.e. 7.49 mm in *Apis mellifera*

adansonii bees in West Africa. It is higher than that observed in the Center of Ivory Coast: 7.38 mm according to Brou *et al.* (2019) [4]. According to Mattu and Verma (1984) [15]; Kouonon *et al.* (2020) [12], bees with long and robust hind legs are better pollen collectors. In addition, honey production is positively correlated with hind leg length of honey bees, particularly the length of the tibia that carries the pollen basket and would define its size (Szabo and Lefkovich, 1988; Brou *et al*, 2019) [23, 4]. The honey bees studied would therefore be good pollen collectors and honey producers.

The average length (8.97±0.14 mm) of the forewings of the studied bees is similar to that of the Central Ivory Coast honey bees (8.97 mm) according to Brou *et al.* (2019) [4]. This value is higher than that of *Apis mellifera* races in Kenya, which varies between 4.57 mm and 5.11 mm according to Raina and Kimbu (2005) [19]; to that of *Apis mellifera sahariensis* (8.96 mm), *Apis mellifera lamarckii* (8.23 mm), *Apis mellifera jemenitica* (8.25 mm), *Apis mellifera litorea* (8.30 mm) and *Lybia* (8.93 mm) in Lybia according to Shaibi *et al.* (2008) [21]; to that of *Apis mellifera anatoliaca* (AR-B: 8.90 mm) in Turkey (Guler, 2010) [9]; to that of the new subspecies of *Apis mellifera* in Ethiopia which ranges from 8.19 mm to 8.78 mm according to Meixner *et al.* (2011) [16]; to that of *Apis mellifera* from the mountain forests of Crimea which ranges from 8.00 mm to 8.10 mm (Bykova *et al*, 2016) [5]; to that of *Apis mellifera* colonies in Nigeria which ranges from 7.98 mm to 8.51 mm (Dukku, 2016) [7] and to that of *Apis mellifera jemenitica* (7.94 mm) in Saudi Arabia according to AL-Kahtani and Taha (2021) [2]. It is however, less than that of *Apis mellifera caucasica* races (CRA-P: 9.15 mm; CRA-C: 9.35 mm) in Turkey according to Guler (2010) [9] and that of *Apis mellifera carnica* (9.14 mm) in Saudi Arabia (AL-Kahtani and Taha, 2021) [2]. The wing length of honey bees is thought to be dependent on the ecological zones where the bees evolve or specific to each subspecies of honey bee.

Furthermore, the average length of the forewings, hindwings and antennae of the studied bees are longer than those of honey bees collected in natural hives in the southwestern part of Ivory Coast (antenna: 3.56 mm; hindwings: 5.36 mm; forewings: 7.67 mm) according to Kouonon *et al.* (2020) [12]. As well as those of honey bees in northeastern Benin (antenna: 3.99 mm - 4.11 mm; hindwing: 5.55 mm - 5.77 mm; forewing: 8.52 mm - 8.70 mm) according to Paraïso *et al.* (2011) [17]. These results assume that the studied bees would be good honey producers and better adapted to beekeeping. Because according to Brou *et al.* (2019) [4], although the amount of honey per hive is mainly attributed to the bee colony and the environment, wing size is an indicator of the honey-producing capacity of a colony. Also, the size of the antennae is important in nutrient foraging and for locating bees. According to Kouonon *et al.* (2020) [12], bees with long antennae travel long distances in search of nutrients and are more easily located.

The average length of the proboscis or tongue or lumen or proboscis (5.18±0.4 mm) of the honey bees studied, is greater than that of honey bees in Central Ivory Coast of 4.88 mm (Brou *et al.*, 2019) [4] and that of the localities in the southwest of Ivory Coast (4.19 mm) (Kouonon *et al.*, 2020) [12], as well as that observed in the northeast of Benin, which varied between 2.67 mm and 3.07 mm (Paraïso *et al.*, 2011) [17] and that of the south of Benin, which varied between 4.34 mm and 5.13 mm (Amakpe, 2010) [3]. This result would confirm the melliferous capacity of bees in the study area. Indeed, a developed proboscis allows the bees to reach more easily and quickly the bottom of the corolla where the nectar glands and stamens containing pollen are housed (Brou *et al.*, 2019) [4]. However, the average proboscis length observed is less than that of *Apis mellifera iberica* (6.31 mm) in southwestern France according to Strange *et al.* (2007) [22]; of *Apis mellifera mellifera* of the European ecotypes: of Provenance: 6.45 mm; of Landes: 6.22 mm; of Essonne: 6.19 mm; of Cevennes: 6.27 mm and of Brittany: 6.29 mm according to Toullec (2008) [25]; of the subspecies of *Apis mellifera* of Crimea which varies from 5.8 mm to 6.85 mm according to Bykova *et al.* (2016) [5]; of the subspecies *Apis mellifera unicolor* (5.67 mm) endemic to Madagascar, Mascarene Islands and Comoros according to Tsiory (2018) [26]; of *Apis mellifera jemenitica* (6.20 mm) and *Apis mellifera carnica* (5.28 mm) in Saudi Arabia (AL-Kahtani and Taha, 2021) [2]. Variation among honey bee proboscis would depend on the depth of the flowers foraged or otherwise be specific to each honey bee ecotype or subspecies. The discoidal transgression and the cubital index are excellent parameters to identify honeybee colonies (Toullec, 2008) [25]. Indeed, according to this author typical black bee colonies have a low cubital index (<2) and a negative discoidal transgression. Mestizo colonies have a mostly high (>2) or sometimes low (<2) cubital index and a very often positive or zero, sometimes negative discoidal transgression. And typical colonies of the Carina variety have a high cubital index (>2) and a positive or zero discoidal transgression. The honey bees studied, have a negative discoidal transgression with an average of -1.37±0.55°C and an average cubital index of 1.70±0.15. Since the average cubital index of *Apis mellifera mellifera* colonies would be low or amount to 1.75; the honey bees studied would therefore be subspecies of *Apis mellifera mellifera* or an ecotype of this subspecies. Black bees with an average cubital index of 1.80±0.07 less than 2 would be a black bee colony of the species *Apis mellifera mellifera*; this value is similar to that of *Apis mellifera mellifera* (1.80) in France according to Strange *et al.* (2007) [22]. The yellow-black bees with a low cubital index of 1.61±0.16 could be a mixed colony of the species *Apis mellifera mellifera*; moreover, this value is closer to that of *Apis mellifera iberica* (1.65) listed in France according to Strange *et al.* (2007) [22]. This interbreeding would be justified by the insemination of the queen, by several males which would come from close colonies during the nuptial flight (Chevalet and Cornuet, 1982; Tessema and Zeleke, 2017; Brou *et al.*, 2019) [4] observable during the periods of swarming**.**

Conclusion

The study on the morphology of honey bees in the study area, identified two types of honey bees. The black honeybees with a mean discoidal transgression of -1.13±0.7°C and a mean cubital index of 1.80±0.07; would be subspecies of the black honeybee *Apis mellifera mellifera*. Yellow-black bees with a mean discoidal transgression of -1.62±0.02°C and a mean cubital index of 1.61±0.16; are thought to be half-breeds of subspecies of *Apis mellifera mellifera*. These honey bees are good pollinators, good honey producers and have an important orientation capacity. They have an average proboscis length of 5.18±0.4 mm; forewings of 8.97±0.14 mm; hindwings of 6.25±0.10 mm; hind legs of 9.63±0.51 mm and antennae of

4.5±0.00 mm.

This study on the morphological characters of the honey bees of the site, is a preliminary study for the identification of the honey bees of this part of the country and their apicultural capacity. It could be added to the genetic (DNA), ecological and behavioral studies, in order to determine in an exhaustive and thorough way, the different species or subspecies of honeybees of the area and other parts of Ivory Coast.

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