

Original Research Paper

Biometrics Assessment Reveals Distinct Population of *Dactylurina staudingeri* (Gribodo, 1893) (Hymenoptera, Apidae, Meliponini) Between two Agroecological Zone of Cameroon.

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Abstract

Dactylurina staudingeri is a widely distributed stingless bee species in West and Central Africa. It provides important pollination services, and its honey is valued for its medicinal properties. However, native population diversity is being poorly investigated. In the context of the expansion of meliponiculture, a biometric study was carried out on samples of *Dactylurina staudingeri* collected in two agro-ecological zones of Cameroon: the agro- ecological zone of the Western Highlands and the Bimodal Rainfall Forest zone. A total of 797 mature worker bees from 49 colonies were collected from the wild. A total of 16 biometric (body length (BL); abdomen length (AL); antenna length (AnL); had length (HL); head width (HW); ocello-ocular distance (OOD); distance between two lateral ocelli (DBO); femur length (FL); metatarsus length (ML); tibia length (TL); tibia width (TW); metatarsus width (ML); right forewing length (RFL); right forewing width (RFW); right hindwing length (RHL); right hindwing width (RHW)) measurements were recorded on each of them using classical biometric methods and data obtained were submitted for test t of Student. The results obtained showed a significant effect of agroecological zone for some measurements, and thus there is a large morphometric variability between bees from the Bimodal Rainfall Forest zone and bees from the Western Highlands. Bees from the Bimodal Rainfall Forest zone have the largest measurements for traits such as abdominal length, head width, body length, antenna length, ocello-dorsal distance, ocello-dorsal ocular distance, tibia length and width, metatarsal length and width. Bees from the Western Highlands had the largest measurements for parameters such as right forewing length and width, head length, hindwing length and width, and femur length. The present study revealed the presence of 3 morphotypes within the *Dactylurina staudingeri* population using principal component analysis.

Keywords: Biometry, stingless bees, *Dactylurina staudingeri*, Cameroon.

Introduction

Stingless bees are eusocial insects in the family *Apidae* and tribe *Meliponini* (Michener, 2000; Eardley, 2004). They are found in all tropical and subtropical regions of the world (Cortopassi *et al.*, 2006; Njoya, 2009). They are critical insects for pollination and honey value chains in tropical and subtropical regions. Stingless bees can thrive in various ecosystems, and due to their relatively smaller size, they pollinate a wide range of plants including ornamental and medicinal plants (Kiatoko *et al.*, 2018; Quezada-Euán, 2018; Bafo, 2019). They are highly resilient and are easy to be nested in artificially designed hives (Heard, 1999). The hive organization of stingless bees is the same as in honeybees. A colony consists of one or several queens (but only one is active), workers, and males also called drones (Kwapong *et al.*, 2010). The products resulting from the exploitation of the hive of stingless bees are diverse. Their honey is very esteemed for its antimicrobial values against infections caused by *Pseudomonas aeruginosa* and some bacteria that depict resistance to antibiotics (Cockburn *et al.*, 2013; Mokaya *et al.*, 2022). Their propolis can be used as a food supplement and as ingredients in traditional medicine and cosmetic industry, antifungal, and antibacterial (Shebu *et al.*, 2016; Carneiro *et al.*, 2016; Márcia *et al.*, 2017; Nicolas *et al.*, 2021; Zulhendri *et al.*, 2021; Costantin *et al.*, 2022; Ozarowski *et al.*, 2022). Their pollen is also used as a food supplement (Carla *et al.*, 2021). They often build their nest on tree branches, above 5 m height (Njoya *et al.*, 2016).

Stingless bees are diverse; they are fifty times more species-rich than honeybees, with over 500 species identified globally (Michener, 2007). In Africa, 36 species grouped in 10 genera are described (Eardley, 2004; Eardley *et al.*, 2010; Gruter, 2020); they are as follows: *Apotrigona* (Moure, 1961), *Axestotrigona* (Moure, 1961), *Cleptotrigona* (Moure, 1961), *Dactylurina* (Cockerell, 1934), *Hypotrigona* (Cockerell, 1934), *Liotrigona* (Moure, 1961), *Meliplebeia* (Moure, 1961), *Meliponula* (Cockerell, 1934), *Plebeiella* (Moure, 1961), *Plebeina* (Moure, 1961). Among these genera, the genus *Dactylurina* holds a core place with 2 species *D. staudingeri* (Gribodo, 1893) in central Africa and *D. schmidtii* (Stadelmann, 1895) in Western Africa. At the ecosystem level, *D. staudingeri* (Gribodo, 1893) is mainly involved in the pollination process because of its large spatial distribution (Tchuengem *et al.*, 2002). However, there are limited data on their population diversity in Cameroon.

In Africa, several countries have embarked on the rational farming activity of stingless bees, i.e. meliponiculture, such as Angola, Burundi, Kenya, and South Africa, for the provision of honey, pollen and propolis (Kiatoko *et al.*, 2016; Bafo, 2019). However, in Cameroon, meliponiculture is just starting because of lack of crucial data and technical skills. Furthermore, stingless bee populations could be at risk due to anthropogenic activities like expansive forest wood logging and the wide utilization of polluting agrochemicals (Lima *et al.*, 2016). These activities lead to the degradation of natural nesting and foraging habitats therefore by causing the loss of biodiversity, and the emergence of new pests and diseases (Bobadoye *et al.*, 2016; Kiatoko *et al.*, 2018; Purkiss and Lach, 2019). Without adequate information on stingless bee species of interest, there are limited options to protect them and harness their potential.

The present study focuses on *D. staudingeri* in the Central African sub-region. The study aims to provide additional and updated information on *D. staudingeri* species and to contribute to the expansion of meliponiculture in Cameroon. More specifically, to evaluate the variability of the biometric characteristics of *D. staudingeri* according to agroecological zone, and to describe the phylogenetic relationships in *D. staudingeri* population in Cameroon.

Materials and methods

Sample and data collection Sampling sites

The study was carried out during two years (from February to April 2019 and from February to April 2020) in two agroecological zones of Cameroon: The Western Highlands and the Bimodal Rainfall Forest zones. The Western Highlands zone, whose coordinates are 4.79°- 6.43° northern and 9.68°-

11.52° eastern has a surface area of 31.192 km² and rainfall between 1500 and 2000 mm/year, with 180 days of rain. The soils are fertile and suitable for agricultural activities, with plateaux enriched with volcanic materials. The main crops grown include coffee, corn, beans, potatoes, and various leafy and root vegetables. The dominant vegetation consists of gallery forests and raffia reliques in the lowlands, with an herbaceous stratum of *Pennisetum purpureum* and *Imperata cylindrica*. More than 80% of the human population of the area comes from the Grassfield communities and 20% from other Regions of the country (Nanganoa *et al.*, 2020). The Bimodal Rainfall Forest zone of Cameroon lies between 2°6" to 4° northern and 10.50° to 16.2° eastern, with the following characteristics: surface area of 165.770km², rainfall between 1500 and 2000 mm/year with two distinct rainy seasons (Moudingo, 2007). The soils are ferrallitic, acidic and clayey, and the main crops are cocoa, coffee, corn, and various fruit trees (Nanganoa *et al.*, 2020). Bees's samples were collected (following a non-invasive approach) in five administrative divisions of the Western Highlands zone (Nde, Hauts-Plateaux, Koung-Khi, Menoua, and Haut-Nkam Divisions) and two divisions of the Bimodal Rainfall Forest zone of Cameroon (Lom-and-Djerem and Haut-Nyong Divisions). The number of sites per division depended on the availability of these bees. The geographical coordinates of each collection point are therefore given in table 1.

Table 1. Geographical coordinates of each collection point according to study area

Divisions	Subdivisions	Latitude	Longitude	Altitude (m)
Nde	Bassamba	05°01.690'	010°36.796'	926
Nde	Bassamba	05°03.352'	010°34.283'	1007
Haut-Nkam	Banka	05°10.720'	010°11.965	1387
Haut-Nkam	Banka	05°10.536'	010°11.965	1387
Haut-Nkam	Banka	05°10.714	010°11.988	1401
Haut-Nkam	Banka	05°10.669	010°11.994	1389

W ter H i g h l a n d Z o n e	Koung-Khi	Bandjoun	05°21.643	010°24.804'	1416
	Koung-Khi	Bandjoun	05°16.553	010°20.205'	1707
	Koung-Khi	Bandjoun	05°20.045'	010°21.019'	1518
	Koung-Khi	Bayangam	05°17.016'	010°26.945	1621
	Koung-Khi	Bayangam	05°16.914'	010°26.750'	1682
	Koung-Khi	Bayangam	05°16.871'	010°26.896'	1636
	Koung-Khi	Bayangam	05°17.286'	010°27.137	1590
	Koung-Khi	Bayangam	05°17.267	010°26.899'	1560
	Koung-Khi	Bayangam	05°17.286'	010°26.888'	1519
	Koung-Khi	Demdeng	05°17.287'	010°27.137	1543
	Koung-Khi	Demdeng	05°19.436	010°27.075'	1567
	Menoua	Dschang	05°27.442	010°05.254'	1480
	Menoua	Dschang	05°27.884	010°05.718'	1447
	Menoua	Dschang	05°26.043'	010°03.302'	1336
	Menoua	Dschang	05°25.206'	010°03.302'	1378
	Menoua	Dschang	05°25.334'	010°03.218'	1405
	Menoua	Dschang	05°26.051'	010°02.378'	1330
	Menoua	Dschang	05°24.467'	010°01.179'	1406
	Menoua	Dschang	05°26.827'	010°04.394'	1402
Z o n e e s t f o r n f a l l R a i d a l i m o B	Menoua	Dschang	05°26.823'	010°04.130'	1395
	Menoua	Dschang	05°26.335'	010°04.225'	1477
	Menoua	Dschang	05°27.627'	010°02.003'	1323
	Menoua	Dschang	05°27.680'	010°01.999'	1308
	Menoua	Dschang	05°27.782'	010°02.076'	1316
	Menoua	Dschang	05°27.780'	010°02.344'	1333
	Menoua	Dschang	05°27.826'	010°02.400'	1346
	Menoua	Dschang	05°27.885'	010°02.394'	1371
	Menoua	Santchou	05°16.552'	010°00.486'	703
	Menoua	Santchou	05°34.291'	010°01.262'	636
	Menoua	Santchou	05°34.256'	010°01.037'	700
	Haut-Nyong	Lomié	03°19.942'	013°35.467'	657
	Haut-Nyong	Lomié	03°19.700'	013°35.350'	623
	Haut-Nyong	Lomié	03°20.303'	013°35.410'	707
	Haut-Nyong	Lomié	03°20.307'	013°35.409'	674
	Haut-Nyong	Lomié	03°20.310'	013°35.413'	687
	Lom-et-Djérem	Bertoua	04°33.214'	013°37.766'	659
	Lom-et-Djérem	Bertoua	04°33.158'	013°37.552'	665
	Lom-et-Djérem	Bertoua	04°33.109'	013°37.426'	656
	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°33.418'	013°37.283'	658
	Lom-et-Djérem	Bertoua	04°33.586'	013°37.983'	673
	Lom-et-Djérem	Bertoua	04°33.650'	013°37.749'	641
	Lom-et-Djérem	Bertoua	04°33.100'	013°37.227'	660

Figure 1 below was created using these coordinates

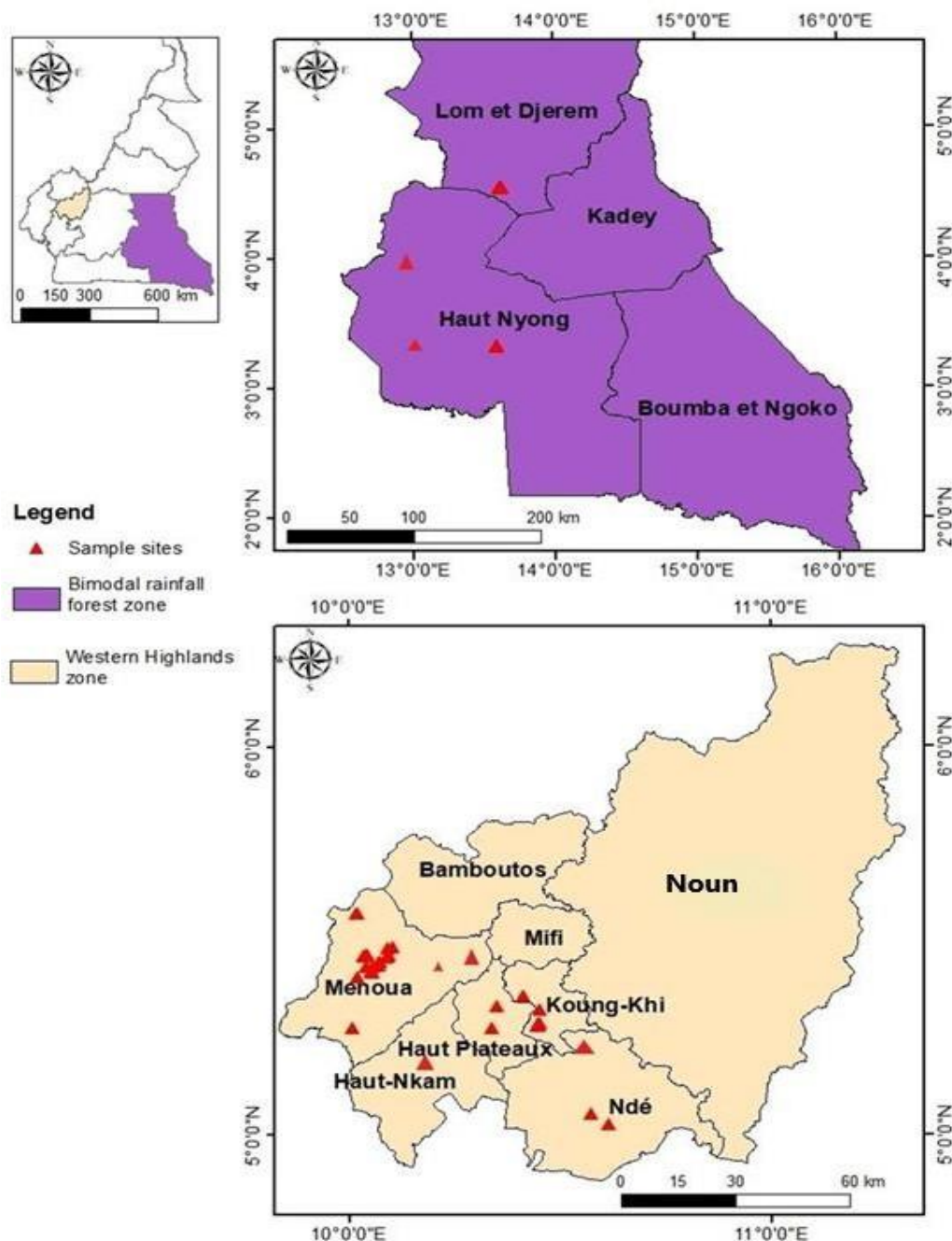


Figure 1. Map of the Study area

Specimen Collection and Taxonomic Identification

The bees were collected at the entrance to their nests usually found hanging from a tree or a building in their natural habitat. The local human populations do not farm stingless bees. A total of 797 worker bees (550 from the Western Highlands zone and 247 from the forest zone with bimodal rainfall) of *D. staudingeri* were collected from 49 colonies, approximately 30 individuals per colony, using a non- probabilistic method, and preserved in 70% alcohol (Franck, 2013) for a two-week period. Measurements were conducted exclusively on worker bees, since haploid males do not accurately represent the genetic diversity of the population. Species identity was confirmed using the classification key established by Michener (2007) which separates the genus *Dactylurina* into two species. *D. staudingeri* in Central Africa and *D. schmidtii* in East Africa. The difference between these two species lies in the villi of the head; the genus *D. staudingeri* has a less hairy head than *D. schmidtii* (Eardley, 2004).



Figure 2. Worker of *Dactylurina staudingeri*

Biometric Data Collection

Measurement step occurred at the National Veterinary Laboratory (LANAVET) Annex of Yaoundé in 2020 and the Biotechnology and Bioinformatics Research Unit at the University of Dschang in 2023 following standardized protocols according to Quezada-Euan *et al.*, 2007; Ndungu *et al.* 2017. Once in the laboratory, the bees were dissected, and their body parts were mounted on slides and examined under a microscope. Measurements were obtained using pre-installed software.

The biometric data were recorded following the protocol described by Quezada-Euan *et al.*, (2007) and Ndungu *et al.*, (2017) using classical biometric methods and included 16 measurements: body length (BL); abdomen length (AL); antenna length (AnnL); had length (HL); head width (HW); ocello-ocular distance (OOD); distance between two lateral ocelli (DBO); femur length (FL); metatarsus length (ML); tibia length (TL); tibia width (TW); metatarsus width (ML); right forewing length (RFL); right forewing width (RFW); right hindwing length (RHL); right hindwing width (RHW) (Fig. 2). These variables were taken after the dissection of the bee had been dissected and placed in a petri dish and then mounted on a Zeiss stereo microscope equipped with a camera and Zen lite measurement software (Stemi 2000- C).



Figure 3. Major body measurements in *Dactylurina staudingeri*

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

Data 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. The degree and significance relationship between different body measurements were determined by Pearson's correlation coefficient. Principal Component Analysis (PCA) was used to investigate the population structure of the *D. staudingeri*

population. Discriminant factor analysis was used to distinguish the different phenotypic types present in the *D. staudingeri* population. Discriminant Factor Analysis (DFA) and Hierarchical Ascending Classification (HAC) were also used to highlight the different phenotypic types and establish clusters of individuals in the population according to their resemblance base on their biometrical measurements.

Results

Biometric Characteristics of *D. staudingeri* Population in Cameroon

Table 2 shows all the biometric characteristics of *D. staudingeri* in the different agroecological zones.

Table 2. Biometric measurements of *D. staudingeri* in Cameroon

Variables	Agro-ecological zones						p
	Western Highlands zone		Bimodal Rainfall forest		All sites		
	X±σ	CV(%)	X±σ	CV(%)	X±σ	CV(%)	
BL	5.703±0.340 ^a	5.961	5.722±0.458 ^a	8.000	5.709±0.380	6.656	ns
AL	3.208±0.322 ^a	10.037	3.286±0.410 ^b	12.447	3.232±0.353	10.922	0.004
HL	1.420±0.065 ^a	4.577	1.399±0.061 ^b	4.360	1.414±0.065	4.597	0.009
HW	1.926±0.059 ^a	3.063	1.959±0.082 ^b	4.185	1.936±0.068	3.512	0.000
DBO	0.344±0.031 ^a	9.012	0.345±0.038 ^a	11.014	0.345±0.033	9.565	ns
OOD	0.331±0.033 ^a	9.700	0.333±0.043 ^a	12.913	0.331±0.037	11.178	ns
AnL	2.171±0.099 ^a	4.560	2.240±0.129 ^b	5.760	2.185±0.111	5.080	0.000
RFL	4.992±0.255 ^a	5.108	4.941±0.229 ^b	4.635	4.976±0.248	4.984	0.007
RFW	1.748±0.098 ^a	5.606	1.735±0.106 ^a	6.110	1.744±0.101	5.791	ns
RHL	3.364±0.118 ^a	3.508	3.358±0.171 ^a	5.092	3.362±0.137	4.075	ns
RHW	0.798±0.051 ^a	6.391	0.795±0.059 ^a	7.421	0.797±0.053	6.650	ns
FL	1.395±0.123 ^a	8.817	1.382±0.067 ^a	4.848	1.391±0.109	7.836	ns
TL	1.949±0.117 ^a	6.003	1.992±0.097 ^b	4.870	1.962±0.113	5.759	0.000
TW	0.701±0.047 ^a	6.705	0.717±0.051 ^b	7.113	0.706±0.051	7.224	0.000
ML	0.658±0.082 ^a	12.462	0.667±0.061 ^a	9.145	0.661±0.076	11.498	ns
MW	0.340±0.032 ^a	9.412	0.345±0.038 ^a	11.015	0.341±0.033	9.677	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; $X \pm \sigma$: mean and standard deviation; n: number of individuals. body length (BL); abdomen length (AL); antenna length (ANL); head length (HL); head width (HW); ocello-ocular distance (OOD); distance between two lateral ocelli (DBO); femur length (FL); metatarsus length (ML); tibia length (TL); tibia width (TW); metatarsus width (MW); right forewing length (RFL); right forewing width (RFW); right hindwing length (RHL); right hindwing width (RHW)

The analysis showed that 43.75% of these variables were significantly different ($p < 0.05$) between the two agro-ecological zones, i.e. abdominal length, head length and width, antenna length, tibia length and width, and right forewing length. In particular, bees from the bimodal rainforest zone showed a high value for most of the characters, 31.25% of the total number for parameters such as abdomen length (3,286 mm vs. 3,208 mm), head width (1.959 mm vs. 1.926 mm), antenna length (2,240 mm vs. 2,171 mm), respectively tibia length and width (1.99mm vs. 1.949mm for the length and 0.717mm vs. 0.701 mm for the width) compared to 12.50% for the Western Highlands zone for parameters such as head length (1,420 mm vs. 1,399 mm) and right forewing length (4,992 mm versus 4,941 mm).

Population means were 3.232 mm for abdominal length, 1.414 mm for head length, 1.936 mm for head width, 2.185 mm for antenna length, 4.976 mm for right forewing length, 1.962 mm for tibial length and 0.706 mm for tibial width.

The correlation between body measurements in *D. staudingeri* in Cameroon are presented in Table 3.

Table 3. Correlation between body measurements in *D. staudingeri* in Cameroon (Central Africa)

Variables	BL	AL	HL	HW	AnL	RFL	RFW	RHL	RHW	FL	TL	TW	ML	MW	DBO	OOD
BL	1.000															
AL	0.659**	1.000														
HL	0.162**	ns	1.000													
HW	0.177**	ns	0.291**	1.000												
AnL	0.148**	0.081*	0.190**	0.300**	1.000											
RFL	0.313**	ns	0.244**	0.352**	0.145**	1.000										
RFW	0.206**	ns	0.114**	0.211**	0.139**	0.425**	1.000									
RHL	0.226**	0.149**	0.237**	0.241**	0.158**	0.268**	0.153**	1.000								
RHW	0.134**	0.123**	ns	ns	ns	0.120**	ns	0.169**	1.000							
FL	0.200**	ns	0.108**	0.235**	0.139**	0.309**	0.278**	0.163**	ns	1.000						
TL	0.184**	ns	0.189**	0.337**	0.164**	0.329**	0.266**	0.220**	ns	0.302**	1.000					
TW	0.064**	ns	0.147**	0.195**	ns	0.153**	0.100**	ns	0.092**	0.117**	0.169**	1.000				
ML	0.143**	-0.137**	0.091*	0.218**	0.144**	0.347**	0.331**	0.114**	ns	0.256**	0.301**	0.113**	1.000			
MW	0.152**	ns	0.123**	0.131**	ns	0.208**	0.119**	0.119**	ns	0.192**	0.198**	0.102**	0.245**	1.000		
DBO	0.098**	0.152**	0.083*	0.134**	0.086**	ns	ns	0.104**	0.087*	0.083*	0.095**	ns	-0.113**	ns	1.000	
OOD	ns	ns	0.085*	0.154**	0.128**	ns	ns	0.072*	ns	ns	ns	0.084*	-0.076*	ns	0.133**	1.000

**Correlation is significant at the 0.05 level (2-tailed); *Correlation is significant at the 0.01 level (2-tailed). body length (BL); abdomen length (AL); antenna length (ANL); head length (HL); head width (HW); ocello-ocular distance (OOD); distance between two lateral ocelli (DBO); femur length (FL); metatarsus length (ML); tibia length (TL); tibia width (TW); metatarsus width (ML); right forewing length (RFL); right forewing width (RFW); right hindwing length (RHL); right hindwing width (RHW)

This table shows that there was a positive correlation ($p < 0.05$) between body length and abdomen length ($r = 0.659$). From the output of the correlation, it was observed that the length of the metatarsus was negatively correlated ($p < 0.05$) with the abdominal length ($r = -0.137$) same as metatarsus length and distance between ocelli ($r = -0.113$), the body length was positively correlated with all the other variables except with ocello-ocular distance which was non-significant.

Phenotypic variability of the *D. staudingeri* population

The accumulated eigenvalue and variance of the different Principal Components in the analysis of the variability observed in the *D. staudingeri* population in Cameroon are presented in Table 4.

Table 4. Analysis of the principal components of *D. staudingeri* in Cameroon.

Principal components	Eigenvalue	Variability (%)	Cumulated Variability
F1	3.346	20.910	20.910
F2	1.704	10.650	31.561
F3	1.367	8.543	40.104
F4	1.052	6.572	46.676
F5	0.980	6.125	52.800

F: Principal component, %: percent

In this table, the first five components explain 52.80% of the phenotypic variability observed in the population of *D. staudingeri* in Cameroon.

Table 5 summarizes the coefficients of the quantitative variables associated with the principal components.

Table 5. Contribution of the variables to explain the phenotypic variability observed in the population of *D. staudingeri* in Cameroon.

Variables	F1	F2	F3	F4	F5
BL	8.592	16.562	17.633	1.551	0.080
AL	1.907	35.445	10.650	2.462	0.939
HL	6.003	0.475	6.498	0.715	5.818
HW	11.352	0.002	8.795	1.484	0.314
AnL	4.816	0.395	5.963	18.117	7.830
RFL	14.297	1.675	1.106	2.109	1.769
RFW	8.806	4.595	2.688	0.412	10.360
RHL	7.492	2.302	1.121	5.056	0.783
RHW	1.086	5.524	0.156	46.059	3.382
FL	8.488	2.030	1.294	0.056	2.178
TL	10.936	1.920	0.651	0.104	0.064
TL	3.252	0.011	4.706	13.658	26.650
ML	7.416	15.019	2.739	0.242	0.001
MW	4.320	1.822	2.527	4.149	33.137
DBO	0.981	9.225	7.475	0.054	6.304
OOD	0.258	2.999	25.998	3.772	0.391

body length (BL); abdomen length (AL); antenna length (ANL); head length (HL); head width (HW); ocello- ocular distance (OOD); distance between two lateral ocelli (DBO); femur length (FL); metatarsus length (ML); tibia length (TL); tibia width (TW); metatarsus width (ML); right forewing length (RFL); right forewing width (RFL); right hindwing length (RHL); right hindwing width (RHW), F1:, F2:, F3:, F4:, F5:, %:

This table allows identifying the variables that can be used to discriminate against the *D. staudingeri* population in Cameroon. It appears that the top three principal components that best explain the total phenotypic variability observed in the *D. staudingeri* population studied are right forewing Length (14.297%), abdomen Length (35.445%), and ocello-ocular distance (25.998%). All these values contribute 75.740% to the explanation of the total phenotypic variability.

Population Structure of *D. staudingeri* in Cameroon

In addition to PCA. HCA has been used to determine the population structure of *D. staudingeri* according to phenotype (Fig. 4) in Cameroon.

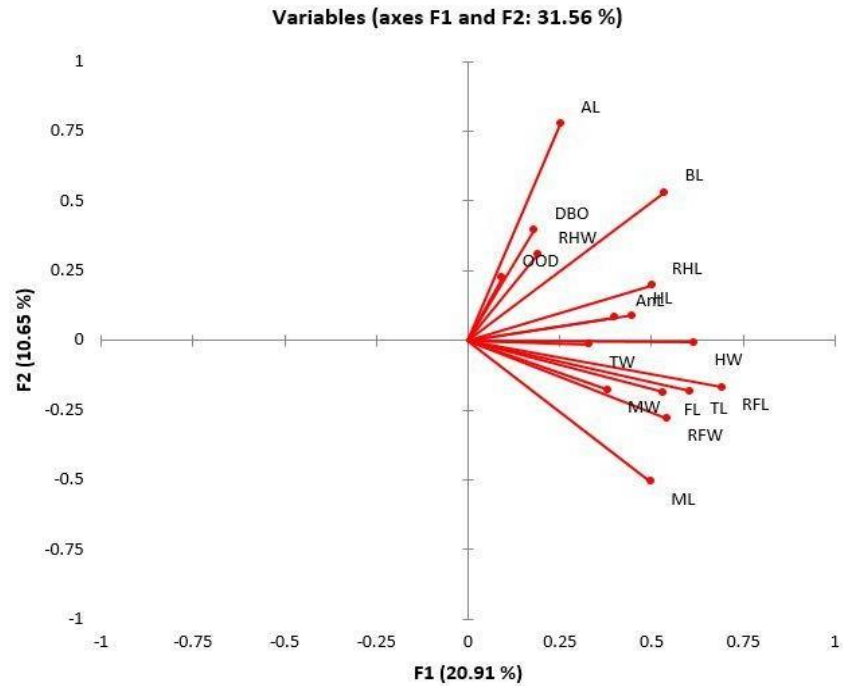


Figure 4: Variable distribution graph of *D. staudingeri* in Cameroon- Central Africa
AnL: Antenna length; *AL:* Abdomen length; *BL:* Body length; *DBO:* Distance between two lateral ocelli; *FL:* Femur length; *HL* and *HW:* Head length and width; *ML:* Metatarsus length. *MW:* Metatarsus width; *OOD:* Ocello-ocular distance; *RFL* and *RFW:* Right forewing length and Right forewing width; *RHL* and *RHW:* Right hindwing length and Right hindwing width; *TL:* Tibia length; *TW:* Tibia width.

Discriminant factor analysis has been used to identify the different phenotypes that exist in the *D. staudingeri* population in Cameroon (Table 6).

Table 6. Average population characteristics of *D. staudingeri* according to phenotypic types in Cameroon- Central Africa

Variable	Morphotype		
	M1	M2	M3
BL	5.560	5.714	5.867
AL	3.134	3.180	3.422
HL	1.624	1.527	1.540
HW	1.948	1.937	1.919
AnL	2.179	2.179	2.188
RFL	4.927	5.038	4.934
RFW	1.708	1.784	1.732
RHL	3.384	3.353	3.350
RHW	0.806	0.791	0.797
FL	1.432	1.523	1.398
TL	1.974	1.988	1.924
TW	0.704	0.720	0.688
ML	0.647	0.688	0.641
MW	0.337	0.344	0.341
DBO	0.346	0.336	0.356
OOD	0.344	0.324	0.329

M1: Morphotype 1; M2: Morphotype 2; M3: Morphotype 3

Table 6 and Fig.4 show that the *D. staudingeri* species in the study area has three morphotypes. Morphotype 2 has higher variables than types 1 and 3.

Phylogenetic relationships between genetic types of *D. staudingeri*

Phylogenetic tree analysis was used to separate the different morphotypes (Fig.5) observed in the *D. staudingeri* population in Cameroon, and the phenotypic distances (Table 7) separating these morphotypes were obtained.

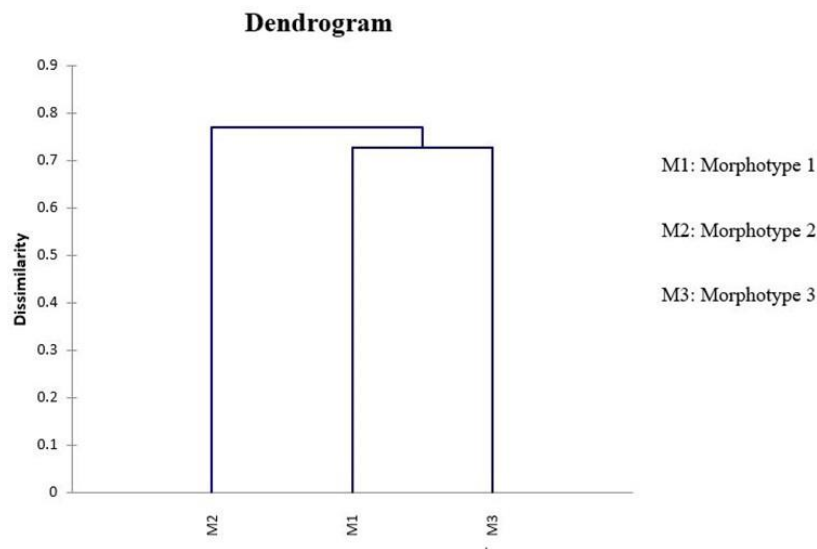


Figure 5. Phylogenetic Dendrogram of the *D. staudingeri* population in Cameroon

Morphotypes 1 and 3 are closer and may seem to have a large number of alleles in common and could therefore have a common origin. Whereas morphotypes 2 and 3 are further apart and seem to have few alleles in common. This is confirmed by Table 7 which shows the phenotypic distances between the phenotypes.

Table 7. Distance between the Barycentre of the phenotypic type classes of *D. staudingeri* in Cameroon-Central Africa

Morphotypes	1	2	3
1	-		-
2	0.2561	-	
3	0.4373	0.3451	0

Discussion

This study is a pioneering work on the biometrics of *D. staudingeri* in Africa, focusing on 16 variables in the Western Highlands of Cameroon, covering five divisions (Nde, Koung-Khi, Hauts-Plateaux, Menoua, and Haut-Nkam), and in the bimodal rainfall forest zone, covering two divisions (Hauts-Nyong and Lom-et-Djerem).

According to Francoy *et al.* (2006), morphometric analyses have proven to be highly effective in identifying African bees.

For this bee species, body length in the population ranges from 5.502 mm to 5.924 mm, which aligns with the results of Eardley (2004), who reported a range of 5.07 to 7 mm. This also corroborates the findings of Sayusti *et al.* (2020), who reported an average body length of 5.600 ± 0.300 mm for *Wallacetrigona incisa*. It is also consistent with the results of Azizi *et al.* (2020) for *Heterotrigona itama* in Belitung, Indonesia, where the body length was 5.040 mm. However, it differs from the findings of Trianto and Purwanto (2020) in Yogyakarta Province and Purwanto and Trianto (2021) in Java Province, Indonesia, where the body length of *Heterotrigona itama* and *Lepidotrigona terminata* was 4.840 mm and 4.610 mm, respectively. Additionally, it differs from the findings of Rasmussen (2013) in India for *Lepidotrigona arcifera*, which had a body length of 4.700 mm, and from Vijayakumar and Jeyarraj (2017) in India for *Tetragonula iridipennis*, which had a body length between 3.25 and 3.84 mm.

Regarding the parameters of tibia length and width, femur length, and metatarsus length and width, the population averages were 1.966 mm, 0.706 mm, 1.461 mm, 0.662 mm, and 0.341 mm, respectively. These results contradict those obtained by Kishan et al. (2017) for *Tetragonula iridipennis*, where the tibia and metatarsus length and width fell within the ranges of 1.31-1.62 mm, 0.43-0.49 mm, 0.42-0.57 mm, and 0.35-0.51 mm; they also differ from Araujo (2004), who reported a femur length of 2.150 mm, and from Rasmussen (2013) for *Lepidotrigona arcifera*, where the tibia length and width and metatarsus width were

1.43 mm, 0.57 mm, and 0.26 mm, respectively. Similarly, Vijayakumar and Jeyaraj (2017) reported tibia length and width ranges of 1.31-1.53 mm and 0.41-0.53 mm, respectively. Mohd Saufi and Karumu (2015) found tibia length and width for *Geniotrigona thoracica* ranging from 1.21-4.36 mm and 0.91-3.81 mm, respectively. However, our results align with those of Purwanto and Trianto (2021) for *Heterotrigona itama*, where the femur length, tibia length and width, and metatarsus length were 1.46 mm, 2.10 mm, 0.67 mm, and 0.69 mm, respectively. Additionally, they align with Trianto and Purwanto (2020) for *Lepidotrigona terminata*, where the tibia and metatarsus length and width were

2.15 mm, 0.74 mm, 0.70 mm, and 0.47 mm, respectively. They also match the findings of Ade et al. (2019) for *Tetragonula laeviceps*, where the metatarsus length and width and tibia width were 0.668 mm, 0.324 mm, and 0.581 mm, respectively.

Regarding the parameters of right forewing and hindwing length and width, the population averages were 4.976 mm and 1.747 mm for the forewing, and 3.362 mm and 0.797 mm for the hindwing. These results are consistent with those of Purwanto and Trianto (2021) for *H. itama*, *Lepidotrigona terminata*, and *Tetragonula drescheri* in Indonesia, where the right forewing length and width were 5.280 mm and 1.770 mm for *H. itama*, 5.270 mm and 2.010 mm for *L. terminata*, and 5.27 mm and 2.21 mm for *T. drescheri*. They also match the results of Trianto and Purwanto (2020), who found 4.550 mm for forewing length and 2.000 mm for forewing width in *Tetragonula sarawakensis*. However, they differ from Purwanto and Trianto (2021) for *T. biroi*, where the values were 3.830 mm for forewing length,

1.210 mm for forewing width, 2.340 mm for hindwing length, and 0.600 mm for hindwing width. They also differ from Ade et al. (2019), who obtained 4.265 mm and 1.493 mm for forewing length and width, respectively, and from Vajayakumar and Jeyaraj (2017), who reported forewing length ranges of 3.280-3.410 mm and hindwing length ranges of 1.180-1.360 mm.

These differences may be due to adaptation to the environment and the availability of food resources. The bimodal rainfall forest zone, compared to the Western Highlands zone, has a greater and well- preserved floral diversity, facilitating resource foraging by bees. According to Novita et al. (2013), changes in temperature or environmental conditions can lead to morphological adaptations in some living organisms as an adjustment for flight and foraging activity.

There is a significant positive correlation between body length and abdomen length ($r = 0.659$). It was observed that metatarsus length was negatively correlated with abdomen length, meaning that as the abdomen length increases, so does the body length. This is advantageous, as a larger bee has a greater pollen collection capacity. The abdomen contains the crop, an organ used for storing liquids such as water, nectar, and other useful substances.

The correlation values obtained between the other measured variables were either significant or non-significant. Body length was positively correlated with all 15 other variables, except for the eye-ocellar distance, which remained almost constant regardless of body size. These correlations indicate which characteristics should be selected if the goal is to improve this species.

PCA results show a high phenotypic variability within *D. staudingeri* populations across different study zones. For this population, the first five principal components explain 52.80% of the total observed variability. These results differ from those of Ndungu (2010) for *Hypotrigona gribodoi* and *Meliponula bocandei*, where the first two components explained 80.85% and 61.99% of the total observed variability, respectively. They also differ from Edzeani (2014) for *Hypotrigona* in Ghana, where the first 11 components explained 98.812% of the total observed variability, with the first three explaining 72.093%.

The variables that can be used to discriminate *D. staudingeri* populations are right hindwing length (46.059%), abdomen length (35.445%), tibia width (26.650%), eye-ocellar dorsal distance (25.998%), and right forewing length (14.297%). These results differ from those of Trianto and Purwanto (2020), who identified tibia length, forewing length with the tegula, and body length as the main discriminating variables.

Discriminant factor analysis identified three distinct morphological types. Phenotypic type 2 exhibited higher measurements than types 1 and 3 for most traits. Types 1 and 3 were closer to each other and shared many characteristics, suggesting a common origin, while types 2 and 3 were more distinct.

Overall, phenotypic type 2 could be used for potential breeding programs to improve this species due to its superior traits.

Conclusion and perspectives

This study aimed to provide updated information on *D. staudingeri* species and to contribute to the expansion of meliponiculture in Cameroon specifically in the Western Highlands and the Bimodal Rainfall Forest zones of Cameroon. The results showed that there was a clear difference in variables between the two

study zones with the bees in the bimodal rainfall forest zone being the largest. The population dendrogram showed the presence of three morphotypes with types 1 and 3 being close to morphotypes 2 and 3.

In order to deepen this investigation, it would be necessary to characterize these stingless bees using molecular tools to confirm the results obtained, to extend this study to other agro-ecological zones in Cameroon and neighboring countries where the species is found, to test and validate a sustainable management program for these stingless bee species for honey production.

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Authors declare that they do not have any conflict of interest

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Ethics

All ethical considerations were taken into account during this study and the bee colonies were left intact in their environment.

References

- Ade E., Tri A., Taruni S. P. 2019.** Morphological characteristics and morphometric of Stingless Bee (Apidae: Hymenoptera) from Banten Province, Indonesia. *Biodiversitas*. **20** (6):1693-1698. <https://doi.org/10.13057/biodiv/d200627>
- Araújo E. D., Costa M., Chaud-Netto J., Fowler H. G. 2004.** Body size and flight distance in stingless bees (*Hymenoptera: Meliponini*): inference of flight range and possible ecological implications. *Brazilian Journal of Biology*, 64 (3B): 563-568. <https://doi.org/10.1590/S1519-69842004000400003>.
- Azizi M. G., Priawandiputra W., Raffiudin R. 2020.** Morphological identification of stingless bees from Belitung. IOP Conf. Series: *Earth and Environmental Science* 457: 012011. <https://doi.org/10.1088/1755-1315/457/1/012011>
- Bafo W. Seifu E. Molapisi M. and Sonno K 2019.** Meliponiculture and physiochemical properties of honey produced by the African stingless bees *Plebeina hildebrandti* Friese in Kalakamati village. Botswana. *Botswana Journal of Agriculture and Applied Sciences*. 13(1):33-42. <https://doi.org/10.37106/bojaas.2019.13>
- Bobadaye BO. Fombong TA. Kiatoko N. Raina S. Teal P. Torto B 2016.** African meliponine bees (*Hymenoptera: Apidea*) maintained in man-made hives as potential hosts for the small hive beetle. *Aethina tumida* Murray (*Coleoptera: Nitidulidae*). Peerj Preprints. 27p. <https://doi.org/10.7287/peerj.preprints.2310v1>
- Blanckenhorn WU. 2000.** The evolution of body size: What keeps organisms small? *Quarterly Review of Biology*. 75(4): 385-407. <https://doi.org/10.1086/393620>
- Carla MS. Samira MPCs. Andreia SN. Fabiane LS. Adriana RB. Carlos ALC 2021.** Chemical and

- microbiological characterization of “samburá” from two stingless bee species. *Journal of Apicultural Research*. <https://doi.org/10.1080/00218839.2021.1918408>
- Carneiro MJ. López BGC. Lancellotti M. Franchi GC. Nowill AE. & Sawaya ACHF 2016.** Evaluación de la composición química y la actividad biológica de los extractos de propóleos de *Tetragonisca angustula* y *Schinus terebinthifolius* Raddi (Anacardiaceae). *Journal of Apicultural Research*. 55(4):315–323. <https://doi.org/10.1080/00218839.2016.1243295>
- Costantin CR. Oliveira HG. Marcia RP 2022.** Evaluation of the antifungal activity of propolis extracts from stingless bees on phytopathogenic fungi. *Research. Society and Development*. 11(16): 1-9. DOI: <http://dx.doi.org/10.33448/rsd-v11i16.38445>
- Cockburn CL. Kwapong PW. Wubah DA. Wubah J. 2013.** Shelf-life and Variances in Antimicrobial Properties of Honey from *Meliponula bocandei* and *Meliponula ferruginea* in central Ghana. *Journal of Young Investigators*. 25(1):10-14.
- Cortopassi-Laurino M. Imperatriz-Fonseca VL. Roubik DW. Dollin A. Heard T. Aguilarf I. Venturieri GC. Eardley C. and Nogueira-Neto P 2006.** Global meliponiculture: challenges and opportunities. *Apidologie* 37: 275-292. <https://doi.org/10.1051/apido:2006027>
- Eardley CD. 2004.** Taxonomic revision of the African stingless bees (*Apoidea: Apidae: Apinae: Meliponini*). *African Plant Protection*. 10(2). 63–96.
- Eardley CD. Kuhlmann M. Pauly A 2010.** Les genres et sous-genres d’abeilles de l’Afrique subsaharienne Abc Taxa 9 : 1-144. http://www.abctaxa.be/volumes/volume-9-les-genres-et-sous-genres-dabeilles-de-abctaxa_vol9lr.pdf/download/index_html
- Edzeani S. 2014.** Species variability in Afrotropical genus *Hypotrigona* (Cockerell) within three districts in the Central Region of Ghana. Thesis submitted Master of Physiology degree in Entomology Department of Entomology and Wildlife of the School of Biological Sciences, University of Cape Coast, 153p.
- Franck A. 2013.** Capture, conditionnement, expédition, Mise en collection des insectes et acariens en vue de leur identification. CIRAD réunion (2008). Réédition 2013. 50p.
- Francoy T. M., Bonatti V., Viraktamath B. R. R. 2016.** Wing morphometrics indicates the existence of two distinct phenotypic clusters within population of *Tetragonula iridipennis* (Apidae: Meliponini) from India. *Insectes Sociaux*. 63(1):109-115. DOI:10.1007/s00040-015-0442-2
- Grüter C. 2020.** Stingless Bees: Thier Behavior. Ecology and Evolution. *School of biology sciences*. University of Bristol. Bristol. UK. 385p.
- Heard TAL. 1999.** The role of stingless bees in crop pollination. *Annual review of entomology* 44 (1): 183-206. <https://doi.org/10.1146/annurev.ento.44.1.183>
- Kiatoko N. Van LF. Raina SK 2018.** Forest degradation influence nesting site selection of Afrotropical stingless bees’ species in a tropical rain forest. Kenya. *African Journal of Ecology*. 56 (3): 669-674. <https://doi.org/10.1111/aje.12491>
- Kiatoko N. Raina SK. Langevelde F 2016.** A vertical compartmented hive design for reducing post-harvest colony losses in three afrotropical stingless bee species. *International Journal of Development Research*. 6(8): 9026-9034. : <https://www.researchgate.net/publication/307593880>
- Kishan M. T., Srinivasan M. R., Vijayakumar K., Natarajan N., Mohan Kumar S. 2017.** Morphometry analysis of stingless bee *Tetragonula iridipennis* Smith (1854). *International Journal of Current Microbiology and Applied Sciences*, 6 (10): 2963-2970. <https://doi.org/10.20546/ijcmas.2017.610.350>
- Kwapong P. Aidoo K. Combey R 2010.** Stingless Bees – Importance. Management. and Utilization: A Training Manual for Stingless Beekeeping. *Unimax Macmillan Ltd.* Accra.
- Lima MAP. Martins GF. Oliveira EE. Guedes RNC 2016.** Agrochemical-induced stress in stingless bees: peculiarities underlying basis. and challenges. *Journal of Comparative Physiology A*. 202:733–747. <https://DOI:10.1007/s00359-016-1110-3>
- Márcia AS. Ana MSP. José ES. 2017.** Pharmacological actions of extracts of propolis of stingless bees (*Meliponini*) *Journal of Apicultural Research*. 2017. <http://dx.doi.org/10.1080/00218839.2016.1260856>
- Michener CD. 2007.** The Bees of the World. 2nd Ed. Baltimore: Johns Hopkins University Press. Maryland. 972 p.
- Michener CD. 2000.** The Bees of the World. *Johns Hopkins University Press*. Baltimore. Maryland. 913 pp.
- Modingo E. 2007.** La situation des forêts au Cameroun. *Société de conservation des forêts au Cameroun. Mouanko-Littoral. Cameroun*. 2007.
- Mohd S. F. N and Kumara T. 2015.** Characterization of nest structure and foraging activity of stingless bee,

- Geniotrigona thoracica* (Hymenoptera: Apidea; Meliponini). Jurnal Teknologi, 77:33 (2015) 69–74.
- Mokaya HO. Kiatoko N. Robert M. Nicolas JV 2022.** Characterization of honey produced by sympatric species of Afrotropical stingless bees (Hymenoptera. *Meliponini*). *Food Chemistry*. 366:130597. <https://doi.org/10.1016/j.foodchem.2021.130597>
- Nanganoa TL. Ngome FA. Suh C. Basga SD 2020.** Assessing Soil Nutrients Variability and Adequacy for the Cultivation of Maize. Cassava. and Sorghum in selected Agroecological Zones of Cameroon. Hindawi. *International journal of Agronomy*. 2020. pp: 20. <https://doi.org/10.1155/2020/8887318>
- Ndungu N. N. 2019.** Discrimination of *Hypotrigona* species (Apidae: Meliponini) in Kenya using nest architecture, cephalic secretions and molecular tools. PhD thesis, Faculty of Natural and Agricultural Sciences, University of Pretoria, Sud-Africa. 90p.
- Ndungu N. Nkoba K. Ciosi M. Salifu D. Nyansera D. Masiga D. Suresh RK. 2017.** Identification of stingless bees (Hymenoptera: Apidae) in Kenya using morphometrics and DNA barcoding. *Journal of Apicultural Research*. 56 (4): 341–343. <https://doi.org/10.1080/00218839.2017.1327939>
- Njoya T. M. (2009).** Diversity of stingless bees in Bamenda Afromontane Forests Cameroon: Nest architecture. behaviour. and labour calendar. PhD Dissertation submitted to Rheinischen Friedrich Wilhelms-University Zu Bonn. 148p.
- Njoya MTM. Wittmann D. Ambebe TF 2016.** Nest architecture of *Dactylurina staudingeri* (Hymenoptera. Apidae. Meliponini) in Cameroon. *International Journal of Research in Agricultural Sciences*. 3(6):335-338.
- Nicolas R. Sartori AA. Honorio MD. Fernanda LC. Santiago BK. Sforcin JM 2021.** Propolis antiviral and immunomodulatory activity: a review and perspectives for COVID-19 treatment. *Journal of Pharmacology* 73(1):281-299. <http://doi:10.1093/jpp/rgaa067>
- Ozarowski M. Karpinski TM. Alam R. Lochynska M 2019.** Pathogen spillover from *Apis mellifera* to a stingless bee. *Proceedings of the Royal Society B* 286. 20191071. <https://doi.org/10.1098/rspb.2019.1071>
- Purkiss T. and Lach L. 2019.** Pathogen spillover from *Apis mellifera* to a stingless bee. *Proceedings of the Royal Society B*. 286 (1908). <https://doi.org/10.1098/rspb.2019.1071>
- Purwanto H. and Manap T. 2021.** Species description, morphometric measurement and identification of stingless bees (Hymenoptera: Apidae: Meliponini) in meliponiculture industry in West Java province, Indonesia, *Serranga*, 26 (1): 13-33.
- Rasmussen C. 2013.** Stingless bees (Hymenoptera: Apidae: Meliponini) of the Indian subcontinent: Diversity, taxonomy and current status of knowledge. *Zootaxa*, 3647(3): 401-428. <https://doi:10.11646/zootaxa.3647.3.1>.
- Ruttner F. 1988.** Biogeography and Taxonomy of Honeybees. Springer-Verlag. Berlin. Heidelberg. RFA.
- Sayusti T., Raffiudin R., Kahono S., Nagir T. 2020.** Stingless bees (Hymenoptera: Apidae) in South and West Sulawesi, Indonésie: morphology, Nest structure, and molecular characteristics. *Journal of Apicultural Research*, 60(1): 143-156. <https://doi.org/10.17576/serangga-2023-2803-20>
- Shehu A. Salwani I. Mohd A.K.R. Harun A. Aziz AA. Haque M. 2016.** Antifungal Properties of Malaysian Tualang Honey and Stingless Bee Propolis against *Candida albicans* and *Cryptococcus neoformans*. *Journal of Applied Pharmaceutical Science*. 6 (2): 44-50. <https://DOI:10.7324/JAPS.2016.60206>
- Slaa EJ. Sánchez LA. Malagodi-Braga KS. Hofstede FE. 2006.** Stingless bees in applied pollination: practice and perspectives. *Apidologie* 37: 293–315. <https://doi.org/10.1051/apido:2006022>
- Tchuenguem FN. Messi J. Pauly A 2002.** Recherches sur l'activité des Apoides sauvages sur le maïs à Yaoundé (Cameroun) et réflexion sur la pollinisation des graminées. *Biotechnologie. Agronomie. Société et Environnement* 6(2):87-98. <https://doi.org/10.1080/00379271.2004.10697411>
- Trianto M. et Purwanto H. 2020a.** Morphological characteristics and morphometrics of Stingless Bees (Hymenoptera: Meliponini) in Yogyakarta, Indonesia. *Biodiversitas*. 21(6): 2619-2628. <https://doi.org/10.13057/biodiv/d210633>
- Vijayakumar K. and Jeyaraj R. 2017.** Morphometric analysis of *Tetragonula irridipennis* (Hymenoptera: Apidae) in Coimbatore district, Tamilnadu, India. *Bulletin of Pure and Applied Sciences*. 36(1): 38-45. www.DOI:10.5958/2320-3188.2017.00006.7
- Quezada-Euan JJG. Paxton RJ. Palmer KA. May Itza W. de J. Tay TW. Oldroyd BP 2007.** Morphological and molecular characters reveal differentiation in a Neotropical social bee. *Melipona beechii* (Apidea: Meliponini). *Apidologie*. 38: 237-258. <https://DOI:10.1051/apido:2007006>
- Quezada-Euan JJG. Nates-Parra G. Marcia MM. Roubik DW. Imperatriz-Fonseca VL. 2018.** The economic and cultural values of stingless bees (Hymenoptera: Meliponini) among ethnic groups of

tropical America. *Sociobiology* 65(4). <https://doi.org/10.13102/sociobiology.v65i4.3447>

Zulhendri F. Chandrasekaran K. Magdalena K. Ravalia M. Kripal K. Fearnley J. Perera CO. 2021.
Antiviral. Antibacterial. Antifungal. and Antiparasitic Properties of Propolis: A Review. *Foods* 10. 1360.
<https://doi.org/10.3390/foods10061360>.