UNIVERSITY OF CAPE COAST

SPECIES VARIABILITY IN AFROTROPICAL GENUS *HYPOTRIGONA* (COCKERELL) WITHIN THREE DISTRICTS IN THE CENTRAL REGION OF GHANA

BY

SANDRA EDZEANI

Thesis submitted to the Department of Entomology and Wildlife of the School of Biological Sciences, University of Cape Coast in partial fulfillment of the requirements for the award of Master of Philosophy degree in Entomology.

JULY 2014

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Name: Sandra Edzeani.

Signature:..... Date:.....

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Name: Prof. Peter Kwapong.

Signature:..... Date:.....

Co-Supervisor's Name: Dr. Rofela Combey.

Signature:..... Date:.....

ABSTRACT

This research was carried out to investigate the species variability within Afrotropical stingless bees of the genus *Hypotrigona* (Cockerell) in three districts in the Central Region of Ghana. *Hypotrigona* species are common visitors to flowering plants in the tropics and subtropics. Structurally, the three known Afrotropical *Hypotrigona* species are very similar and pose difficulty in accurately identifying existing species. In view of this taxonomic impediment, this present study aimed at investigating variation within the Afrotropical genus *Hypotrigona* based on their nest entrance characteristics, the geo-morphometry of their wings and the traditional morphometry of the bees within three districts in the Central Region of Ghana. The research was conducted from April 2013 to November 2013. A total of 5441 bees were sampled from 68 colonies. Using nesting characteristics, geometric morphometrics and traditional morphometrics, three probable species were observed in the study. Precisely 2027 individual right forewings of *Hypotrigona* bees were assessed using geometric morphometrics. Statistical analyses (PCA, CVA, DFA, correlation and Procrustes ANOVA) conducted on the right forewings were statistically significant $p < 0.0001$. All three protocols used for assessing variability identified three different species within the genus as *Hypotrigona gribodoi*, *Hypotrigona araujoi* and *Hypotrigona ruspolii*. Nest entrance characteristics were found to be highly variable in terms of level of aggression and entrance tube length. All three protocols; were effective in distinguishing species within the genus *Hypotrigona* and are recommended for taxonomic analysis of the Afrotropical *Hypotrigona* species.

ACKNOWLEDGEMENTS

Many thanks to Prof. Peter Kwapong, my principal supervisor for the immense contribution towards this work. I wish to express my profound gratitude to Dr. Rofela Combey my co-supervisor, for the assistance provided during the statistical analyses and the encouragements throughout the entire work. I am grateful to Dr. Rofela Combey for the enlightenment on the geometric morphometric protocol as well as her guidance in accurately applying the taxonomic keys used for the traditional morphometry on the genus *Hypotrigona*. My gratitude to the Department of Entomology and Wildlife for making the department's museum accessible throughout the entire work. Many thanks to Dr. Ameyaw of the Department of Biomedical and Forensic Science for providing assistance in acquiring laboratory equipment's for this work. I am very thankful to my family especially to my mum and dad Mr. and Mrs. Felix Edzeani for the financial and emotional support throughout the entire work. Not forgetting my siblings Mrs. Enyonam Gbeti, Ms. Hellen Edzeani and Mr. Daniel Edjeani for their numerous encouragements. I express my thanks to Mr. Rockson Aheto for the support provided in making the work a success. I also thank Mr. Richard Adu and Mr. Jacob Anooson for the assistance they provided on the field during the sampling period.

DEDICATION

To the Edzeani family of Takoradi

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ACRONYMS

A/AET	Absent Entrance Tube
CVA	Canonical Variate Analysis
df	Degrees of Freedom
DFA	Discriminant Function Analysis
L/LET	Long Entrance Tube
M/MET	Medium Entrance Tube
MS	Mean Squares
NED	Nest Entrance Diameter
NETL	Nest Entrance Tube Length
PCA	Principal Component Analysis
S/SET	Small Entrance Tube
SBG	Science Botanical Garden
SS	Sum of Squares
UCC	University of Cape Coast

CHAPTER ONE

INTRODUCTION

Background to the Study

Stingless bees belong to the subfamily Meliponinae, one of the three subfamilies of the family Apidae, order Hymenoptera, which are known to be a large group of eusocial bees (Michener, 2007). Stingless bees can be found in most tropical and subtropical regions of the world; Australia, Africa, Southeast Asia and tropical America (Heard, 1999; Kwapong, Aidoo, Combey and Karikari, 2010).

They are amongst the oldest evolved bees to have been found preserved inside pieces of amber 80 million years ago (Michener and Grimaldi, 1988; Engel, 2000; Engel and Michener, 2013). Stingless bees are said to have evolved before the continents drifted apart from each other, thus, accounting for their presence in all tropical parts of the world (FAO, 2009). They are variable in body size ranging from 2 to 14 mm. Stingless bees can be distinguished from other bees by important taxonomic features such as; the reduction and weakness of the wing venation, and the presence of the penicillum (a brush of long stiff setae located anteriorly) (Sanchez, Kraus and Hernandez, 2007). In addition, they form part of a group of bees with a non-functional sting apparatus (Sanchez et al., 2007). Nevertheless, members have evolved other highly efficient ways of colony defense (Sanchez et al., 2007). This is mainly based on nest architecture, biting,

pulling of hair and the use of mandibular gland secretions as a chemical weapon (Sanchez et al., 2007). Eardley (2004) described six genera of African stingless bees namely *Cleptotrigona, Dactylurina, Meliponula, Plebeina, Hypotrigona* and *Liotrigona.* It is estimated that 400 to 500 different species of stingless bees occur throughout the world and new species are discovered every year (Ruttner, 1988; Danaraddi, Viraktamath, Basavanagoud and Bhat, 2009; Lima, Silvestre and Balestieri, 2013). Estimated numbers of known species so far are 50 in Africa, 300 species in the Americas, 60 in Asia and 10 in Australia (FAO, 2009). Recent studies by Kwapong et al (2010) records 9 species in Ghana. The different species of stingless bees are diverse in size ranging from 2 to 14 mm with some as small as the tiny sweat bees whereas others are slightly bigger than the European honey bee (FAO, 2009). The number of bees in a colony can range from some few hundreds to more than a hundred thousand depending on the species (FAO, 2009).

They nest both in the soil and in wooden materials, (Michener, 2007; Eardley, 2004). The nest design differs among species as well as the habitat in which they are found (Njoya, 2009). They may be found in rock cervices, wall cavities, old rubbish bins, water meters, termite mounds (active or abandoned), anthills, gullies and storage drums (Roubik, 2006). Stingless bees are active throughout the year though less active in cooler climate, with some species presenting diapause (Ribeiro, 2002; Alves, Imperatriz-Fonseca and Santos-Filo, 2009). They are true generalists and collect pollen and nectar from a vast array of plants (Heithaus, 1979; Biesmeijer et al., 2005).

Like all other bees, stingless bees even though small in size are efficient pollinators of agricultural and forest crops as well as tropical and subtropical forest trees which plays a major role in maintaining and conserving biodiversity (Daily et al., 1997; Njoya, 2009). Studies in Ghana, Australia, Japan and Mexico have shown promising results where stingless bees pollinated strawberries and other crops equally as the honey bees (Kakutani, Inoue, Tezuka and Maeta, 1993; Blanche, Ludwig and Cunningham, 2006; Palma et al., 2008; Kwapong et al., 2010). Aside pollination, stingless bees store hive products such as honey and beebread in pots made of plant resins (Vit, 1999; Kwapong et al., 2010). The castes of most stingless bees consist of the queen, drones and workers (Bassindale and Harrison Matthews, 2009). Stingless bees have developed a variety of communication mechanisms to effectively allocate the workers of a colony to different tasks (Wilson, 1971). Foragers of stingless bees not only come to rewarding food source, but also recruit nest mates to do so (Friedrich, Hrncir and Jarau, 2008). This is done using thoracic vibrations and body contacts within the nest, pilot flight as well as footprint secretions and pheromone marks deposited on the field (Angilar, Fonseca and Biesmeijer, 2005; Nunes-Silva, Hrncir and Imperatriz-Fonseca, 2008).

The genus *Hypotrigona* is among the six genera described by Eardley (Eardley, 2004). Generally, the body length of the *Hypotrigona* worker bee ranges from 2 to 3 mm. Their scutum is densely punctate and not shiny, with the dorsal view of scutellum although short, concealing the metanotum (Moure, 1961; Eardley, 2004). The lateral view of the propodeum shows that, the subhorizontal

part is longer than the subvertical region (Eardley, 2004). Further, the hind tibia is rounded at the posterodistal end (Moure, 1961; Eardley, 2004). These are taxonomic characters that distinguish the genus *Hypotrigona* from the other stingless bee species (Eardley, 2004; Kajobe 2007). Members of this genus are cluster builders and nest in cavities which are either tubular or planiform (Lima et al., 2013).

Although three species of the genus *Hypotrigona* have been identified worldwide, taxonomists are unable to adequately distinguish among the species (Eardley, 2004). The species of *Hypotrigona* are difficult to separate based on morphology (Eardley, 2004). Eardley and Kwapong (2013) suggest the need for advanced modern protocols in the identification of species of the genus *Hypotrigona*. Over the years, nesting biology and nest architecture, level of aggression among others has enabled the identification of several stingless bees' species (Woyke, 1992; Roubik, 2006; Kwapong et al., 2010).

One modern protocol that has successfully categorized organisms into their appropriate taxonomic groups is the geometric morphometric technique (Bookstein, 1991). Geometric morphometrics is based on the description of shape in space on the Cartesian coordinates (Bookstein, 1991). It is also a procedure for abstracting and comparing biological forms and it is widely used in systematics (Bookstein, 1991). The protocol has shown promising results in evolutionary biology and ecology as well as in other biological disciplines such as anthropology, paleontology, embryology, cellular biology and the medical sciences (Rohlf and Slice, 1990; Rohlf, 1999; Sansom, 2009; Slice and Ann,

2009). Geometric morphometric technique was used to efficiently discriminate subspecies of *Apis mellifera* into four major evolutionary branches (Ruttner, Tassencourt and Louveaux, 1978; Ruttner, 1988). Combey, Teixeira, Bonatti, Kwapong and Francoy (2013) used geometric morphometrics to reveal morphological differences within four African stingless bee species. Members of the genus *Hypotrigona* were reported as one of the nine stingless bees found in Ghana (Kwapong et al., 2010). However, information on characterization of the genus into various species was limited. Nesting characteristics such as, diameter of tube entrance, entrance tube length, the level of aggression at the tube entrance among others were successfully used to segregate members of the sister genus *Trigona* (Pooley and Michener 1969; Danaraddi et al., 2009). Nest architecture, entrance tube length and level of aggression of organisms including stingless bees is species specific (Pooley and Michener, 1969; Franck et al., 2004; Danaraddi et al., 2009; Lima et al., 2013). Thus, Pooley and Michener (1969) reported the entrance tube length of *Trigona gribodoi* to be short with a range of (6 to 25 mm). There is therefore the need to incorporate nesting characteristics, geometric morphometrics and traditional morphometry in this study to more accurately identify the various species of the genus *Hypotrigona* (Bookstein, 1991; Rohlf, Loy and Corti, 1996; Smith, Crespi and Bookstein, 1997).

Statement of the Problem

Three species have been identified worldwide as the Afrotropical species of the genus *Hypotrigona* namely; *Hypotrigona gribodoi*, *Hypotrigona ruspolii* and *Hypotrigona araujoi* (Michener 1959; Moure 1961; Eardley, 2004). The three

species were previously considered as *Hypotrigona gribodoi* due to the close morphological similarities existing among them (Moure, 1961; Wille and Michener 1973; Michener, 1974). In recent years, molecular data and improved traditional morphometrics have allowed accurate separation of the species to be possible and reduced the inconsistencies.

However, the molecular methods require expensive reagents and laboratory equipment while the traditional morphometrics require high level taxonomic capacity to separate this group of closely related bees. There is therefore the need to explore other cheap modern morphometric techniques that employ recent advances in statistical analysis and image recognition software that easily segregates possible variations within this group. In addition, this present study assesses nest behaviour variability as a possible tool for a more practical and precise analyses for species identification.

Purpose of the Study

The purpose of the study was to explore alternative protocols to enhance the accurate separation of species within the genus *Hypotrigona*.

Objective of the Study

The project aims at investigating species variation within the Afrotropical genus *Hypotrigona* based on their nest entrance characteristics, the geo-morphometry of their wings and the traditional morphometry of the bees within three districts in the Central Region of Ghana.

Research Questions

1. What is the extent of species variability within the genus *Hypotrigona* from the study area?
2. Do all species of *Hypotrigona* construct nest entrance tubes?
3. How variable are the nest entrances of species of the genus *Hypotrigona*?
4. Does nest entrance variability correspond to species variability within the genus *Hypotrigona*?
5. Is the level of nest entrance aggression species specific?
6. Does wing geometric morphometrics correspond to variability within the genus *Hypotrigona?*

Hypothesis

1. Nest entrance characteristics do not correspond to species variability in the genus *Hypotrigona*.
2. There is species variability within the genus *Hypotrigona* in the three districts.
3. Geometric morphometric protocol could not distinguish variation within the genus *Hypotrigona* in the three districts.
4. Nest entrance characteristics do not correspond to species variability within the genus.

Significance of the Study

Stingless bees are common visitors to flowering plants in the tropics and subtropics (Heard, 1999). They are known to visit the flowers of approximately 90 crop species and are confirmed to be effective and important pollinators of

crops (Heard, 1999; Kwapong et al., 2010). Stingless bees are also economically important for production of medicinal honey, various hive products as well as their value in aesthetics. Their importance in ecotourism has also been emphasized (Kwapong et al., 2010). Accurate identification of key species in any ecological area is thus relevant for the maximization of species importance.

Structurally, the three known Afrotropical *Hypotrigona* species are very similar and have for many years been misidentified by various authors (Portugal-Araujo, 1955; Moure, 1961; Wille and Michener 1973; Michener, 1974). The difficulty in applying clearly defined morphological features and the limited taxonomic capacity in many institutions compounded this taxonomic impediment.

In addition, the taxonomy of many stingless bees species is sometimes ambiguous, as species names have changed overtime and different authors have applied different schemes of classification and phylogeny (Wille 1983; Roubik, 1992; Michener, 2000; Eardley, 2004). Venturieri (2009) discussed landscape alteration on bee nest to have direct impact on community density and nest structure. Nest architecture and nesting behaviour have been reported as useful in taxonomic studies (Rasmussen and Camargo, 2008; Lima et al., 2013). However, the architecture of the nest entrance of most stingless bees is species-specific (Pooley and Michener, 1969; Franck, Cameron, Good, Rasplus and Oldroyd, 2004: Lima et al., 2013). Thus incorporating nesting characteristics data to morphological data would possibly provide more robust form of differentiating existing species.

CHAPTER TWO

LITERATURE REVIEW

Stingless Bees

Stingless bees (Meliponini) are a large monophyletic group of highly eusocial bees (Michener, 1974) found in abundance in warm humid forests around the globe. They are indispensable pollinators within tropical ecosystems (Roubik, 1989) and vary widely in both individual and colony size. They share the presence of corbicula, a pollen-carrying structure on the hind legs, with the other corbiculate bees. The corbiculate bees include the highly eusocial honey bee (Apini), primitively eusocial bumble bee (Bombini) and the most solitary orchid bees (Euglossini) Michener (2000).

Even though stingless bees and honey bees both exhibit highly eusocial behaviour (Michener, 1974), including perennial colonies of workers and a single queen, the two tribes have evolved their peculiar kind of sociality independently (Cameron and Mardulyn, 2001; Kawakita, Ascher, Sota, Kato and Roubik, 2008; Whitfield, Cameron, Huson and Steel, 2008). Stingless bees are the only group of social bees that have left an imprint in fossil record spanning most of the Cenozoic. They produce lots of hive products such as honey, bee wax, propolis, pollen and royal jelly which are not well known. As a result Meliponini honey is not included in the international standards for honey (Codex, 2001). Nevertheless, research on the hive products of stingless bees has unearthed the fact that they

have several medicinal uses. Aside this, stingless bees have recently been of much importance to the global world because of their ability to efficiently pollinate a wide range of crops because of their generalist nature. Honey bees used to be the subject of interest because of their pollination efficacy with a wide range of them known to be efficient and effective pollinators of many plant species (FAO, 2007). Population of honey bees are declining rapidly, causing a global concern for pollination services (Kearns, Inouye and Waser, 1998; Biesmeijer et al., 2006). Significant reduction of bee population in the ecosystem could seriously threaten food security and biodiversity (FAO, 2007). There is therefore the need to encourage and promote the culture and conservation of stingless bees.

TAXONOMY OF STINGLESS BEES

Three proposed biogeographical hypothesis of stingless bees, based on the distribution of a putative *Plebeia* lineage, with extant taxa in the Neotropical, Afrotropical and Australasian regions (Camargo and Wittmann, 1989). The authors proposed a Gondwanan origin in which the South American taxa became separated from the Afrotropical taxa during the opening of the Atlantic Ocean.

Stingless bees belong to the family Apidae and tribe Meliponini. The classification of stingless bees has been presented differently by different authors (Sakagami, 1982). Wille (1979) was the first to distinguish common characters of the African Meliponini, indicating them as the ancestral group and placing them into five genera. Camargo and Pedro (1992) carried out a major revision of African Meliponini genera and those of non-African origin. The African taxa showed remarkable external similarities to that from the Americas (Michener,

2007). The African *Dactylurina* resembles the *Trigona*; African *Plebeina* resembles *Plebeia*, *Liotrigona* resembles *Trigonisca* and African *Meliponula* resembles *Melipona*. The African genera and the several groups of stingless bees from other continents appeared to exhibit parallel evolution with members having acquired similar characteristics independently, though coming from related ancestral lineage (Wille, 1979). In Ghana, nine species of stingless bees are known (Kwapong et al., 2010) and they occur in five genera, *Dactylurina* (Cockerell), *Meliponula* (Cockerell), *Hypotrigona* (Cockerell), *Cleptotrigona* (Moure) and *Plebeina* (Moure).

Taxonomy of the Genus *Dactylurina* (Cockerell)

Trigona (*Dactylurina*) Cockerell, 1934 was designated as the group name; however, the type species was originally designated as *Trigona staudinger* Gribodo. Species in the genus build vertical combs which are surrounded with bitumen in exposed nests on tress and are easily identifiable (Michener, 2000). This genus has two species, *Dactylurina schmidti* (Stadelmann) and *Dactylurina staudingeri* (Gribodo). They are also characterized by laterally compressed metasoma and a partly convex corbicula (Plate 1). However, the two species have different facial vestiture (Eardley, 2004).

Plate 1: *Dactylurina* species on a flower (Kwapong et al., 2010)

Taxonomy of the Genus *Meliponula* (Cockerell)

There are 3 subgenera in *Meliponula* (Michener, 2000) these are *Meliponula* (*Meliponula*) Cockerell the type species was described by Spinola with an original designation as *Meliponula bocandei* in 1853. Cockerell worked on this specimen in 1934 and placed it in *Trigona* (*Meliponula*) Cockerell, later on some other authors (Wille, 1979; Wille, 1983; Michener, 1900 and Michener, 2000) worked on the species and maintained the name *Meliponula* (*Meliponula*) Cockerell. The second subgenus is *Meliponula* (*Axestotrigona*). Moure (1961) worked on the type species and assigned the name *Axestotrigona* Moure. The type species was originally designated *Meliponula ferruginea.* Last but not the least in this group is *Meliponula* (*Meliplebeia*) (Moure, 1961).

Meliponula bocandei is characterized by the presence of an orange vestiture, a largely black face, yellowish-orange scutellum and a spoon shaped hind tibia (Plate 2). It was first designated as *Melipona bocandei* Spinola 1853. Researchers like Friese (1909), Cockerell (1934) and Moure (1961) confirmed the species name.

Plate 2: *Meliponula bocandei* worker picking floral resources (Kwapong et al., 2010)

Taxonomy of the Genus *Cleptotrigona* (Moure)

The type species by original designation was *Lestrimelitta* Friese 1903. It was renamed by Moure 1961 as *Lestrimelitta* (*Cleptotrigona*) Moure. Subsequent works finally settled on the genus name *Cleptotrigona.* Species of this genus are robber stingless bees that forage in the nests of other stingless bees (Eardley, 2004). They are not known to visit flowers, and are easily identified by their small body size which is dark brown in colour, no corbicula, and has a large shiny head and black velvety vestiture on the scutum (Plate 3).

Plate 3: *Cleptotrigona* bee species (Ruggiero and Ascher, 2013)

Taxonomy of the genus *Plebeina* (Moure)

Moure (1961) renamed the species as *Plebeina* the original type species was *Melipona denoiti* Vachal. The genus has one variable species (Plate 4) and these nests in cavities in terrestrial termite nests (Eardley, 2004).

Plate 4: *Plebeina* worker bee collecting floral resources (Martins, 2014)

Taxonomy of the Genus *Hypotrigona* (Cockerell)

Cockerell was the first to erect *Hypotrigona* as a genus in 1934. Several taxonomists confirmed it as a valid genus including Moure (1961) who acknowledged Cockerell as the sole author of this group. Although there are some taxonomic disparities among the group, members of this genus are distinguished from other genera based on sub-horizontal region of propodeum which is longer than the subvertical part (Eardley, 2004). There is the roundness of the upper apical part of the hind tibia (Eardley, 2004). Metanotum is shifted backwards possibly to increase the size of the thorax providing for larger flight muscles (Plate 5) (Moure 1961). The pterostigma of the genus *Hypotrigona* is greatly enlarged while the marginal cell is shortened and widened at its base (Moure, 1961).

Plate 5: *Hypotrigona* bee species (Ruggiero and Ascher, 2013)

History and classification of genus *Hypotrigona*

The history and classification of this genus of closely related bees follows this chronology in zoological records. The holotype *Trigona gribodoi* was erected by Magretti in 1884. Until the early 1900's, several taxonomic studies considered bees in the genus *Hypotrigona*as *Trigona* species (Friese, 1909a; Cockerell, 1910; Strand, 1911a, 1911b; Cockerell, 1919; Friese, 1921). In 1934, Cockerell erected *Hypotrigona* as a valid subgenus and placed it under the genus *Trigona* based on the male genitalia which is rectigonal and unique among other Meliponini.

Members of the genus *Hypotrigona* have complete dorsal opening of their gonocoxites and a large membranous basal bulb of the penis valve (Michener, 1990; Michener, 2007). The male gonostyli are freely articulated but do not break off easily. The gonostyli of workers are minute to papilliform, not flattened and separated along their lengths, with several setae but without minute hairs. The worker sting stylus is a blunt convexity and not acute as in most African genera of Meliponini. In workers the upper apical angle of the hind tibia is absent (Michener, 2007). There were several misidentifications of members of this subgenus in several taxonomic works from the mid-1900's to the late 1900's (Cockerell, 1934; Bassindale, 1954; Araujo, 1955a, 1955b; Araujo and Kerr, 1959; Michener, 1959). It was Moure (1961) who erected the *Hypotrigona* as a valid genus and indicated this genus's close resemblance to the genus *Liotrigona*. Moure (1961) found clear differences between the two genera (Table 1).

Table1: Differences between *Hypotrigona* and *Liotrigona* bees

Character	*Hypotrigona*	*Liotrigona*
Scutum, dorsal view	Densely punctate, not shiny	Sparsely punctate, glabrous
Scutellum, dorsal view	Conceals metanotum	Metanotum exposed
Propodeum, lateral view	Subhorizontal part longer than subvertical region	Subhorizontal part shorter than subvertical part
Worker hind tibia: posterodistal corner	Rounded	Angulate
Worker hind tibia: distal end	At most slightly concave	Distinctly concave
Worker hind tibia: Corbicula	Less than half as long as hind tibia	More than half as long as hind tibia
Worker hind tibia: median width	Slightly more than one-third length	Distinctly less than one-third length.

Michener (2000, 2007) provided a comprehensive classification of the world's species, though his work was very limited when it came to the African *Hypotrigona* species. Eardley (2004) could not distinguish between the species of *Hypotrigona* since the workers resemble each other. There are possibly sibling species of *Hypotrigona* that have not yet been recognized (Eardley, 2004).

There stands, however, three species known worldwide within this genus that is *Hypotrigona gribodoi*, *Hypotrigona araujoi* and *Hypotrigona ruspolii*. *Hypotrigona gribodoi* (Magretti) 1884 was erected by Moure (1961) after assigning the group *Trigona* to a valid genus name *Hypotrigona,* although the

type specimen was originally erected by Magretti in 1884 as *Trigona gribodoi* it officially became a species under the genus *Hypotrigona* after Moure (1961). This was also confirmed by Michener (1990). *Trigona braunsi* was also established by Kohl (1894), but was later thought to be *Melipona (Trigona) braunsi* (Kohl) (Vachal 1903). This was however unjustified yet Friese (1909) also confirmed the species name. Sommeijer, Houtekamer and Bos, (1984) pointed out that there were no differences between the species *gribodoi* and *braunsi,* and stated that they could be synonymy. The species name *gribodoi* is most preferred because of the spelling discrepancy in *braunsi* (Eardley, 2004).

There were two forms of *H. braunsi* recognized by Araujo and Kerr (1959) that they identified to be *H. braunsi* sensu stricto with a vernacular name 'cassusso'. The other form was later described by Michener in Portuguese vernacular as 'landula' which he also called *H. araujo* in 1959 thus; the second species of this genus was described.

The third species of the genus is *Trigona ruspolii* Magretti (1898), which was erected as *Trigona magretti* by Friese (1900). It was later on described as *Hypotrigona magretti* (Friese) by Medler (1980). This same species was also classified as *Melipona (Trigona) bouyssoui* by Vachal (1903). It was later confirmed that, *ruspolii, magretti* and *bouyssoui* are synonym thus *Hypotrigona ruspolii* (Magretti) was finally confirmed by Moure (1961).

The taxonomy of this genus is thus, very difficult and species names have changed several times. Many of the authors could not clearly distinguish among the species. For example Eardley (2004) could not separate the workers of *H.*

gribodoi and *H. braunsi*. Guiglia (1955) in considering the imaginary line on the hind tibia of these species concluded that *H. gribodoi* resembles *H. ruspolii*. The main reason why the taxonomy of this species is troublesome is because most of their descriptions were based on length and ratios only (Michener, 1959).

Even though the *Hypotrigona* bees are faced with taxonomic issues, these are widespread throughout tropical Africa. And are very good competitors in the area of acquiring nectar from diverse plant species (Kajobe and Roubik, 2006). Kajobe and Roubik (2006) reported the ability of this same species to have more sugar concentration in their nectar resource collected than other smaller bees within the Meliponini family.

Reproduction in Stingless Bees

Nest architecture, entrance shape and population size are very diverse and characteristic for each species (Roubik, 2006; Rasmussen and Camargo, 2008). In highly eusocial bees (honey bees and stingless bees), the formation of new colonies occurs through swarming. However, the swarming process in stingless bees is gradual occurring in smaller numbers (Oliveira, Menezes, Soares and Imperatiz-Fonseca, 2012). Colonies of stingless bees are characterized by morphologically distinct female castes (gynes or queens, and workers), division of reproductive labour between the castes and generation overlap (Michener, 1974). At individual level, most reproduction aims to increase the number of worker bees in the colony, which is needed to produce new colonies. Colony growth by production of workers also increases the number of gynes and males that can be reared by the colony (Chinh, 2004). When swarming, stingless bee workers from

the mother nest choose a new cavity and start to prepare it by cleaning and bringing nest materials from the mother nest. While some build the colony entrance and food pots, others start foraging or continue visiting the mother nest.

The relative numbers and positions of colonies within an area have inevitably shaped the evolution of stingless bees. When the new nest is ready, more workers fly together with a virgin queen pursued by hundreds of drones who would be waiting in anticipation (Velthuis, Koedam, Imperatriz-Fonseca, 2005) for a single mating (Peters, Queller, Imperatriz-Fonseca, Roubik and Strassmann, 1999; Palmer, Oldroyd, Quezada-Euan, Paxton and May-Itza, 2002) to perform the nuptial flight soon after arrival at the new nest. However, contact between mother and daughter nest can last from few days to several months depending on resource availability (Wille and Orozco, 1975; Inoue, Sakagami, Salmah and Yamane, 1984; Engels and Imperatriz-Fonseca, 1990; van Veen and Sommeijer, 2000). The queen may be killed, or indeed, several queens are killed by predators on such mating attempts (Paxton, Bego, Shah and Mateus, 2003).

Stingless bees have a different life cycle as compared to honeybees. There can be two or more queens laying eggs in the same nest (Kerr, 1950). Queens are produced regularly, but most of them are killed and never allowed to produce eggs (Wenseleers, Ratnieks, Ribeiro, Alves and Imperatriz-Fonseca, 2005). Some queens may be imprisoned in special cells as reserves. Replacement of egg-laying queen does not happen every year. The queen lays eggs in a special way first; a completed cell is half filled with honey and pollen by workers. One or more workers lay an egg in the cell and the queen is encouraged to come near where,

she eats the workers egg from the cell and lays her own eggs instead of proceeding to another cell. Workers then close the cell by bending the upper collar of the cell against the center. The cell is closed until an adult emerges. This process is called mass provisioning system which differs from honeybees where, the honeybee larvae are fed continuously as they develop. Stingless bee queens can provide 10 to 100 cells with eggs a day depending on the species (Michener, 2000). The cell is torn down when a fully developed bee leaves the brood; the materials are reused to build new cells. Fertilized eggs develop into worker bees or queens whereas unfertilized eggs from queens or workers develop into drones. Sometimes an egg laying worker lays her eggs into cells containing a queen's egg. This male egg develops faster into larvae and then adult and leaves the cell.

STINGLESS BEES AND NESTING SITE SELECTION

Stingless bees are known to be generalists with regards to selection of nesting sites (Hubbell and Johnson, 1977; Roubik, 1989). They nest in felled trees, in bush that has been burnt or trampled or cut down by man or other animals. Earthen banks of road cuts, paths, fields, and in banks made by rushing water, have often provided the opportunities to study bee nesting biology (Michener, 1974; Roubik, Moreno, Vergara and Wittmann, 1986). The several hundreds of stingless bee species existing worldwide differ considerably in colony size, in body size and colour (Drumond, Bego and Melo, 1995; Michener, 2000). The bees vary considerably in their nest architecture with different designs in brood cells arrangements. Brood cells are arranged in horizontal or vertical combs, semi combs or in clustered cells. The nests can either be constructed in

crevices, trees or in the ground (Wille and Michener, 1973; Roubik, 2006). Sakagami, Inoue and Salmah, (1990) noted that the elaboration of their nest entrance is generally species specific. Kajobe and Roubik (2006) affirm that attributes of the nests are useful in taxonomic studies especially in equatorial tropical Africa where little has been studied. Some previous studies on nest biology of stingless bees have been carried out by (Sakagami, 1982; Eltz, Bruhl, Van der kaars and Linsenmair, 2002; Eltz, Bruhl, Imiyabir and Linsenmair, 2003; Slaa, 2003; Roubik, 2006; Kajobe and Roubik, 2006). Stingless bees have evolved adaptive nest construction strategies which have resulted in sophisticated nest architecture in many species while others lack certain structural components (Schwarz 1948; Nougueira-Neto and Sakagami, 1966; Kerr, Pisani and Aily , 1967; Camargo 1970; Imperatriz-Fonseca, Ferreira de Souza and Nogueira Neto, 1972; Wille and Michener 1973; 1974, Roubik 1979; 1980; 1983a; Camargo and Wittmann 1989).

Many species, particularly those of the moist tropics, are unable to withstand chilling temperatures (Michener 1974). One major component of the nests of *Hypotrigona* species and other stingless bees in general is the excellent insulation especially with the exposed nests. Nests in large trunks or in soils are particularly well insulated. Roubik (1983b) observed that the nests of many stingless bee species are yet to be described. This observation is especially important for equatorial Africa where very little studies have been done on stingless bees.

An essential characteristic of insect societies is their unconventional cooperation, but at the same time their colonies are also vulnerable to both interspecific and intraspecific social parasitism. This is because social parasites may benefit nutritionally and/or reproductively from the resources stored in the host colony and by the rearing of their offspring by their hosts (Nash and Boomsma, 2008). To keep parasites out, social insect colonies are defended by entrance guards, which admit nestmates but exclude intruders (Wilson, 1971). Guards can generally discriminate nestmates from non-nestmates by using chemical cues, which are believed to be colony-specific odours that are partially genetically determined and partially environmentally acquired (Breed, 1983; Stuart, 1988; van Zweden and d'Ettorre, 2010).

Although most studies on nestmate recognition have confirmed the ability of social insects to discriminate nestmates from non-nestmates (Breed, Butler and Stiller, 1985; Gamboa, 1986; Breed and Page, 1991; Singer and Espelie, 1992; Inoue, Roubik and Suka, 1999; Buchwald and Breed, 2005; Jones et al., 2012), some have also shown this guarding system to be imperfect. For instance, in the Western honeybee, *Apis mellifera*, guards will accidentally allow 10 to 50% of alien workers to enter their hives (Downs and Ratnieks, 2000). The fact that the colonies guarding systems are often imperfect opens the door for reproductive parasitism. Indeed, over the past decade, several cases of intraspecific worker parasitism have been discovered in various groups of social insects, including bumblebees (Birmingham, Hoover, Winston and Ydenberg, 2004; Lopez-Vaamonde, Koning, Brown, Brown and Bourke, 2004; Takahashi, Martin, Ono

and Shimizu, 2010) and honeybees (Nanork, Paar, Chapman, Wongsiri and Oldroyd, 2005; Nanork et al., 2007; Chapman et al., 2009; Chapman, Beekman and Oldroyd, 2010). Despite, intraspecific worker parasitism among Meliponini bees, there is also intraspecific queen parasitism (Van Oystaeyen et al., 2013).

A long-term genetic study on *Melipona scutellaris* had earlier confirmed the occurrence of intraspecific queen parasitism in the genus *Melipona*, with data showing unrelated queens frequently invading and taking over colonies in which the mother queen happened to die, and with 25% of all colony take-overs being undertaken by alien queens (Wenseleers Alves, Francoy, Billen and Imperatriz-Fonseca, 2011). The occurrence of intraspecific queen parasitism in Melipona bees may be linked with overproduction of queens in this genus (Kerr, 1950; Wenseleers and Ratnieks, 2004; Santos-Filho, Alves, Eterovic, Imperatriz-Fonseca and Kleinert, 2006).

It is suggested that queen overproduction is the result of self-determination over caste fate (Bourke and Ratnieks 1999; Ratnieks, 2001). Thus females enhance their own inclusive fitness by developing into a queen, which leads up to 20% of all females developing into queens (Ratnieks and Wenseleers, 2008). In swarm-founding species of *Melipona*, producing this large number of queens is not beneficial to the colony as it would be in species with solitary foundresses. This is because the ability to found colonies by swarming is limited by the number of workers, and females that develop into queens will compromise the production of new workers (Michener, 1974; Ratnieks, 2001). Indeed, confirmation for queens being produced in excess of colony needs is provided by

the fact that workers kill many of the newly emerged gynes soon after they enclose from their cells (Silva, Zucchi and Kerr, 1972; Koedam, Monge and Sommeijer, 1995; Wenseleers and Ratnieks, 2004). Workers also chase many of the gynes out of the colony (Sommeijer and De Bruijn, 2003). For example, in *M. favosa*, it has been demonstrated that 43% of all gynes are killed by the workers and that the remaining 57% are chased out of the colony by the workers (Sommeijer, De Bruijn, Meeuwsen and Slaa, 2003b). The fact that opportunities for *Melipona* queens to supersede their mother queen or head a new daughter swarm are so limited suggests that their queens will also be strongly selected to try to seek alternative strategies to reproduce, such as taking over other colonies (Sommeijer, De Bruijn and Meeuwsen, 2003a; Wenseleers et al., 2011).

Nesting and Diversity of Stingless Bees

Stingless bees have inhabited the tropics for over 65 million years longer than *Apis*, the stinging honey bees (Camargo and Pedro, 1992; Michener, 2000). Where both groups make honey in perennial nests founded by a swarm of sterile workers and a queen, and colonies occasionally produce male bees. Nevertheless, stingless bees have 50 times more species and, as emphasized here, differ from *Apis* in many biologically significant ways.

Meliponini cannot migrate unlike honey bees; they produce brood in the manner of solitary bees, with an egg placed on top of a food mass in a sealed cell. In general, stingless bee colonies make far less honey, and therefore have less economic appeal, compared to honey bees (Michener, 1974). In contrast to *Apis,* meliponines generally have no sting, mate only once, do not use water to cool

their nest or pure wax to build. They cannot freely swarm to reproduce (but rather makes a new domicile) and the males feed on flowers, whereas gravid queens cannot fly (Biesmeijer et al., 2005). The nest is the central place from which stingless bees mate, forage and pass through life stages. Nests are immobile and potentially long-lived. Distribution (spatial arrangement) of colony resources and 'stress sources' have much significance, which is a primary evolutionary response of meliponines to such critical factors defines their nesting biology (Roubik, 1989; Camarago and Pedro, 2002 a, b).

Nesting biology of Stingless bees

Nests are notable points of bee activity which are highly visible facets of stingless bee behaviour, (Michener, 1974; Roubik, 2006). Colonies are active every day (Roubik, 1989; Hansell, 1993). The individual species of Meliponini are recognizable from the nest entrances and sometimes from the particular site. Inside the nest, there are different shapes and arrangements of brood cells. Pollen and honey are stored in separate pots (Plate 6) (Roubik, 2006).

Plate 6: Exposed hive content of stingless bees with arrows (a) indicating honey pots (b) pollen pots (c) brood cell (Kwapong et al., 2010)

Architectural innovations may occur in a taxon after its divergence from ancestors but distinct species may converge due to the similarity of nesting material or available sites (Roubik, 2006). Some large genera display considerable species-level variation in the nesting habit, likely produced by adaptive radiation. Large variation occurs, for example within the Neotropical genus *Plebeia*. Some *Plebeia* build regular pancake-like stack of brood cells separated by pillars and arranged in circular combs, whereas the smallest species do not build combs but instead make loose chains of cells or clusters (Roubik, 2006). Bee size appears decisive because, among tiny Meliponini, clustered cells are the architectural rule among phylogenetically diverse bees (Michener, 2000).

GEOMETRIC MORPHOMETRIC TECHNIQUE

Geometric morphometrics is the description of shape in a Cartesian coordinate system (Bookstein, 1991; Rohlf and Marcus, 1993). It could also be described as the quantitative representation and analysis of morphological shape using geometric coordinates instead of measurements (Polly, 2012). This technique is based on a well-established theory of shape (Kendall, Barden, Carne and Le, 1999). There are two different ways of obtaining data for geometric morphometrics; the landmark based and the outline based methods (Polly, 2012).

Types of Geometric Morphometrics

Landmark based geometric morphometrics are points that can be placed on a biologically or geometrically homologous point on the structure. There are

semi-landmarks which are also points placed arbitrarily using algorithm, often by defining endpoints at biologically homologous points and placing a specified number of semi-landmarks between them (Polly, 2012).

Outline based geometric morphometrics on the other hand are perimeters delimited by many points. Points classified as Cartesian coordinates (x, y, z), are often converted into angles. With outline data acquisition, many points are required to represent a shape (Polly, 2012).

In place of the distances and angles as in traditional morphometrics, geometric morphometrics uses the coordinates of the points on the samples for example the wing as landmarks (Tofilski, 2008). Many methods have been developed in the identification of the Africanized bees (Francoy et al., 2008) such as the analysis of isozymes (Contel, Mestriner and Martins, 1977; Del Lama, Figueiredo, Soares and Del Lama, 1988), cuticular hydrocarbons (Ferreira-Caliman, Zucchi and Nascimento, 2012), mitochondrial DNA polymorphism (Hall and Muralidharan, 1989; Smith, Taylor and Brow, 1989; Sheppard, Rinderer, Mazzoli, Stelzer and Shimanuki, 1991a; Sheppard, Soares, De Jong and Shimanuki 1991b; Segura, 2000) and nuclear DNA (Hall, 1988; Clarke, Rinderer, Franck, Quezada-Euan and Oldroyd, 2002; Whitfield et al., 2006). All these techniques are very important in taxonomic classification; however, biochemical and molecular methods are very expensive and require specific reagents and laboratory equipment's.

Geometric morphometrics on the other hand is less expensive and has shown promising result in recent years. Francoy et al (2008) used the geometric

morphometric technique in the identification of Africanized honey bees using the only wing features of which about 99.2% of the colonies sampled were correctly identified. With the discriminant function and cross-validation correctly identified 97.8% of the colonies. Geometric morphometrics seeks to address potentially serious problems of most traditional approaches by focusing on data and methods that completely and efficiently archive the geometric information recorded from the specimens in a sample (Rohlf and Marcus, 1993; Slice, 2005, 2007). Slice and Ann (2009) used this protocol in the classification of crania in forensic science and further determine the sexes of the samples they observed. Lopes de Carvalho et al, (2011) in using geometric morphometric protocol in conjunction with conventional morphometry on the right forewings and the hindwings were able to identify the maternal source of *Melipona scutellaris* workers from a polygyne colony with five queens.

In conclusion, landmark based geometric morphometrics will help draw a clear line between the species of *Hypotrigona* using the right forewing.

Other morphometric software

Morphometric software is currently available as a stand-alone package with graphical user interfaces or as packages of routines of programming environments such as Matlab or R (Claude, 2008). This spectrum associated with an inherent trade-off of user-friendliness in competition with flexibility of the systems where users are allowed to do some of the programming themselves (Klingenberg, 2011).

MorphoJ however, aims to take a transitional position in this spectrum. It is based on graphical user interface and therefore does not require users to have programming skills. It also aims to provide a maximum flexibility by offering a wide range of analysis (often with several options such as pooled within group analysis) and an integrated user environment that facilitates combining different methods in the analysis of the shape data. This software contains a number of unique features and fully takes into account the symmetry of landmark configurations throughout the analyses. MorphoJ contains some advance tools for analyzing modularity and integration of shape.

It is a programme package for geometric morphometric analysis for two and three dimensional landmark data. This programme is written in pure java and can therefore run on any computer. It is a programme that is designed for the analysis of actual biological data (Klingenberg, 2011). MorphoJ does not work in isolation but operates with the collections of data in the form of projects, datasets and data matrices. In addition to these variables, it also works with identifiers, classifiers and covariates.

Identifiers are a sequence of characters that serve to identify the specimen. MorphoJ obtains identifiers with the coordinate data when these are imported initially, and passes them along with the data during analyses. Classifiers provide information about properties of the observations such as the sex, age (juvenile or adult), origin, habitat, patient or control or any other resounding criterion that may be used for defining the membership of the groups of observations (Klingenberg,

2011). The last but not the least tool that MorphoJ needs in acquiring its data is the file type from which MorphoJ can import coordinate data.

CHAPTER THREE

MATERIALS AND METHODS

Study Area

The research was conducted in the field and the laboratory. The field work was carried out in selected localities within three districts in the Central Region of Ghana from April 2013 to November 2013. The sampling sites were the University of Cape Coast Science Botanical Garden (Cape Coast Municipal Assembly), Mfuom community (Twifo Heman-Lower Denkyira District) and Kwesi Gyan community (Mfantsiman Municipal Assembly) (Figure 1).

The sites were selected based on preliminary surveys conducted to locate *Hypotrigona* colonies centered on the nest architecture and morphology as reported by (Schwarz, 1948; Sakagami, 1978). Nesting substrates of *Hypotrigona* bees were identified form many places including, wall cavities and bamboo internodes (Wille and Michener, 1973; Roubik, 2006).

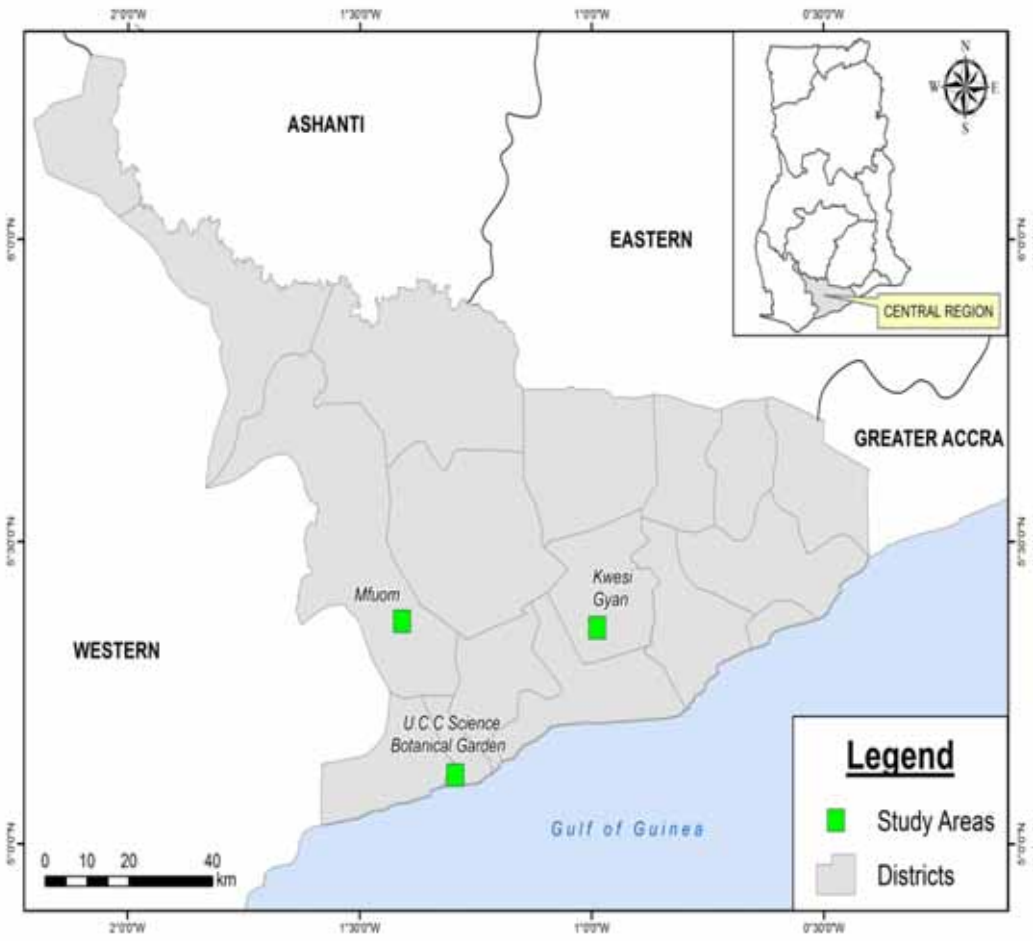


Figure 1: Map of Central Region of Ghana indicating study sites

DESCRIPTION OF STUDY SITES

Site One: UCC Science Botanical Garden

The UCC Science Botanical Garden (05° 7' N; 01° 17' W; 17m elevation) is located west of the Science Faculty Complex within the University with an approximate size of (40634.776 m^2). It was established in 1970 by the then Department of Botany to facilitate scientific research. There are lot of forest patches due to logging activities and construction works within the garden (Plate 7). This study site is found in the Cape Coast Municipal Assembly. The garden is occupied with forest trees, grasses and shrubs.

Site Two: Mfuom Community Area

Mfuom (05° 22’ N; 01° 24’ W; 161m elevation) in the Twifo Heman-Lower Denkyira District is a community surrounded by a forest. The area is characterized by hilly terrain and forest higher up the slope with trees such as *Eucalyptus* sp, *Senna siamea* and many more. However, there were lots of forest fragmentation due to bush fire, farming, construction for human settlements and logging activities by the indigenes (Plate 8). This site was also characterized with old mud houses.

Site Three: Kwesi Gyan Community Area

The study site is found within the Mfantsiman Municipal Assembly (05° 21’ N; 000° 59’ W; 39m elevation) and is about 8 kilometers from the Mankessim market. Kwesi Gyan is also a community surrounded by a forest with farming as the main occupation in the community (Plate 9).

Plate 7: Foot path within the Science Botanical Garden

Plate 8: Mfuom community showing study area

Plate 9: Logging and farming activities within Kwesi Gyan community

DATA COLLECTION

Survey/ Sampling of *Hypotrigona* Bees from the Study Sites

A survey for *Hypotrigona* species nests was conducted within the month of April, 2013 to ascertain the presence of the bees within the various sites. Two days were spent at each site to scout for the bees from nesting substrates as reported by (Wille and Michener, 1973; Roubik, 2006). Assistance was sought from local farmers and research assistants during the survey. Entire communities were surveyed for the presence of bees of the genus *Hypotrigona*. Once possible nests were located, substrates were marked for easy identification in subsequent visits.

At each sampling site, visual observations of the external nest entrance characteristics of *Hypotrigona* colonies were made for presence/ absence of nest entrance tubes (Plate 10, 11 and 12). Entrance tube characteristics in terms of tube length from substrate as well as diameter of entrance tube were also measured (Plate 10). Tube lengths were measured using a piece of thread which was then transferred unto a measuring rule and recorded. Likewise, a pair of mathematical set divider was used to obtain measurements of the diameter of the nest entrances and then transferred unto the measuring rule and recorded (Plate 10). In addition, other behavioral characteristics such as the level of aggression when disturbed and substrate types were also recorded. Using the collector as an intruder to the entrance of the colony, bee aggressiveness was observed for 5 minutes.

Hypotrigona species used for geometric morphometrics and traditional morphometry were obtained by hanging plain transparent polythene bags at the

Plate 10: Assessing nest entrance tube characteristics from colonies.

entrance tube of the nest of each colony marked at the study sites (Plate 11). It is important to sample these cluster building bees from the point of the nest entrance. This is to ensure that each colony sampled contains only members derived from the same queen with the same gene pool. Most stingless bee queens mate once in their lifetime, hence, members of the colony possess similar genetic make-up (Chinh 2004). Thus, this procedure eliminates any form of introduced variability to that colony. Collected bees were killed in soapy water on the field and preserved in 90% ethanol. Specimens were brought to the Entomology Museum University of Cape Coast for further laboratory analyses.

Plate 11: Collecting *Hypotrigona* bees with plain transparent polythene bags.

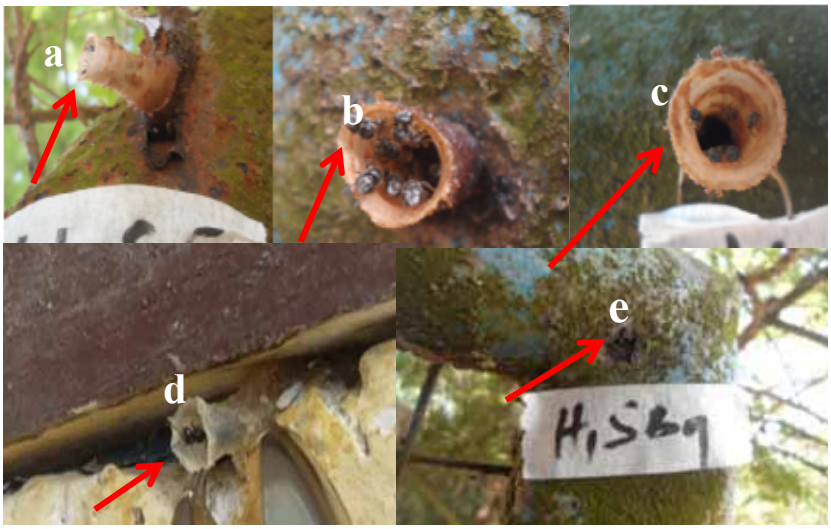


Plate 12: Various nest entrances of *Hypotrigona* species: (a, b, c) with opaque entrance tubes. (d) transparent nest entrance tube (e) no nest entrance tube protrusion.

Identification of collected species of *Hypotrigona*

Preserved bees from the field were sent to the Department of Entomology and Wildlife Museum University of Cape Coast for identification and further analyses on the samples. Specimens were verified as belonging to the genus *Hypotrigona* using taxonomic keys (Eardley, 2004). Characters examined were, the sub-horizontal region of propodeum, sub-vertical part of the propodeum and the upper apical part of hind tibia. The presence of a densely punctate, not shiny scutum at the dorsal view and scutellum concealing the metanotum were considered as well (Eardley, 2004).

GEOMETRIC MORPHOMETRIC APPROACH

In order to assess variability of species within the genus *Hypotrigona*, wing geometric morphometrics technique was employed. A total of two thousand and twenty seven (2027) right forewings of *Hypotrigona* worker bees were analyzed. Thus, 30 specimens per colony from 68 colonies within the three experimental sites. The right forewings of worker bees were dissected and relaxed in 90% ethanol to enable the even spread of the wings on microscope slides (Plate 13). Plain nail polish was used to secure the wings on the microscope slide preparation (Plate 13). Terminologies used in this study were adapted from (Bookstein, 1991; Rohlf, 2010).

Mounted wings were then photographed with a digital camera attached to a stereomicroscope (Plate 13 and 14). This was done to generate images of the

wings for detailed geometric morphometrics analyses. A Jpeg image file was created for the wing images acquired.

Plate 13: Laboratory work on the wings of *Hypotrigona* species

Plate 14: Set up for image acquisition using the stereomicroscope

The thin-plate spline (TPS) programme (a Computer programme) was employed in morphometric data acquisition from the wings of the bees. This contains two software programmes (TPS Utility and TPS_Digitize 2) used in the data collection and analyses process. TPS Utility version 1.58 programme (Plate 15) enabled an input file to be created for data acquisition on the 2027 images on the wings prepared (Rohlf, 2012). Furthermore, a second software programme TPS_Digitize 2 (Plate 16) read the data acquired from the TPS Utility Programme (Plate 15). TPS_Digitize 2 retrieved all images and allowed for landmark digitization (Rohlf, 2010). The vein junctions were used as landmarks for geometric morphometrics data acquisition. A landmark is any point that can be placed on a biologically or geometrically homologous point on the surface (Rohlf, 2010). Eight homologous landmarks were plotted on the vein junctions because of the reduction of venation within this genus (Eardley, 2004). In using TPS Dig_2 version 2.17 software (Plate 16) the landmarks were aligned according to generalised orthogonal least-squares procedures (Rohlf and Slice, 1990). This program allows for visualization in 2-Dimensions shape differences of the specimens based on deformation of average shape of all specimens. This average shape is also called the consensus (or reference) shape.

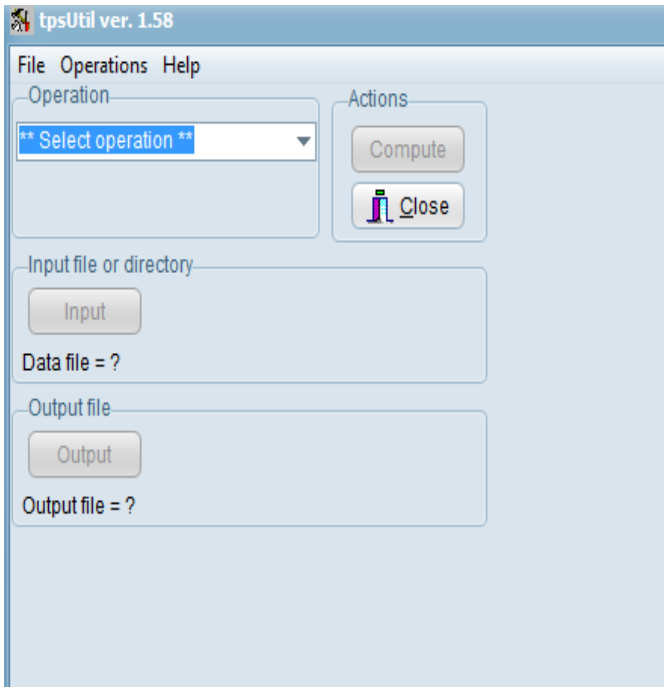


Plate 15: An interface of thin-plate spline TPS Utility programme for data acquisition.

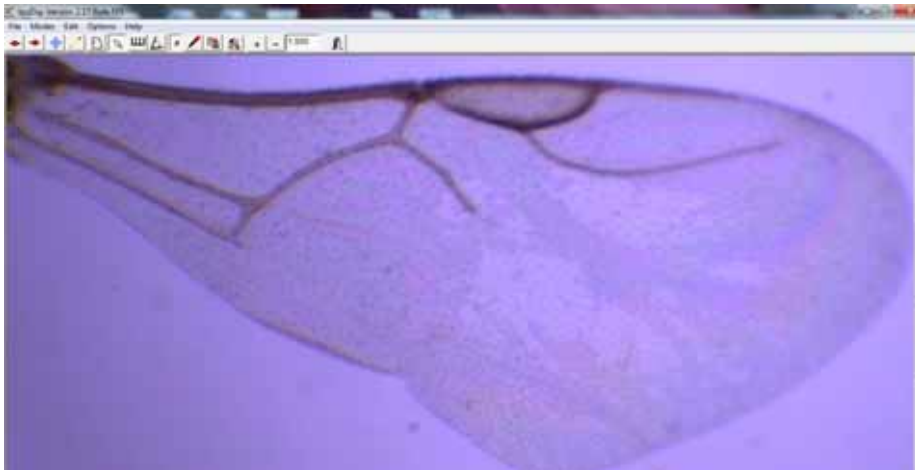


Plate 16: A display of the thin-plate spline Tps_Digitize 2 programme for landmark digitization**.**

Collecting Landmark Data from the vein Intersections on the Wings

During the process of landmark data acquisition, the input file that was created to collect landmarks was used TPS Utility. TPS_Digitize 2 programme (Plate 16) was opened to upload the input file containing the images into it. The

TPS_Digitize landmark symbol on the TPS_Dig 2 menu bar was clicked and that allowed for the placement of landmarks on each of the 2027 forewings. Unlike a generalized be wing with elaborate venation (Plate 17a), *Hypotrigona* bees have reduced venation. Thus landmarks were placed on eight marked positions (Plate 17b). In order to minimize variation across specimens due to random error, corresponding landmarks were placed in the same position for each specimen (Plate 17b). Landmarks were added in sequence starting from landmark 1 to landmark 8. Whenever an image was fully digitized, it was immediately saved by clicking on the file menu bar. The saved data were then imported into MorphoJ software version 1.05e for analyses (Klingenberg, 2011).

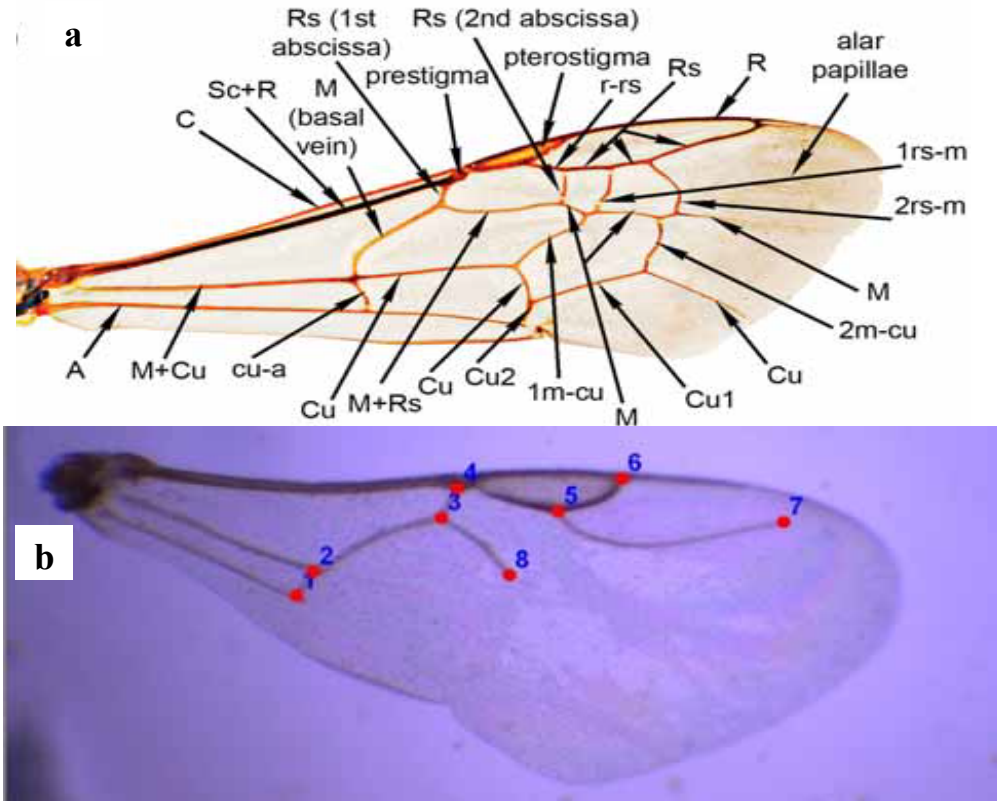


Plate 17: (a) A generalized bee wing (Eardley, Kuhlmann and Pauly, 2010) (b) forewing of *Hypotrigona* species with eight homologous landmarks.

TRADITIONAL MORPHOMETRY

Confirmatory tests on the results from geometric morphometric analyses were done using traditional morphometry (Michener, 1959; Moure 1961). Specimens were placed on a graticule under a stereomicroscope. The characters assessed included measurements of the entire body length, head width, eye length, upper and lower interocular distances, hamuli number, forewing length and scape (Plate 18, 19, 20 and 21). A total of 30 adult workers of each colony were examined.

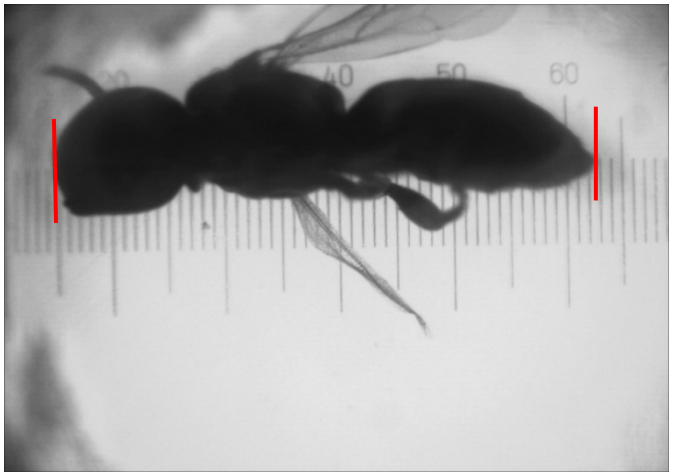


Plate 18: Determining the entire body length of the *Hypotrigona* worker bee.

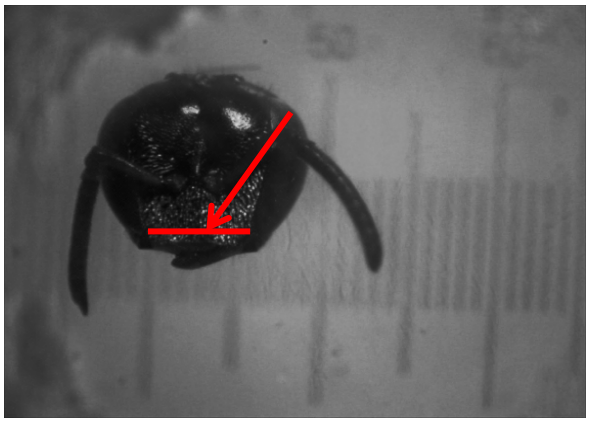


Plate 19: Length of the lower interocular distance of *Hypotrigona* bee

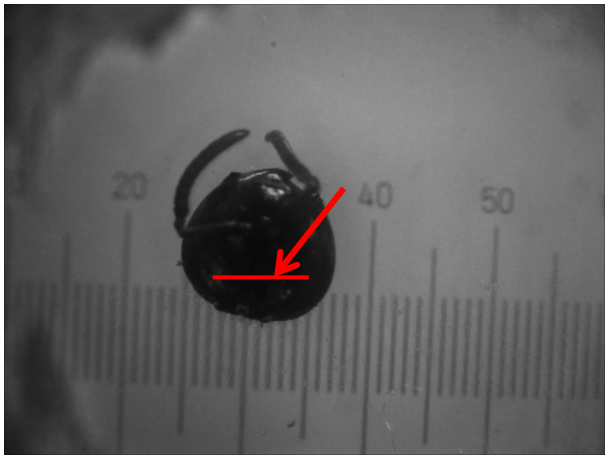


Plate 20: Length of the upper interocular distance worker bee.

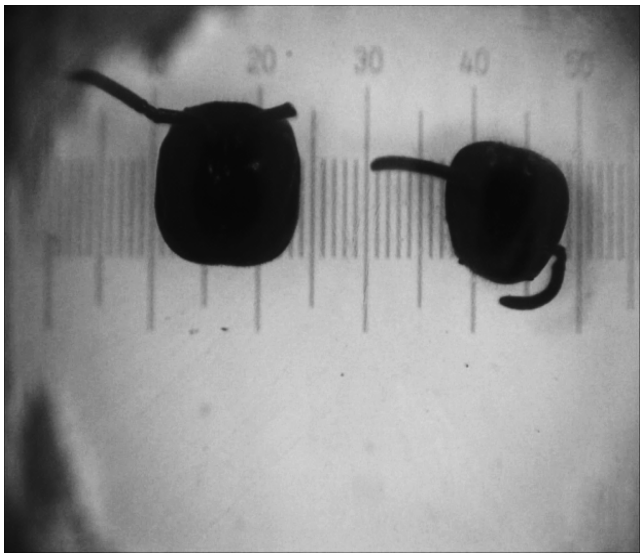


Plate 21: Measuring and/ comparing head shape of *Hypotrigona* species.

STATISTICAL ANALYSIS

Microsoft Excel for windows and Statistical Package for the Social Sciences (SPSS) version 16 tools were employed in analyzing the nesting characteristics of *Hypotrigona* species from the three study sites.

All Cartesian coordinates on the wing vein junctions from the landmark data (Wiley, Amenta and Alcantara, 2005) were imported to MorphoJ software version 1.05e (Klingenberg, 2011) for geometric morphometric analyses. MorphoJ is a statistical software tool that analyzes datasets in geometric morphometrics. Data was subjected to several analyses such as Procrustes superimposition, principal component analyses and many more to assess variability within the genus with 90% confidence interval.

Procrustes Superimposition

A generalized Procrustes superimposition was performed on configuration of the wings, in order to analyze the shape change exclusively. Information such as the size, position and orientation of the configurations were not included in the analyses (Kendall, 1977; Rohlf and Slice, 1990). Shape can be mathematically defined as the entire geometric information about a landmark configuration except its position, orientation, and scale (Dryden and Mardia 1998; Klingenberg, 2011). Firstly, the shape of all configurations was scaled to a unit centroid size said to be the sum of squared distances between each landmark and the centroid. Secondly, the configurations were moved so that the centroid overlaps, thereby taking the information of position. Finally the configurations were rotated so that the sum of the squared distances among each landmark was minimized. This final step

enabled the orientation to be dealt with maintaining the shape change for subsequent analysis.

Data Outlier Analysis on the Dataset

In addition, a data outlier analysis was performed on the dataset, which inspected the dataset for coordinates that were distant from other observations (Grubbs, 1969; Klingenberg and Monteiro, 2005).

There are additional concepts concerning variables used by MorphoJ to provide external information relating to landmark configuration being analyzed. These are the identifiers and classifiers. Identifiers are a sequence of characters that serves to identify the specimen whiles classifiers provide information about the properties of observations (Klingenberg, 2011). The study associated classifiers with the ranges in entrance tube lengths and experimental sites as identifiers for the specimens.

Beside the outliers, morphometric analyses generated covariance matrices (also known as the dispersion matrix) which were used to generalize the notion of variance to multiple dimensions (Wasserman, 2004; Klingenberg, 2011).

Principal Component Analysis (PCA)

MorphoJ was used to generate covariance matrices from datasets after Procrustes superimposition. The data obtained were used as variables in multivariate analysis such as principal component analysis (PCA) and regression residuals (Klingenberg, 2008, 2009).

In addition, principal component analysis and ordination analysis were performed to determine the main features of shape variation in the samples and also performed ordination analysis and the arrangement of wings in morphospace respectively (Shlens, 2003; Klingenberg, 2011).

Canonical Variate Analysis (CVA)

Canonical variate analysis (CVA) is a type of ordination analysis, which maximizes the separation of specified groups (Klingenberg, 2011). This analysis can be applied to several populations at once and was ran for the whole dataset with "nest entrance tube length" as the classifier variable. Outputs included statistical analyses with estimates of the significance (p values) and extent (Procrustes Distance) of between population morphometric variation as well as graphical representations of the deformation function between the average shape of the whole sample (N= 2027). The average shape of each population; and plots of the spread of the specimens on axes representing any two of the canonical variates, essentially projecting two dimensions of the multi-dimensional shape space onto the X and Y axes (Fig 10). Between group (population) shape differences were considered significant if $p \leq 0.05$ and highly significant if $p < 0.0001$ (Klingenberg and Mclntyre, 1998; Klingenberg, Barluenga and Meyer, 2002; Klingenberg, 2011).

Discriminant Function Analysis and Cross-validation test

The discriminant function analysis (DFA) and cross-validation indicate whether groups can be distinguished reliably (Klingenberg, 2011). This analysis

can only be performed on two groups at a time and was ran for each possible pairwise combination of sites. The discriminant function produced was validated using 1000 random permutations. Outputs included Procrustes distance, significance (parametric p value and p-value for 1000 permutations) and accuracy of the DFA in separating the original data (% separated). Pairwise shape differences between populations represented by the discriminant function were considered significant if $p \leq 0.05$ and highly significant if $p < 0.0001$ (Klingenberg, 2008: Klingenberg, 2011). Procrustes ANOVA was used to assess the relative amounts of variation among individuals, and of measurement error.

Confirmatory analysis on the level of variation was established with correlation and regression analysis on the dataset. Visualization of the different wings was done using a wireframe which is one of the packages in MorphoJ and consists of a set of connecting lines between landmarks in a graph that helps the viewer to visualize landmark configuration (Klingenberg, 2008). Using traditional morphometry the individual groupings were categorized accordingly.

CHAPTER FOUR

RESULTS AND DISCUSSION

Results

The results presented here are the findings of the scientific investigations on the genus *Hypotrigona*: their nesting characteristics (entrance tube length and diameter), geometric morphometrics and traditional morphometry analysis within the three districts in the Central Region of Ghana.

HYPOTRIGONA BEE SPECIES AND THEIR NESTING CHARACTERIS TICS

Nest Substrate Characteristics

The total number of *Hypotrigona* bees assessed from nest entrance in the three study sites was five thousand four hundred and forty-one (5441). Thus within the three localities, total number of species collected in the UCC Science Botanical Garden (n=1383), Mfuom community (n=3136) and Kwesi Gyan community (n=922) (Table 2).

Regarding the substrate used, 11 nests were found without entrance tube protrusions on different substrate within the three study sites. The study observed six different nesting substrates for species of the genus *Hypotrigona* within the three localities (Table 2). Pooley and Michener (1969) reported that tube lengths in stingless bees are species specific with *Trigona gribodoi* having a tube length

of (6-25 mm). In the present study, the length of the nest entrance tube has been categorized into four different groups: absent (0 mm); short (1-25 mm); medium (26-50 mm) and long (>51 mm).

Six different materials have been identified as preferred nesting substrates for the genus *Hypotrigona* and these include: wooden window frame (WWF), cavity in metal pole (CMP), crack in wall (CW), crack in mud houses (CMH), cavity in meter board (CMB) and bamboo internode (BI). Among these substrates, the wooden window frames and bamboo internodes were the most preferred compared to the other substrates in all three experimental sites.

Table 2: Populations of *Hypotrigona* species sampled from various nests within the three experimental sites

Experimental sites	Nesting substrate	Population
UCC Science Botanical Garden	Crack in wall (CV)	50
	Bamboo internodes (BI)	563
	Cavity in metal pole (CMP)	770
Mfuom community	wooden window frame (WWF)	2381
	Crack in wall (CV)	452
	Bamboo internodes (BI)	108
	Wooden meter board (WMB)	195
Kwesi Gyan community	Crack in mud house (CMH)	232
	Bamboo internodes (BI)	690
Total		5441

Table 3: Number of *Hypotrigona* colonies found inhabiting different substrates within the three localities

Nesting habitat	No. of colonies within Localities			Total
	UCC Science Botanical Gardens	Mfuom Community	Kwesi Gyan Community	
Crack in wall (CW)	1	4	0	5
Bamboo internodes (BI)	8	2	11	21
Cavity in metal pole (CMP)	8	0	0	8
Wooden window frame (WWF)	0	28	0	28
Wooden meter board (WMB)	0	3	0	3
Crack in mud house (CMH)	0	0	3	3
Total	17	37	14	68

Collections from the UCC Science Botanical Garden provided three nesting substrates for the *Hypotrigona* species (Table 4). Cavity in metal poles offered the highest mean nest entrance tube length (NETL) for *Hypotrigona* species. In all 17 colonies were surveyed within the UCC Science Botanical Garden.

Table 4: Nesting characteristics (mean nest entrance diameter and tube length) of *Hypotrigona* species collected from the UCC Science Botanical Garden

Nesting substrates	Mean Nest entrance diameter (NED)/ mm	Mean Nest entrance tube length (NETL)/ mm
Crack in wall (CW)	6.1 ± 0.0	12.0 ± 0.0
Bamboo internodes (BI)	6.1 ± 0.4	16.5 ± 4.9
Cavity in metal pole (CMP)	6.6 ± 0.5	38.6 ± 13.2

Mfuom community provided more nesting substrates for the genus *Hypotrigona* than the UCC Science Botanical Garden (Table 4). Specimens from (WWF) and (WMB) gave equal numbers of mean (NETL) of 13.3mm with (BI) recording the least mean (NETL) (Table 5)

Table 5: Measurements of nesting characteristics (mean nest entrance diameter and tube length) of *Hypotrigona* species within the Mfuom community

Nesting substrates	Mean (NED) mm	Mean (NETL) mm
Wooden window frame (WWF)	5.5 ± 0.3	12.3 ± 1.5
Crack in Wall (CW)	5.2 ± 0.3	10.3 ± 3.8
Bamboo internodes (BI)	6.5 ± 1.1	9.5 ± 4.0
Wooden meter board (WMB)	6.0 ± 0.5	13.3 ± 3.0

Within this community (Table 6) two nesting substrates were observed for species of the genus *Hypotrigona*; these were substrates on bamboo internodes and crack in mud houses. Although bamboo internode presented the largest nest entrance tube diameter, this did not reflect in the length of the nest entrance tube length.

Table 6: Mean external nest characteristics of the genus *Hypotrigona* within the Kwesi Gyan community

Nesting substrates	Mean (NED) mm	Mean (NETL) mm
Bamboo internodes (BI)	5.1 ± 0.5	9.7 ± 6.1
Crack in mud house (CMH)	4.1 ± 0.5	17.3 ± 4.9

This is a representation of the activities within the three study areas (Table 7), where (WWF) recorded the highest number of colonies whereas (WMB) and (CMH) showed equal colony numbers of *Hypotrigona* species. Different nest entrance tube lengths (NETL) were recorded from the localities as well.

Table 7: Nesting characteristics of the genus *Hypotrigona* within the three study sites

Nesting Habits	No of Colonies	Dimensions mean + se	
		Nest Entrance Diameter (mm)	**Nest Entrance Tube length (mm)**
Wooden Window Frame (WWF)	28	5.5± 0.3	12.3 ± 1.5
		(3-8)	(4.5-30)
Bamboo Internodes (BI)	21	5.1 ± 0.3	16.1 ± 2.9
		(2-8)	(9-45)
Cavity in Metal Pole (CMP)	8	6.6 ± 0.5	38.6 ± 13.6
		(4.8-8)	(7-105)
Wooden Meter Board (WMB)	3	6.0 ± 0.5	13.3 ± 2.9
		(5.1-7)	(9-19)
Crack inWall (CW)	5	5.4 ± 0.3	10.6 ± 2.9
		(4.7-6.1)	(11-18)
Crack in Mud Houses (CMH)	3	5.1 ± 0.5	9.7 ± 6.1
		(4.4-6)	(8-21)

Nest Entrance tube Characteristics

Generally it was observed that, nest entrance characteristics vary within the genus. In all three sites, there were few colonies that did not possess nest entrance tubes, while greater number of colonies sampled possessed nest entrance tubes of varied diameter and length (Figure 2). Among the preferred nest substrates, generally diameter of entrance tubes was not significant although in few instances, large diameter tubes were observed within colonies from cavity in metal poles. Cavity in metal pole (CMP) recorded the highest length in entrance tube within the three localities, followed by colonies from bamboo internode (BI) with the least entrance tube length recorded from crack in mud houses (CMH).

The ANOVA test for nest entrance tube length from the various sites (Table 8), exhibited a significant p value of 0.012, indicating possible variation among the genus *Hypotrigona* from the sampling sites.

Table 8: Variability within the genus using the nest entrance tube length

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2387.196	2	1193.598	4.157533	0.019994	3.138142
Within Groups	18661.03	65	287.0928			
Total	21048.23	67				

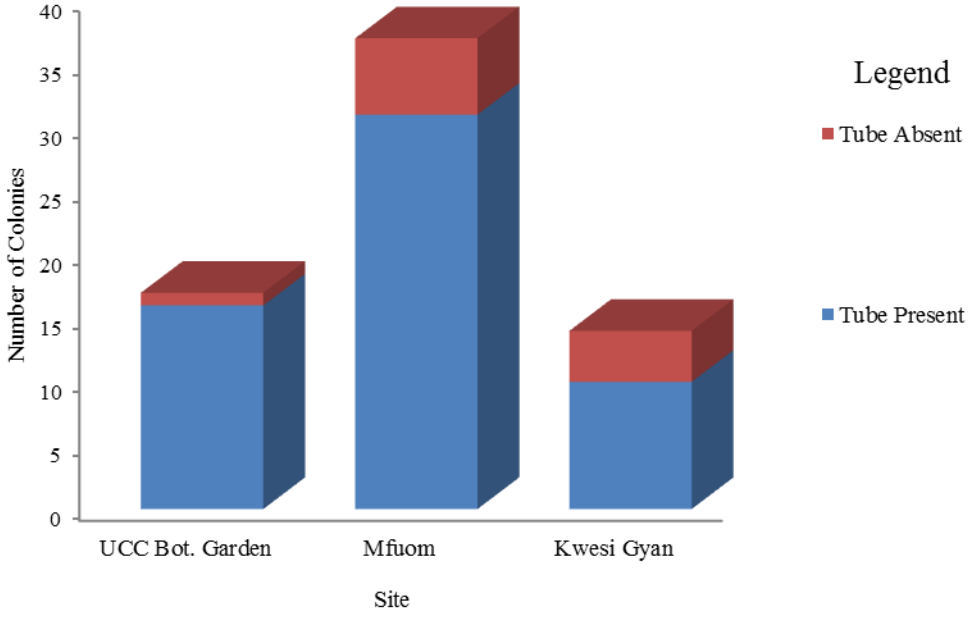


Figure 2: Nest entrance characteristic's within three sites showing presence or absence of entrance tube

In accordance with ranges within nest entrance tube length, four categories of tube length were observed in this study (Figure 3). There was a significant difference among tube length within the three study areas suggesting four possible groups within the genus *Hypotrigona* in the Central Region of Ghana.

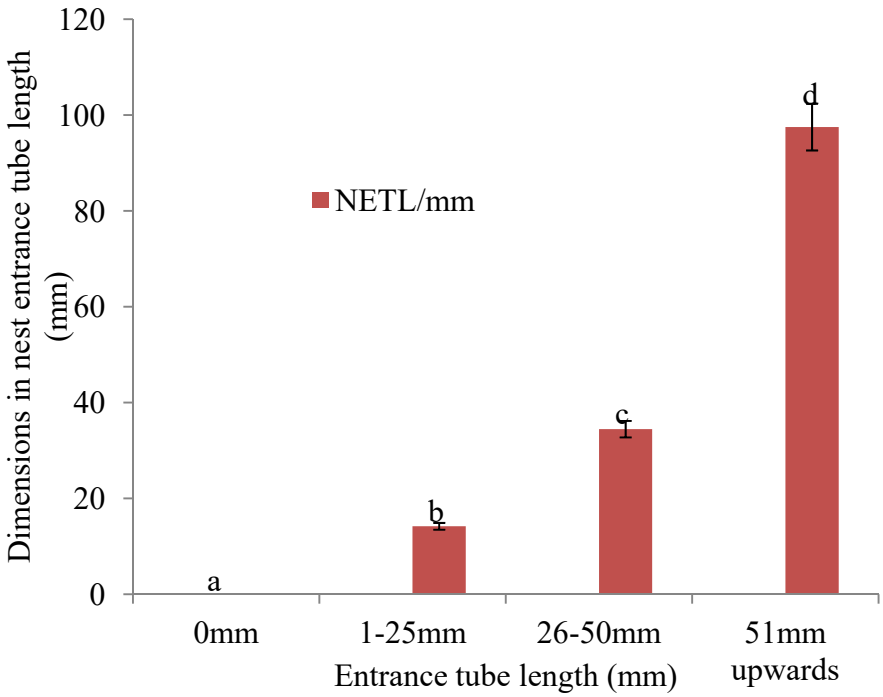


Figure 3: Variations in nest entrance tube lengths of the genus within the districts

GEOMETRIC MORPHOMETRICS ANALYSES

Geometric morphometrics techniques employed in this present study was able to detect possible variability within the genus *Hypotrigona*.

In all a total of 2027 right forewings of the genus *Hypotrigona* were used in the classification model to generate shape coordinates for wing morphometric analyses. Eight anatomical landmarks generated 12 principal components (PC's) of the wing shape. All statistical analyses conducted showed significant differences within the species from the three study sites.

In geometric morphometrics, Procrustes distance between the mean shapesafter they have been superimposed indicated that four of the eight landmarks accounts for most variations within the genus. For each landmark, the blue circle indicates the location of the landmark for the average shape and the

black dots indicate the locations for individual wings with red numbers indicating. There were lot of clustering of individual wings about the mean landmark, however, landmark 5, 6, 7 and 8 showed slight deviations about the mean landmark (Table 9, Figure 4).

Table 9: Average coordinates of shape after Procrustes superimposition

Landmark	Axis 1 (x)	Axis 2 (y)
1	-0.44519615	-0.19315225
2	-0.40653647	-0.13203639
3	-0.13334008	0.02455224
4	-0.09619907	0.10763362
5	0.12999012	0.07047551
6	0.26765791	0.16125792
7	0.63406135	0.07501711
8	0.04956239	-0.11374774

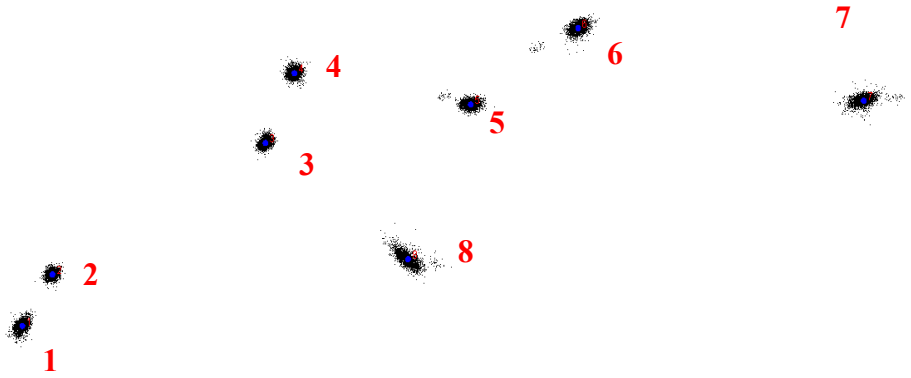


Figure 4: Landmark configuration of all wings in the dataset from the three localities after Procrustes superimposition

Data outlier assessed deviation of samples from the normal multivariate curve. The blue curve indicates the normal multivariate curve while red curve shows the distribution of the distances in the dataset. The red curve stretched out at the top of the diagram, proved that there were few specimens that deviate very strongly from the others (Appendix A).

Principal component analysis explored the main features of shape variation from the dataset since data outliers showed deviation of specimens.

On the wing morphometric analysis of three populations, the Principal component analysis (PCA) on eight anatomical homologous landmarks generated 12 principal components. The first eleven eigenvalues greater than one accounted for 98.812% variability among the samples tested with the twelfth value, giving 100% variability among samples analyzed. The major contributors to the variability within the genus are from the first three factors (PC1, PC2 & PC3), which contributed to 72.093% of the total variability. Each of the three factors contributed 38.684%, 23.610% and 9.799% respectively (Figure 5; Appendix B).

On the PCA score graphical representation, samples were crowded about the centroid, however, a few were pulled further away from the centroid with some scattered just around the centroid. Some specimens from Mfuom community with medium entrance tube length were distinctly pulled further away from the centroid while most of the specimens from all sampled location clustered around the centroid (Figure 6). Letters A, M, S, L or AET, MET, SET and LET representing absent and medium, short and long entrance tubes respectively.

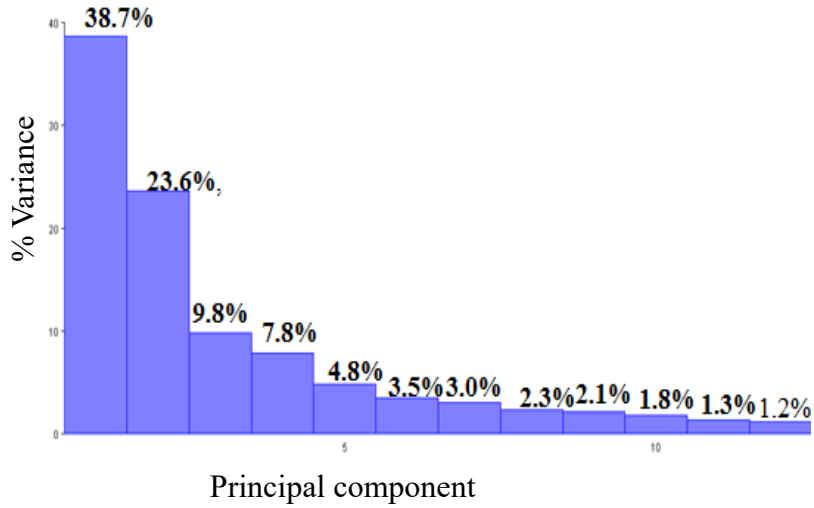


Figure 5: Percentage variation from the Principal Component Score for all specimens from the three districts

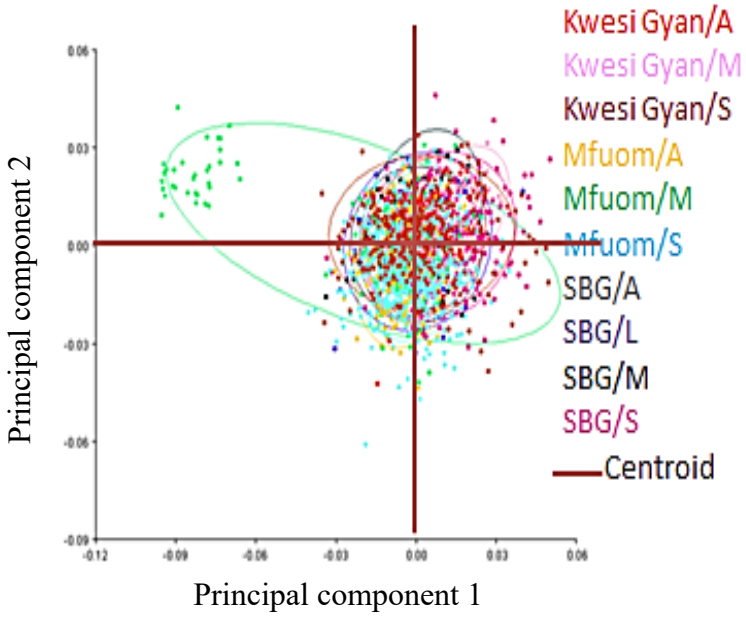


Figure 6: Scatterplot of Principal component scores showing scattering of specimen about the centroid with possible distinct sub-group

In order to confirm the extent of variability, a more detailed analysis was conducted that is the Canonical variate analysis (CVA). This method was used to find the shape features that best distinguish among multiple groups of specimen (Figure 7). During the CVA, some specimens from Mfuom with medium entrance tubes were clustered farther away from the centroid as compared to the rest of the specimens.

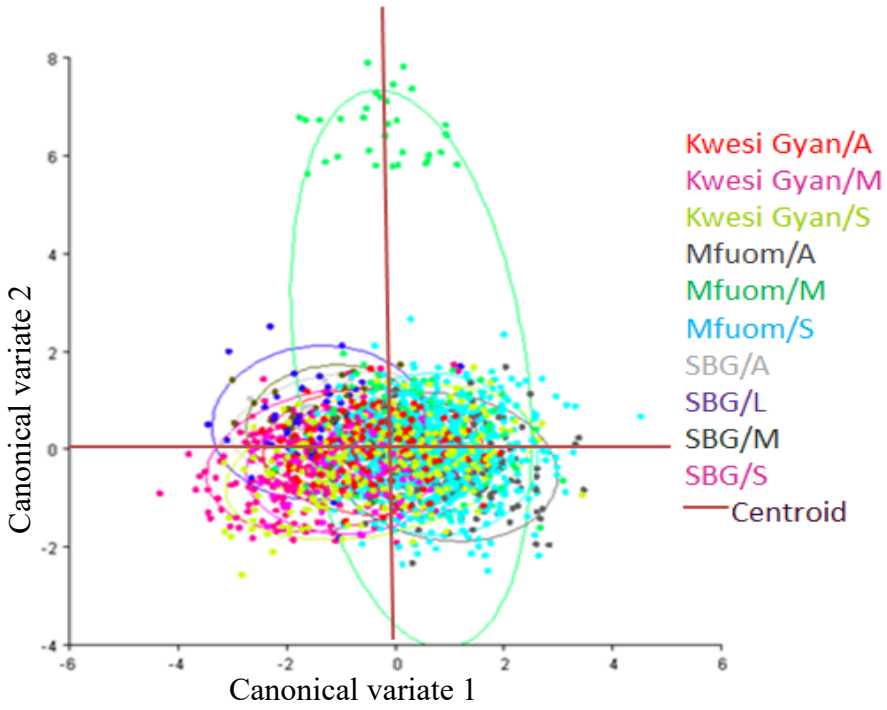


Figure 7: Scatterplot of Canonical variate analysis showing cluster of specimens about the centroid from the three study sites

The first five factors of the CVA scores greater than one, contributed most to the variation although, there were overlaps this accounted for 98.64%. The first three components contributed 87.6% of the variability within the genus; that is (CV1, CV2 and CV3), with the variables being 58.171%, 21.987% and 7.395% respectively (Table 10).

Table 10: Components of Canonical variate analysis showing the degree of variation among specimens

Canonical variate	Eigenvalues	Variance%	Cumulative %
1	0.68000299	58.171	58.171
2	0.25701926	21.987	80.158
3	0.08644068	7.395	87.553
4	0.05029281	4.302	91.855
5	0.04244414	3.631	95.486
6	0.03688131	3.155	98.641
7	0.00885081	0.757	99.398
8	0.00538739	0.461	99.859
9	0.00164479	0.141	100

Once CVA exhibited variability among specimens from the three study sites, discriminant function analysis and cross-validation test were conducted on the wing coordinates with regards to the entrance tube length and the sites of collection.

Discriminant function analysis (DFA) and cross-validation test showed highly significant difference within the populations with P-value of < 0.0001. Colonies tested showed variation in terms of nest entrance characteristics.

With the DFA, two groups are usually assessed at a time showing some percentage of variability among the groups assessed. A percentage score of >75% in both DFA and Cross-validation test will allow for separating the two groups being assessed as belonging to different groups altogether with their p-values indicating the level of significance of the groups being assessed. However, any value below the 75% mark is not good enough to permit separating the groups under examination (Klingenberg, 2009, 2011).

The discriminant score between two districts with absent entrances tube differed from each other (Figure 8; Appendix C). Kwesi Gyan recorded 82% of the variability whereas Science Botanical Garden recorded 90% of the variability and this is a firm ground in on which specimens collected from those two sites could be separated. Further, cross-validation test showed positive result where Kwesi Gyan recorded 74.2% and Science Botanical Garden recorded 76.7% of variability (Figure 9). The groups were highly significant with p <0.0001.

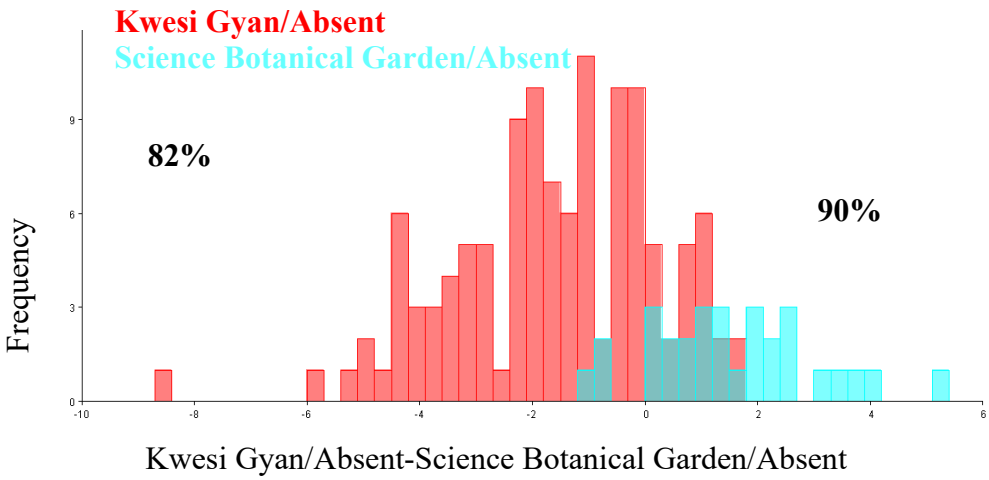


Figure 8: Discriminant score between samples collected with absent entrance tube

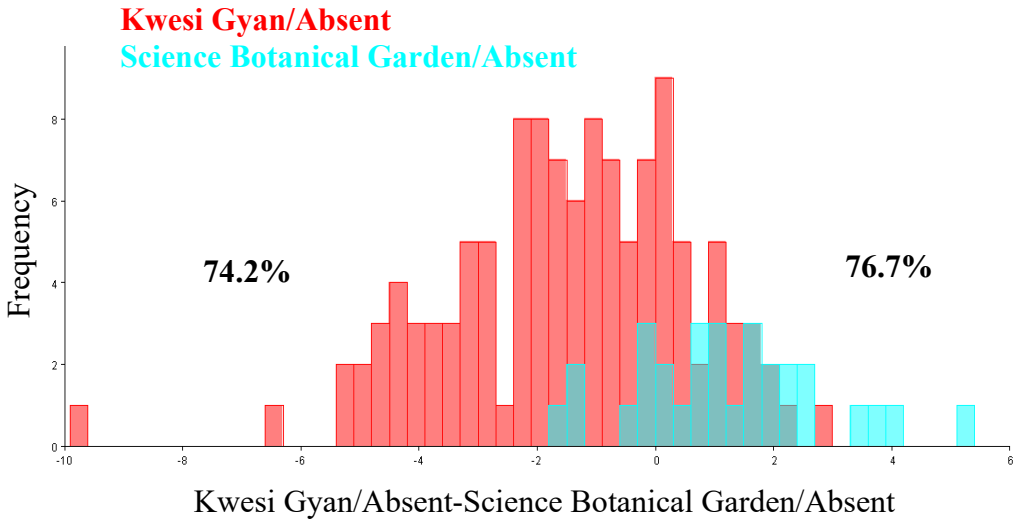


Figure 9: Cross-validation score for samples with absent entrance tube from two sites

DFA between samples from Kwesi Gyan and Mfuom communities indicated a discriminant score 88.3% of variation within Kwesi Gyan community while Mfuom community represented a score of 83% (Figure 10). These values indicate some degree of variation among the samples collected. The cross-validation test performed on nest entrance with absent tube length from the two

communities spelt out the variation with Kwesi Gyan recording 85% of the variation and Mfuom registering 82% (Figure 11; Appendix E).

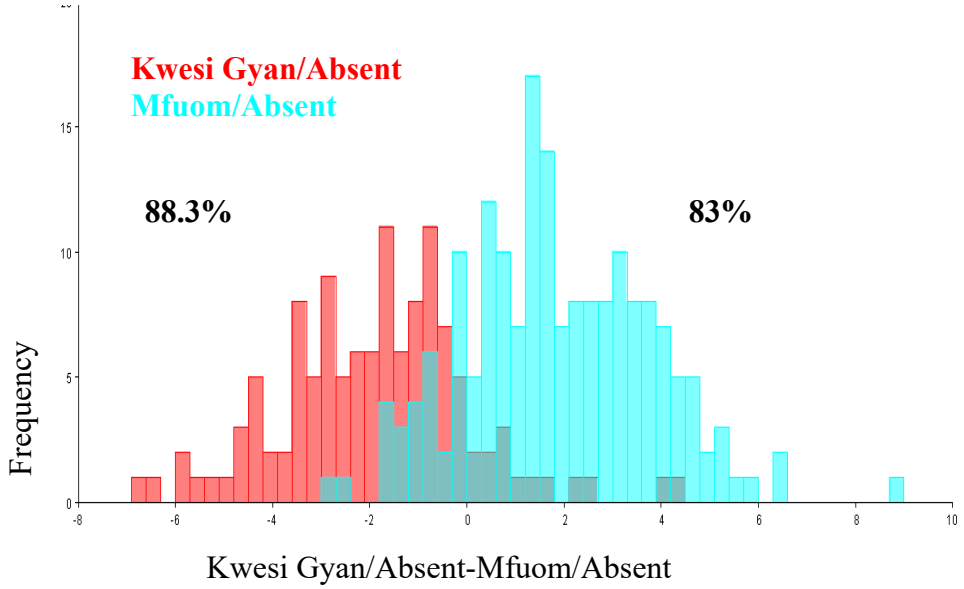


Figure 10: Discriminant score showing variation among species collected from two districts with absent nest entrance tube length

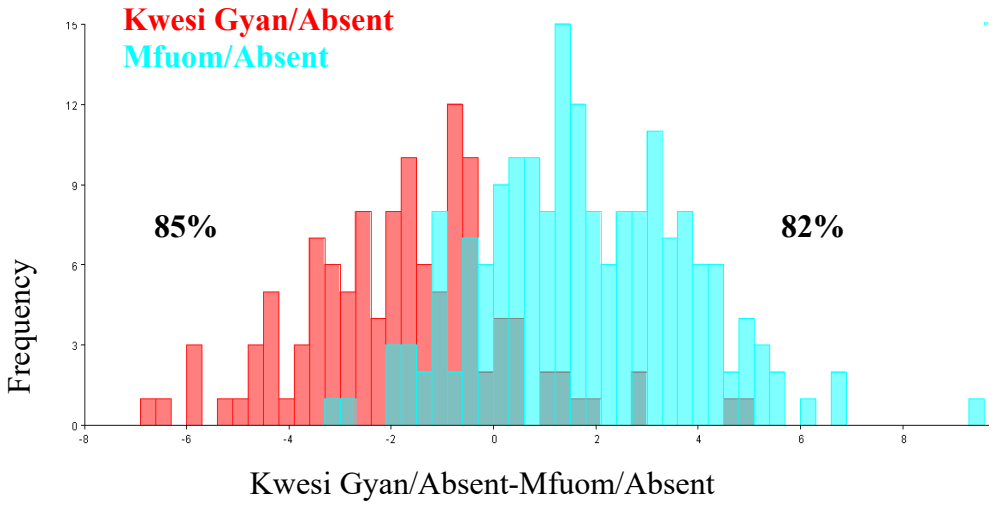


Figure 11: Cross-validation score confirming the degree of variation among the two communities

Specimens collected from Mfuom and Science Botanical Garden recorded the greatest level of variation within colonies with absent entrance tube length. There was 91.7% variation among the Mfuom collections and as much as 93% variability among the Science Botanical Garden for discriminant score. However, cross-validation test also made things clearer by giving 88.9% and 93% variability among collections from Mfuom and UCC Science Botanical Garden respectively (Figure 12 & 13; Appendix D)

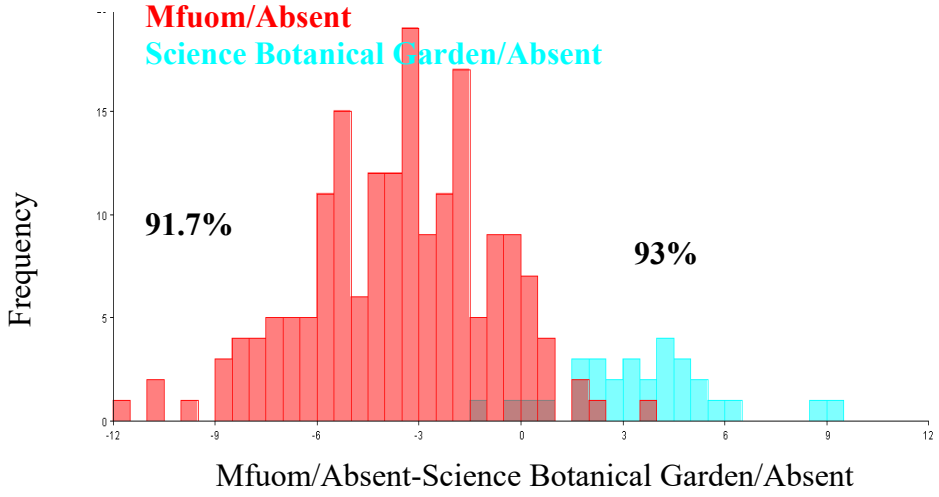
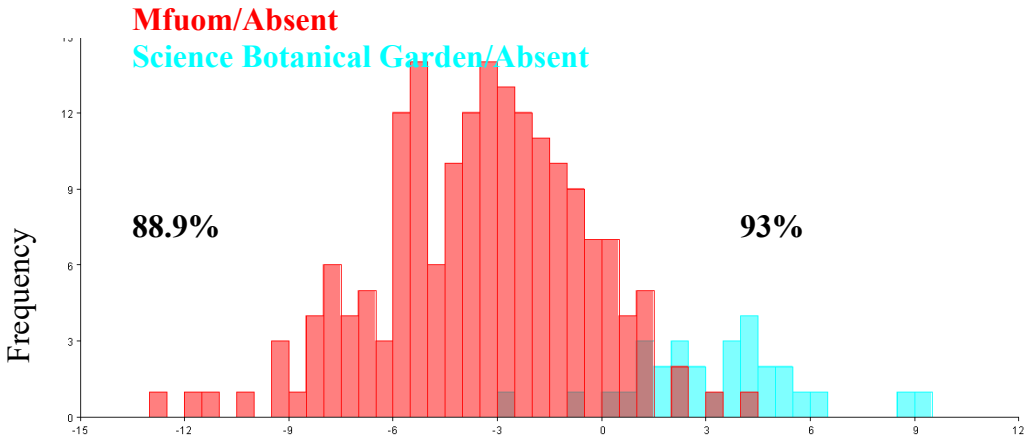


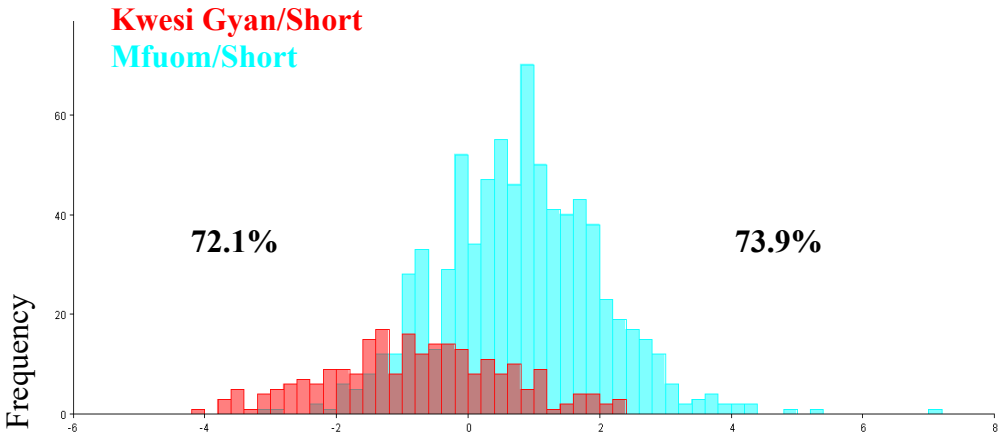
Figure 12: Discriminant scores from Mfuom and Science Botanical Garden with absent entrance tubes



Mfuom/Absent-Science Botanical Garden/Absent

Figure 13: Cross-validation score showing the degree of variation within the two sites

For nesting tubes with short length within Mfuom and Kwesi Gyan districts, the discriminant score was 72.1% and 73.9% respectively (Figure 14). A cross-validation score did not show much deviation from the 70th mark but also registered 70% and 72% respectively (Figure 15 and Appendix F).



Kwesi Gyan/Short-Mfuom/Short

Figure 14: Discriminant score of tubes with short length from Kwesi Gyan and Mfuom communities

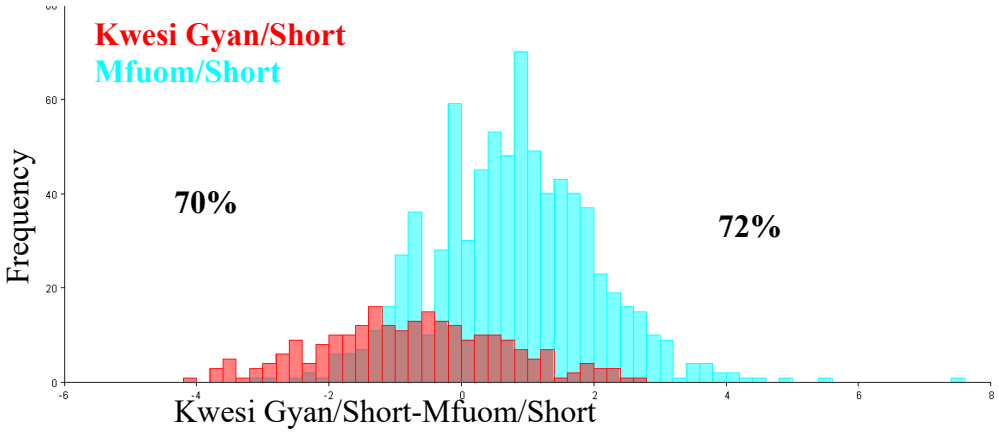


Figure 15: Cross-validation score for tubes with short length within Mfuom and Kwesi Gyan sites

The discriminant score among samples collected within Mfuom and Science Botanical Garden study areas, recorded 86.1% and 80.3% respectively of the variation. Cross-validation test within the two sites showed much variation within the two sites that is 85.7% within Mfuom collections and 78.9% among samples from the Science Botanical Garden (Figure 16, 17 and Appendix H).

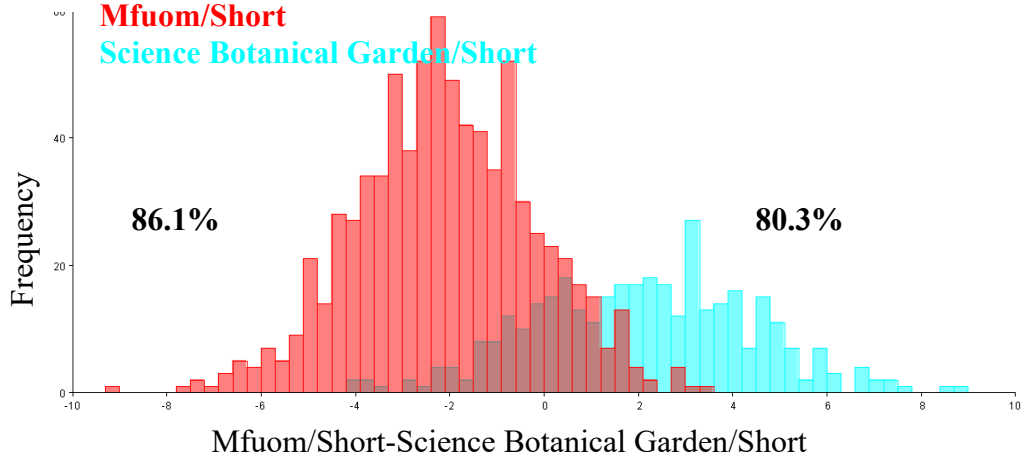


Figure 16: Discriminant score for sample collected with short tube length from Mfuom and Science Botanical Garden

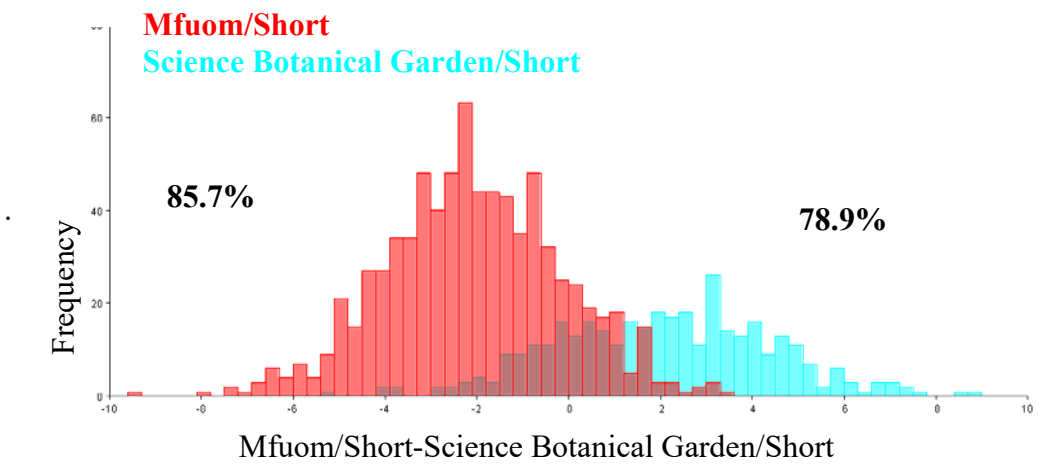


Figure 17: Cross-validation score showing variation among samples with short tube length

Variation among samples collected from Kwesi Gyan district with short entrance tube length 69.2% (Figure 18) and 67.9% (Figure 19). That for samples collected from Science Botanical Garden was 73% (Figure 18) as against 71.9% (Figure 19 and Appendix G).

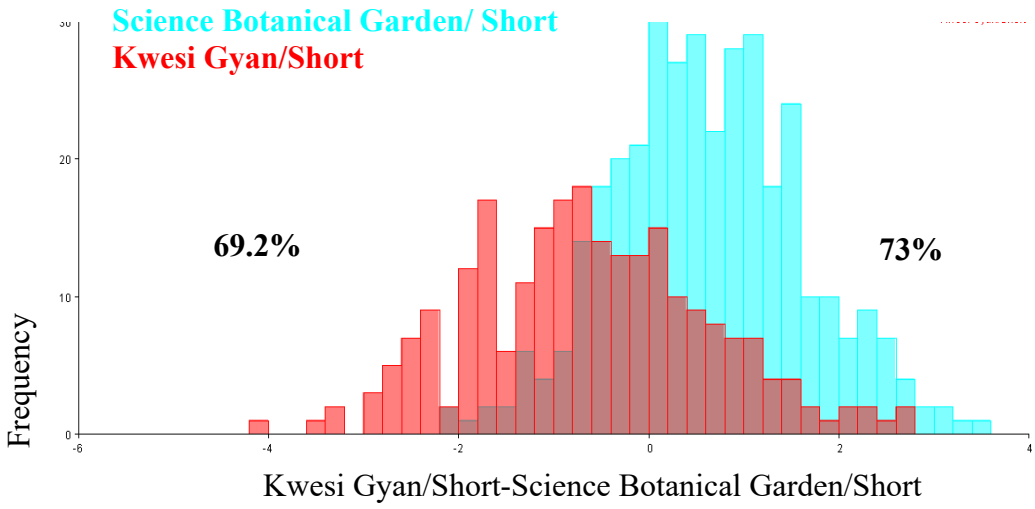


Figure 18: Discriminant test for samples collected from sites with short entrance tube length

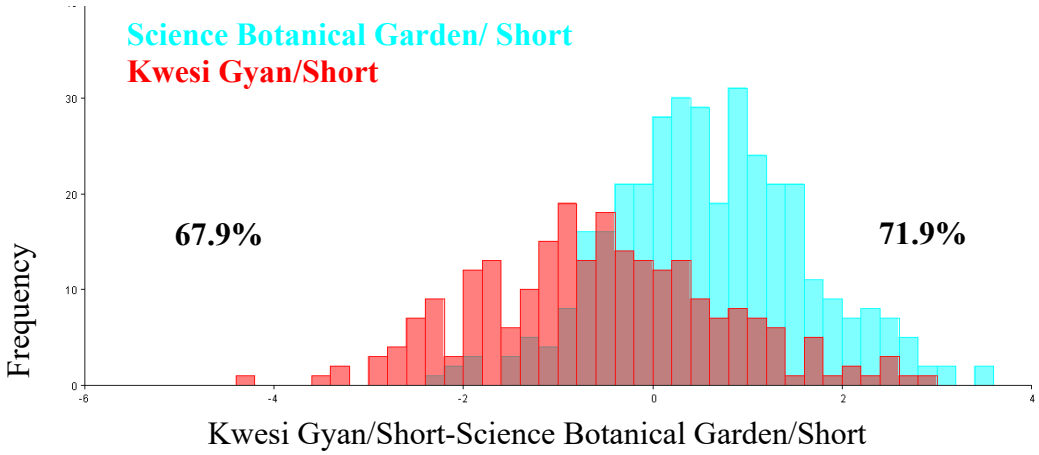


Figure 19: Cross-validation score displaying samples from two sites with short entrance tube length

Thus from the graphical representation, the discriminant function tests within the two study sites were, Kwesi Gyan recorded 85% and Mfuom was 85.3% (Appendix I). In performing a cross-validation test on the specimens collected from these sites, Mfuom still recorded much variation than Kwesi Gyan; however, the variations were all within the 80% range (Figure 20 and 21)

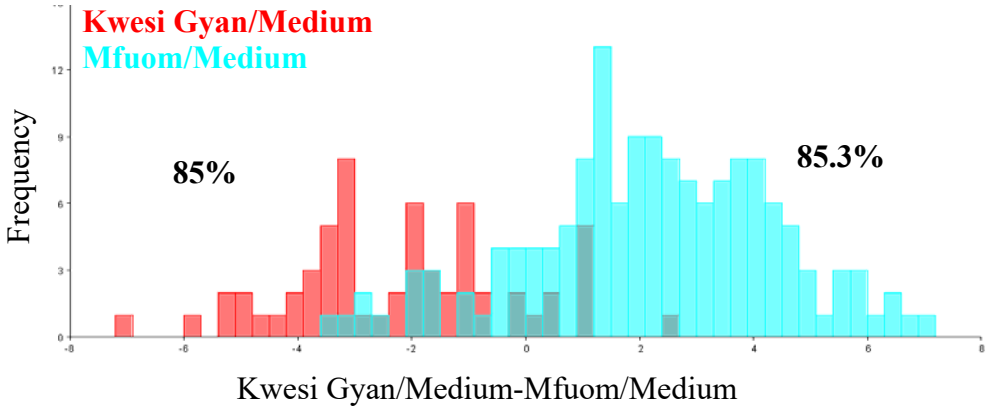


Figure 20: Discriminant score for samples from sites with medium entrance tube length

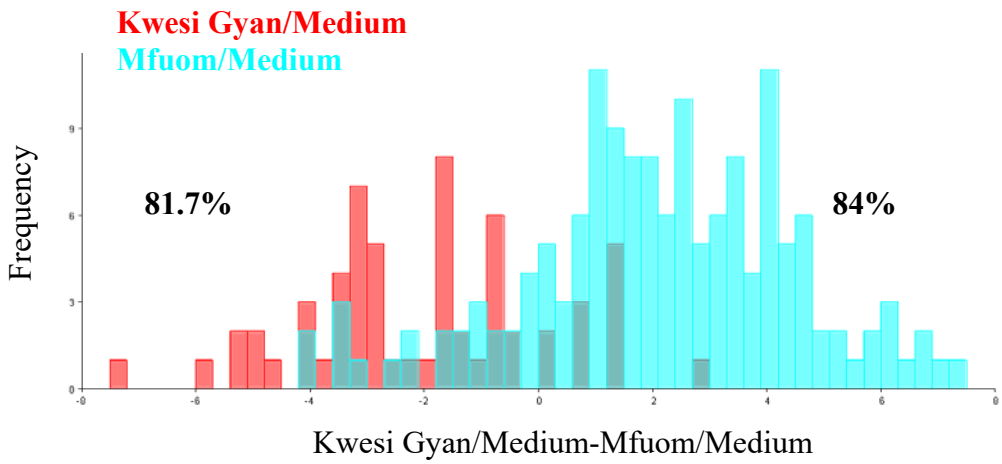


Figure 21: Cross-validation score from sites with medium entrance tube length

Collections from these two sites Mfuom and Science Botanical Garden shows variations with the discriminant score (Figure 22) and much variation with the cross-validation score (Figure 23 and Appendix J).

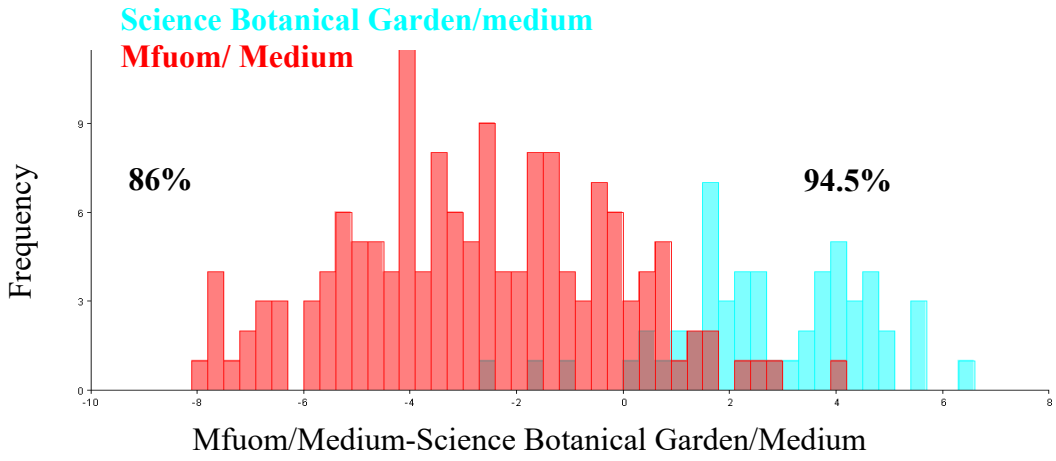


Figure 22: Discriminant score for samples collected from Mfuom and Science Botanical Garden

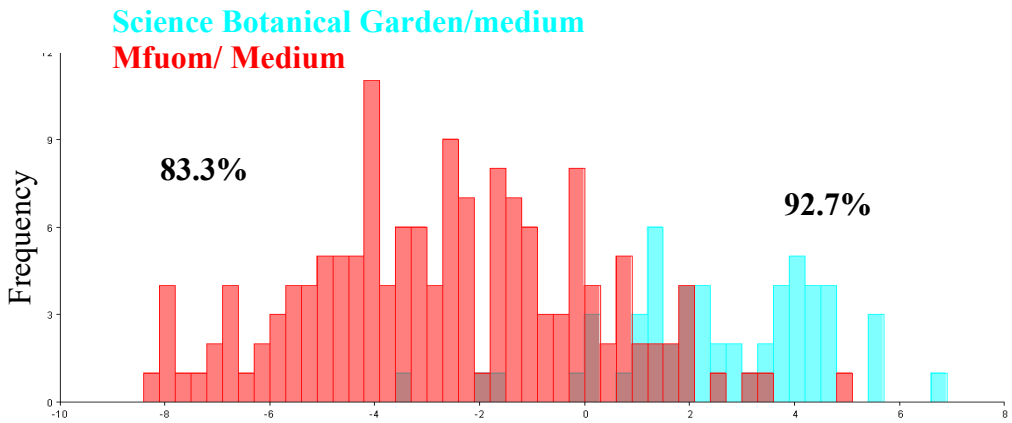


Figure 23: Cross-validation score for samples collected from medium entrance tube length

Specimens collected from Kwesi Gyan and Science Botanical Garden with medium entrance tube lengths, had a significant p-value of <0.0001 and a discriminant score of 90% for Kwesi Gyan community and 92.7% for the Science Botanical Garden (Figure 24). The cross-validation score also give 88.3% variation for the Kwesi Gyan community and 89.1% for the Science Botanical Garden (Figure 25 and Appendix K).

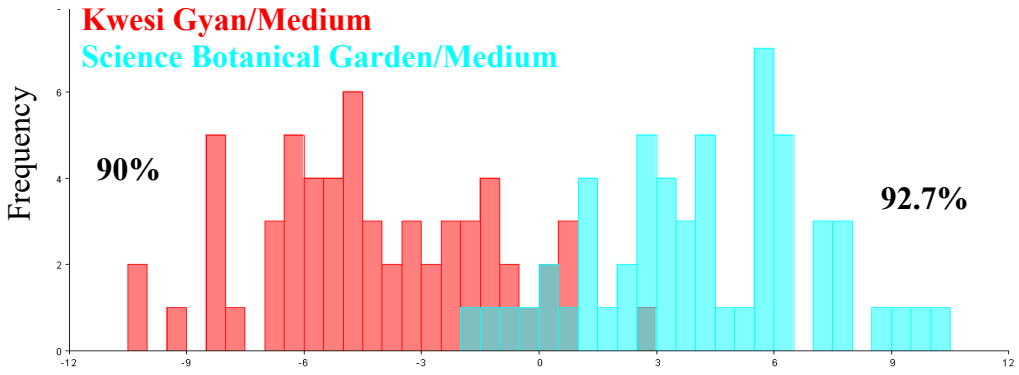


Figure 24: Discriminant score for samples from Kwesi Gyan and Science Botanical Garden with medium entrance tube length

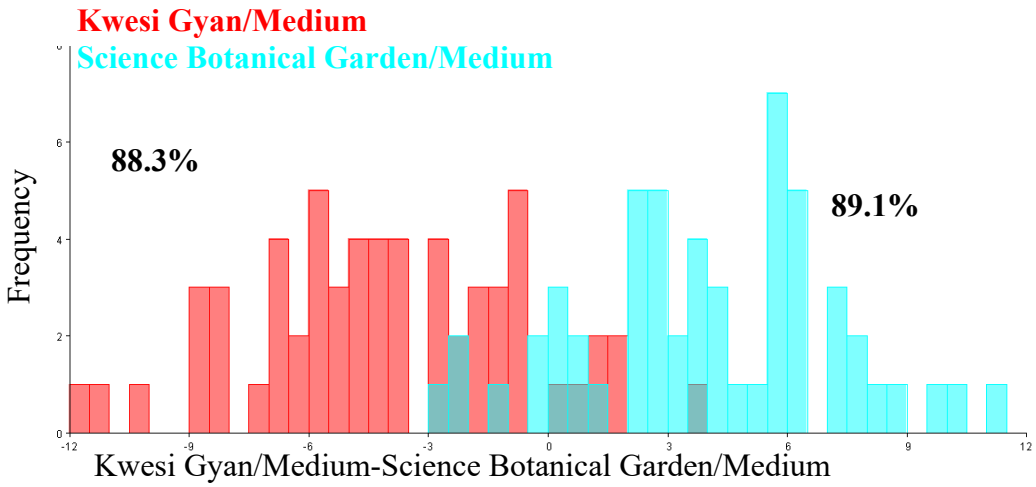


Figure 25: Cross-validation score for medium entrance tube length within two districts

The last set of comparison was performed using the UCC Science Botanical Garden with long entrance tube length against the other experimental sites with medium entrance tube lengths. This was done as only one site was observed having a long nest entrance tube length.

Collections from these two study sites with different nest entrance tube length had greater percentages of DFA and cross-validation score with a highly significant p-value <0.0001 (Figure 26, 27 and Appendix L).

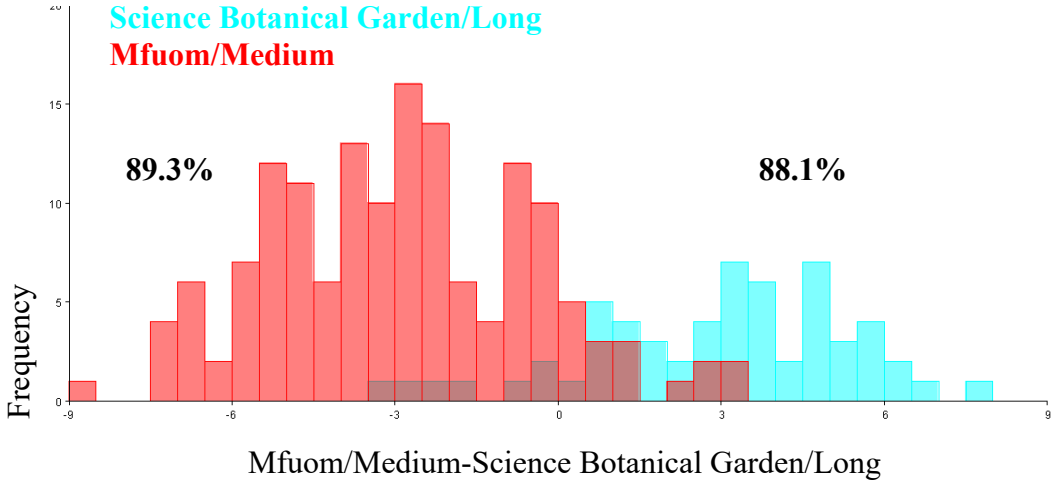


Figure 26: Discriminant score for samples collected from two experimental sites with different entrance tube lengths.

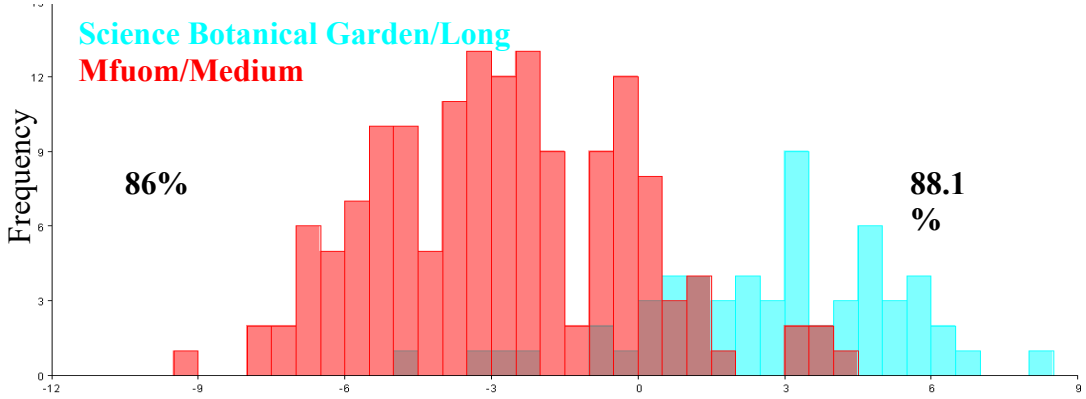


Figure 27: Cross-validation score showing the rate of variation among samples collected from two experimental sites.

Variability with regards to percentage score was great for samples within these experimental sites. The p-values were both significant for the comparison and for 1000 permutations (Appendix M).

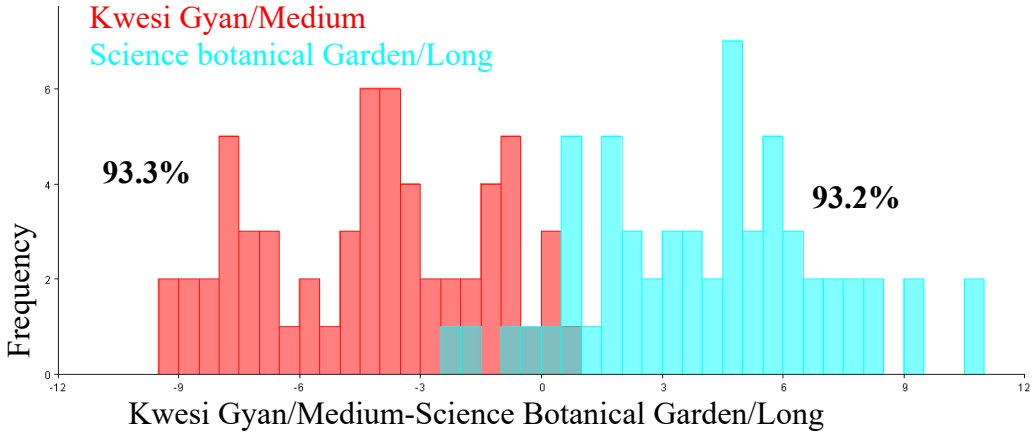


Figure 28: Discriminant score showing variation among samples from two experimental sites with different entrance tube lengths

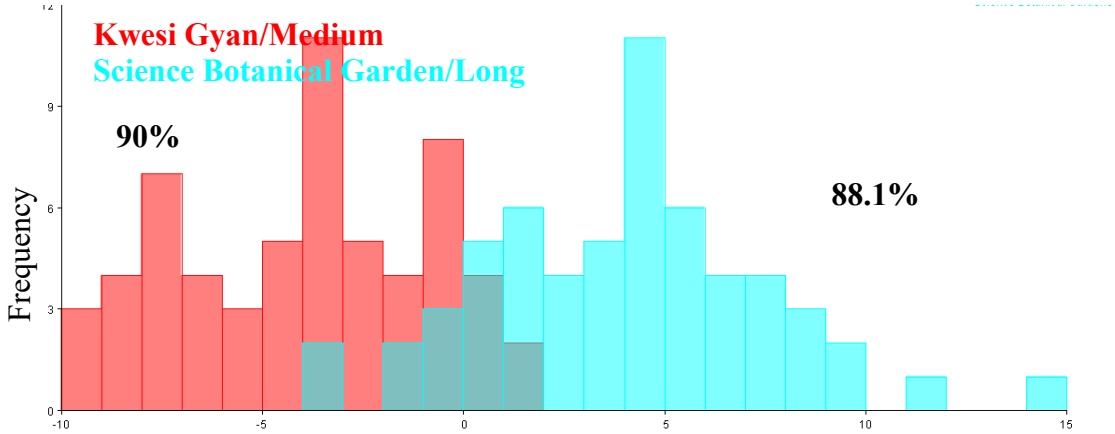


Figure 29: Cross-validation score for samples from two study sites with different entrance tube length

Samples collected from the same study sites with differences in nest entrance tube length showed high percentage scores for both DFA and cross-validation scores with $p < 0.0001$ which is highly significant (Figure 30, 31 and Appendix N).

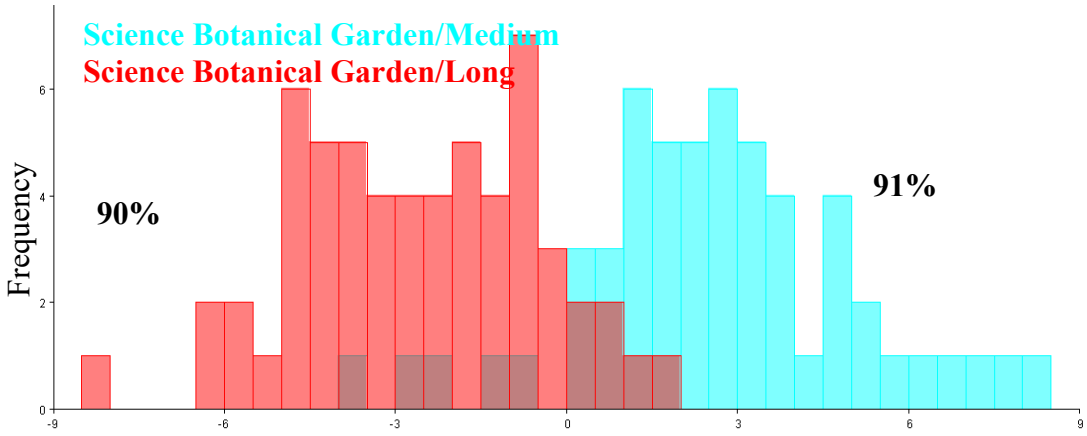


Figure 30: Discriminant score for samples from the same experimental sites with different entrance tube lengths

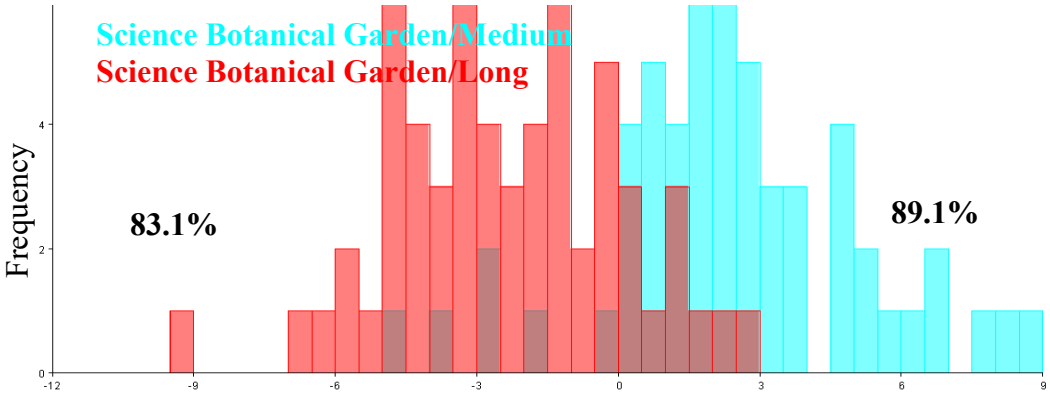


Figure 31: Percentage variability among samples from the same experimental site with different entrance tube lengths

The correlation analysis between the nest entrance tube length and the site against the Procrustes coordinates resulted in a positive relationship among the parameters tested on the genus *Hypotrigona*. The dispersion of individual points was aggregated about the centroid with a few of the dispersions just above or

below this mark. The coefficient r= 0.828 and the correlation determinant was 69% (Figure 32).

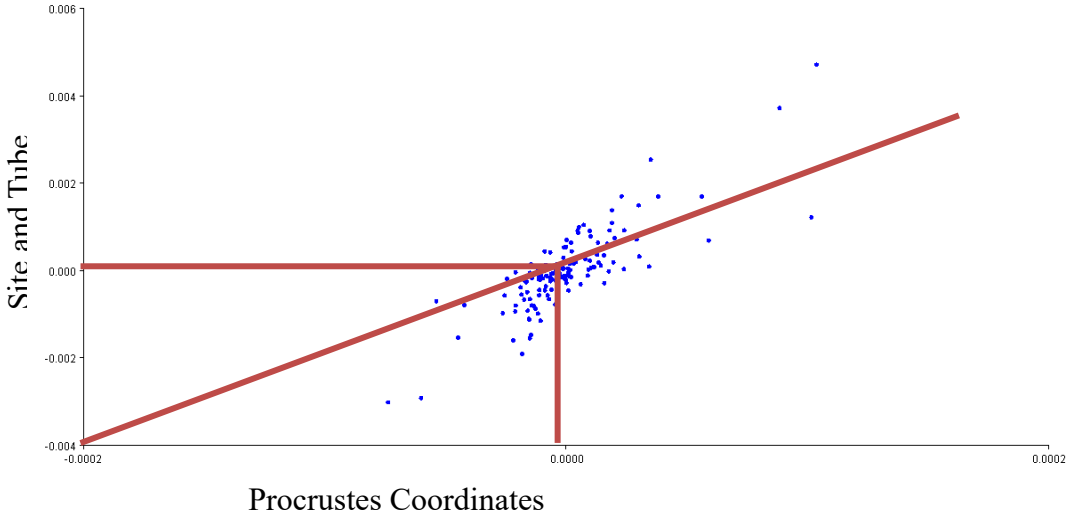


Figure 32: Correlation graph showing the relationship between site and tube entrances within the study sites

Mean squares (MS) are the amount of variation from the one higher level in the hierarchy. The (F) value represents the comparison of each MS to the one lower level of MS which could be the source of error. Procrustes ANOVA estimated the variation at each level (individual) from the deviation from the mean shape which corresponded to the one higher level in the hierarchy. The analysis of variance, using Procrustes sums of squares (SS) as a measure of overall variation in shape, showed that individual variation was significant. The p-values of both Procrustes ANOVA and Centroid size were <0.0001 (Table 11, 12). The measurement error was negligibly small compared to the source of variation dealt in the analysis. There was a positive correlation based on the structure of the wings and the experimental site.

Table 11: Specimens centroid size from three localities within the study areas

Centroid Size					
Effect	SS	MS	df	F	P (param)
Individual	1306220.368	163277.546	8	48.88	<0.0001
Residual	6740583.397	3340.229632	2018		

Table 12: Procrustes ANOVA for the genus *Hypotrigona* sampled from the three Districts

Shape, Procrustes ANOVA						
Effect	SS	MS	df	F	P (param)	Pillaitr
Individual	0.059365	0.000619	96	11.9	<0.0001	0.44
Residual	1.33901	0.0000553	24216			

In accordance with the regression analysis, three groups of clustering were exhibited (Figure 33). This confirms the variability encountered in all the analyses. Here again, some collections from Mfuom with medium entrance tube length were pooled further away from the other two groups.

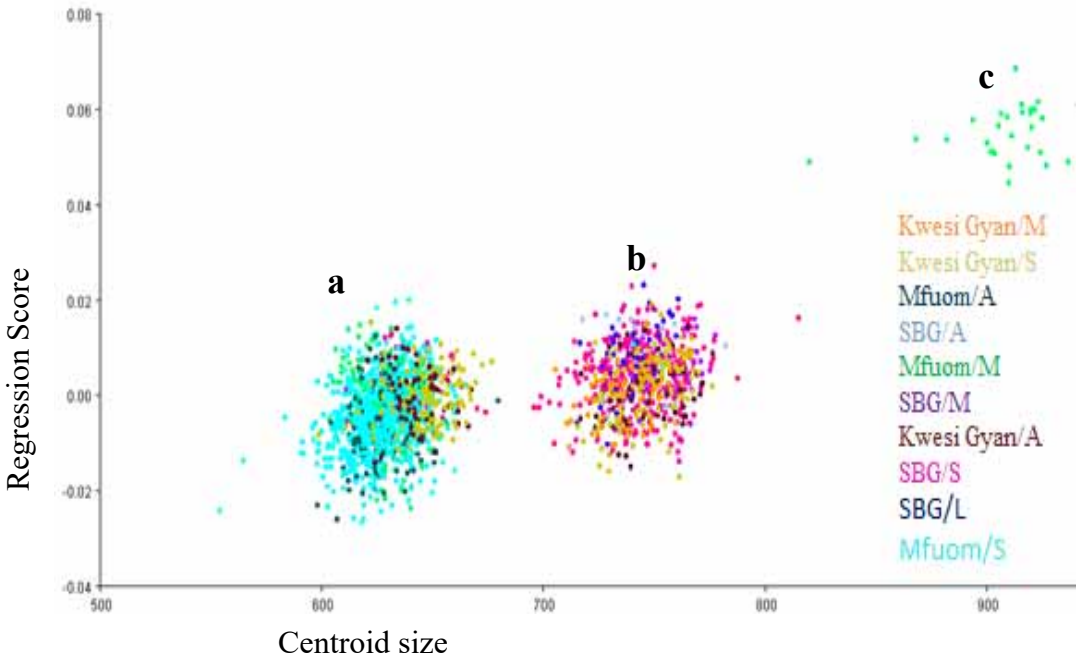


Figure 33: Regression scatterplot showing the degree of variation of points about the centroid mark

Regarding the result of the groups established by the regression graph (Figure 33), individual wings belonging to each of the three groups were displayed to explain the extent of variability within the genus. A wire frame showing shape changes also depicted the rate at which the wing shape varied from each other (Figure 34 to 39). The alphabets (a, b and c) represented the respective groupings from the regression graph.

The graphical output in (Figure 34) is an expansion from (Figure 33) showing the landmark of a wing belonging to group (a) with a wireframe graph giving a visual representation of the change in the wing shape (Figure 35).

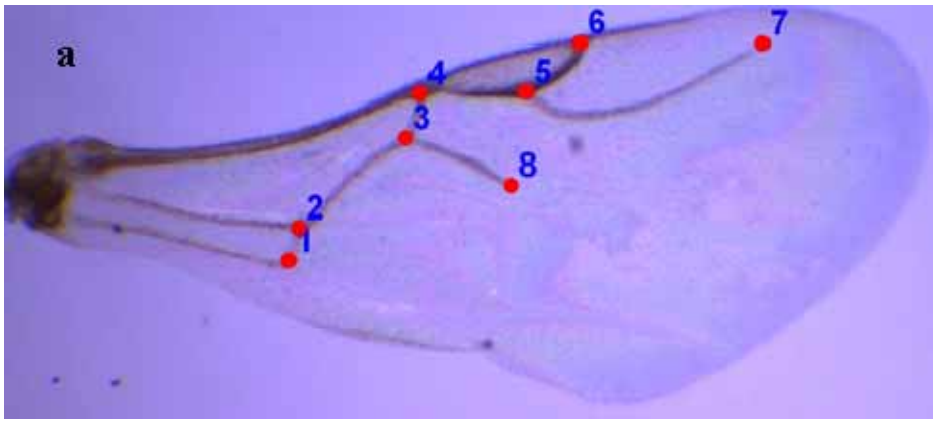


Figure 34: Example of wing belonging to group (a)

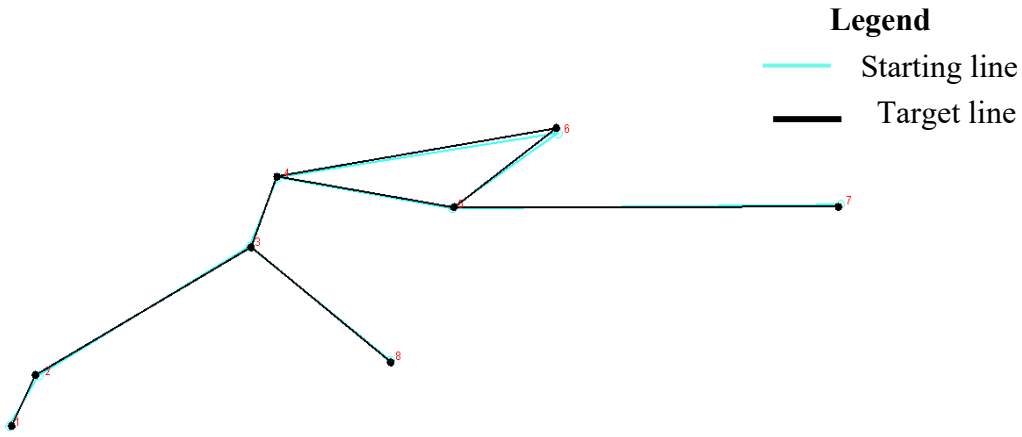


Figure 35: Wireframe shape change within group (a)

This is a representation of the shape change in wings belonging to group (b) from figure 33, showing the landmark and the extent to which the shape changes using a wireframe graph (Figure 36, 37).

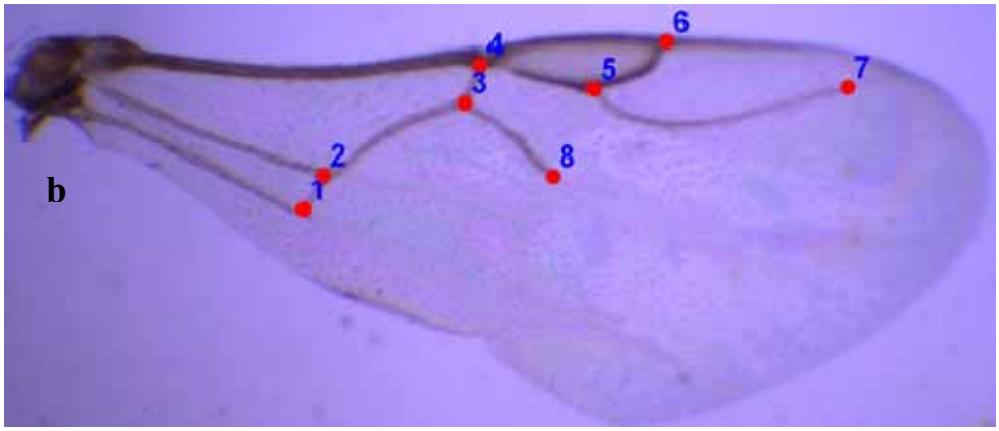


Figure 36: Forewing belonging to group (b) from the regression graph

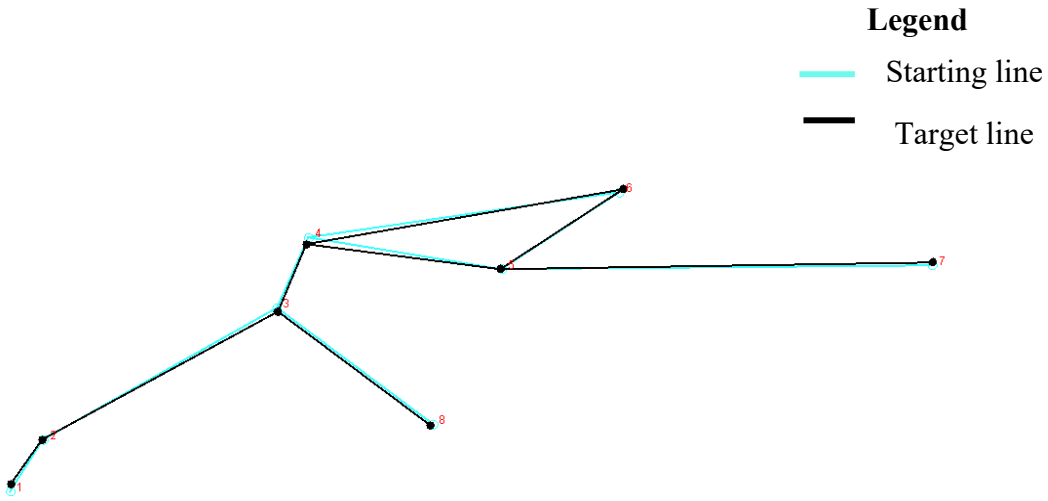


Figure 37: Wireframe graph showing the shape change of wings in group (b)

This is a forewing belonging to the last group (c) from the regression analysis (Figure 33) showing the position of the landmarks with a wireframe graph giving a visual representation of how shapes in group c vary from the average shape (Figure 38, 39).

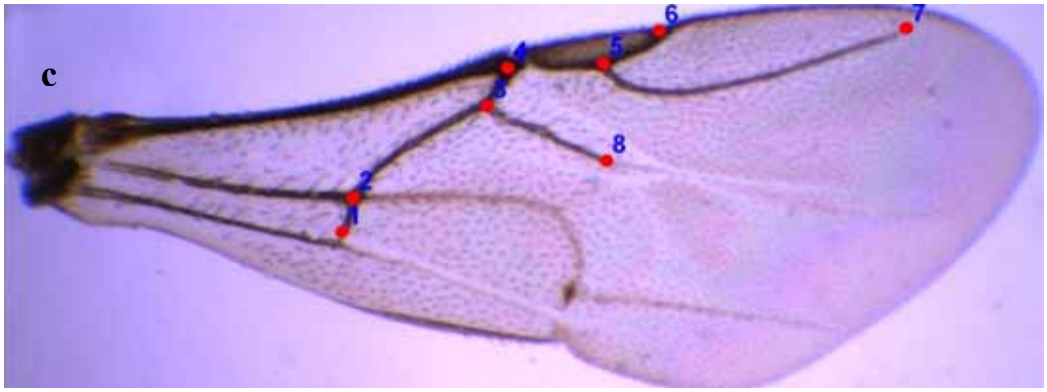


Figure 38: Forewing of *Hypotrigona* species representing group (c)

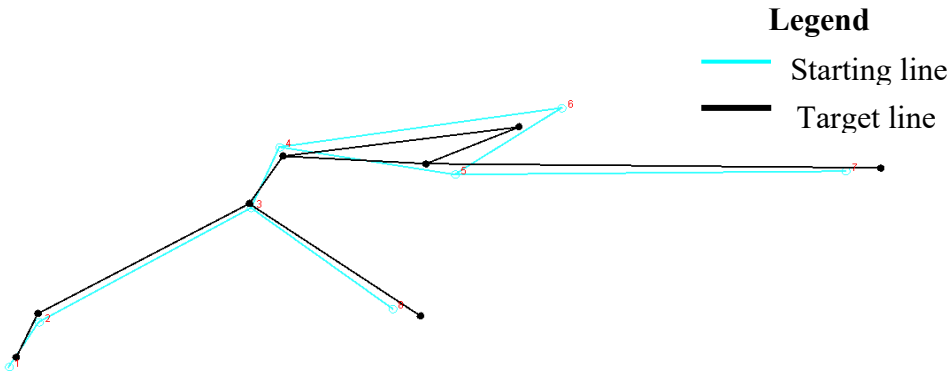


Figure 39: Wireframe shape change on group (c).

TRADITIONAL MORPHOMETRY ON THE ENTIRE BEES

The regression graph analysis indicates that three species could be recognized. The biological features for distinguishing these species included measurement of the entire body length, head width among others.

The differences between the three species are shown in (Table 13).

Table 13: Characters for distinguishing species within the genus *Hypotrigona* from the three study sites

Characters for identification	*Hypotrigona gribodoi*	*Hypotrigona araujoi*	*Hypotrigona ruspolii*
Entrance of nest tube length	0-45mm	27-30mm	0-105mm
Level of Aggression	Less aggressive	Non-Aggressive	Highly aggressive
Entire body length	2.8-3.3mm	4.0-4.8mm	3.4-3.6mm
Head width	1.2mm	1.4mm	1.2mm
Eye length	0.6-0.7mm	1.1-1.3mm	0.9-1.0mm
Upper Interocular distance	0.4-0.6mm	1.0-1.1mm	0.7-0.8mm
Lower interocular distance	0.4-0.5mm	0.7-0.8mm	0.52-0.6mm
Scape	0.30-0.36mm	0.45-0.53mm	0.45-0.53mm
Hamuli number	5	6	5
Forewing length.	2.4-2.8mm	3.0-3.5mm	2.4-2.8mm
Head shape	Heart shaped	Rectangular shaped	Heart shaped

Discussion

This section seeks to draw explanations from the results in previous chapter giving possible reasons to the outcomes.

***Hypotrigona* Species in all three Study Sites**

Three species of bees were identified within the genus, namely; *Hypotrigona gribodoi*, *Hypotrigona araujoi* and *Hypotrigona ruspolii*. Among the three species, *H. gribodoi* and *H. ruspolii* were the most dominant species in three

sites while *H. araujoi* seems to occur in only Mfuom community. These three *Hypotrigona* species have been recorded as the only species of this genus in Ghana and Africa at large (Moure, 1961: Michener, 1959; Eardley, 2004; Kwapong et al., 2010; Njoya, 2009). In Eardley (2004) for instance, all three species were differentiated based on integumental colour distribution and vestiture (hair). In addition to the above mentioned features, the presence or absence of an imaginary line on hind femur as well as species distribution and list of host plants visited were used to separate species. Moure (1961), separated species within the genus based on lengths and ratios. In spite of the existence of these taxonomic keys, their use is quiet difficult in separating species within the genus. Hence the need to explore alternative protocols to augment the accurate separation of species within the genus. In the present study however, the three species were separated based on lengths and ratios, level of aggression and nest entrance tube length as well as using the differences in wing shape to effectively categorize members of this genus. This permits the easy identification of species within the genus especially in the field.

ASSESSING NEST ENTRANCE CHARACTERISTICS

Using Aggression as a Character for Distinguishing Species

The social regulation of aggression plays an important role in competitive interactions among animals including bee communities (Moynihan, 1998; Reitz and Trumble, 2002). In social bees especially members of the tribe Meliponini, group aggression plays a vital role during nest defense (Michener 1974; Breed, Robinson and Page. 1990; Hölldobler and Wilson 1990; Kwapong et al., 2010).

The level of aggression at the nest entrance is through biting the body parts of intruders, entering any available hole and by mass attack (Kirchner and Friebe, 1999).

This present study assessed levels of nest entrance aggression as a species specific character. Aggression among species seems to be highly variable among the three species. Highly aggressive behaviour was mostly distinct among members of *Hypotrigona ruspolii,* whereas members of *H. gribodoi* exhibited less aggressive nest entrance behaviour. In *H. araujoi* however, a non-aggressive behaviour seems to be exhibited among members. Defense in *H. araujoi* therefore could possibly involve the use of their large body size as a barrier to prevent intruders from gaining assess into the nest. *Hypotrigona araujoi* also appears to defend nest entrances with only one or two entrance guards as is observed in other stingless bee species with non-aggressive workers (Couvillon, Wenseleers, Imperatriz-Fonseca, Nogueira-Neto and Ratnieks, 2007).

Variation among Species Using the nest Entrance tube Length

In terms of the nest entrance tube lengths, bees of this genus may or may not build entrance tubes as was found in members of the species *H. gribodoi* and *H. ruspolii*. In cases where entrance tube was present, as was observed in all colonies of the species *H. araujoi*, tube appeared transparent exiting from nest substrates. This character of nest entrance type has been reported by several authors ascertaining it as a tool for distinguishing small-sized genera of stingless bees. Among colonies of *H. gribodoi* and *H. ruspolii* that lack entrance tubes, propolis linings are known to be present within the interior portion of the nest to

provide the needed security into or out of the nest (Wille and Michener, 1973; Njoya, 2009).

Variation in the length, shape, camouflage and firmness of the entrance tube indicates a defense role in many stingless bee species (Njoya, 2009). It is important to note that, entrance tubes are highly variable in length among the three species when present. Thus *H. ruspolii* possesses the longest nest entrance tubes when present ranging from 0 -105 mm whereas that of *H. gribodoi* was 0 - 45 mm. Even though some nest of *H. araujoi*, exhibited no nest entrance tube protrusion, those with entrance tubes were of medium length that ranges 27 - 30 mm. All nest cavities observed in this study had only a single narrow entrance tube, which confirms studies on some other stingless bee groups (Moritz and Crewe, 1988). However, in certain instances, multiple entrance tubes have been observed among the *Hypotrigona* bees (Njoya, 2009).

The greater number of colonies that had entrance tubes was observed as straight tunnels at the nest sites with very slight curves. This allowed easy movement of foragers into and out of the nest. Whiles the presence of guards and resin deposits on the entrance tube curtailed intruders from invading the nest of these bees as reported in several studies (Camargo and Pedro, 2003; Couvillon et al., 2007).

Pooley and Michener (1969) suggested entrance tube length as a species specific character. However in this work, entrance tube length alone was unable to capture variation among the three species within the study sites. Hence the use of other protocols to capture this variability was necessary.

Using nest Entrance tube diameter for Distinguishing Species within the Genus

Nest entrance characteristics and nesting habits are thought to be useful in taxonomic studies (Rasmussen and Camargo, 2008) and ecological studies of stingless bees (Michener, 1990; Camargo and Pedro, 2003). The diameter of entrance tube though varies was not significant among the three species in this study. One possible reason for this outcome could be an adaptive measure for excluding non-nest mates from invading the colony. However, in this study two species were observed nesting within the same nest that is *H. araujoi* and *H. gribodoi* species. It could be that both species use a common entrance but within the substrates there are possibly different nest in which they live. Alternatively, different species may co-exist together in a hive. Nonetheless, this lies with the intruder rather than nest inhabitants (Kirchner and Friebe, 1999). Although Breed and Page (1991) reported that non-nestmate intraspecific encounters occur in *Melipona scutellaris*, *M. quadrifasciata* and *M. rufiventris.* Which usually resulted in the death of one of these species; this was not observed among the *Hypotrigona* species.

Many of the colonies encountered in the study had a number of guards at the entrance tube which examined foragers to the nest. Nest of some *H. gribodoi* bees had few guards at the entrance tubes which would retreat in and out of nest. Guards at the entrance of the nest probably were for protection and security reasons to ensure the continuity of that colony.

Preference for Nesting Substrates among Species

Concerning the nesting substrates, stingless bees are considered as generalists in the selection of nesting sites (Roubik, 1989; Hubbell and Johnson, 1977, Lima et al., 2013). Roubik (1983a) reports that nests of several stingless bees are undescribed. In this work, nest of species of the genus *Hypotrigona* were built in artificial structures. Thus wooden window frames (WWF), cavity in metal poles (CMP), crack in walls (CW), crack in mud houses (CMH), bamboo internodes (BI) and cavity in meter boards (CMB) were the substrates used. Species of this genus nested in artificial structures possibly due to the disturbances in their natural habitats, compelling the bees to adapt to other nesting strategies.

Venturieri (2009) reported that *Trigona* bees are found in all kinds of nesting substrates which was also observed in this study. Ricketts (2004) and Brosi et al. (2007, 2008), also reported that Meliponini species including the genus *Hypotrigona* will even nest in human-dominated habitats neighbouring their natural forest habitats that have experienced high degrees of disturbance. This attribute might contribute to an increase in nest biomass of one or more species by offering them more available nesting sites in the area. Similar incidence was observed in this research, where two study sites, Mfuom and Kwesi Gyan communities had bees in human settlement areas possibly as a result of farming and logging activities in these areas.

Winfree, Ratnieks and Kremen, (2007) reported on anthropogenic land use which may be compatible with the conservation of many, but not all bee species.

Nevertheless the most preferred among these substrates were the wooden window frames (WWF) and bamboo internodes (BI) to the other nesting substrates. All three species were observed nesting in any of the substrates encountered in the study, however, *H. araujoi* nest was located within a wooden window frame (WWF). Whereas *H. gribodoi* and *H. ruspolii* nested in any of the six substrates observed within this study.

Species within the genus were observed to construct nests in aggregation within a particular site. Perhaps as a result of limited availability of suitable nesting site especially in degraded areas that lacks sufficient natural cavities. Therefore in situations of this nature, presence of cavities and crevices in construction materials of farm houses can permit high concentrations of colonies (Starr and Sakagami, 1987). Another possible reason for colony aggregation in a particular site could be due to the availability of nesting sites for long duration and short swarming distances (Danaraddi et al., 2009).

Testing the Efficiency of Protocols to Capture Variations among Colonies

In the present study, geometric morphometrics technique was able to capture variability among species of the genus *Hypotrigona* within the three study sites based on the intersections of the vein junctions on the wing. The wing morphometrics technique together with the nest entrance characteristics successfully discriminated among the species within the study sites. Variability encountered in the study could be due to environmental conditions pertaining to the different study sites. Many studies have highlighted variation in wing shape as likely driven by environmental pressures (Alpatov 1929; Verma, Mattu and Daly,

1994; Hepbrun, Radloff and Oghiakhe, 2000). The environment is proposed to influence phenotypic variation among *Hypotrigona* species (Castanheira and Contel, 2005; Owen, 2009). Nonetheless these study sites have been subjected to anthropogenic destruction which as a result clumps species together in those areas. Zeder, Emshwiller, Smith and Bradley, (2006) confirms the actions of human disturbance to disperse or maintain species in the ecosystem.

Regarding the wing geometric morphometrics technique, some species from Mfuom with medium entrance tubes were polarized away from the centroid in both PCA and CVA graphical output exhibiting the degree of variability in the wing pattern of members of the species *H. araujoi*. However, the clustering of closely related species about the centroid in PCA and CVA, suggests possibly other species within the genus. Alternatively, there could be two different species with similar pattern in the morphometry of the wing. Meanwhile discriminant function analysis, cross-validation tests, correlation analysis, ANOVA and regression analysis, captured the differences within this genus showing statistically significant p-values (<0.0001). Confirmatory test based on traditional morphometry and nest entrance characteristics indicated the other groups as *H. gribodoi* and *H. ruspolii*.

In some bee species, there can be some adaptive constrains accounting for variability such as the genetic variation, foraging behaviour, flying mechanism and pollen load (Aytekin, Terzo, Rasmont and Çağatay, 2007). Thus in the studied genus *Hypotrigona*, variation encountered could possibly be due to these factors as well as species genetic make-up.

Although, some insect species have large differences between the wings of males and workers, as in *Apis mellifera*, the wings of the different stingless bees are visually indistinguishable (Francoy, Silva, Nunes-Silva, Menezes and Imperatriz-Fonseca, 2009). However, the use of geometric wing morphometric technique was able to capture small variations within the species from the different study sites. The series of analyses conducted in the present study strongly refute the null hypothesis on the geometric morphometric technique. This establishes the technique as an effective tool for discriminating species of the Afrotropical genus *Hypotrigona* just as it has been employed with bees from other parts of the world (Mendes, Francoy, Nunes-Silva, Menezes and Imperatriz-Fonseca, 2007; Francoy et al, 2009; Francoy, Grassi, Imperatriz-Fonseca, May-Itzá and Quezada-Euán, 2011). The technique has enabled the separation of four Africanized stingless bees (Combey et al., 2013).

It is in view of this that the geometric morphometric wing technique uses the pattern of venation on the wings of insects to categorize organisms into their various groups. The present study analyzed wings of species from the genus *Hypotrigona* in the three study sites drawing conclusions from the results, three main groups of *Hypotrigona* species exist within the study sites. Variability among the various species was confirmed using traditional morphometry (Michener, 1959; Moure, 1961).

Eardley and Kwapong (2013, p 261) reported that, “nest architecture and host plant preference which pose logistical problems in gathering materials for taxonomic revision”. There is therefore the need to incorporate other modern

techniques to successfully categorize organisms. This study used geometric wing morphometrics technique, nest entrance characteristics and traditional morphometrics in segregating the various species within the genus. Thus, suggesting the use of wing venation pattern of insects as an important source of diagnostic characteristic at all taxonomic levels (Rehn, 2003).

The use of geometric wing morphometrics technique together with nest entrance characteristics as well as traditional morphometry has confirmed the three existing species within the genus *Hypotrigona* in Ghana. Thus *Hypotrigona gribodoi*, *H. ruspolii* and *H. araujoi* providing easy and fast methods of recognizing these species both in the field and in the laboratory. There is therefore the need to emphasize that this protocol is viable enough in capturing the thinnest variation among species, thereby making it an important tool in the field of morphometrics.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

Overview of the study

The study sought to investigate species variability in Afrotropical genus *Hypotrigona* (Cockerell) within three districts in the central region of Ghana. A survey was conducted to identify various nesting sites of the *Hypotrigona* bees. At each sampling site, visual observations of the external nest entrance characteristics of *Hypotrigona* colonies were made for presence/ absence of nest entrance tubes. The level of aggression when disturbed was observed. In addition plain polythene bags were used to sample *Hypotrigona* worker bees for geometric and traditional morphometric analyses.

The right forewings of worker bees were dissected and relaxed in 90% ethanol. This was to ensure to the even spread of the wings on microscope slides for landmark digitization for geometric morphometric analyses. The entire body length, head width, eye length, upper and lower interocular distances, scape, hamuli number, forewing length and head shape were used for the traditional morphometric analyses.

Key Findings

1. It was found from the study that the species within the genus *Hypotrigona* were variable.
2. The study also revealed that some worker bees constructed entrance tubes whereas some did not.
3. The study also brought to fore that all three techniques applied to determine variability within the genus were viable.

Conclusion

The following conclusions are deduced from this present study.

Three species of bees were identified within the genus, namely; *Hypotrigona gribodoi* (Magretti), *Hypotrigona araujoi* (Michener) and *Hypotrigona ruspolii* (Magretti). Among the three species, *H. gribodoi* and *H. ruspolii* were the most dominant species in the three study sites while *H. araujoi* seems to occur only within the Mfuom community.

In terms of nest entrance characteristics, *H. gribodoi* and *H. ruspolii* may or may not construct entrance tubes however; *H. araujoi* constructs nest entrance tubes. It is important to note that tube entrances are highly variable in length among the three species when present. *H. ruspolii* possesses the longest nest entrance tubes when tube is present. The diameter of nest entrance tubes though varied showed no significant differences among the three species.

Aggressive behaviour is variable among the three species. Highly aggressive behaviour was also observed within members of *H. ruspolii* while less aggressive

behaviour was observed among the members of *H. gribodoi*. In *Hypotrigona araujoi*, members are non-aggressive.

In this present study, geometric morphometrics techniques applied on the wings detected possible variability within the genus *Hypotrigona*. This confirms the protocol as a sound alternative tool for assessing variability within the genus.

Recommendations:

1. Further studies should be done to determine whether variations in the wings of the genus *Hypotrigona* have genetic basis or are mere reflections of phenotypic and environmental variation.
2. In the field, researchers could identify different *Hypotrigona* species using the characters listed in (Table 13).
3. The use of geometric morphometric should be encouraged as a viable tool to augment the research of taxonomist who rely on traditional morphometry and molecular marker techniques.
4. There is the need to encourage the conservation and culture of the *Hypotrigona* bee species and not destroy their hives.
5. Humans should desist from activities that endanger nests and colonies of *Hypotrigona* bees.

REFERENCES

Angilar, I., Fonseca, A. & Biesmeijer, J. C. (2005). Recruitment and communication of food sourcelocation in three species of stingless bees (Hymenoptera, Apidae, Meliponini), *Apidologie, 36*, 313–324.

Alpatov, W. W. (1929). Biometrical studies on variation and races of the honey bee *Apis mellifera* L. *Quarterly Review of Biology*, *4*, 1–58.

Alves, D. A., Imperatriz-Fonseca, V. L. & Santos-Filho, P. S. (2009). Production of workers, queens and males in *Plebeina remota* colonies (Hymenoptera, Apidae, Meliponini), a stingless bee with reproductive diapause. *Genetics and Molecular Research*, *8*(2), 672-683.

Araujo, V. (1955a). Notas sobre colonias de Meliponineos de Angola – Africa. *Dusenia, 6*, 96–114.

Araujo, V. **(**1955b). Colmeiaspara 'abelhassemferrao' 'MELIPONINI'.*Boletim do Instituto de Angola*, *7*, 9–50.

Araujo, V & Kerr, W. E. (1959). A case of sibling species among social bees. *Revista Brasileira de Biologia., 19*, 224–228.

Aytekin, A. M., Terzo, M., Rasmont, P. & Çağatay, N. (2007). Landmark based geometric morphometric analysis of wing shape in *Sibiricobombus* Vogt (Hymenoptera: Apidae: *Bombus* Latreille). *Ann. Soc. Entomol*., *43*(1), 95-102.

Bassindale, R. (1954). The biology of the stingless bee *Trigona* (*Hypotrigona*) *gribodoi* Magretti (Meliponidae). Proceedings of the Zoological Society *London*, *125*, 49–62.

Bassindale, R. & Harrison Matthews, L. (2009). The biology of the Stingless bee *Trigonu* (*Hypotrigona*) *gribodoi* Magretti (Meliponidae).Proceedings of the Zoological Society of London, *125*(1), 49–62.

Biesmeijer, J. C., Giurfa, M., Koedam, D., Potts, S. G., Joel, D. M. & Dafni A. (2005). Convergent evolution: floral guides, stingless bee nest entrances, and insectivorous pitchers, *Naturwissenschaften*, *92*, 444–450.

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemu¨ ller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J. & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science 313*, 351-354.

Birmingham, A. L., Hoover, S. E., Winston, M. L. & Ydenberg, R. C. (2004). Drifting bumble bee (Hymenoptera: Apidae) workers in commercial greenhouses may be social parasites. *Canadian Journal of Zoology*, *82*, 1843-1853.

Blanche, K. R., Ludwig, J. A. & Cunningham, S. A. (2006). Proximity to rainforest enhances pollination and fruit set in macadamia and longan orchards in north Queenland, Australia. *Journal of Applied Ecology*, *43*, 1182-1187.

Bookstein, F. L. (1991*). Morphometric Tools for Landmark Data: Geometry and Biology*. New York, Cambridge University Press.

Bourke, A. F. G. & Ratnieks, F. L. W. (1999). Kin conflict over caste determination in social Hymenoptera. *Behavioral Ecology and Sociobiology*, *46*, 287-297.

Breed, M. D. (1983). Nestmate recognition in honey bees. *Animal Behaviour*, *31*, 86-91.

Breed, M. D., Butler, L. & Stiller, T. M. (1985). Kin discrimination by worker honey bees in genetically mixed groups. *Proceedings of the National Academy of Sciences* U.S.A, *82*, 3058-3061.

Breed, M. D., Robinson, G. E. & Page Jr., R. E. (1990). Division of labor during honey bee colony defense. *Behavioral Ecology and Sociobiology*, *27*, 395-401.

Breed, M. D. & Page, R. E. (1991). Intraspecific and interspecific nestmate recognition in Melipona workers (Hymenoptera, Apidae). *Journal of Insect Behavior*, *4*, 463- 469.

Brosi, B. J., Daily, G. C. & Ehrlich, P. (2007). Bee community shifts with landscape context in a tropical countryside. *Ecological Applications*, *17*, 418-430.

Brosi, B. J., Daily, G. C., Shih, T. M., Oviedo, F. & Duran, G. (2008). The effects of forest fragmentation on bee communities in tropical countryside.*Journal of Applied Ecology*, *45*, 773-783.

Buchwald, R. & Breed, M. D. (2005). Nestmate recognition cues in a stingless bee, *Trigona fulviventris*. *Animal Behaviour*, *70*, 1331-1337.

Camargo, J. M. F. (1970). Nests and biology of some species of Meliponídeos (Hymenoptera: Apidae) in the region of Porto Velho, Rondonia Territory, Brazil. *Revista de Biologia Tropical*, *16*, 207-239.

Camargo, J. M. E. (1974). Introduction to immunofluorescence techniques. *Rev Bras Clin Pathol*., *10*(3), 87-107.

Camargo, J. M. F. (1980). The Partamona group their kinds, distribution and geographical differentiation (Meliponinae, Apidae and Hymenoptera). *Amazon Acta*., *10*(4), 175.

Camargo, M. F. & Wittmann, D. (1989). Nest architecture and distribution of the Primitive stingless bees, *Mourella caerulea* (Hymenoptera, Apidae, of some Meliponinae): Evidence for the Origin of Plebeia on the Gondwana continent. *Stud. Neotrop. Fauna and Environ*., *24*(4), 213- 229.

Camargo, J. M. F. & Pedro S. R. M. (l992). Systematics, phylogeny and biogeography of the Meliponinae (Hymenoptera, Apidae): a mini-review, *Apidologie, 23*, 509–522.

Camargo, J. M. F. & Pedro S. R. M. (2002a). Mutualistic association between a tiny Amazonian stingless bee and a wax-producing scale insect, *Biotropica., 34*, 446–451.

Camargo, J. M .F. & Pedro S. R. M. (2002b). Uma especie nova de *Schwarzula*da Amazonia, Iheringia, *Ser. Zool, 92*, 101–112.

Camargo, J. M. F. & Pedro, S. R. M. (2003). Meliponini neotropicais: o genero Partamona Schwarz, 1939 (Hymenoptera, Apidae, Meliponini) – bionomia e biogeografia. *Rev. Bras. Entomol*., *47*, 311–372.

Cameron, S. A & Mardulyn, P. (2001). Multiple molecular datasets suggest independent origins of highly eusocial behaviour in bees (Hymenoptera: Apinae). *Systematic Biology, 50*, 194-214.

Castanheira, E. A. & Contel, E. P. B. (2005). Geographic variation in *Tetragonisca angustula* (Hymenoptera, Apidae, Meliponini) *Apic Res*., *44*, 101-105.

Chapman, N. C., Nanork, P., Gloag, R., Wattanachaiyingcharoen, W., Beekman, M. & Oldroyd, B. (2009). Queenless colonies of the Asian red dwarf honey bee (Apis florea) are infiltrated by workers from other queenless colonies. *Behavioral Ecology*, *20*, 817-820.

Chapman, N. C., Beekman, M. & Oldroyd, B. P. (2010). Worker reproductive parasitism and drift in the western honeybee *Apis mellifera*. *Behavioral Ecology and Sociobiology*, *64*,419-427.

Chinh, T. X. (2004). Reproduction in eusocial bees (Apidae: Apini, Meliponini) A Thesis submitted to Utrecht University. Bee Research and Development Centre. Hanoi, Vietnam. pp 11-12.

Clarke, K. E., Rinderer, T. E., Franck, P., Quezada-Euan, J. G. & Oldroyd, B. P. (2002). The Africanization of honeybees (*Apis mellifera* L.) of the Yucatan: A study of a massive hybridization event across time. *Evolution, 56*, 1462–1474.

Claude, J. (2008). *Morphometrics with R. Springer*, New York.

Codex (2001). *Revised Codex Standard for honey*. Codex Stan 12-1981. Codex Alimentarius Commission. FAO/OMS. Rome, Italy, pp 7.

Cockerell, T. D. A. **(**1910). New and little-known bees. Transactions of the American Entomological. *Society, 36*, 199–249.

Cockerell, T. D. A. **(**1919). Bees in the collection of the United States National Museum. 3. Proceedings of the United States. *National Museum, 55*, 167–221.

Cockerell, T. D. A. **(**1934). Some African Meliponine bees. Revue de Zoologieet de Botanique Africaines, *26*, 46–62.

Combey, R., Teixeira, J. S. G., Bonatti, V., Kwapong, P & Francoy, T. M. (2013). Geometric morphometrics reveals morphological differentiation within four African stingless bee species. *Annals of Biological Research*, *4*(11), 93-103.

Contel, E. P. B., Mestriner, M. A. & Martins, E. (1977). Genetic-Control and Developmental Expression of Malate-Dehydrogenase in *Apis mellifera*, *Biochem.Genet*., *15*, 859–876.

Couvillon, M. J., Wenseleers, T., Imperatriz-Fonseca, V. L., Nogueira-Neto, P. & Ratnieks, F. L. W. (2007). Comparative study in stingless bees (Meliponini) demonstrates that nest entrance size predicts traffic and defensivity. *J. Evol*., *21*, 194-201.

Daily, G. C., Alexander, S., Ehrlich, P. R., Goulder, L., Lubchenco, J., Matson, P. A., Mooney, H. A., Postel, S., Schneider, S. H.., Tilman, D. & Woodwell, G. M. (1997). Ecosystem services: benefits supplied to human societies by natural ecosystems. *Issues in Ecology*.1-16.

Danaraddi, C. S., Viraktamath, S., Basavanagoud, K. & Bhat, A. R. S. (2009). Nesting habits and structure of stingless bee, Trigona iridipennis Smith at Dharwad, Karnataka. *Karnataka J. Agric. Sci*., *22*(2), 310-313.

Del Lama, M. A., Figueiredo, R. A., Soares, A. E. E. & Del Lama, S. N. (1988). Hexokinase polymorphism in *Apis mellifera* and its use for Africanized honeybee identification, *Rev. Bras. Genet, 11*, 287–297.

Downs, S. G. & Ratnieks, F. L. W. (2000). Adaptive shifts in honey bee (*Apis mellifera* L.) guarding behavior support predictions of the acceptance threshold model. *Behavioral Ecology*, *11*, 326-333.

Drumond, P. M., Bego, L. R., & Melo, G. A. R. (1995). Nest architecture of the stingless bee Plebeia and related consideration (Hymenoptera, Apidae, Meliponinae). *Iheringia. Série Zoologia.*, *79*, 39-45.

Dryden, I. L. & Mardia, K. V. (1998). *Statistical shape analysis*. Wiley, Chichester. pp 1-47.

Eardley, C. D. (2004). Taxonomic revision of the African stingless bees (Apoidea: Apidae: Apinae: Meliponini). *African Plant Protection, 10*, 63-96.

Eardley, C. D., Kuhlmann, M. & Pauly, A. (2010). The Bee Genera and Subgenera of sub-Saharan Africa. A series of manual dedicated to capacity building in taxonomy and collection managements .*Abc Taxa*., *7*, 18.

Eardley, C. D. & Kwapong, P. (2013). Taxonomy as a Tool for Conservation of African Stingless bee and their honey. In: *Pot-Honey*. A legacy of

stingless bees. Vit. P, Pedro, S. R. M and Roubik, D (eds). Springer, pp 261-263.

Eltz, T. C., Bruhl, S., Van der kaars & Linsenmair. K. E. (2002). Determinants of stingless bee nest density in lowland dipterocarp forests of Sabah, Malaysia. *Oecologia, 131*, 34.

Eltz, T., Bruhl, C. A., Imiyabir, Z. & Linsenmair K. E. (2003). Nesting and nest trees of stingless bees (Apidae: Meliponini) in lowland dipterocarp forests of Sabah, Malaysia, withimplications for forest management. *Forest Ecology and Management, 172*, 301-313.

Engels, W. & Imperatriz-Fonseca, V. L. (1990). Caste development, reproductive strategies and control of fertility in honeybees and in stingless bees, In: Engels, W. (ed.), Social Insects, an evolutionary approach to caste reproduction. Springer, pp. 167–230.

Engel, M. S. (2000). A new interpretation of the oldest fossil bee (Hymenoptera: Apidae). *American Museum Novitates*, *3296*, 1-11.

Engel, M. S. & Michener, C. D. (2013). Geological history of stingless bees (Apidae: Meliponini): Stingless bees process honey and pollen in cerumen pots. *Natural History Museum*, pp 1-7.

FAO. (2007). Pollinators: Neglected biodiversity of importance to food and agriculture CGRFA-11/07/Inf.15. Food and Agriculture Organization of United Nations, Rome.

FAO. (2009). Non-wood forest products, bees and their role in forest livelihoods.Food and Agriculture Organization, Rome.

Ferreira-Caliman, M. J., Zucchi, R & Nascimento, F. S. (2012). Cuticular Hydrocarbons Discriminate Distinct Colonies of *Melipona marginata* (Hymenoptera, Apinae, Meliponini).*Sociobiology, 59*(3), 1-10.

Franck, P., Cameron, E., Good, G., Rasplus, J. Y., Oldroyd, B. P. (2004). Nest architecture and genetic differentiation in a species complex of Australian stingless bees. *Mol. Ecol*., *13*, 2317-2331. In *Sociobiology*, *60*(4), 421-428 (2013) 427.

Francoy, T. M., Wittmann, D., Drauschke, M., Muller, S., Steinhage, V., Bezerra-Laure, M. A. F., De Jong, D. & Gonçalves, L. S. (2008). Identification of Africanized honey bees through wing morphometrics: two fast and efficient procedures. *Apidologie, 39*, 1-7.

Francoy, T. M., Silva, R. A. O., Nunes-Silva, P., Menezes, C. & Imperatriz-Fonseca, V. L. (2009). Gender identification of five genera of stingless bees (Apidae, Meliponini) based on wing morphology. *Genetics and Molecular Research, 8*(1), 207-214.

Francoy, T. M.; Grassi, M. L.; Imperatriz-Fonseca, V. L.; May-Itzá, W. J. & Quezada-Euán, J. J. G. (2011). Geometric morphometrics of the wing as a tool for assigning genetic lineages and geographic origin to *Melipona beecheii* (Hymenoptera: Meliponini). *Apidologie*, 1297-9678.

Friedrich, G. Hrncir, B. M. & Jarau, S. (2008). Signals and cues in the recruitment behavior of stingless bees (Meliponini). *J. CompPhysiol A*., *194*, 313-327.

Friese, H. (1900). NeuArten der Bienungattungen Melipona Ill. Und *Trigona* Jar. *Térmeszetrajzi Fuzetek*, *23*, 381–394.

Friese, H. (1903). New Meliponidae. II. *Journal of Systematic Hymenopterologie and dipterology*, *3*, 359-361.

Friese, H. **(**1909). 8. Hymenoptera. 5. Apidae. In: *B. Y. Sjostedts. Kilimanjaro-Meru Expedition. Stockholm*, 119–168.

Friese, H. **(**1909a). Die Bienen Afrikas nach dem Stande unserer heutigen Kenntnisse. In: *Zoologische und Anthropologische Ergebenisseeiner Forschungsreise im westlichen und zentralen Südafrika ausgeführt in den jahren*. Band 2 (L Schultze), Denkschriften der Medizinisch-Naturwissenschaftlichen Gesellschaftzu Jena, *14*, 83–476, pls IX–X.

Friese, H. **(**1921). Ergebnisse der Zweiten Deutschen Zentral-Afrika-Expedition.*Apidae.HamburgischeWissenschaftZeitung*, **1**: 1091–1102.

Gamboa, G. J. (1986). The evolution and ontogeny of nestmate recognition in social wasps.*Annual Review of Entomology*, *31*, 431-454.

Grubbs, F. E. (1969). Procedures for detecting outlying observations in samples. *Technometrics, 11*(1), 1-21.

Guiglia, D. (1955). Missionedel Prof. Guiseppe Scorteccain Migiurtinia, Hymenoptera. *Annali del Museo Civico di Storia Naturale di Genova 68*, 306–311.

Hall, H. G. (1988). Distinguishing African and European honeybees using nuclear DNA restriction fragment polymorphisms Fla. *Entomol*., *71,* 294-299.

Hall, H. G. & Muralidharan, K. (1989). Evidence from mitochondrial DNA that African honey bee spread as continuous maternal lineages, *Nature, 339***,** 211– 213.

Hansell, M. H. (1993). The ecological impact of animal nests and burrows. *Funct. Ecol*., *7*, 5–12.

Heard, T. A. (1999). The role of stingless bees in crop pollination. *Annual review of Entomology*, *44*, 183-206.

Heithaus, E. R. (1979). Flower-feeding specialization in wild bees and wasp communities in seasonal Neotropical habitats. *Oecologia*., *42,* 179-194.

Hepburn, H. R., Radloff, S. E. & Oghiakhe, S. (2000). Mountain honeybees of Africa. *Apidologie*, *31*(2), 205–221.

Hölldobler, B. & Wilson, E. O. (1990). The ants. Cambridge, Massachusetts, Belknap Press of Harvard University Press, 732.

Hubbel, S. P. & Johnson, L. K. (1977). Competition and nest spacing in a tropical stingless bee community.*Ecology, 58*, 949-963.

Imperatriz-Fonseca, V. L., Ferreira de Souza, S. C. & Nogueira Neto, P. (1972). Subterranean nest structure of a stingless bee *Paratrigona subnuda* Moure (Meliponinae, Apidae, Hymenoptera). *Cienc Cult*., *24*, 662–666

Inoue, T., Sakagami, S. F., Salmah, S. & Yamane, S. (1984). The process of colony multiplication in the Sumatran stingless bee *Trigona* (Tetragonula). *laeviceps. Biotropical, 16*, 100–111.

Inoue, T., Roubik, D. & Suka, T. (1999). Nestmate recognition in the stingless bee Melipona panamica (Apidae, Meliponini).*Insectes Sociaux*, *46*, 208-218.

Jones, S. M., van Zweden, J. S., Grüter, C., Menezes, C., Alves, D. A., Nunes-Silva, P., Czaczkes, T., Imperatriz-Fonseca, V. L. & Ratnieks, F. L.

(2012). The role of wax and resin in the nestmate recognition system of a stingless bee, *Tetragonisca angustula*. *Behavioral Ecology and Sociobiology*, *66*, 1-12.

Kajobe, R. & Roubik, D. W. (2006). Honey-making bee colony abundance and predation by apesand humans in a Uganda Forest Reserve. *Biotropical, 38*(2), 1-9.

Kajobe, R. (2007). Botanical sources and sugar concentration of the nectar collected by two stingless bee species in a tropical African rain forest.*Apidologie, 38*, 110-121.

Kakutani, T., Inoue, T., Tezuka, T. & Maeta, Y. (1993). Pollination of strawberry by stingless bee, *Trigona minangkabau*, and honey bee, *Apis mellifera*: an experimental study of fertilization efficiency *Res. Popul. Ecol*., *35*, 95-111.

Kawakita, A., Ascher, J. S., Sota, T., Kato, M. & Roubik, D. W. (2008). Phylogenetic analysis of the corbiculate bee tribes based on 12 nuclear protein-coding genes (Hymenoptera: Apoidea: Apidae). *Apidologie, 39*,163-175.

Kearns, C. A., Inouye, D. W. & Waser, N. M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics*, *29*, 83-112.

Kendall, D. (1977). The diffusion of shape. *Advances in Applied Probability*, *9*(3), 428-430.

Kendall, D. G., Barden, D., Carne, T. K. & Le, H. (1999). Shape and shape theory, John Wiley & Sons, Chichester.

Kerr, W. E. (1950). Genetic determination of castes in the genus Melipona. *Genetics*, *35*, 143-152.

Kerr, W. E., Pisani, J. F. & Aily, D. (1967). The application of modern systematics of the genus *Melipona* sp, with the division into two subgenera (Hymenoptera, Apoidea) principles. *Roles Avulsos Zool*., *20*, 135-145.

Kirchner, H. K. & Friebe, R. (1999). Nest mate discrimination in the African stingless bee *Hypotrigona gribodoi* Magretti (Hymenoptera: Apidae). *Apidologie*, *30*, 293-298.

Klingenberg, C. P. & Mclntyre, G. S. (1998). Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. *Evolution*, *52*, 1363–1375.

Klingenberg, C. P., Barluenga, M. & Meyer, A. (2002). Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution*, *56*, 1909–1920.

Klingenberg, C. P. & Monteiro. L. R. (2005). Distances and directions in multidimensional shape spaces: implications for morphometric applications. *Syst. Biol.*, *54*, 678–688.

Klingenberg, C. P. (2008). Morphological integration and developmental modularity. *Annual Review of Ecology, Evolution and Systematics, 39*, 115-132.

Klingenberg, C. P. (2009). Morphometric integration and modularity in configurations of landmarks: tools for evaluating a-priori hypotheses. *Evolution and Development, 11*, 405-421.

Klingenberg, C. P. (2011). MorphoJ: An integrated software package for geometric morphometrics. *Molecular Ecology Resources11*, 353-357.

Koedam, D., Monge, I. A. & Sommeijer, M. J. (1995). Social interactions of gynes and their longevity in queenright colonies of Melipona favosa (Apidae: Meliponinae). *Netherlands Journal of Zoology*, *45*, 480-494.

Kohl, F. F. (1894). To Hymenopteran fauna Africa. *Annals of K. K. Natural History Court Museum, Vienna 9*, 279-350.

Kwapong, P. K., Aidoo, K., Combey, R. & Karikari, A. S. (2010). *Stingless bees: Importance, management and utilization*. Unimax Macmillan, Press, Accra, 5-36.

Lima, F. V. O., Silvestre, R. & Balestieri, J. B. P. (2013). Nest Entrance Types of Stingless Bees (Hymenoptera: Apidae) in a Tropical Dry Forest of Mid-Western Brazil. *Sociobiology*, *60*(4), 421-428.

Lopes de Carvalho, C. A., da Silva Santos, W., Nunes, L. A., de Almeida Souza, B., Almeida de Carvalho Zilse, G & Marcos de Oliveira Alves, R. (2011). Offspring Analysis in a Polygyne Colony of *Melipona scutellaris* (Hymenoptera: Apidae) by Means of Morphometric Analyses *Sociobiology, 57*(2), 347-354.

Lopez-Vaamonde, C., Koning, J. W., Brown, R. M., Jordan, W. C. & Bourke, A. F. G. (2004). Social parasitism by male-producing reproductive workers in a eusocial insect.*Nature*, *430*, 557-560.

Magretti, P (1884). Risultatidi raccolte imenotterologiche nell'Africa Orientale. *Annali del Museo Civico di Storia Naturale di Genova*, *21*, 523–636.

Magretti, P. (1898). Imeotteri. Della Seconda Spedizione di Don Eugeiodei Principi Ruspolinei Paesi Galla e Somali. *Annali del Museo Civico di Storia Naturale di Genova,* 39, 25–36.

Martins, D. J. (2014). Our friends the Pollinators. A handbook of Pollinator Diversity and Conservation in East Africa. pp 10.

Medler, J. T. (1980). Insects of Nigeria. Check list and bibliography. *Memoirs of the American Entomological Institute 30*, 1–919.

Mendes, M. F. M., Francoy, T. M., Nunes-Silva, P., Menezes, C. & Imperatriz-Fonseca, V. L. (2007). Intra-populational variability of *Nannotrigona testaceicornis* Lepeletier 1836 (Hymenoptera, Meliponini) using relative warp analysis, *J. Biosci*., *23*(1), 147–152.

Michener, C. D. **(**1959). Sibling species of *Trigona* from Angola (Hymenoptera, Apidae). *American Museum Novitates,* 1–5.

Michener, C. D. (1974). The social behaviour of the bees: a comparative study. Belknap Press of Harvard University Press, Cambridge, Massachusetts .pp 404.

Michener, C. D. & Grimaldi, D. A. (1988). A *Trigona* from late Cretaceous amber of New Jersey (Hymenoptera: Apidae: Meliponinae). *American Museum Novitates*, *2917*, 1-10.

Michener, C. D. (1990). Classification of the Apidae.University of Kansas *Science Bulletin, 54*, 75-153.

Michener, C. D. (2000). The bees of the world.Johns Hopkins University Press. Baltimore, Maryland.pp 913.

Michener, C. D. (2007). The bees of the world.Johns Hopkins University Press.Baltimore, Maryland. 2nd (ed) pp 816.

Moritz R. F. A. & Crewe, R. M. (1988). Air ventilation in nest of two African stingless bees *Trigona denoiti* and *Trigona gribodoi*. *Springer Link*, *44*, (11-12) 1024-1027.

Moure, C. M. F. (1961). A Preliminary Supra-specific Classification of Old World Meliponine Bees (Hymenoptera, Apidae). *Studia Ent*., *4*, 1-4.

Moynihan, M. (1998). The social regulation of competition and aggression in animals. Washington, Smithsonian Institution Press, 158.

Nanork, P., Paar, J., Chapman, N. C., Wongsiri, S. & Oldroyd, B. P. (2005). Asian honeybees parasitize the future dead. *Nature*, *437*, 829.

Nanork, P., Chapman, N. C., Wongsiri, S., Lim, J., Gloag, R. S. & Oldroyd, B. P. (2007). Social parasitism by workers in queenless and queenright *Apis cerana* colonies. *Molecular Ecology*, *16*, 1107-1114.

Nash, D. R. & Boomsma, J. J. (2008). Communication between hosts and social parasites. *In: Sociobiology of Communication: an Interdisciplinary Approach*, pp. 55-79. Oxford: Oxford University Press.

Njoya, M. T. M. (2009). Diversity of stingless bees in Bamenda Afromontane Forests Cameroon: Nest architecture, behaviour and labour calendar. Dissertation submitted to Rheinischen Friedrich-Wilhelms-University Zu Bonn. pp 14-25.

Nougueira-Neto, P. & Sakagami, S. F. (1966). Nest structure of a subterranean stingless bee *Geotrigona mombuca* Smith (Meliponinae, Apidae, Hymenoptera). *An. Acad. Bras. Ciênc*., *38*(1),187-194.

Nunes-Silva, P., Hrncir, M. & Imperatriz-Fonseca, V. L (2008). Thoracic vibrations in stingless bees: is body size important for efficient buzz pollination? In *Proc. 23rd International Congress of Entomology, Durban, Africa doSul*.

Oliveira, C. R., Menezes, C., Soares, E. E. A. & Imperatiz Fonseca, L. V. (2012). Trap-nests for stingless bees (Hymenoptera, Meliponini).*Apidologie, 10*, 12-52.

Owen, R. E. (2009). Applications of Morphometrics to the Hymenoptera, particularly Bumble bee (Bombus: Apidae). Morphometrics. pp 1-30.

Palma, G. J. G., Javier. M., Quezada-Euan, V., Melendez-Ramirez, J., Irigoyen, G. R., Valdovinos-Nunez, G. R. & Rejon, M. (2008). Comparative Efficiency of *Nannotrigona perilampoides, Bombus impatiens* (Hymenoptera: Apidae), and Mechanical Vibration on fruit production of

Enclosed Habanera Pepper. *Journal of Economic Entomology*, *101*,132-138.

Palmer, K. A., Oldroyd, B. P., Quezada-Euan, J. G., Paxton, R. & May-Itza, W. de, J. (2002). Paternity frequency and maternity of males in some stingless bee species, *Mol. Ecol*., *11*, 2107–2113.

Paxton, R. J., Bego, L. R., Shah, M. M. & Mateus, S. (2003). Low mating frequency of queens in the stingless bee *Scaptotrigona postica* and worker paternity of males, *Behav. Ecol*. *Sociobiol., 53*, 174–181.

Peters, J., Queller, D. C., Imperatriz-Fonseca, V. L., Roubik, D. W. & Strassmann, J. E. (1999). Mate number, kin selection and social conflicts in stingless bees and honeybees, *Proc. R. Soc. London B., 266*, 379–384.

Polly, P. D. (2012). Geometric morphometrics. An Introduction. Department of Geology, Indiana (Biology and Anthropology) University, 1001E. 10th Street, Bloomington, IN 47405, USA.

Pooley, A. C. & Michener, C. D. (1969). Observations on nests of stingless honey bees in Natal (Hymenoptera: Apidae).

Portugal-Araujo, V. (1955). Notas sobre colonias de meliponineos de Angola-Africa. *Dusenia*, *9*, 297-350.

Ratnieks, F. L. W. (2001). Heirs and spares: caste conflict and excess queen production in Melipona bees. *Behavioral Ecology and Sociobiology*, *50*, 467-473.

Ratnieks, F. L. W. & Wenseleers, T. (2008). Altruism in insect societies and beyond: voluntary or enforced? *Trends in Ecology & Evolution*, *23*, 45-52.

Rasmussen, C. & Camargo, J. M. F. (2008). A molecular phylogeny and the evolution of nest architecture and behavior in *Trigona*. sp. (Hymenoptera: Apidae: Meliponini).*Apidologie,* 39, 102–118.

Rehn, A.C. (2003). Phylogenetic analysis of higherlevel relationships of Odonata. *Syst. Ent.,* 28, 181-239.

Reitz, S. R. &Trumble, J. T. (2002).Competitive displacement among insects and arachnids.*Annual Review of Entomology 47*, 435-465.

Ribeiro, M. F. (2002). Does the queen of *Plebeia remota* (Hymenoptera, Apidae, Meliponini) stimulate her workers to start brood cell construction after winter? *Insects Sociaux*., *49*, 38-40.

Ricketts, T. H. (2004**)**. Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology*, *18*, 1262-1271.

Rohlf, F. J. & Slice, D. (1990). Extension of the Procrustes method for the optimal superimposition of landmarks. *Syst. Zool., 39*, 40–59.

Rohlf, F. J. & Marcus, L. F. (1993). A Revolution in Morphometrics. *Trends in Ecology and Evolution, 8*(4), 129-132.

Rohlf, F. J., Loy, A. & Corti, M. (1996). Morphometric analysis of Old World Talpidae (Mammalian, Insectivora) using partial-warp scores. *Systematic Biology, 45*, 344-362.

Rohlf, F. A. (1999). Shape Statistics: Procrustes Superimpositions and tangent spaces. State University of New York at Stony Brook. *Journal of Classification*, *16*, 197-223.

Rohlf, F. J. (2010). *Tps Dig*, digitize landmarks and outlines, version 2.16. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F. J. (2012). *Tps Util*, file utility programme. Version 1.58. Department of Ecology and Evolution, State University of New York at Stony Brook.

Roubik, D. W. (1979). Nest and colony characteristics of stingless bees from French Guiana (Hymenoptera: Apidae). *Journal of Kansas Entomological Society, 52*, 443-470.

Roubik, D. W. (1983a). Nest and colony characteristics of stingless bees from Panama. *Journal of Kansas Entomological Society, 56*, 327-355.

Roubik, D. W. (1983b). Experimental community studies: time–series tests of competition between African and Neotropical bees. *Ecology, 64*, 971-978.

Roubik, D. W., Moreno, J. E., Vergara, C. & Wittmann, D. (1986). Sporadic food competition with the African honey bee: projected impact on Neotropical social bees. *J. Trop. Ecol., 2*, 97–111.

Roubik, D. W. (1989). Ecology and natural history of tropical bees. Cambridge University press, Cambridge, New York. pp 514.

Roubik, D. W. (1992). Stingless bees (Apidae: Meliponinae): a guide to Pananmanian and Mesoamericana species and their nests, In: *Insects of Panama and Mesoamerica* 495-524. Oxford University Press.Oxford UK.

Roubik, D. W. (2006). Stingless bee nesting biology. A review: *Apidologie, 37*, 124-143.

Ruggiero, M. & Ascher, J. (2013). ITIS Bees: World Bee Checklist. In: *Species 2000 and IT IS Catalogue of life*, (Roskov, Y., Kunze, T., Orrell, T., Abucay, L., Culham, A., Baily, N., Kirk, P., Bourgoin, T., De Wever, A. eds. Digital resource at www.catlogueoflife.org/col. Species 2000 Naturalis, Leiden, the Netherlands.

Ruttner, F., Tassencourt, L. & Louveaux, J. (1978). Biometrical-statistical analysis of the geographic variability of *Apis mellifera* L. *Apiodologie*, *9*, 363-381.

Ruttner, F. (1988). Biogeography and taxonomy of honey bees. Springer Verlag, Berlin, pp 281.

Sakagami, S. F. (1978). *Tetragonula* stingless bees of the Continental Asia and Sri Lanka (Hymenoptera, Apidae*). Journal of Faculty of Science*, Hokkaido University, Series VI, *Zoology*, *21*, 165-247.

Sakagami, S. F. (1982). Stingless Bees. *In: Social Insects*, **3**: 45-52. (ed) H. R. Herman.Academic Press, New York.

Sakagami, S. F., Inoue, T. & Salmah, S. (1990). Stingless bees of Sumatra. Natural History of Social Wasps and Bees in Equatorial Sumatra (eds S. F. Sakagami, R. Ohgushi & D. W. Roubik*).* Hokkaido University Press, Sapporo, Japan.pp 125–138.

Sanchez, D., Kraus, F. B. & Hernandez, M. D. (2007). Experience, but not distance, influences the recruitment precision in the stingless bee *Scapotrigona mexicana*. *Natur wissenschaften*., *94*, 567-573.

Sansom, R. S. (2009). Phylogeny, classification, and character polarity of the Osteostraci (Vertebrata).*Journal of Systematic Paleontology*, *7*, 95-115.

Santos-Filho, P. D. S., Alves, D. D. A., Eterovic, A., Imperatriz-Fonseca, V. L. & Kleinert, A. D. M. P. (2006). Numerical investment in sex and caste by stingless bees (Apidae: Meliponini): a comparative analysis. *Apidologie*, *37*, 207-221.

Schwarz, H. E. (1948). Stingless bees (Meliponidae) of the Western Hemisphere. *Bull. Am. Mus. Nat. hist*., *90*, 1-546.

Segura, J. A. L. (2000). Highly polymorphic DNA markers in an Africanized honey bee population in Costa Rica, Genet. *Mol. Biol*., *23*, 317–322.

Shlens, J. (2003). A tutorial on Principal Component Analysis.Derivation, discussion and singular value decomposition, pp 1-16.

Sheppard, W. S., Rinderer, T. E., Mazzoli, J., Stelzer, J. A. & Shimanuki, H. (1991a). Gene flow between African and European derived honey bee populations in Argentina, *Nature, 321*, 674–679.

Sheppard, W. S., Soares, A. E. E., De Jong, D. & Shimanuki, H. (1991b). Hybrid status of honey bee populations near the historic origin of Africanization in Brazil, *Apidologie, 22*, 643–652.

Silva, D. L. N., Zucchi, R. & Kerr, W. E. (1972). Biological and behavioural aspects of the reproduction in some species of Melipona (Hymenoptera, Apidae, Meliponinae). *Animal Behaviour*, *20*, 123-132.

Singer, T. L. & Espelie, K. E. (1992). Social wasps use nest paper hydrocarbons for nestmate recognition. *Animal Behaviour*, *44*, 63-68.

Slaa, J. (2003). Foraging ecology of stingless bees: from individual behaviour to community ecology. Dissertation Utrecht University, Dissertation, Elinkwijk, Utrecht. The Netherlands. pp 181.

Slice, D. E. (2005). Modern Morphometrics in Physical Anthropology.Springer.

Slice, D. E. (2007). Geometric morphometrics.*Annual Review of Anthropology, 36*, 261-281.

Slice, D. E. & Ann, R. (2009). Geometric Morphometric Classification of Craniafor Forensic Scientists North Carolina State University Raleigh, North Carolina, USA.

Smith, D. R., Taylor, O. & Brow, W. M. (1989). Neotropical Africanized honey bees have African mitochondrial DNA, *Nature, 339*, 213–215.

Smith, D. R., Crespi, B. J. & Bookstein, F. L. (1997). Fluctuating asymmetry in the honey bee, *Apis mellifera*, effects of ploidy and hybridization. *J. Evol. Biol*., *10*, 551-574.

Sommeijer, M. J., Houtekamer, J. L. & Bos, W. (1984). Cell construction and egg-laying in *Trigona nigra* pauper with a note on the adaptive significance of oviposition behaviour of stingless bees. *Insectes Sociaux 31*, 199–217.

Sommeijer, M. J. & De Bruijn, L. L. (2003). Why do workers of Melipona favosa chase their sister-gynes out of the nest? *Proceedings of the Section Experimental and Applied Entomology: Netherlands Entomological Society*, *12*, 45-48.

Sommeijer, M. J., De Bruijn, L. L. M. & Meeuwsen, F. (2003a). Reproductive behaviour of stingless bees: solitary gynes of Melipona favosa (Hymenoptera: Apidae, Meliponini) can penetrate existing nests. *Entomologische Berichten*, *63*, 31-35.

Sommeijer, M. J., De Bruijn, L. L. M., Meeuwsen, F. & Slaa, E. J. (2003b). Reproductive behaviour of stingless bees: nest departures of non-accepted gynes and nuptial flights in Melipona favosa (Hymenoptera: Apidae, Meliponini). *Entomologische Berichten*, *63*, 7-13.

Spinola M. (1853). Compterendu des Hyménoptères in-éditis provenants du voyage entomologiquede M. Ghilianidans le Para en 1846. Memorie della Reale .*Accademia delle Scienze di Torino*, *13*(2), 92–94.

Starr, C. K. & Sakagami, S. F. (1987). An extraordinary concentration of stingless bee colonies in the Philippines.Leiden, pp 610.

Strand, E. **(**1911a). Faunistische und systematische Notizen über afrikanische Bienen. *Wiener Entomologische Zeitung, 30*, 135–159.

Strand, E. **(**1911b). Apidae. Wissenschaftliche Ergebnisse der Deutschen Zentral-Afrika, Bd. 3. Leipzig, Klinkhandte Biermann. pp 135–166.

Stuart, R. J. (1988). Collective cues as a basis for nestmate recognition in polygynous leptothoracine ants. *Proceedings of the National Academy of Sciences*, U.S.A. *85*, 4572-4575.

Takahashi, J., Martin, S. J., Ono, M. & Shimizu, I. (2010). Male production by nonnatal workers in the bumblebee, *Bombus deuteronymus* (Hymenoptera: Apidae). *Journal of Ethology*, *28*, 61-66.

Tofilski, A. (2008). Using geometric morphometrics and standardmorphometry to discriminate three honeybee subspecies *Apidologie, 39*, 558–563.

Vachal, J. **(**1903). Hyménoptères du Congo Francais Rapportés par l'ingénieur J. Bouyssou. *Mellifera*. *Annales de la Société entomologique de France, 72*, 358–400.

Van Oystaeyen, A, Alves, D. A, Oliveira, R. C, do Nascimento, D. L, do Nascimento, F. S, Billen, J. & Wenseleers, T. (2013). Sneaky queens in *Melipona* bees selectively detect and infiltrate queenless colonies. *Animal Behaviour, 86*, 603-609.

van Veen, J. W & Sommeijer, M. J. (2000). Colony reproduction in *Tetragonisca angustula* (Apidae, Meliponini).*Insectes Soc., 47*, 70–75.

van Zweden, J. S. & d'Ettorre, P. (2010). Nestmate recognition in social insects and the role of hydrocarbons.*Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology, 11*, 222-243.

Velthuis, H. W., Koedam, K. & Imperatriz-Fonseca, V. (2005). The males of *Melipona* and other stingless bees, and their mothers, *Apidologie, 36*, 169–185.

Venturieri, G. C. (2009). Effect of landscape change on the structure of the stingless bee community (Hymenoptera: Apidae) in Meta. *Colombia Mol Res., 8*(2), 684-689.

Verma, L. R., Mattu, V. K. & Daly, H. V. (1994). Multivariate morphometrics of the Indian honeybee in the northwest Himalayan region.*Apidologie*, *25*, 203–223.

Vit, P. (1999). A draft for stingless bee money regulations.*Annual meeting of the International Honey Commission*. Dijon, France.

Wasserman, L. (2004). All of Statistics: A concise Course in Statistical inference. ISBN 0-387-402721.

Wenseleers, T., Alves, D. A., Francoy, T. M., Billen, J. & Imperatriz-Fonseca, V. L. (2011). Intraspecific queen parasitism in a highly eusocial bee.*Biology Letters*, *7*, 173-176.

Wenseleers, T. & Ratnieks, F. L. W. (2004). Tragedy of the commons in Melipona bees.*Proceedings of the Royal Society B*, *271*, 310-312.

Wenseleers, T.; Ratnieks, F. L. W.; Ribeiro, M. D. F.; Alves, D. D. A. & Imperatriz-Fonseca, V. L. (2005). Working-class royalty: Bees beat the caste system. *Biology Letters 1*(2), 125–128.

Whitfield, C. W., Behura, S. K., Berlocher, S. H., Clark, A. G., Johnston, J. S., Sheppard, W. S., Smith, D. R., Suarez, A. V., Weaver, D. & Tsutsui, N. D. (2006). Thrice out of Africa: Ancient and recent expansions of the honey bee, *Apis mellifera*. *Science, 314*, 642–645.

Whitfield, J. B., Cameron, S. A., Huson, D. H & Steel, M. A. (2008). Filtered Z-closure super networks for extracting and visualizing recurrent signal from incongruent gene trees.*Systematic Biology, 57*, 939-947.

Wiley, D. F., Amenta, N. & Alcantara, D. A. (2005). Evolutionary morphing. Proceedings of the IEEE Visualizations, 431–438.

Wille, A. & Michener, C. D. (1973). The nest architecture of stingless bees with special references to those in Costa Rica.*Revista de Biological Tropical 21*, 271-278.

Wille, A. & Orozco, E. (1975). Observations on the founding of a new colony by *Trigona cupira* (Hymenoptera: Apidae) in Costa Rica. *Rev Biol Trop., 22*, 253–287.

Wille, A. (1979). Phylogeny and relationships among the genera and subgenera of the stingless bees (Meliponine) of the world.*Rev. Biol Trop*., *27*,241-77.

Wille, A. (1983). Biology of the stingless bees.*Annual Review of Entomology*, *28*, 41-64.

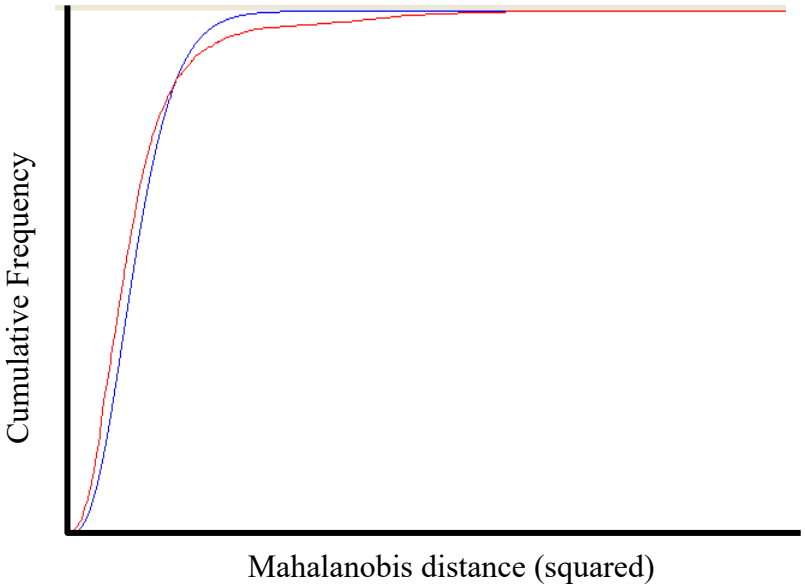
Wilson, E. O. (1971). The Insect Societies. Cambridge, Massachusetts: Harvard University Press.

Winfree, R., Griswold, T. & Kremen, C. (2007). Effect of Human Disturbance on Bee Communities in a Forested Ecosystem. *Conservation Biology*, *21*(1), 213-223.

Woyke, J. (1992). Diurnal and seasonal variation in defensive behaviour of African bees *Apis mellifera adansonii* in Ghana. *Apidologie, Springer* Verlag (Germany) *23*(4), 311-322.

Zeder, M. A., Emshwiller, E., Smith, B. D. & Bradley, D. G. (2006). *Trends in Genetics*, *22*, 139–155.

APPENDIX A



Appendix 1: Level of deviation from the normal multivariate curve (data outliers) from samples from the three study areas

APPENDIX B

Principal components scores generated from PCA analysis in 2-Dimensions using eight homologous landmarks.

Axis	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
x1	-0.229	-0.1873	0.2528	0.1398	-0.099	-0.081	-0.288	-0.024	0.5323	-0.137	0.165	0.1978
y1	-0.1855	0.0606	0.2885	-0.324	0.0207	0.0411	-0.227	0.126	0.0317	-0.063	-0.203	-0.545
x2	-0.1121	-0.1476	0.1863	0.2467	0.0195	-0.141	0.4002	-0.077	-0.507	0.2573	-0.097	-0.208
y2	-0.1353	0.0867	0.1271	-0.154	0.075	0.0333	0.0342	-0.044	-0.268	0.1046	0.186	0.7065
x3	0.1004	0.0826	-0.372	-0.094	0.322	-0.058	0.0911	0.6254	0.1139	-0.028	-0.38	0.1669
y3	0.0122	0.1371	-0.226	0.1225	-0.167	-0.178	0.4288	0.1522	0.3876	0.3071	0.468	-0.203
x4	0.0845	0.1115	-0.331	-0.081	0.505	0.3157	-0.014	-0.512	0.0461	-0.111	0.243	-0.166
y4	0.1093	0.0792	0.0672	0.3486	0.0058	-0.242	0.3025	-0.271	0.1285	-0.579	-0.359	0.0713
x5	0.3629	0.011	-0.068	-0.335	-0.246	-0.538	-0.209	-0.324	0.0298	0.2756	-0.165	0.046
y5	-0.0825	0.1036	-0.125	0.3457	-0.086	0.3835	-0.235	-0.171	0.1295	0.5013	-0.435	0.0618
x6	0.5497	-0.1247	0.4693	0.1432	-0.06	0.3946	0.0641	0.2009	0.0386	0.0152	0.116	0.0039
y6	0.1137	0.1063	0.0701	0.2835	0.3735	-0.333	-0.464	0.1913	-0.267	-0.024	0.29	-0.098
x7	-0.5885	-0.3281	-0.001	-0.022	0.0768	-0.059	0.056	0.0162	0.0017	0.007	0.001	-0.009
y7	0.0108	0.0584	0.2261	-0.548	0.0971	0.1347	0.2746	-0.039	0.0687	-0.022	-0.033	0.042
x8	-0.168	0.5825	-0.136	0.003	-0.519	0.166	-0.1	0.0949	-0.255	-0.28	0.117	-0.032
y8	0.1573	-0.632	-0.428	-0.074	-0.32	0.1602	-0.113	0.0557	-0.21	-0.224	0.086	-0.035

APPENDIX C

Discriminant analysis for Kwesi Gyan and Science Botanical Garden study areas with absent tube length.

Comparison: Kwesi Gyan/Absent -- Science Botanical Garden/Absent

Difference between means:
Procrustes distance: 0.00816730
Mahalanobis distance: 1.7777
T-square: 75.8464, P-value (parametric): <.0001
P-value for permutation tests (1000 permutation runs):
Procrustes distance: 0.0070
T-square: <.0001
Classification/misclassification tables
Group 1: Kwesi Gyan/Absent
Group 2: Science Botanical Gardens/Absent
From discriminant function:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	98	22	120
Group 2	3	27	30

From cross-validation:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	89	31	120
Group 2	7	23	30

APPENDIX D

Variability among species from Mfuom and Science Botanical Gardens with absent tube length

Discriminant Function Analysis 'H3Discriminant function ...'
Comparison: Mfuom/Absent -- Science Botanical Gardens/Absent

Difference between means:
Procrustes distance: 0.01782904
Mahalanobis distance: 2.6709
T-square: 183.4414, P-value (parametric): <.0001
P-values for permutation tests (1000 permutation runs):

Procrustes distance: <.0001

T-square: <.0001

(Note: The permutation test using the T-square statistic is equivalent to a test using Mahalanobis distance.)

Classification/misclassification tables
Group 1: Mfuom/Absent
Group 2: Science Botanical Gardens/Absent
From discriminant function:

True Group	Allocated to Group 1	Group 2	Total
Group 1	165	15	180
Group 2	2	28	30

From cross-validation:

True Group	Allocated to Group 1	Group 2	Total
Group 1	160	20	180
Group 2	2	28	30

APPENDIX E

Discriminant Function Analysis and cross-validation score among species from Kwesi Gyan and Mfuom communities with absent entrance tubes.

Comparison: Kwesi Gyan/Absent -- Mfuom/Absent

Difference between means:
Procrustes distance: 0.01314279
Mahalanobis distance: 1.9371
T-square: 270.1643, P-value (parametric): <.0001
P-values for permutation tests (1000 permutation runs):

Procrustes distance: <.0001

T-square: <.0001

(Note: The permutation test using the T-square statistic is equivalent to a test using Mahalanobis distance.)

Classification/misclassification tables
Group 1: Kwesi Gyan/Absent
Group 2: Mfuom/Absent
From discriminant function:

True Group	Allocated to Group 1	Group 2	Total
Group 1	106	14	120
Group 2	31	149	180

From cross-validation:

True Group	Allocated to Group 1	Group 2	Total
Group 1	102	18	120
Group 2	33	147	180

APPENDIX F

Variability among the two communities with short entrance tube length.

Discriminant Function Analysis 'H5Discriminant function ...'
Comparison: Kwesi Gyan/Short -- Mfuom/Short

Difference between means:
Procrustes distance: 0.00890674
Mahalanobis distance: 1.2569
T-square: 289.6926, P-value (parametric): <.0001
P-value for permutation test (1000 permutation runs):
Procrustes distance: <.0001
T-square: <.0001
(Note: The permutation test using the T-square statistics is equivalent to a test using Mahalanobis distance)
Classification/misclassification tables
Group 1: Kwesi Gyan/Short
Group 2: Mfuom/Short
From discriminant function:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	173	67	240
Group 2	203	574	777

From cross-validation:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	168	72	240
Group 2	212	565	777

APPENDIX G

The extent of variability within Kwesi Gyan and Science Botanical Garden with short entrance tubes

Discriminant Function Analysis 'H2Discriminant function ...'
Comparison: Kwesi Gyan/Short -- Science Botanical Garden/Short

Difference between means:
Procrustes distance: 0.00950225
Mahalanobis distance: 1.1092
T-square: 176.3685, P-value (parametric): <.0001

Classification/misclassification tables
Group 1: Kwesi Gyan/Short
Group 2: Science Botanical Garden/Short
From discriminant function:

True Group	Allocated to Group 1	Group 2	Total
Group 1	166	74	240
Group 2	96	260	356

From cross-validation:

True Group	Allocated to Group 1	Group 2	Total
Group 1	163	77	240
Group 2	100	256	356

APPENDIX H

Variability among species from two different sites with short entrance tubes

Discriminant Function Analysis 'H7Discriminant function ...'
Comparison: Mfuom/Short -- Science Botanical Garden/Short

Difference between means:
Procrustes distance: 0.01501409
Mahalanobis distance: 2.0375
T-square: 1013.5179, P-value (parametric): <.0001
P-values for permutation tests (1000 permutation runs):

Procrustes distance: <.0001

T-square: <.0001

(Note: The permutation test using the T-square statistic is equivalent to a test using Mahalanobis distance.)

Classification/misclassification tables
Group 1: Mfuom/Short
Group 2: Science Botanical Garden/Short
From discriminant function:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	669	108	777
Group 2	70	286	356

From cross-validation:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	666	111	777
Group 2	75	281	356

APPENDIX I

Species with medium entrance tubes from two communities showing variability among members

Discriminant Function Analysis 'H4Discriminant function ...'
Comparison: Kwesi Gyan/Medium -- Mfuom/Medium

Difference between means:
Procrustes distance: 0.03404321
Mahalanobis distance: 2.1038
T-square: 189.6776, P-value (parametric): <.0001
P-values for permutation tests (1000 permutation runs):

Procrustes distance: <.0001

T-square: <.0001

(Note: The permutation test using the T-square statistic is equivalent to a test using Mahalanobis distance.)

Classification/misclassification tables
Group 1: Kwesi Gyan/Medium
Group 2: Mfuom/Medium
From discriminant function:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	51	9	60
Group 2	22	128	150

From cross-validation:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	49	11	60
Group 2	24	126	150

APPENDIX J

Medium entrance tubes from Mfuom and Science Botanical Gardens with variation among species

Discriminant Function Analysis 'H6Discriminant function ...'
Comparison: Mfuom/Medium -- Science Botanical Gardens/Medium

Difference between means:
Procrustes distance: 0.02175347
Mahalanobis distance: 2.3386
T-square: 220.0888, P-value (parametric): <.0001

Classification/misclassification tables
Group 1: Mfuom/Medium
Group 2: Science Botanical Gardens/Medium
From discriminant function:

True Group	Allocated to Group 1	Group 2	Total
Group 1	129	21	150
Group 2	3	52	55

From cross-validation:

True Group	Allocated to Group 1	Group 2	Total
Group 1	125	25	150
Group 2	4	51	55

APPENDIX K

Variation in medium entrance tube length in two study areas

Discriminant Function Analysis 'H Discriminant function ...'
Comparison: Kwesi Gyan/Medium -- Science Botanical Gardens/Medium

Difference between means:
Procrustes distance: 0.01854714
Mahalanobis distance: 2.9041
T-square: 242.0207, P-value (parametric): <.0001
P-values for permutation tests (1000 permutation runs):

Procrustes distance: <.0001

T-square: <.0001

(Note: The permutation test using the T-square statistic is equivalent to a test using Mahalanobis distance.)

Classification/misclassification tables
Group 1: Kwesi Gyan/Medium
Group 2: Science Botanical Gardens/Medium
From discriminant function:

True Group	Allocated to Group 1	Group 2	Total
Group 1	54	6	60
Group 2	4	51	55

From cross-validation:

True Group	Allocated to Group 1	Group 2	Total
Group 1	53	7	60
Group 2	6	49	55

APPENDIX L

Comparing two different nest entrance tube length within two experimental sites

Discriminant Function Analysis 'Discriminant function ...I'

Comparison: Mfuom/Medium -- Science Botanical Gardens/Long

Difference between means:

Procrustes distance: 0.02186344

Mahalanobis distance: 2.4066

T-square: 245.2488, P-value (parametric): <.0001

P-values for permutation tests (1000 permutation runs):

Procrustes distance: <.0001

T-square: <.0001

(Note: The permutation test using the T-square statistic is equivalent to a test using Mahalanobis distance.)

Classification/misclassification tables

Group 1: Mfuom/Medium

Group 2: Science Botanical Garden/Long

From discriminant function:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	134	16	150
Group 2	7	52	59

From cross-validation:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	129	21	150
Group 2	7	52	59

APPENDIX M

DFA and cross-validation score from samples with two different entrance tube lengths within two experimental sites

Discriminant Function Analysis 'Discriminant function ...J'

Comparison: Kwesi Gyan/Medium -- Science Botanical Garden/Long

Difference between means:

Procrustes distance: 0.01721781

Mahalanobis distance: 2.8906

T-square: 248.5658, P-value (parametric): <.0001

P-values for permutation tests (1000 permutation runs):

Procrustes distance: <.0001

T-square: <.0001

(Note: The permutation test using the T-square statistic is equivalent to a test using Mahalanobis distance.)

Classification/misclassification tables

Group 1: Kwesi Gyan/Medium

Group 2: Science Botanical Gardens/Long

From discriminant function:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	56	4	60
Group 2	4	55	59

From cross-validation:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	54	6	60
Group 2	7	52	59

APPENDIX N

DFA and cross-validation score for samples from the same experimental site with different nest entrance tube lengths

Discriminant Function Analysis 'Discriminant function ...K'

Comparison: Science Botanical Gardens/Long -- Science Botanical Gardens/Medium

Difference between means:

Procrustes distance: 0.00808942

Mahalanobis distance: 2.2850

T-square: 148.6267, P-value (parametric) : <.0001

P-values for permutation tests (1000 permutation runs):

Procrustes distance: 0.0010

T-square: <.0001

(Note: The permutation test using the T-square statistic is equivalent to a test using Mahalanobis distance)

Classification/misclassification tables

Group 1: Science Botanical Gardens/Long

Group 2: Science Botanical Gardens/Medium

From discriminant function:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	53	6	59
Group 2	5	50	55

From cross-validation:

True	Allocated to		
Group	Group 1	Group 2	Total
Group 1	49	10	59
Group 2	6	49	55