

Original Research Paper

Biometrics reveals distinct population of *Meliponula ferruginea* (Lepeletier, 1841) and *Meliponula bocandei* (Spinola, 1853) (Hymenoptera, Apidae, Meliponini) in Cameroon-Central Africa

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Abstract

The Western Highlands and the bimodal rainfall forest zone of Cameroon are rich in flora. This plant diversity, combined with favorable agro-ecological conditions, makes these areas an ideal environment for the establishment and development of stingless bees, particularly those of the *Meliponula* genus. As well as playing an essential role in pollination, these bees are renowned for their ability to produce honey, which is highly prized for its therapeutic properties. With the rise of meliponiculture in these regions, these insects are the focus of increasing attention, but they remain largely unstudied despite their economic, ecological and therapeutic importance. Therefore, in order to fill this knowledge gap, a biometric study was carried out on 485 worker bees divided into two species, *Meliponula ferruginea* (395 individuals) and *Meliponula bocandei* (90 individuals), collected in 7 divisions of the western highlands and the bimodal rainfall forest zone. A total of 16 biometric measurements were then taken using a stereomicroscope and the conventional biometry method. The data obtained were subjected to a one-way analysis of variance using XLSTAT 2014 and SPSS 2010 statistical software. With $P < 0.05$. The various results obtained show that for *Meliponula ferruginea*, all the measurements were influenced by locality. In contrast, for *Meliponula bocandei*, only body length was influenced by locality. Multivariate analyses such as AFD, ACP and CAH were used to identify 3 morphotypes within each species. The results obtained from this biometric study are therefore needed to assess the degree of homogeneity within the population of *Meliponula bocandei* and *Meliponula ferruginea* in these regions, which will be used during the domestication process of these bees, particularly when selecting colonies for propagation.

Keywords: Biometry, stingless bees, *Meliponula bocandei*, *Meliponula ferruginea* Cameroon

Introduction

Like other corbiculate bees, stingless bees are highly eusocial insects, representing approximately 70% of all bee species worldwide (Hrncir *et al.*, 2016). They play an essential role in food security and biodiversity maintenance through the process of pollination (FAO 2013; FAO 2020). These bees are primarily distributed in the Neotropics (77%), the Indo-Malay/Australian region (16%), and the African tropics (7%). The exact number of species is unknown, increases with scientific advancements, and varies among authors.

Approximately 550 species grouped into 56 genera have been identified worldwide. Among them, about 36 species grouped into 10 genera are endemic to Africa, including *Apotrigona* (Moure, 1950),

Axestotrigona (Moure, 1961), *Cleptotrigona* (Moure, 1961), *Dactylurina* (Cockerell, 1934), *Hypotrigona* (Cockerell, 1934), *Liotrigona* (Moure, 1961), *Meliplebeia* (Moure, 1961), *Meliponula* (Cockerell, 1934), *Plebeiella* (Moure, 1961), and *Plebeina* (Moure, 1961) (Eardley, 2004; Cortopassi et al., 2006; Michener, 2007; Pauly and Vereecken, 2013; Grüter, 2020).

Moreover, the genera *Meliponula*, *Axestotrigona*, and *Apotrigona* are often grouped by some authors (Njoya, 2009). This study focused on *Meliponula bocandei* and *Meliponula ferruginea*, characterized by their large size (5–7 mm), the coloration of their abdomen ranging from brown with dark bands to completely black for *M. ferruginea*, and orange body coloration for *M. bocandei*. These bees also exhibit high honey production, with yields of up to 5 kg for *M. bocandei* and 2 kg for *M. ferruginea*. This honey is sold at prices ranging from 14 to 28 euros per kilogram (Kajobe, 2006; Macharia, 2008; Njoya, 2009).

Their ecological success is attributed to the division of labor, which ensures the efficient organization of their society (Wilson, 1990). A nest is made up of 3 castes, namely the queen, the males and the workers, each of which has clearly defined functions within the nest (Bassindale and Harrison, 1955; Robinson, 2009). Unlike domesticated bees, stingless bee nests can house one or multiple queens, but only one is fertile. Additionally, unlike honeybees, where swarming occurs as a singular event, the swarming process in stingless bees is gradual and may span several weeks (Hartfelder et al., 2006).

Stingless bees produce various products with numerous therapeutic properties. Their honey, besides being a significant source of income, exhibits a wide range of effects, including antibacterial (Cockburn et al., 2013), antidiabetic (Ali et al., 2020), and anti-inflammatory properties (Biluca et al., 2020). Propolis serves as a dietary supplement and is also used in traditional medicine (Suelen et al., 2019; Ngaini et al., 2021). In Burkina Faso, honey is commonly used to relieve menstrual pain (Meda et al., 2004).

Beyond their therapeutic roles, stingless bees also hold cultural and economic significance. In Tanzania, meliponine honey is used during circumcision rites (Héger et al., 2023). Conversely, among the Guayakis in the Chaco region of Argentina, women avoid consuming honey during menstruation, as it is considered a bad omen for the community (Vit et al., 2015).

Despite the importance of these bee species, their biodiversity is declining due to several factors, including deforestation, nest excavation by humans, nest predation by insects, lizards, and other parasites, excessive use of insecticides and herbicides, and bushfires (Kiatoko et al., 2018; Toledo-Hernandez et al., 2022). To mitigate the loss of their biodiversity, methods such as the production of artificial queens using in vitro techniques and colony multiplication strategies are being considered. It would therefore be necessary to understand the biometric and molecular characteristics of each species. However, several shortcomings limit the progress of these techniques, particularly the lack of morphometric and molecular data for several species. As a result, various characterization methods, such as classical and geometric morphometric, have been used to establish systematic relationships between species (Kerr and Cunha, 1976). While biometric data are available for American, Asian and Australian species, there is little information on African species, particularly those from Cameroon.

Although some authors have studied nest architecture, few studies have focused on the comparative systematics of morphotypes using biometric measurements, and this lack of information complicates long-term exploitation or conservation efforts (Njoya et al., 2009; Nkoba, 2012). Ndungu et al. (2010) identified 2 morphotypes in the *M. ferruginea* population and 3 morphotypes in the *M. bocandei* population in Kenya using classical morphometry and wing geometry, but these conclusions remain limited and restricted to this area. In addition, the lack of long-term monitoring of these bee populations prevents an estimate of their morphological or genetic traits under the effect of anthropogenic and environmental factors, thus limiting their sustainable exploitation and also their conservation (César et al., 2022). This study therefore aims to improve knowledge of the diversity of *M. ferruginea*, a species widely used in African rainfed cultivation, on the basis of biometric characteristics in order to support its conservation and sustainable use. More specifically, it seeks to describe the biometric traits of these species, providing an overview of the population structure of *M. ferruginea* and *M. bocandei* in Cameroon.

Materials and Methods

Study period and areas

The study was conducted between February 2019 and May 2023 in the agroecological zone of the western highlands and the bimodal rainfall forest zone of Cameroon (Figure 1). Specifically, it focused on the West region (N 5°27'49.74" and E 10°48'0.02") and the East region (N 3°57'1.85" and E 13°54'51.84"). These zones are characterized as follows:

Western Zone: Rainfall ranges between 1.500 and 2.000 mm per year, with about 180 rainy days. The region has very fertile soils, ideal for agricultural activities such as cocoa, coffee, maize, beans, potatoes, and vegetable farming. The dominant vegetation includes gallery forests and raffia palm forests in lowlands, along with herbaceous layers composed of *Pennisetum purpureum* and *Imperata cylindrica*.

Eastern Zone: Similarly, rainfall ranges from 1.500 to 2.000 mm annually. The soils are ferralitic, acidic, red, clayey, deep, and rich in organic matter, suitable for cultivating cacao, coffee, cassava, plantains, maize, oil palm, and pineapple. The vegetation comprises dense evergreen forests and semi-deciduous dense forests dominated by *Sterculiaceae* and *Ulmaceae*.

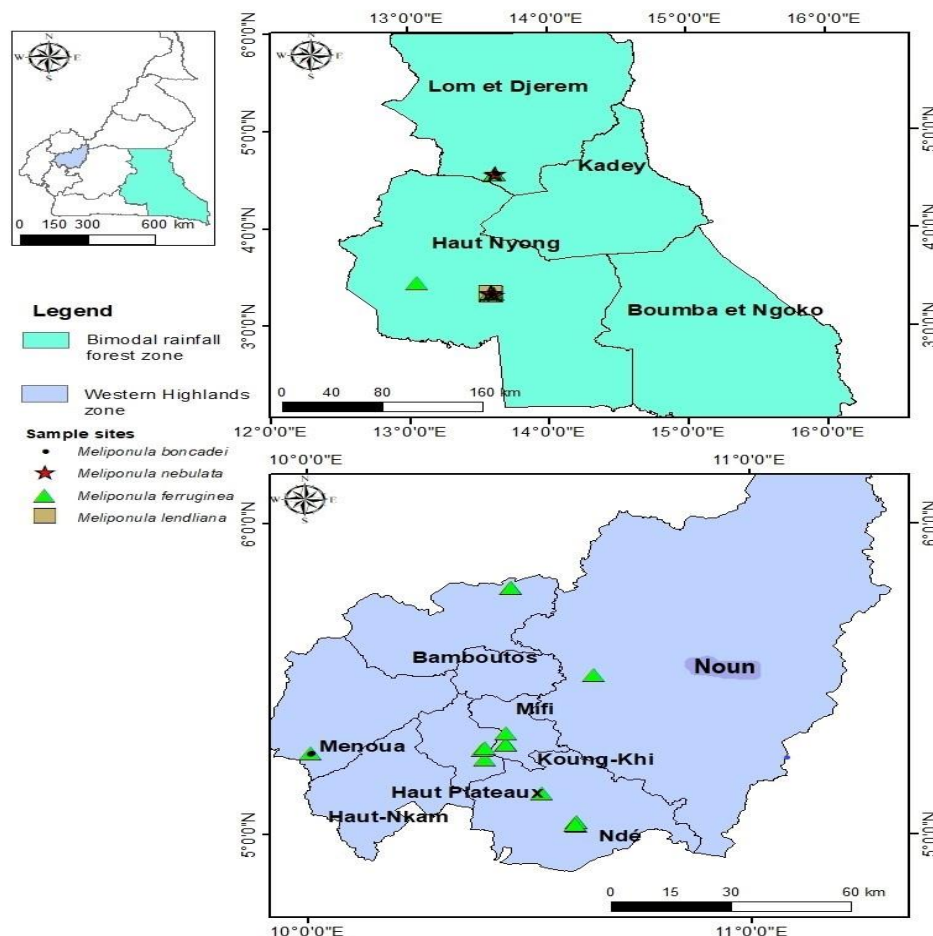


Figure 1: Study area

Selection of Nesting Sites, Species Identification, and Worker Collection

In each locality, access to nesting sites was facilitated using the non-probabilistic snowball sampling method. Species identification was performed using existing literature and Eardley's taxonomic key (2004). Once a nest was located, workers were collected by sealing the nest entrance to ensure only workers were

captured. From each nest, 30 worker bees were collected (Toullec, 2008). Tables 1 and 2 provide the geographic coordinates of the collection sites for *M. ferruginea* and *M. bocandei*.

Table 1: Geographical coordinates of the Different Collection Sites for *Meliponula ferruginea*

Zones	Divisions	Subdivisions	Latitude	Longitude	Altitude (m)
Western highlands zone	Ndé	Bassamba	05°01.707'	010°36.376'	932
	Ndé	Bassamba	05°02.326'	010°36.347'	945
	Ndé	Bassamba	05°02.324'	010°36.349'	950
	Ndé	Bassamba	05°01.812'	010°36.299'	935
	Ndé	Banganaté	05°07.793'	010°31.640'	1319
	Hauts-Plateaux	Bangou	05°14.405'	010°23.997'	1846
	Hauts-Plateaux	Bangou	05°16.227'	010°23.693'	1805
	Koung-Khi	Bayangam	05°16.686'	010°24.019'	1790
	Koung-Khi	Bayangam	05°17.286'	010°26.888'	1519
	Koung-Khi	Bayangam	05°17.297'	010°26.906'	1559
	Koung-Khi	Bayangam	05°17.318'	010°26.900'	1557
	Koung-Khi	Demdeng	05°19.534'	010°26.976'	1549
	Menoua	Santchou	05°15.572'	010°00.506'	718
	Menoua	Santchou	05°30.638'	009°98.820'	719
	Bamboutos	Gallim	05°77.423'	010°37.657'	1309
	Bamboutos	Gallim	05°77.520'	010°37.680'	1318
Bimodal rainfall forest zone	Haut-Nyong	Lomié	03°26.238'	010°03.096'	654
	Haut-Nyong	Lomié	03°19.942'	010°35.467'	657
	Haut-Nyong	Lomié	03°19.700'	013°35.350'	623
	Haut-Nyong	Lomié	03°18.871'	013°35.298'	662
	Haut-Nyong	Lomié	03°19.700'	013°36.125'	684
	Haut-Nyong	Lomié	03°19.670'	013°36.056'	697
	Haut-Nyong	Lomié	03°20.225'	013°35.426'	678
	Lom-et-Djérem	Bertoua	04°33.097'	013°37.384'	658
	Lom-et-Djérem	Bertoua	04°33.090'	013°37.299'	665
	Lom-et-Djérem	Bertoua	04°33.100'	013°37.227'	660

Table 2: Geographical coordinates of the different collection sites for *Meliponula bocandei*

Zones	Divisions	Subdivisions	Latitude	Longitude	Altitude(m)
Western highlands zone	Menoua	Santchou	05°15.532'	010°00.408'	714
	Menoua	Santchou	05°15.601'	010°00.627'	722
Bimodal rainfall forest zone	Haut-Nyong	Lomié	03°19.700'	013°35.260'	699
	Haut-Nyong	Lomié	03°20.305'	013°35.418'	738
	Haut-Nyong	Lomié	03°19.423'	013°35.269'	652
	Lom-et-Djérem	Bertoua	04°33.581'	013°37.985'	686
	Lom-et-Djérem	Bertoua	04°33.692'	013°37.763'	652

Worker bees conditioning

Once collected, the worker bees were placed in 30 ml plastic vials and preserved in 70% ethanol. They were stored at -20°C (Ndungu et al., 2017). Measurements were performed on worker bees as males, being haploid, are not representative of the population.

Biometric data collection

In the laboratory, biometric data were collected using a Zeiss stereo microscope equipped with a camera and Zen measurement software with a precision of 0.05 mm. Sixteen body measurements, as illustrated in Table 3, were recorded (Ruttner, 1988; Sakagami, 1978; Divya, 2016). The measurements, defined and shown in Figure 2, were taken following a well-established protocol.

Table 3: Symbols and definition of different measurements

Variables	Symbol	Definition
Body length	BL	Distance from the top of the head to the end of the abdomen
Abdomen length	AL	Distance from the base of the abdomen to the trunk
Head length	HL	Is measured along the midline from the base of the head to the top of the mandibles in the vertical plane
Head width	HW	Maximum distance through the eyes
Antenna length	AnL	Sum between length of flagellum, pedicel and scape
Distance between Dorsal Ocelli	DBO	Shortest space between the lateral ocelli on the dorsal surface of the head
Dorsal Ocello Ocular Distance	OOD	Shortest distance between the edge of the lateral ocelli and the compound eye
Femur length	FL	Distance from the third segment of the leg
Tibia length	TL	Distance from the fourth segment of the leg
Tibia width	TW	Maximum distance between the extremity of tibia
Metatarsal length	ML	Distance from the fifth segment of the leg
Metatarsal width	Mw	Maximum distance between the two ends of the metatarsus
Right forewing length	RfL	Distance from the base of the coastal sclerite to the apical point
Right forewing width	RfW	Maximum distance of this wing
Right hind wing length	RHL	Distance from the base of coastal sclerite to the apical point of this wing
Right hind wing width	RHW	Maximum distance of this wing

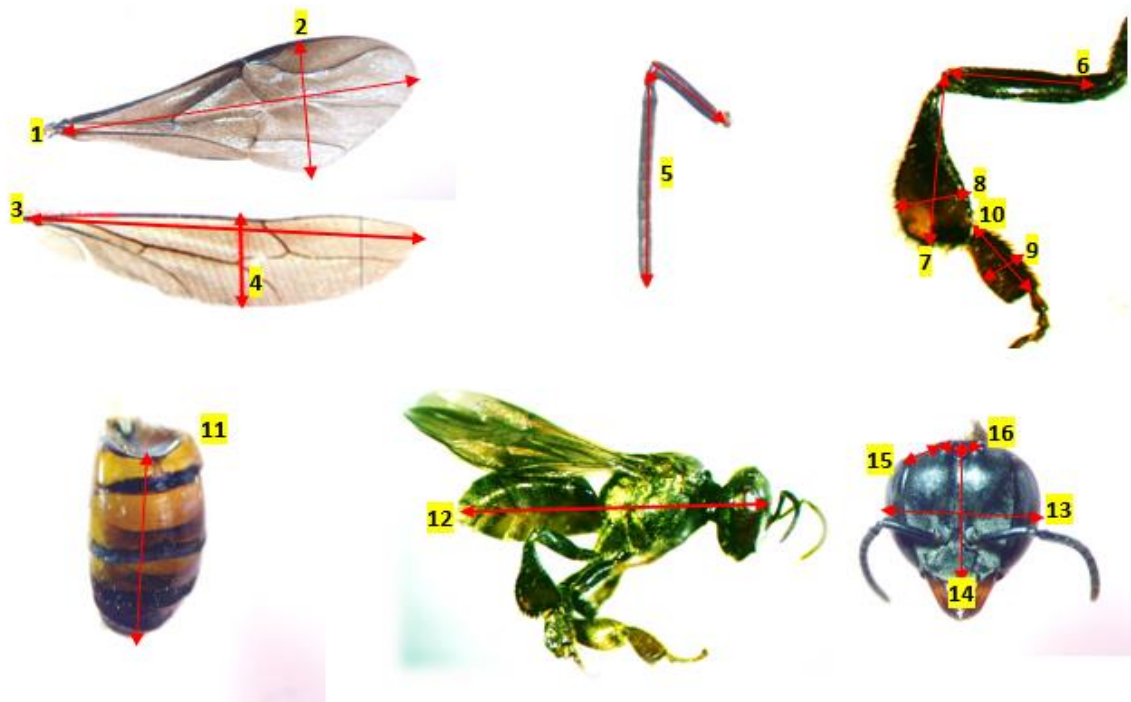


Figure 2: Major body measurements in *Meliponula* bees

1 and 2: Right forewing length and width; 3, 4: Right hindwing Length and Width; 5: Antenna length, 6, 7, 8, 9 and 10: Femur length, Tibia length, Tibia Width, Metatarsus Length, and Width, respectively; 11: Abdomen length, 12: Body length, 13, 14: Head Length and Width, 15, 16: Distance Between two lateral Ocelli, Ocello-ocular Distance respectively

Statistical analyses

Statistical analyses were performed using XLSTAT 2022 and SPSS 20.0. Student's t-test was applied to compare the different biometric variables between the two agroecological zones. Pearson's correlation coefficient determined a correlation between different variables. Principal component analysis (PCA) was used to visualize the distribution of individuals in the different populations of *M. ferruginea* and *M. bocandei* in order to illustrate the variability of the characters measured and to identify groupings within populations. The dendrogram resulting from the hierarchical ascending classification will provide a graphic representation of the grouping of individuals of each species according to similarities, thus forming distinct morphotypes on the basis of their biometric characteristics. By observing the levels where the branches of the dendrogram meet, it will be possible to recognize homogeneous groups of individuals: the closer two individuals are, the more their branches will meet in the tree. In addition, discriminant factor analysis (DFA) will be used to separate the different morphotypes more distinctly and identify the variables that have led to the differentiation of these different groups within each species.

Results

Biometric characteristics of Meliponula (Axestotrigona) ferruginea and Meliponula bocandei in Cameroon

Table 4 presents the biometric measurements of *M. ferruginea* in Cameroon respectively.

Table 4: Biometric measurements of *Meliponula ferruginea* in Cameroon Central-Africa

Features	Bimodal Rainforest zone	Western Highlands zone	All sites	T-test
	$\bar{X} \pm \sigma$	$\bar{X} \pm \sigma$	$\bar{X} \pm \sigma$	
BL	6.121±0.489 ^a	5.712±0.462 ^b	5.916±0.475	**
AL	2.711±0.428 ^a	2.490±0.326 ^b	2.600±0.377	**
HL	2.044±0.101 ^a	1.933±0.137 ^b	1.988±0.119	**
HW	2.648±0.113 ^a	2.492±0.211 ^b	2.570±0.162	**
DBO	0.504±0.038 ^a	0.481±0.056 ^b	0.492±0.047	**
OOD	0.394±0.045 ^a	0.373±0.048 ^b	0.383±0.046	**
AnL	2.785±0.150 ^a	2.638±0.221 ^b	2.711±0.185	**
RFL	5.603±0.203 ^a	5.460±0.170 ^b	5.531±0.186	**
RFW	2.043±0.094 ^a	1.958±0.135 ^b	2.000±0.114	**
RHL	4.122±0.157 ^a	3.922±0.245 ^b	4.022±0.201	**
RHW	0.996±0.080 ^a	0.952±0.094 ^b	0.974±0.087	**
FL	1.607±0.074 ^a	1.533±0.141 ^b	1.570±0.107	**
TL	2.178±0.106 ^a	2.130±0.125 ^b	2.154±0.115	**
TW	0.860±0.074 ^a	0.846±0.068 ^b	0.853±0.071	**
ML	1.069±0.087 ^a	0.987±0.113 ^b	1.028±0.100	**
MW	0.564±0.063 ^a	0.542±0.066 ^b	0.553±0.064	**

a.b: Intra-class means of the same line followed by the same letter are not significantly different at the 5% level; p<0.05; NS : p>0.05 ; CV: coefficient of variation; $\bar{X} \pm \sigma$: mean and standard deviation; n: number of individuals

This table shows that for *M. ferruginea*, at $p < 0.05$, the biometric measurements were influenced by locality. Bees from the bimodal rainfall forest zone were larger than those from the western highlands. The average body measurements in millimeters (mm) for bees from the East region were:

BL: 6.121, AL: 2.711, HL: 2.044, HW: 2.648, DBO: 0.504, OOD: 0.394, AnL: 2.785, RFL: 5.603, RFW: 2.043, RHL: 4.122, RHW: 0.996, FL: 1.607, TL: 2.178, TW: 0.860, ML: 1.069, MW: 0.564. For bees from the West region, the measurements were:

BL: 5.916, AL: 2.600, HL: 1.988, HW: 2.570, DBO: 0.492, OOD: 0.383, AnL: 2.711, RFL: 5.531, RFW: 2.000, RHL: 4.022, RHW: 0.974, FL: 1.570, TL: 2.154, TW: 0.853, ML: 1.028, MW: 0.553.

Table 5 presents the biometric measurements of *M. bocandei* in Cameroon respectively.

From this table, it appears that only body length was influenced by locality. The average body length was 7.943±0.449 mm, with bees from the bimodal rainfall forest zone being larger than those from the western highlands. The average measurements (mm) for bees from the forest zone were:

BL: 8.111, AL: 3.784, HL: 2.890, HW: 3.465, DBO: 0.594, OOD: 0.776, AnL: 3.594, RFL: 7.312, RFW: 2.679, RHL: 5.367, RHW: 1.410, FL: 2.227, TL: 2.719, TW: 1.053, ML: 1.410, MW: 0.786.

For bees from the western highlands, the measurements were:

BL: 7.776, AL: 3.683, HL: 2.894, HW: 3.459, DBO: 0.580, OOD: 0.767, AnL: 3.672, RFL: 7.215, RFW: 2.656, RHL: 5.366, RHW: 1.417, FL: 2.179, TL: 2.689, TW: 1.086, ML: 1.366, MW: 0.770.

Table 5: Biometric measurements of *Meliponula bocandei* in Cameroon Central-Africa

Features	Bimodal Rainforest zone	Western Highlands zone	All sites	T-test
	$\bar{X} \pm \sigma$	$\bar{X} \pm \sigma$	$\bar{X} \pm \sigma$	
BL	8.111±0.558 ^a	7.776 ± 0.340 ^b	7.943±0.449	**
AL	3.784±0.474	3.683 ± 0.300	2.129±0.387	NS
HL	2.890±0.071	2.894 ± 0.062	2.892±0.066	NS
HW	3.465±0.073	3.459 ± 0.046	3.462±0.059	NS
DBO	0.594±0.053	0.580 ± 0.035	0.587±0.044	NS
OOD	0.776±0.049	0.767 ± 0.066	0.771±0.106	NS
AnL	3.594±0.215	3.672 ± 0.163	3.633±0.189	NS
RFL	7.312±0.215	7.215 ± 0.217	7.263±0.216	NS
RFW	2.679±0.084	2.656 ± 0.087	2.667±0.085	NS
RHL	5.367±0.174	5.366 ± 0.117	5.366±0.145	NS
RHW	1.410±0.067	1.417 ± 0.063	1.413±0.065	NS
FL	2.227±0.116	2.179 ± 0.102	2.203±0.109	NS
TL	2.719±0.109	2.689 ± 0.108	2.704±0.108	NS
TW	1.053±0.073	1.086 ± 0.088	1.069±0.080	NS
ML	1.410±0.105	1.366 ± 0.145	1.388±0.125	NS
MW	0.786±0.045	0.770 ± 0.054	0.778±0.049	NS

a,b: Intra-class means of the same line followed by the same letter are not significantly different at the 5% level; p<0.05; NS : p>0.05 ; CV: coefficient of variation; $\bar{X} \pm \sigma$: mean and standard deviation; n: number of individuals

Correlation between Biometric Measurements of Meliponula ferruginea and Meliponula bocandei in Cameroon (Central Africa)

Table 6 and 7 shows the correlation between biometric measurements of *Meliponula ferruginea* and *Meliponula bocandei* in Cameroon respectively

Table 6: Correlation between different measurements of *Meliponula ferruginea* in Cameroon (Central Africa)

Variables	BL	AL	HL	HW	AnL	RFL	RFW	RHL	RHW	FL	TL	TW	ML	MW	DBO	OOD
BL	1															
AL	0.743	1														
HL	0.403	0.165	1													
HW	0.405	0.108	0.659	1												
AnL	0.402	0.144	0.649	0.705**	1											
RFL	0.439	0.287	0.427	0.435**	0.454**	1										
RFW	0.325	0.104	0.496	0.455**	0.467**	0.475**	1									
RHL	0.447	0.170	0.623**	0.663**	0.537**	0.573**	0.638**	1								
RHW	0.325	0.156	0.503**	0.492**	0.475**	0.362**	0.433**	0.567**	1							
FL	0.413	0.121	0.598**	0.680**	0.635**	0.474**	0.550**	0.628**	0.576**	1						
TL	0.139	0.096	0.290**	0.294**	0.310**	0.392**	0.233**	0.272**	0.179**	0.246**	1					
TW	0.115	0.015	0.244**	0.246**	0.174**	0.243**	0.159**	0.218**	0.171**	0.177**	0.345**	1				
ML	0.398	0.175	0.589**	0.639**	0.581**	0.468**	0.453**	0.553**	0.428**	0.606**	0.292**	0.200**	1			
MW	0.229	0.015	0.506**	0.490**	0.482**	0.309**	0.386**	0.469**	0.440**	0.537**	0.294**	0.372**	0.398**	1		
DBO	0.340	0.139	0.527**	0.522**	0.508**	0.308**	0.356**	0.459**	0.432**	0.533**	0.080	0.178**	0.456**	0.454**	1	
OOD	0.133	0.101	0.328**	0.361**	0.299**	0.213**	0.225**	0.210**	0.253**	0.338**	0.251**	0.233**	0.367**	0.253**	0.198**	1

**Correlation is significant at the 0.05 level (2-tailed); *Correlation is significant at the 0.01 level (2-tailed)

Table 7: Correlation between different measurements of *Meliponula bocandei* in Cameroon (Central Africa)

Variables	BL	AL	HL	HW	AnL	RFL	RFW	RHL	RHW	FL	TL	TW	ML	MW	DDO	DOOD
BL	1.000															
AL	0.841	1.000														
HL	0.180	0.062	1.000													
HW	0.409	0.259	0.217	1.000												
AnL	-0.076	0.003	-0.107	0.158	1.000											
RFL	0.317	0.253	0.145	0.625	0.056	1.000										
RFW	0.151	0.145	-0.007	0.461	0.145	0.457	1.000									
RHL	0.340	0.261	0.111	0.699	0.213	0.564	0.380	1.000								
RHW	0.089	0.128	-0.134	0.200	0.166	0.082	0.156	0.074	1.000							
FL	0.152	-0.088	0.148	0.441	0.062	0.316	0.139	0.382	0.235	1.000						
TL	0.083	0.037	0.055	0.285	0.004	0.245	0.281	0.180	0.061	0.001	1.000					
TW	0.008	0.053	0.067	0.260	-0.006	0.065	0.071	0.190	0.242	0.158	0.101	1.000				
ML	0.035	0.076	0.126	0.200	-0.150	0.212	0.123	0.139	0.0003	0.133	-0.021	-0.148	1.000			
MW	0.089	0.001	-0.119	0.248	-0.029	0.207	0.197	0.321	0.312	0.273	0.169	0.337	-0.015	1.000		
DDO	0.285	0.231	0.115	0.236	-0.022	0.166	0.078	0.095	-0.093	0.032	-0.005	0.067	0.003	-0.204	1.000	
DOOD	0.103	0.122	-0.109	0.144	0.156	0.156	0.057	0.256	0.154	0.095	0.069	0.032	0.045	0.165	-0.316	1.000

**Correlation is significant at the 0.05 level (2-tailed); *Correlation is significant at the 0.01 level (2-tailed)

For *M. ferruginea* (Table 6), the biometric measurements were linearly correlated, with all correlations between body parameters being positive at a 5% significance level. The highest positive correlation coefficient ($r=0.743$) was observed between body length and abdomen length.

For *M. bocandei* (Table 7), both positive and negative correlations were observed. The highest positive correlation ($r=0.841$) was also found between body length and abdomen length.

Phenotypic Variability of Meliponula ferruginea and Meliponula bocandei Populations in Cameroon (Central Africa)

Tables 8 and 9 summarize the eigenvalues and cumulative variance obtained from the principal component analysis (PCA) of the populations of *Meliponula ferruginea* and *Meliponula bocandei* in Cameroon, covering the Western Highlands and the bimodal rainfall forest zone.

Table 8: Principal component analysis of *Meliponula ferruginea* in Cameroon (Central Africa)

Principal component	Eigen value	Variability (%)	Cumulated Variability
F1	6.928	43.301	43.301
F2	1.564	9.777	53.079
F3	1.248	7.803	60.883
F4	0.905	5.661	66.544
F5	0.850	5.317	71.861
F6	0.684	4.279	76.141
F7	0.585	3.659	79.800
F8	0.503	3.146	82.946
F9	0.489	3.057	86.004
F10	0.440	2.751	88.756
F11	0.405	2.531	91.288
F12	0.377	2.356	93.644
F13	0.355	2.221	95.865
F14	0.288	1.800	97.666
F15	0.197	1.236	98.902
F16	0.175	1.097	100.000

F: Principal component

This table shows that, in the *M. ferruginea* population, principal component one has an eigenvalue of 6.928 and explains 43.30% of the total genetic variability observed. This shows that there are several morphological differences between individuals that could be summarized by a factorial axis. Furthermore, the first two components explain 53.08% of the total variability and the first three components 60.88% of the variability, showing a concentration of information on two or three axes. This shows that the representation of biometric diversity through these two or three axes is reliable.

In contrast to *M. ferruginea*, the first principal component explained 23.67% of the variance, illustrating a population with a more dispersed biometric structure. Furthermore, the first three components only account for 44.96% of the variance. To achieve 50% of the observed variability, it is necessary to group together at least the first four components, which account for 50.06%.

Structure of the Meliponula ferruginea and Meliponula bocandei Populations in Cameroon (Central Africa)

Figures 3 & 4 show the Variable distribution and figures 5 & 6 the population structure of *M. ferruginea* and *M. bocandei* populations based on PCA.

Table 9: Principal component analysis of *Meliponula bocandei* in Cameroon (Central Africa)

Principal component	Eigen value	Variability (%)	Cumulated Variability
F1	3.786	23.667	23.667
F2	1.960	12.253	35.920
F3	1.446	9.039	44.960
F4	1.296	8.100	53.060
F5	1.202	7.518	60.578
F6	1.103	6.894	67.473
F7	0.959	5.998	73.471
F8	0.800	5.002	78.474
F9	0.703	4.397	82.872
F10	0.635	3.971	86.843
F11	0.558	3.487	90.331
F12	0.446	2.791	93.122
F13	0.417	2.608	95.731
F14	0.357	2.232	97.963
F15	0.238	1.488	99.451
F16	0.087	0.548	100.000

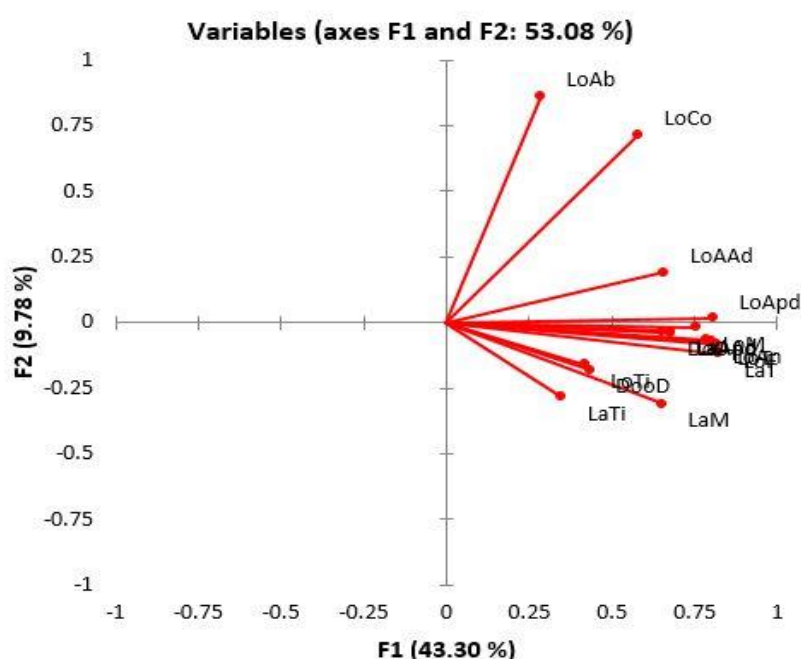


Figure 3: Variable distribution of *Meliponula ferruginea* of Cameroon

For *M. ferruginea* (figure 3), several interesting relationships are visible: Variables like anterior wing length and posterior right-wing length are close to each other, indicating a strong positive correlation.

Other variables are positioned at wider angles or are perpendicular to each other, indicating either no correlation or opposite relationships. These relationships help understand which variables jointly influence the principal components F1 and F2 and their impact on the overall data structure.

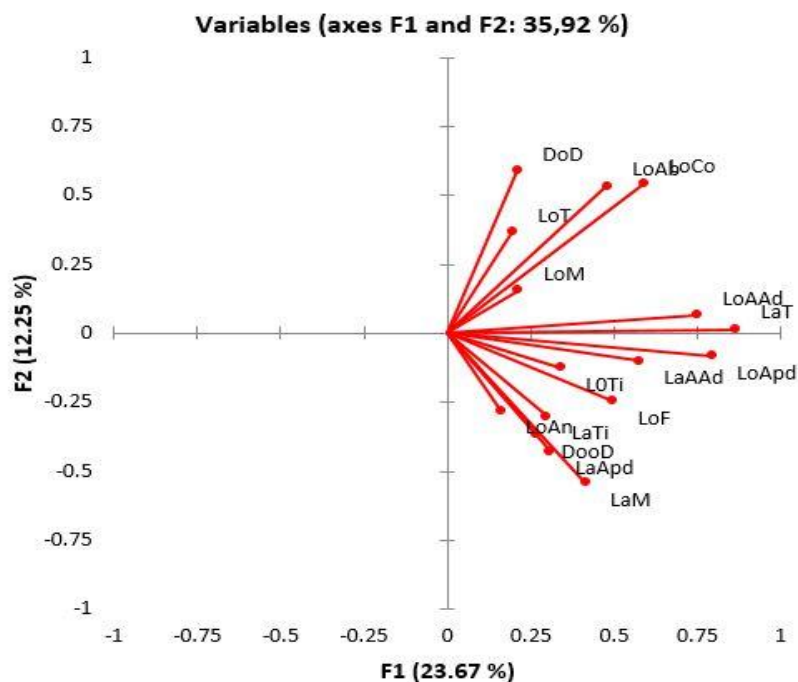


Figure 4: Variable distribution of *Meliponula bocandei* of Cameroon

For *M. bocandei* (Figure 4), the F1 and F2 axes explained 23.67% and 12.25% of the total variability (35.92%). The main relationships are as follows a strong positive correlation between anterior wing length (RFL) and posterior right-wing length (RHL) on the F1 axis, Independence (absence of correlation) between variables such as ocellar-dorsal distance (OOD) and posterior right-wing length (RHL), visible by their perpendicular positioning on the axes. These analyses highlight the phenotypic diversity and the more dispersed structure of the population, which requires several axes to illustrate the variability observed, suggesting a more homogeneous diversity or one that is difficult to detect.

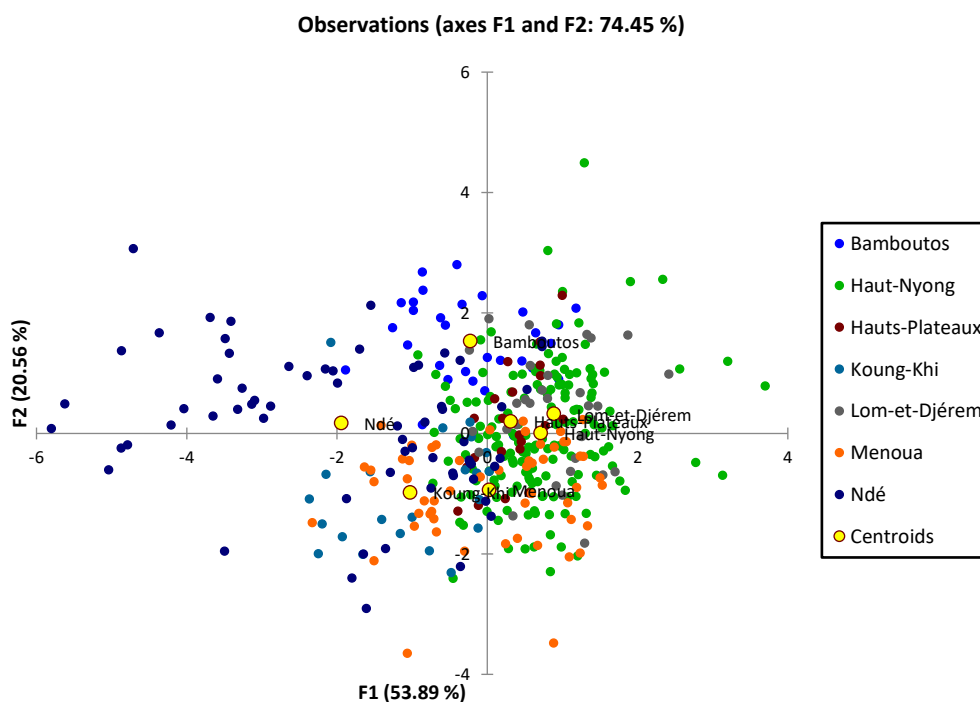


Figure 5: Population structure of *Meliponula ferruginea* in Cameroon (Central Africa)

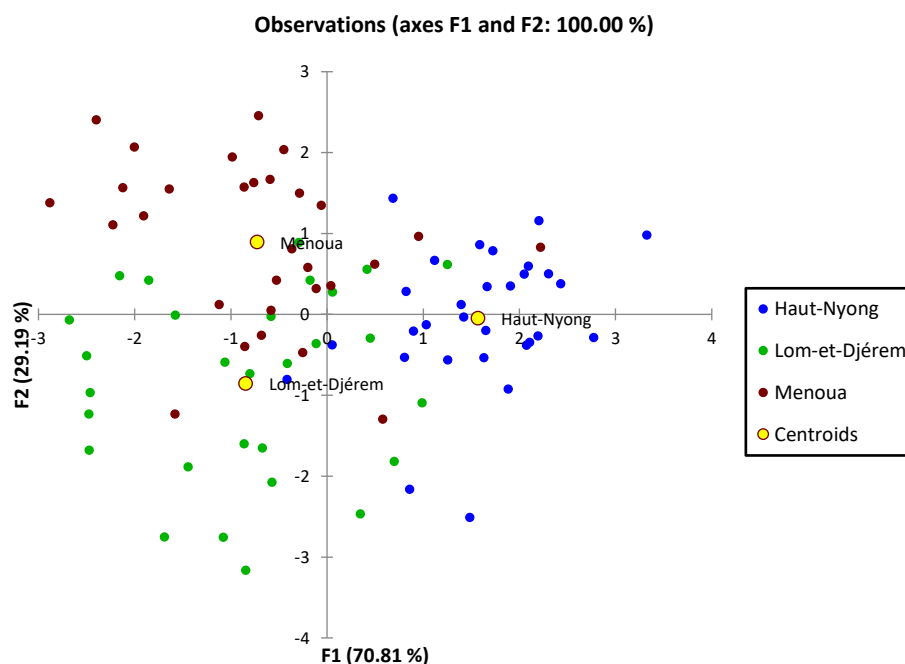


Figure 6: Population structure of *Meliponula bocandei* in Cameroon (Central Africa)

Figure 5 shows the population structure of *Meliponula ferruginea*. The F1 and F2 axes together explain 74.45% of the total variance. However, the F1 axis (53.89%) provides half of the information contained in the data. In addition, there is a distinction between groups but also an overlap between groups in certain zones, showing that certain distinctions are not clear-cut.

Figure 6 shows the population structure of *Meliponula bocandei*. This figure shows that the F1 and F2 axes together explain 100% of the total variance. However, the F1 axis (70.81%) provided more than half of the information contained in the data. The centroids are separated from each other, suggesting the existence of geographically distant morphotypes. Individuals from Haut-Nyong are found in the right half of the centroid, whereas those from Lom-et-Djerem and Menoua are found in the left and upper part of the centroid.

Phylogenetic Relationship of Meliponula ferruginea and Meliponula bocandei in Cameroon (Central Africa)

Additionally, discriminant factor analysis was used to identify the different morphotypes within the populations of *Meliponula ferruginea* and *Meliponula bocandei*. Figures 5 and 6 show the dendrograms for the populations of *M. ferruginea* and *M. bocandei*.

This figure 7 shows the 3 morphotypes that exist within the *M. ferruginea* population named M1, M2 and M3. It illustrates that morphotypes M1 and M2 are closer with a low dissimilarity unlike morphotype 3 which has a high dissimilarity. This may suggest that morphotypes 1 and 2 share similar biometric characteristics unlike morphotype 3 which is more distinct.

In contrast to *M. ferruginea*, analysis of figure 8 above shows that in the *M. bocandei*, morphotypes 2 and 3 are closer together, suggesting that they share common biometric characteristics, unlike morphotype 1 which is further apart from M2 and M3. This could reflect a different geographical origin showing that each *M. ferruginea* and *M. bocandei* follow different evolutionary dynamics depending on their morphological traits. Furthermore. Tables 10 and 11 present the distances between the centroids of the morphotypes in the populations of *M. ferruginea* and *M. bocandei*.

Table 10: Distance between the barycentres of the morphotype of *Meliponula ferruginea* in Cameroon (Central Africa)

Distances between the morphotype centroids	1	2	3
1	0		
2	0.954	0	
3	1.945	1.487	0

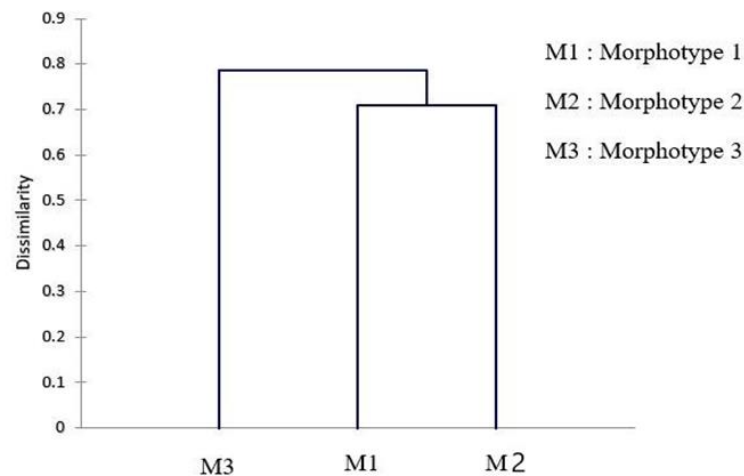


Figure 7: Dendrogram *Meliponula ferruginea* of Cameroon

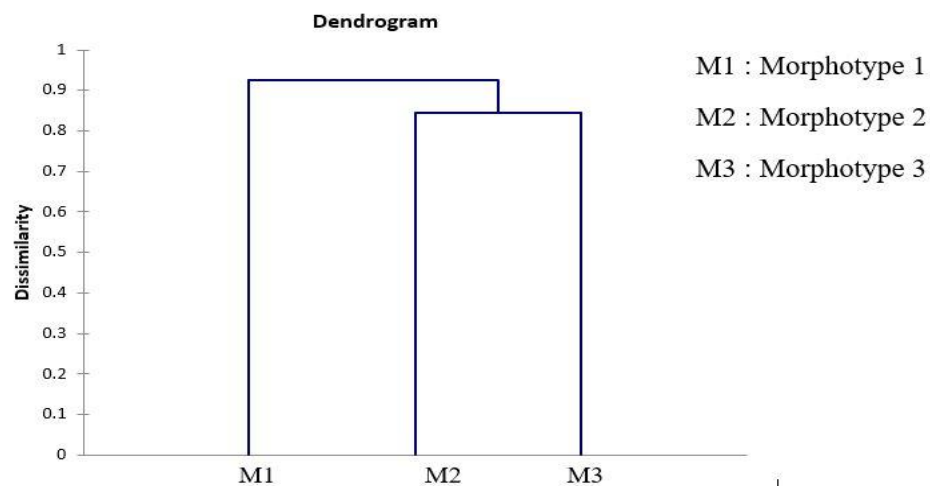


Figure 8: Dendrogram *Meliponula bocandei* of Cameroon

In the case of *M. ferruginea*, the distance between morphotypes 3 and 2 is the largest, while in *M. bocandei*, the distance between morphotypes 3 and 1 is the largest. These morphotype 3 is the furthest away from the others, particularly morphotype 1 (1.945) and is more distinct from the other two.

Table 11: Distance between the barycentres of the morphotype of *Meliponula bocandei* in Cameroon (Central Africa)

Distances between the morphotype centroids	1	2	3
1	0		
2	0.533	0	
3	0.694	0.328	0

Morphotypes 1 and 2 are closer together (0.533), while 3 is further apart with less variation than morphotypes 1 and 2.

3. Discussion

Several species of stingless bees are present in Cameroon, including *Meliponula bocandei* and *Meliponula ferruginea* which are the most exploited for their honey by local populations. However, little information is available on the biometric and genetic characteristics of these African stingless bees. In order to distinguish between these different species, a biometric study was carried out on these two species.

All the measurements of the *M. ferruginea* population were influenced by locality. As a result, the average body length was between 5.441 and 6.391 mm. These results are close to those found by Eardley (2004) with bees that were 5.100 and 5.900 mm long. The observed variability may be due to the adaptation of these bees to the activity of collecting, storing and transporting pollen and propolis (Grula et al., 2021). As bees in the western highlands are smaller than those in the bimodal rainfall forest zone, the small size of these bees may be due to adaptation to foraging behavior as well as genetic factors. Smaller bees visit more flowers per tree than larger bees (Pereboom and Biesmeijer 2003; Greenleaf et al., 2007). In addition, the head length of *M. ferruginea* ranged from 1.869 to 2.107 mm. These results are also close to those of Eardley (2004) where the average head length ranged from 1.900 to 2.100 mm.

For *M. bocandei*, there were no significant differences for most biometric characteristics with the exception of body length which was significantly greater in the bimodal rainfall zone (8.111 ± 0.558 mm) than in the western highlands (7.776 ± 0.340 mm). The average body length is between 7.494 and 8.392 mm, which is greater than or equal to the average length of the bees found by Eardley (2004). This could be explained by low morphological plasticity or adaptation to similar climatic conditions (Maleszka, 2018). Regarding head length, the average was between 2.826 and 2.958 mm these results differ from those of Eardley. (2004) who had the average head length of 2.600 mm. The size of the head parameters could be associated with the foraging activity of the bees, which is also linked to the type of vegetation present in the area (Kerfoot, 1967).

The average forewing length for *M. ferruginea* was between 5.345 and 5.717 mm and for *M. bocandei* between 7.047 and 7.479 mm. For *M. ferruginea*, these results are also close to those found by Eardley (2004), which were 5.400 and 6.000 mm for *M. ferruginea* and 6.600 mm for *M. bocandei*. The variability observed could be the result of ecological adaptations such as the type of flight, the distance travelled in search of food and local environmental conditions (Hepburn et al., 2001).

In addition, the mean leg length of *M. ferruginea* in both areas ranged from 4.430 to 5.074 mm and the leg length of *M. bocandei* ranged from 5.953 to 6.637 mm. These results are greater than or equal to the leg lengths of *M. bocandei* found in the Kakamega. Mwingi and Coast forests of Kenya where the bees had an average leg length of 5.360 mm; 5.350 mm; 5.270 mm respectively (Ndungu. 2010). As the pollen storage space is smaller in small stingless bee species, a longer hind leg results in an enlarged pollen basket and therefore the bee collects more pollen (Munyuli. 2014).

With regard to correlations, all the correlation coefficients for *M. ferruginea* were positive. The largest correlation coefficient being between body length and abdomen length (0.743). On the other hand, in

M. bocandei, positive and negative correlation coefficients were noted. The largest correlation coefficient also being between the length of the body and the length of the abdomen (0.841). For *M. bocandei* species, these results are close to those found by Ndungu. (2010) in which there were negative weights from -0.840 to 0.477 and positive second component weights from 0.09 to 0.573. These results may be due to biological variations, in particular intra-specific variability and environmental factors such as climate, habitat type and the phenotypic plasticity of these bees to adapt to local conditions, or also to methodological variations such as the number of components retained for the study and the choice of selection of certain variables.

Discriminant factor analysis also revealed that the degree of intra-genetic variation is higher than the degree of inter-genetic variation in *M. ferruginea* and *M. bocandei*. This could be linked to movement and/or crossover between different nests. Bees without stingers during swarming migrate over short distances of a few hundred meters between colonies (Araujo *et al.*, 2004; Roubik, 2006).

The phylogenetic tree obtained following this analysis shows that in *M. ferruginea*, morphotypes 1 and 2 are closer and genetic types 2 and 3 are further apart, and in *M. bocandei*, morphotypes 2 and 3 are closer, whereas morphotypes 1 and 3 are further apart.

These distant genetic types could have the same origin or belong to the same subgroup which then differentiated to give distinct genetic types. In similar studies carried out on populations of *M. bocandei* from the Kakamega forest in Kenya, the PCA was used to divide the populations into two subgroups - the first two components contributed 61.990% of the total variability but when the data were subjected to the canonical axis of correlations, the populations separated into three distinct groups (Ndungu. 2010). Furthermore, in populations of *Melipona beecheii* from Yucatan and Campeche in Mexico, the first three components explained 92.860% of the total variability (May-Itza *et al.*, 2012). Similarly, morphobiometric studies on populations of *M. bocandei* and *M. ferruginea* in Ghana showed that, in general, SFM had enabled 97.010% of samples to be classified into ecozones. In the case of *M. ferruginea*, 97.870% of the SFM scores were used to distinguish bees from three ecozones. In *M. bocandei*, the score was 92.450% (Combey *et al.*, 2013).

Morphometric analyses have made it possible to distinguish the different stingless bee species present in the western highlands and the bimodal rainfall forest zone of Cameroon. However, these results will be extended by molecular characterization studies.

The results of the various characterization works of stingless bees made by the biometrics approach make it possible to distinguish the different species, provided that the variables are well-chosen and that the samples are representative of the geographical areas. , this approach has limitations because it does not allow on its own to distinguish the subspecies or critical species in which the recorded variabilities could nevertheless be due to reversible ecological adaptations such as the plasticity of foraging behaviour depending on climatic conditions or floral availability, the increase in temperature during larval development which could increase the size of certain body parameters, the density of the brood which can also affect the size of the bees. Therefore, it is necessary to supplement the results with molecular analyses such as mitochondrial DNA sequencing, analysis of the nuclear genome which varies little with environmental factors.

Conclusion and Perspectives

Among the stingless bees found in Cameroon. *Meliponula ferruginea* and *Meliponula bocandei* represent a significant but underexplored and poorly documented resource. The analyses conducted have shown that there is variability within these two bee populations, which could contribute to their improvement: conservation and efficient exploitation. However, due to certain morphological similarities between different species, it would be essential to complement this study with molecular identification work and extend these studies to other agroecological zones to better identify these bees.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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